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Long-term dietary pattern of fecal donor correlates with butyrate production and markers of protein fermentation during in vitro fecal fermentation

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ABSTRACT

Diet influences gut microbiota composition. Therefore, we hypothesized that diet would impact the extent of dietary fiber utilization and the types of metabolic end-products produced by the microbiota during in vitro fecal fermentation. By obtaining long-term dietary records from fecal donors, we aimed to determine the correlations between dietary intake variables and dietary fiber degradation and short-/branched-chain fatty acid (BCFA) and ammonia production during in vitro fecal fermentation. Eighteen subjects completed 1-year diet history questionnaires and provided fecal samples that were used for in vitro fermentation of a whole wheat substrate. The percentage of dietary fiber fermented was not correlated with nutrient intakes; however, butyrate production was correlated with fecal donor intake of many nutrients of which principal component analysis revealed were mostly contributed by grain-, nut-, and vegetable-based foods. Negative correlations were found for propionate with intake of total carbohydrate, added sugar, and sucrose and for ammonia and BCFA production with intake of unsaturated fats. Thus, our analysis did not support our first hypothesis: the percentage of dietary fiber fermented during in vitro fermentation was not correlated with dietary records. However, production of butyrate; BCFA; ammonia; and, to a lesser extent, propionate was correlated with the diet records of fecal donors, thus supporting our second hypothesis. These results suggest that diets high in plant-based foods and high in unsaturated fats are associated with microbial metabolism that is consistent with host health.

1. Introduction

The human gut is colonized by the gut microbiota, a complex and dynamic microbial community whose collective genome exceeds the size of the human genome by 2 orders of magnitude [1]. The gut microbiota is involved in host energy harvest and storage [2], immune response [3], and development of metabolic syndrome [4].

Previous reports have focused on how diet changes the gut microbiota composition [5-8]. One study found that the fecal microbiota from children in Burkina Faso consuming a diet high in dietary fiber contained an enrichment of *Prevotella* and *Xylanibacter*, which contain species that are evolved to efficiently use cellulose and xylans, compared with children in the European Union consuming more refined diets [5]. In another cross-sectional study, long-term intake of protein and animal

Abbreviations: BCFA, branched-chain fatty acid; DHQ, diet history questionnaire; FFQ, food frequency questionnaire; MUFA, monounsaturated fatty acid; NHANES, National Health and Nutrition Examination Survey; NA, not applicable; PUFA, polyunsaturated fatty acids; PC, principal component; PCA, principal component analysis; Sat. fat, saturated fat; SCFA, short-chain fatty acid.

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fat led to enrichment in *Bacteroides* compared with long-term intake of carbohydrates, which is associated with *Prevotella* [8]. Controlled trials have shown that diet can rapidly alter the types of bacteria that appear in the feces. Russell et al [7] showed a decline in *Roseburia/Eubacterium rectale* when subjects were on a high-protein, low-carbohydrate diet compared with a maintenance diet. The susceptibility of these butyrate producers to carbohydrate intake was confirmed in subjects consuming diets devoid of carbohydrate [6].

Although knowledge on the compositional changes in the gut microbiota is of interest, the products of gut microbiota metabolism are of at least equivalent importance. For instance, short-chain fatty acids (SCFAs), the major metabolites produced by gut microbiota, have been suggested to be important regulators of energy balance, gut inflammation signaling, and insulin sensitivity [7]. Conversely, ammonia and branched-chain fatty acid (BCFA) productions have been implicated in having undesirable effects on host health [7,9].

Past approaches to quantify these metabolites have included analysis of fecal samples for the compounds themselves or for genes involved in their production [7,10,11]. In addition, others have drawn conclusions about metabolite production based on the proportions of bacteria present [12,13]. However, given that these metabolites are absorbed and metabolized by the colonic epithelia cells, these approaches either do not reflect the actual production or are only semiquantitative [14,15].

When addressing changes in the gut microbiota in response to diet, a wealth of research has concentrated on host benefits of prebiotic oligosaccharide consumption (eg, fructans, galacto-oligosaccharides) [16]. These prebiotics are highly fermentable by the gut microbiota and result in desirable shifts in gut microbiota composition. However, intake of these oligosaccharides in most diets (1-4 g/d) [17] is dwarfed by the quantities of complex and poorly fermented dietary fibers (eg, cellulose, cross-linked arabinoxylan, and complex pectic substances) that typically make up the substrates for gut bacteria (11-20 g/d) [18]. Although these dietary fibers are generally poorly fermented by gut bacteria, it could be that adequate and continuous dietary exposure of these materials to the gut microbiota would cause compositional shifts such that the microbiota are more able to use these substrates with subsequent benefits to the host [5].

Unfortunately, quantitative measures of fermentation of specific dietary fibers and resulting SCFA production and by the gut microbiota in humans are not practical in vivo. Although conditions in in vitro batch fermentations are very different from conditions in the large intestine (eg, no absorption of metabolites, water, minerals, or other nutrients; no pH control [except buffering]; no mucus layer; etc), batch in vitro fecal fermentation models may be an ideal way to evaluate both the microbial production of certain metabolites as well as utilization of complex substrates because compounds are not absorbed and can easily be quantified [19]. Furthermore, in our previous study, we found that changes in the gut microbiota during 12 hours of in vitro fermentation were similar to changes reported in the literature for human trials [20].

We hypothesized that diet of the fecal donor would impact the extent of dietary fiber utilization and the types of metabolic end-products produced by the microbiota during in vitro fecal fermentation. To test our hypothesis, we assessed long-term dietary patterns of subjects using a food frequency questionnaire (FFQ) and then used stool samples collected from these individuals as a source of bacteria for in vitro fermentation using predigested whole wheat flour as a substrate. We then quantified carbohydrate fermented, SCFA, BCFA, and ammonia produced during fermentation and correlated the results with intake of nutrients and food groups of the stool donors. This research may allow us to identify dietary strategies to alter the production of metabolites by the gut microbiota in a manner that is consistent with human health.

2. Methods and materials

2.1. *Subjects, dietary records, and stool sample collection*

Individuals who were 19 years or older, considered themselves to be in generally good health with no digestive diseases or dietary restrictions (excluding voluntary dietary restrictions), and had not taken antibiotics in the last 6 months were recruited using electronic and paper advertisements on campus at the University of Nebraska-Lincoln. Individuals who met these criteria were invited to participate in this study.

Subjects completed the past-year (long-term) food intake diet history questionnaire (DHQ) II online [21]. The DHQ II is a validated FFQ consisting of 134 food items and 8 dietary supplement questions [22]. The survey included questions about seasonal differences in food intakes. Each subject received a unique login and password and completed the DHQ II after brief instructions on how to properly complete the questionnaire. Subjects' responses were analyzed using Diet*Calc software (Bethesda, MD, USA) [23]. This software used food frequency responses from subjects to estimate daily food intakes and serving sizes based on combined results from the National Health and Nutrition Examination Surveys (NHANES) conducted in 2001 to 2002, 2003 to 2004, and 2005 to 2006. Daily nutrient and food group intakes were generated using the DHQ Database [24], which computed nutrient estimates using data from the USDA Nutrient Database for Standard Reference [25] and the Nutrient Data System for Research [26]. The My Pyramid Equivalents Database [27] was used to calculate daily servings from various food groups.

At the time subjects received their login and password information for the DHQ II, they received stool collection materials and instructions on how to use them. Subjects were instructed to bring a stool sample to the laboratory within 2 hours of defecation. The stool collection materials included a collection container with tight-fitting lid that fit under the toilet seat (Commode Specimen Collection System; Fisher Scientific, Pittsburgh, PA, USA), an insulated cooler with cold packs, and an anaerobic gas-generating tablet (Anaerocult C; BD GasPak, Franklin Lakes, NJ, USA), which subjects placed inside the collection container immediately after defecation and before securing the lid and packing in the cooler. Immediately upon receiving stool samples from subjects, study personnel transferred the sample to an anaerobic hood (Bactron X; Sheldon Manufacturing, Cornelius, OR, USA) where it was