Autophagic punctum

Addendum to: Zumbrun SD, Melton-Celsa AR, Smith MA, Gilbreath JJ, Merrell DS, O’Brien AD. Dietary choice affects Shiga toxin-producing Escherichia coli strain that produces Stx2*. We further demonstrated that mice on an HFD not only had elevated levels of butyrate in the gut but also had enhanced expression of the Stx receptor, Gb3, in or on the mouse gut epithelia and kidney. We theorize that the enhanced virulence of Stx2+ O157:H7 in mice fed an HFD results from two factors. First, we hypothesize that high fiber diets increase local and systemic levels of butyrate and that these elevated butyrate concentrations lead to more Gb3-expressing colonic and renal tubular epithelial cells, respectively. The enrichment of Gb3 on cells in the gut results in more Stx2 binding to these enterocytes and more transfer of Stx2 into the bloodstream. This increased toxaemia, in turn, leads to more Stx2 that is available to bind to the renal tubules now enriched in Gb3. More tubular necrosis then ensues that ultimately causes more morbidity and mortality in HFD-fed mice than in low fiber diet (LFD)-fed animals. An HFD reduces the population of commensal Escherichia coli species in the gut,* therefore, we speculate that a niche is made available for the incoming STEC and facilitates the increased colonization by Stx2+ O157:H7 that we observed in the HFD mice. A model of the two-pronged proposed mechanism of the enhanced virulence of STEC is shown in Figure 1.

**Fiber, Butyrate, and Gb3**

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When a healthy diet turns deadly

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The health benefits of a high fiber diet (HFD) result in part from the action of metabolic end products made by gut commensals on the host epithelium. Butyrate is one such beneficial metabolite; however, butyrate paradoxically enhances the capacity of Escherichia coli-produced Shiga toxin type 2 (Stx2) to kill tissue culture cells. We recently showed that mice fed an HFD exhibited increased butyrate in gut contents and had an altered intestinal microbiota with reduced numbers of Escherichia species. Furthermore, mice fed an HFD and infected with Stx-producing *E. coli* (STEC) were colonized to a higher degree, lost more weight and succumbed to infection at greater rates compared with STEC-infected low fiber diet animals. The HFD animals showed higher levels of the Stx receptor globotriaosylceramide (Gb3) in both the gut and kidneys. We speculate that an HFD that leads to increased intestinal butyrate and Gb3 in the intestines and kidneys may explain the higher rate of the hemolytic uremic syndrome in females over males.

**Introduction**

Shiga toxin (Stx)-producing *E. coli* (STEC) are food- and water-borne pathogens that cause bloody diarrhea. The hemolytic uraemic syndrome (HUS) occurs as a sequel of STEC infection in 4–30% of the cases.* Possible host-related influences on which patients will develop the HUS are not defined apart from younger age* and female gender.* However, we recently showed that when the food regimen is altered in mice to a high fiber diet (HFD), there was an increase in butyrate in the gut, and the animals became more susceptible to infection by an *E. coli* O157:H7 strain that produces Stx2.* We further demonstrated that mice on an HFD not only had elevated levels of butyrate in the gut but also had enhanced expression of the Stx receptor, Gb3, in or on the mouse gut epithelia and kidney. We theorize that the enhanced virulence of Stx2+ O157:H7 in mice fed an HFD results from two factors. First, we hypothesize that high fiber diets increase local and systemic levels of butyrate and that these elevated butyrate concentrations lead to more Gb3-expressing colonic and renal tubular epithelial cells, respectively. The enrichment of Gb3 on cells in the gut results in more Stx2 binding to these enterocytes and more transfer of Stx2 into the bloodstream. This increased toxaemia, in turn, leads to more Stx2 that is available to bind to the renal tubules now enriched in Gb3. More tubular necrosis then ensues that ultimately causes more morbidity and mortality in HFD-fed mice than in low fiber diet (LFD)-fed animals. Second, an HFD reduces the population of commensal *Escherichia coli* species in the gut,* therefore, we speculate that a niche is made available for the incoming STEC and facilitates the increased colonization by Stx2+ O157:H7 that we observed in the HFD mice. A model of the two-pronged proposed mechanism of the enhanced virulence of STEC is shown in Figure 1.

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Keywords: butyrate, *Escherichia coli* O157:H7, globotriaosylceramide, microbiota, colon, kidney, hemolytic uraemic syndrome, Stx, diet

Abbreviations: STEC, *Shiga toxin-producing Escherichia coli*; HFD, high fiber diet; HUS, hemolytic uremic syndrome; Stx, *Shiga toxin*; Gb3, globotriaosylceramide; SCFA, short-chain fatty acids; NSP, non-starch polysaccharide; RS, resistant starch; EAEC, enteraggregative *E. coli*
fiber diet increases fecal butyrate and the concentrations of other short-chain fatty acids (SCFA). The specific fiber source plays a considerable role, however, in the amount of butyrate produced. What is popularly known as “dietary fiber” is the non-starch polysaccharide (NSP), or non-α-glucan polysaccharide. NSP includes plant material such as pectins, guar and cellulose. Fiber in the human diet is typically NSP. Another fiber type is resistant starch (RS). RS is a type of starch that resists digestion in the small bowel and is thus available as a substrate for fermentation in the large bowel. Examples of RS include corn, peas, beans, cracked grains, potatoes and bananas. In general, the amount of butyrate produced in the gut for a given fiber type is governed by the fermentability of a given fiber substrate and the quantity of the fiber present in the gut. For example, pigs fed white rice, which contains low fiber content (RS type) have a distal colonic butyrate pool of 0.06 mol, compared with 0.47 mol in pigs fed brown rice, which has high fiber content (RS type). In the latter example, most of the white rice was digested in the stomach and small intestines, while the brown rice was more resistant to digestion and provided fermentable material to the colon for production of SCFA such as butyrate.

Butyrate has a profound effect on cell morphology and function and acts as a primary energy source for colonic enterocytes. In fiber-rich diets, crypt deepening occurs, and crypt duplication can take place, events that increase the number of crypts per unit length and total crypt depth in response to diet. Hence, butyrate levels alter the mucosa, although the possible effect of butyrate-mediated anatomical and physiological changes in the gut on STEC colonization is unknown. However, butyrate and other SCFA are most concentrated on the right side of the colon and levels fall progressively toward the distal colon. On note, STEC-induced pathology occurs in that same region (the ascending and transverse colon). Indeed, in STEC infections, the cecum and right colon are described as markedly abnormal, exhibiting edema, erosion, hemorrhage and surface ulceration, whereas the descending colon typically has mild or no changes. We speculate that the reason for the apparent co-localization of the highest levels of intestinal butyrate and STEC-evoked pathology is due to butyrate-mediated increased levels of Gb3 expression in the same region. Despite the profound effect of butyrate on Gb3 expression, additional effects of the butyrate cannot be ruled out. Butyrate and other SCFA produced in the large bowel are rapidly absorbed and pass into the portal vein, encounter the liver and then circulate through the body to the kidney. The kidney naturally expresses high levels of Gb3, and we found that an HFD can increase those levels further.

A disparity exists between those who develop the HUS following STEC infection and those who do not, i.e., children <10 years old are 10 times more likely on average to acquire that serious sequela. While there is some evidence of age-related changes in intestinal bacterial populations, we are not aware of any studies that assess general butyrate content as a function of age. We therefore asked whether an inherent difference in the capacity to produce butyrate exists between children and adults by assessing
the butyrate content in stool from those groups. The amount of butyrate measured in stool is likely an underestimate of the overall colonic concentration of that fatty acid because greater than 95% of gut butyrate is absorbed systemically by the time digesta reaches the anus.10 We found that although children exhibit a slight trend toward more abundant butyrate, there was no significant difference in butyrate content in the stool of children compared with adults in this study. Figure 2.

Another example of host-related differences found during STEC outbreaks is that in two recent large, produce-associated outbreaks, women were more likely to develop the infection than children or men.3,21 Could the disparity in STEC cases for these two outbreaks simply be attributed to the healthier eating habits of women compared with men?22 Maybe so for the disparity in the number of STEC infections between females and males, but, for the HUS, women do appear to be generally more susceptible to that sequela.6 Further, in one of the produce examples—the sprout-linked German outbreak due to an unusual Stx2+ enteropathogenic E. coli—30% of the STEC cases in women resulted in the HUS, whereas just 15% of the infected men developed the HUS.23 So, if healthier eating habits alone cannot explain the difference in the rate of the HUS between females and males where else can we look for answers? A study by Lampe, et al. suggests that there may be gender differences in colonic function. In that study, despite equal fiber intakes by men and women, mean fecal transit times were consistently faster and fecal weights greater for men than women on all diets.24 This longer transit time resulted in enhanced digestibility in women and seemed to be primarily due to greater digestion of wheat bran and vegetable fiber diets.25 The increased digestion in women would lead to more gut butyrate as would the diet rich in fiber. Therefore, the reason females are more likely to develop the HUS than males may be due to their higher concentrations of intestinal butyrate that subsequently lead to an unusual Stx2+ enteropathogenic E. coli.6—30% of the STEC cases in women resulted in the HUS; whereas patients are symptom-based, and the use of antibiotics or anti-motility agents are generally contraindicated.26 However, intravenous volume expansion may reduce the likelihood of anuria and the HUS.26 In contrast, for diarrhea due to cholera, oral rehydration (ORS) therapies can shorten the duration and volume of diarrhea. The addition of guar gum or amylase-resistant starch to ORS may reduce diarrheal symptoms that those carbohydrates are fermented in the colon, the SCFA are then absorbed, and as a consequence, net fluid absorption is increased and the volume and duration of diarrhea is decreased.26 We caution however, that the use of starch- or guar gum-supplemented ORS for the treatment of non-cholera diarrhea mediated by STEC might be deleterious for the patient as the predicted increase in SFCA in the colon could lead to increased levels of Gb3 in the colon and the kidney and, consequently, could increase the susceptibility of the patient to the effects of the Stxs.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

We thank Dr Phil Tan (University of Washington, St. Louis, MO) for providing pediatric stool samples; Dr Louise Teel (Uniformed Services University, Bethesda, MD) for providing adult stool samples; and Kenneth Gable for assistance with GC/MS analysis. This work was supported by NIH grant R37 AI020148 to ADO.

References

1. Mood PS, Shaffer S, Davis V, McCray LF, Brono JS, Shapiro C, Griffin PM, Tanner RV. Food-related illness and death in the United States. Emerg Infect Dis 1999; 5:607-20; PMID:10515177; http://dx.doi.org/10.3201/eid0505.990502

2. Renz PC, Ordinio E, Liao H, Wells GA, McLain JN. A prospective study of exposure to verotoxin-producing Escherichia coli among Canadian children with hemolytic uremic syndrome. The CPKDRC co-investigators. Epidemiol Infect (1993) 110:1-7. PMID:8432313; http://dx.doi.org/10.1017/S0950268800018415

3. California Food Emergency Response Team. California Department of Health Services, U.S. Food and Drug Administration. Investigation of an Escherichia coli O157:H7 outbreak associated with Dole pre-packaged spinach. California Department of Health Services, 2007.

4. Johnson RV, Charlton R, Wilson JR, Read SC, Rubin E, Ranwijk S, et al. Growing concerns and more outbreaks involving non-O157:H7 enteropathogenic Escherichia coli. J Food Prot 1996; 59:112-22.

5. Barten Birkhed G, Jonass TE, Vigar DJ, Long C, Marcus R, Smith B, Thomas S, Zander S, Fuller AE, Israel S, et al. FoodNet Working Group. Diarrheal disease active surveillance network (FoodNet), 1994-2005. J Infect Dis 2011; 204:263-7; PMID:22076957; http://dx.doi.org/10.1093/infdis/jir263

Figure 2. Butyrate content in the stool of uninfected children and adults. Butyrate content in the stool of children <10 years old is not statistically different from adults >25 y old. Butyrate was extracted from frozen stool samples and measured by acidic extraction followed by gas chromatography/mass spectroscopy as described previously.7 Error bars represent standard error of the mean.
14. Gilbreath JJ, Merrell DS, O’Brien AD. Dietary choice affects Shiga toxin-producing Escherichia coli O157:H7 colonization and disease. Proc Natl Acad Sci U S A 2013; 110:E2126-33; PMID:23690602;
dx.doi.org/10.1146/annurev-physiol-021909-135817

23. Troost FJ, Brummer RJ. Review article: the role of dietary fiber in human nutrition, Third Edition. Boca Raton, FL: CRC Press LLC, 2001.

24. Macfarlane GT, Macfarlane AJ, Park: Flinders University of South Australia, 1995.

25. Older SD, Melton-Celsa AR, Smith MA, Runyon SD, Metcalf CR, Suster S, Weiss LM, ed. Modern Surgical Pathology. Philadelphia: Saunders, 2009.