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Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice

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ABSTRACT

Obesity is characterized by altered gut homeostasis, including dysbiosis and increased gut permeability closely linked to the development of metabolic disorders. Milk oligosaccharides are complex sugars that selectively enhance the growth of specific beneficial bacteria in the gastrointestinal tract and could be used as prebiotics. The aim of the study was to demonstrate the effects of bovine milk oligosaccharides (BMO) and *Bifidobacterium longum* ssp. *infantis* (*B. infantis*) on restoring diet-induced obesity intestinal microbiota and barrier function defects in mice. Male C57/BL6 mice were fed a Western diet (WD, 40% fat/kcal) or normal chow (C, 14% fat/kcal) for 7 wk. During the final 2 wk of the study, the diet of a subgroup of WD-fed mice was supplemented with BMO (7% wt/wt). Weekly gavage of *B. infantis* was performed in all mice starting at wk 3, yet *B. infantis* could not be detected in any luminal contents when mice were killed. Supplementation of the WD with BMO normalized the cecal and colonic microbiota with increased abundance of *Lactobacillus* compared with both WD and C mice and restoration of *Allobaculum* and *Ruminococcus* levels to that of C mice. The BMO supplementation reduced WD-induced increase in paracellular and transcellular flux in the large intestine as well as mRNA levels of the inflammatory marker tumor necrosis factor α. In conclusion, BMO are promising prebiotics to modulate gut microbiota and intestinal barrier function for enhanced health.
Key words: prebiotic, obesity, gut homeostasis

INTRODUCTION

Impaired intestinal barrier function and microbiota dysbiosis are involved in a wide range of long-term conditions, including digestive and extra-digestive diseases (Sanz et al., 2010; Bischoff et al., 2014). This has stimulated research to find efficient ways to prevent or treat altered gut homeostasis. Among extra-digestive pathologies, obesity and its downstream complications such as metabolic syndrome and cardiovascular risks, constitute a major economic burden to our societies (Finkelstein et al., 2010). In the last decade, research on the role of the gut microbiota in host physiology, behavior, and metabolism has provided new insights into the causal mechanisms in obesity. Intestinal dysbiosis has been observed in obese patients and in animal models of obesity (Ley et al., 2005, 2006; Hamilton et al., 2015). Transfer of the gut microbiota from obese mice into lean germ-free mice recapitulates the obese phenotype (Turnbaugh et al., 2006), suggesting that the gut microbiota may play a role in the development or maintenance of obesity. Changes in gut barrier function are also seen in obesity and have been proposed to connect microbial dysbiosis to obesity (Raybould, 2012). The gut microbiota and barrier function are highly responsive to gut environment (Kashyap et al., 2013) and are able to be targeted by nutritional or pharmacological means. Thus pre-clinical and clinical trials with probiotics or prebiotics have been used to treat obesity and related co-morbidities.

Milk oligosaccharides are complex sugars that selectively enhance the growth of specific beneficial bacteria in the gastrointestinal system (Smilowitz et al., 2014). Bovine milk is a source of oligosaccharides that resemble human milk oligosaccharides (**HMO**), though with different structures and a much lower concentration than human milk (Chichlowski et al., 2011). The majority of the molecules are more simple in structure compared with those found in human milk (Zivkovic and Barile, 2011). New methods for separation and concentration

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of bovine milk oligosaccharides (**BMO**) from bovine milk industrial streams such as whey permeate are now available. Because whey permeate is a byproduct in the production of whey protein concentrate and is readily available, it is an attractive source of oligosaccharides for potential application in human nutrition (Zivkovic and Barile, 2011; Barile and Rastall, 2013). Our group has shown that BMO enhance the growth of *Bifidobacterium longum* ssp. *infantis* (*B. infantis*) in vitro (D. A. Mills, unpublished data). *Bifidobacterium longum* ssp. *infantis* is a neonate gut commensal that has been shown to confer both intestinal and extra-intestinal health benefit to the host: it modulates barrier function and protects epithelial cells against cytokine or chemical-induced inflammation (Ganguli et al., 2013; Konieczna et al., 2013; Miyauchi et al., 2013; O'Hara et al., 2006). Previous studies from our laboratories have demonstrated that *B. infantis* grown on HMO affected intestinal epithelial function in vitro, compared with lactose-grown control culture (Chichlowski et al., 2012; Wickramasinghe et al., 2015).

Our objective was to establish the proof-of-concept that a combination of dietary BMO and *B. infantis* can reverse the gut microbial dysbiosis and altered gut permeability induced by ingestion of the Western diet (**WD**). To that aim, we gavaged diet-induced obese mice weekly with fresh culture of *B. infantis* and supplemented their diet with BMO. The BMO-supplemented mice exhibited improved gut barrier function and microbiota after 2 wk compared with nonsupplemented obese mice; however, we did not detect *B. infantis* in the luminal contents of the gut 1 wk after the last gavage. This suggests that BMO itself could prove to be beneficial in the treatment of obesity-related gut dysfunction.

MATERIALS AND METHODS

Diets

Bovine milk oligosaccharides were extracted from whey by Hilmar Ingredients (Hilmar, CA). The product was lipid- and protein-free and contained 80% carbohydrates from which lactose represented only 0.02%. Composition of the BMO was obtained by nano-chip HPLC QToF (Figure 1A). The WD+BMO diet was formulated by substituting inulin and part of corn starch by 7% of BMO in a WD (40% fat, #5TJN, TestDiet, St. Louis, MO). The control diet was regular laboratory chow (**C**, #5001, LabDiet, St. Louis, MO). Composition of diets is presented in Figure 1B and ingredient composition of WD and WD+BMO diets in Supplemental Table S1 (https://doi.org/10.3168/jds.2016-11890).

B. infantis Culture

The *B. infantis* ATCC 15697 was grown by a food-grade commercial facility (Culture Systems Inc., Mishawaka, IN) as described previously (Underwood et al., 2013) and stored at −80°C. Purity and number of viable bacteria per gram were confirmed by the investigators every 6 mo by culture. Cultures from stocks frozen at −80°C were grown anaerobically at 37°C in a semisynthetic de Man, Rogosa, Sharpe broth (Becton Dickinson, Franklin Lakes, NJ) supplemented with 1% (wt/vol) L-cysteine hydrochloride. After centrifugation, bacteria were re-suspended in PBS before gavage.

Animals

Animals were maintained and handled in accordance with protocols approved by the Institutional Animal Care and Use Committee (University of California, Davis) under #16076. Male C57/BL6 mice (18.6 ± 0.3 g, n = 18, Harlan, San Diego, CA) were acclimatized to the animal facility and fed the C diet ad libitum. All animals were housed individually at 22°C with a 12:12 h light-dark cycle. After 1 wk of acclimation, 12 mice were switched to the WD diet for a total of 5 wk to establish obesity. At that time, 6 mice continued on the WD, whereas 6 mice switched to the WD+BMO diet for another 2 wk. All animals were gavaged once a week with 0.2 mL of 10^9 cfu/mL of *B. infantis* solution, starting at wk 3. Food and water were provided ad libitum. Body weight and food intake were measured weekly. The protocol is summarized on Figure 1C.

At experiment completion, mice were euthanized by cardiac puncture under deep anesthesia induced with isoflurane. Blood was collected via cardiac puncture in heparinized tubes. Plasma was obtained after centrifugation (4°C; 1,500 × *g*, 10 min) and stored at −20°C. Luminal contents of the cecum and proximal colon were flash frozen in liquid nitrogen and stored at −80°C. Segments of cecum and proximal colon were collected and stored in Krebs buffer for immediate measure of intestinal permeability in Ussing chambers. Sections of proximal colon were flash frozen in liquid nitrogen and stored at −80°C until RNA extraction.

Ussing Chamber Analysis

Gut tissue was opened along the mesenteric border and mounted in Ussing chambers (Physiologic Instruments, San Diego, CA), exposing 0.5 cm^2 of tissue surface area to 2.5 mL of oxygenated Krebs-glucose (10 m*M*) and Krebs-mannitol (10 m*M*) at 37°C on the serosal and luminal sides, respectively. Tissue conductance was measured using a voltage/current clamp (VCC