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Effect of lactulose-derived oligosaccharides on intestinal microbiota during the shift between media with different energy contents

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ABSTRACT

The microbiological and metabolic changes of an overweight-associated colonic microbiota after reducing *in vitro* the carbohydrate supply and its supplementation with oligosaccharides derived from lactulose (OsLu) were evaluated using a dynamic simulator of the gastrointestinal tract. The differentiation and stability of the microbial communities within each colon compartment were reached after two weeks of feeding the system with a high energy (HE) medium based on fructose and readily fermentable starches. The effect of reducing the energy content (low-energy medium, LE) and the supplementation with OsLu caused minor variations in bacterial counts, except for Enterobacteriaceae. The LE medium caused an effect on the microbial metabolic activity that was characterized by an absence of net butyrate production and an increase in ammonium content. This shift from fermentative to proteolytic metabolism was not observed when the LE medium was supplemented with OsLu. This oligosaccharide mixture was mainly metabolized in the proximal colonic compartment. The results obtained in this study indicate that the substitution in the diet of easily digestible carbohydrates by OsLu maintains the fermentative functionality of the intestinal microbiota, allowing the net production of butyric acid with potential beneficial effects on health, and avoiding a full transition to proteolytic metabolism profiles.

1. Introduction

Dietary habits involving high energy intake are related to the development of overweight and obesity. Monosaccharides and disaccharides such as fructose and sugar alcohols (sorbitol, lactitol and other polyols), widely used for the formulation of processed foods or beverages, can reach the large intestine when overfeeding of these sugars occurs (Payne, Chassard, & Lacroix, 2012). There is epidemiological evidence that sugar-sweetened beverages increase the risk of overweight and obesity, at all ages, and that obese individuals are reported to consume significantly more protein and sugars and lower fibre than normal-weight subjects (Lafontan, Visscher, Farpour-Lambert, & Yumuk, 2015; Requena et al., 2013). Related to it, there is an increased interest in understanding the possible effects of high energy diets in the intestinal microbiota. However, the highly personalized human microbiota shows a smaller dietary influence as the inter-individual variation decreases systematic effects (Wu et al., 2011; David et al., 2014). Salonen et al. (2014) described that studies from 14 obese males consuming fully controlled diets supplemented with resistant starch or non-starch polysaccharides and a weight-loss diet revealed that the diet explained around 10% of the total variance in microbiota composition, which was substantially less than the inter-individual variance. All these studies have noted strong individuality of the responses, the extent of which appears to depend on the initial microbiota composition (Korpela et al., 2014). The fact that the broad phylum level changes between Bacteroidetes and Firmicutes have not been found consistently (Ley, 2010; Ravussin et al., 2012) may indicate that relevant changes associated to diet-induced obesity could involve lower taxonomic levels within these phyla (Cox & Blaser, 2013).

In view of the fact that prebiotics are well-recognized to influence the gut microbiota composition, they could be consumed as part of a weight management diet. Genetically obese mice and diet-induced obese mice and rats (Alligier et al., 2014; Everard et al., 2011; Pyra, Saha, & Reimer, 2012), as well as overweight and obese adults (Parnell & Reimer, 2009) have all been reported to exhibit reduced fat mass following consumption of prebiotics. Indeed, subjects consuming diets rich in fructo-oligosacharides (FOS) and galacto-oligosacharides (GOS) show lower risk of overweight (Pérez-Cornago et al., 2015). Sarbini, Kolida, Deaville, Gibson, and Rastall (2014) described the potential of a novel dextran oligosaccharide for obesity management through *in vitro* experimentation. The degree of branching of the compound identified it as a slower-fermenting nutrient that was considered to be advantageous for obese individuals, as energy would be made available more gradually. Recently, the enzymatic synthesis of oligosaccharides derived from lactulose (OsLu) has been aimed for the production of a group of more slowly fermenting prebiotics (Cardelle-Cobas, Martínez-Villaluenga, Villamiel, Olano, & Corzo, 2008). In addition, the

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compounds have demonstrated to be selectively fermented by bifidobacteria and lactobacilli and to increase the concentration of short chain fatty acids (Cardelle-Cobas et al., 2012).

In this study we have used the dynamic simulator of the gastrointestinal tract described by Barroso, Cueva, Peláez, Martínez-Cuesta, and Requena (2015). The model simulates the gastric and small intestine digestion and is equipped with three-stage continuous reactors for reproducing the colon region-specific microbiota and its metabolism. The stabilization period in this study has been adapted to simulate an obese-associated microbiota by using a high energy-content medium. Changes in microbiological and metabolic characteristics were assessed after lowering the energy content and the supplementation with OsLu used as a potential prebiotic.

2. Materials and methods

2.1. Dynamic simulator of the gastrointestinal tract

The dynamic gastrointestinal simulator SIMGI was used in the operating mode to work with the units simulating the small intestine (SI) and the ascending (AC), transverse (TC) and descending colon (DC) regions (Barroso et al., 2015; Fig. S1). Therefore, the three colon reactors were filled and pre-conditioned with the nutritive medium that feed the system during the stabilization period. In this case, the setup was made to recreate an obese-associated microbiota. For this purpose, a starting high energy (HE) medium was used as described by Payne, Chassard, Banz, and Lacroix (2012), which was characterized by a high content of high-glycaemic index carbohydrates (digestible starch) and simple carbohydrates (fructose). The HE medium contained arabinogalactan (1 g/L), pectin from apple (2 g/L), xylan (1 g/L), potato starch (6 g/L), maize starch (4 g/L), fructose (6 g/L), glucose (0.4 g/L), yeast extract (3 g/L), peptone (1 g/L), mucin (4 g/L) and L-cysteine (0.5 g/L); that is 45% more fermentable carbohydrates than the standard nutritive medium (Barroso et al., 2015) to create the HE medium. The AC, TC and DC units were inoculated with 20 mL of a fresh 20% (w/v) faecal sample from an overweight volunteer, homogenized in anaerobic conditions with sodium phosphate buffer (0.1 M, pH 7.0), containing 1 g/L sodium thioglycolate as reducing agent, as described by De Boever, Deplancke, and Verstraete (2000). The development and stabilization of the microbial community until steady-state conditions in the three colon units was approached by feeding the small intestine with nutritive medium (75 mL, pH 2) mixed with pancreatic juice (40 mL of a solution of 12 g/L $NaHCO_3$, 6 g/L oxgall dehydrated fresh bile and 0.9 g/L porcine pancreatine) three times a day during 14 days (Van den Abbeele et al., 2010). The small intestine digestion was performed during 2 h at 37 °C and the content of the vessel was automatically transferred to the following colon compartment (AC) at a flow rate of 5 mL/min, which simultaneously activated the transit of colonic content between the AC, TC and DC compartments at the same flow rate. The overall residence time of the colon compartments was 76 h. All the vessels were maintained under anaerobic conditions by continuously flushing N_2. The stabilization of the microbial community until steady-state conditions was evaluated by sampling and measuring the production of short chain fatty acids (SCFA) and ammonium over time (see below). Stability was reached when rates of change of the parameters measured dropped below 10% for each colon compartment (Barroso et al., 2015).

After the two-week stabilization period of the colonic microbiota, the SIMGI was subjected to a 1-week experiment consisting in removing the maize starch and fructose content and reducing the potato starch content to 1.5 g/L (low energy medium; LE) and adding 10 g/L of an oligosaccharide mixture derived from lactulose (OsLu), obtained such as it will be described below. Finally, a 1-week wash-out period was included at the end of the experiment by feeding the SIMGI daily with the LE medium. During the whole study, samples were collected daily at regular time points from the three colon vessels and stored at −20 °C until further analysis.

2.2. Synthesis of oligosaccharides derived from lactulose (OsLu)

OsLu were synthesized following the method described by Anadón et al. (2013) by using a commercial preparation Duphalac (Abbott Biologicals B.V., Barcelona, Spain), containing 670 g/L lactulose and the β-galactosidase from *Aspergillus oryzae* (16 U/mL; Sigma-Aldrich, St. Louis, MO, USA). Enzymatic reactions were performed at 50 °C and pH 6.5 in an orbital shaker at 300 rpm for 24 h. In order to eliminate monosaccharides, the mixture of oligosaccharides (20%, w/v) was treated with fresh *Saccharomyces cerevisiae* (1.5%, w/v) (Levital, Paniberica de Levadura S.A., Valladolid, Spain) at 30 °C for 48 h in an orbital shaker (300 rpm). Mono- and disaccharides as well as OsLu were analysed by GC with a flame ionization detector (GC-FID) as described by Montilla, Van de Lagemaat, Olano, and Del Castillo (2006).

2.3. Microbiological analyses

2.3.1. DNA extraction and purification

Microbial DNA extraction of the samples taken from the AC, TC and DC compartments was performed as described by Moles et al. (2013). Briefly, samples (1 mL) were centrifuged (10,000 ×g, 10 min, 4 °C) and the pellet (suspended in 200 mM Tris–HCl pH 7.5, 0.5% SDS, 25 mM EDTA, 250 mM NaCl and 3 M sodium acetate) was incubated with 20 mg/mL lysozyme and 5 mg/mL lysostaphin (Sigma-Aldrich). Bacterial lysis was completed by mixing with glass beads. The DNA was extracted with phenol/chloroform/isoamyl-alcohol, precipitated by adding 0.6 volumes of isopropanol and finally resuspended in DNase, RNase free water (Sigma-Aldrich). The DNA yield was measured using a NanoDropH ND-1000 UV spectrophotometer (Nano-Drop Technologies).

2.3.2. Quantitative PCR (qPCR)

The quantitative microbiological analysis of samples was carried out by qPCR experiments that were analysed using SYBR green methodology in a ViiA7 Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). Primers, amplicon size, annealing temperature for total bacteria, *Bacteroides*, *Bifidobacterium*, *Enterobacteriaceae*, *Lactobacillus*, *Prevotella*, the specific phylogenetic groups *Blautia coccoides-Eubacterium rectale* Cluster XIVa, *Ruminococcus* Cluster IV and *Clostridium leptum* subgroup specific cluster IV have been described previously (Barroso et al., 2013). DNA from *Escherichia coli* DH5α, *L. plantarum* IFPL935, *Bifidobacterium breve* 29M2 and *Bacteroides fragilis* DSM2151 were used for quantification of total bacteria, *Lactobacillus*, *Bifidobacterium* and *Bacteroides*, respectively. For the rest of groups analysed, samples were quantified using standards derived from targeted cloned genes using the pGEM-T cloning vector system kit (Promega, Madison, WI, USA), as described previously (Barroso et al., 2013). For the analysis of *Akkermansia* (primers AM1: CAGCACGTGAAGGTGGGGAC and AM2: CCTTGCGGTTGGCTTCAGAT), *Faecalibacterium* (Fprau 07: CCATGAATTGCCTTCAAAACTGTT and Fprau 02: GAGCCTCAGCGTCAGTTGGT) and *Roseburia* (Ros-F1: GCGGT RCGGCAAGTCTGA and Ros-R1: CCTCCGACACTCTAGTMCGAC), the samples were quantified using standards derived from clones obtained from the faecal inoculum, amplified with the mentioned primers and using the conditions described by Collado, Derrien, Isolauri, De Vos, and Salminen (2007); Sokol et al. (2008), and Ramirez-Farias et al. (2009), respectively. The PCR amplicons were cloned using the pGEM-T cloning vector system kit (Promega) as described previously (Barroso et al., 2013). The correctness of the *Akkermansia*, *Roseburia* and *Faecalibacterium* inserts was confirmed by sequence analysis.

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