The pleiotropic effects of prebiotic galacto-oligosaccharides on the aging gut

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SUBJECT AREAS
General Microbiology

KEYWORDS
Gut Microbiome, Prebiotics, Bifidobacterium, Intestinal Permeability, Host-Microbiota Interactions, Diet, Antibiotics, Metagenomics, Transcriptomics, Organoids
Abstract

Background: Prebiotic galacto-oligosaccharides (GOS) have an extensively demonstrated beneficial impact on intestinal health. In this study, we determined the mechanistic impact of GOS diets on hallmarks of gut aging: microbiome dysbiosis, inflammation, and intestinal barrier defects (“leaky gut”). We also evaluated if short-term GOS feeding influenced how the aging gut responded to antibiotic challenges, since these interventions are common and relevant in older adults. Finally, we assessed the ability of colonic organoids to reproduce in vivo responses to GOS.

Results: Old animals had a distinct microbiome characterized by lower diversity, increased ratios of non-saccharolytic versus saccharolytic bacteria and lower abundance of O-Glycosyl hydrolases. GOS treatment increased abundance of non-saccharolytic (Akkermansia muciniphila) and saccharolytic bacteria (species of Bacteroides and Lactobacillus), and increased the abundance of β-galactosidases and β-glucosidases in young and old animals. Clyndamicin treatment reduced the abundance of beneficial bacteria including Bifidobacterium and Lactobacillus, while increasing Akkermansia, Clostridium, Coprococcus, Enterococcus, Bacillus, Bacteroides, and Paenibacillus. Prebiotics impacted the effects of the antibiotics decreasing the abundance of Akkermansia in the GOS-antibiotic groups compared to the control-antibiotics groups. GOS reduced the age-associated increased intestinal permeability via increased MUC2 expression and mucus biosynthesis. Transcriptomics analysis of colon from old animals fed GOS diets showed increased expression of genes involved in small molecule metabolic processes and specifically the respirasome, which could indicate an increased oxidative metabolism and energetic efficiency. In young mice, GOS induced expression of binding-related genes and the galectin gene Lgals1, a β-galactosyl-binding lectin that bridges molecules by their sugar moieties, forming a signaling and adhesion network. Further analysis showed higher expression levels of genes in focal adhesion, PI3K-Akt and ECM-receptor interaction pathways. GOS reduced the expression of TNF in old animals, and altered serum levels of inflammatory biomarkers IL-6, IL-17, IP-10 and Eotaxin. Stools from young mice exhibiting variable bifidogenic response to GOS, injected into colon organoids in the presence of prebiotics, reproduced the response and non-response phenotypes.
Conclusions: GOS modulation of intestinal homeostasis likely occurs through direct GOS-host interactions via modulation of host gene expression and mucus production, as well as through interactions mediated by the gut microbiota that result in increased or restored saccharolytic potential.

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.
The aging gut has a distinct microbiome (a) Phylogenetic diversity and species richness in stool samples from old (n=12) and young mice (n=12), (b) Unweighted Unifrac Principal Coordinate Analysis (PCoA) plot of samples, (c) Relative abundance of saccharolytic, non-saccharolytic and unclassified bacteria by age, (d) Comparative analysis of differences in abundance of specific genera between young and old animals.
Prebiotic GOS and antibiotics decrease microbiome diversity in the gut. GOS increase the abundance of prebiotic-metabolizing enzymes (a) Experimental design outlining timepoints T1 (post standardization), T2 (post specialized diet), T3 (post antibiotics in water), T4 (post clindamycin IP injection), and T5 (pre-sacrifice) (N=48) (b) Impact of the different treatments on phylogenetic diversity. GOS reduced diversity in both old and young animals. Diversity recovered at T3 but declined again at T4 after IP clindamycin, (c) Relative abundance of saccharolytic, non-saccharolytic, and bacteria of undetermined metabolism over time. Line colors are as in 2b, (d) Whole Genome Shotgun (WGS) sequencing of selected stool samples showed that GOS treatment increased the abundance of betaglucosidases/ cellobioses of the GH1 family of glycosyl hydrolases, and beta-galactosidases of the GH1, Gh2, and GH42 families. These enzymes are essential to metabolize GOS in the gut.
Unweighted Unifrac PCoA plots and differences in relative abundance of specific bacteria at different timepoints and treatments (a) The T1-T2 PCoA plot (Left) revealed baseline
differences between old and young mice and how GOS induced a convergence of young and old samples in Cluster 3 (OC=old mice, control diet, OG= old mice, GOS diet, YC=young mice, control diet, YG=young mice, GOS diet). The middle panel shows the prebiotic impact on young (dark purple) and old (red) mice compared to the control diet (light purple = young control, light pink = old control). The right panel shows composed differences in relative abundances by age, diet and time point. (b) The T2-T3 PCoA plot (left) and genus-level analysis (middle) showed a minor impact of the antibiotic treatment in water. Middle panel: Control and GOS diets as before. Light blue dots represent no antibiotic treatment, dark blue represent antibiotic treatment. (c) The T3-T4 PCoA plot (left) and genus-level analysis (middle) represent samples immediately after clindamycin IP injection. The graphs did not show dramatic impacts to the microbiome, which were clearly visible in (d) the T4-T5 PCoA and taxa plots. At this timepoint transition a new cluster (Cluster 5), which encompass only antibiotic-treated control animals, was observed.
Figure 4

(a) Old mice had higher intestinal permeability measured by FITC-dextran assays than young animals. Intestinal permeability was reduced in GOS fed mice (i) via increased expression of MUC2. Expression of TFF3 and RELMb tended to increase in the GOS groups but differences were not statistically significant (ii). Paraformaldehyde vapor fixation and subsequent PAS staining showed increased mucus thickness in old mice fed the prebiotics diet (iii). (b) Inflammatory biomarkers were modulated by GOS and antibiotics. A 2 x 2 x 2 ANOVA showed increased serum IL-6 and IL-17 in antibiotic treated mice regardless of age or diet (i). Similarly, serum IL-13 was higher in young mice regardless of diet or antibiotics (ii). Eotaxin (iii) and IP-10 (iv) were higher in the GOS group within the no-antibiotic animals but lower in the GOS group that received antibiotics regardless of age. No significant differences in serum IL-6 were observed by age or diet in the no-antibiotics group. Within the animals that received antibiotics, old mice had a non-significant increased concentration of the cytokine while mice on the GOS diet had higher levels of IL-6 regardless of age (v). Finally, expression of TNFa quantified by RT-qPCR was higher in old animals compared to young and reduced by GOS treatment in old animals.
STRING network analysis [135] of expression data from colon showed different GOS effects on the intestinal epithelium of old and young mice. (a) GOS induced expression of binding-related genes (GO:0005488) in young mice while inducing (b) small molecule metabolic processes genes (GO:0044281) in old animals. Network nodes represent predicted proteins. Splice isoforms or post-translational modifications are collapsed so each node represents all the proteins produced by a single, protein-coding gene locus. The confidence cutoff for showing interaction links was 0.900 (highest). The lower panel in figure (a) shows the most represented GO categories within binding-related genes in our transcriptomics data. (c) A heatmap of expression data revealed that GOS act as a modulator of the immune system in old and young mice. The heatmap was generated using ClustVis [136]. Rows were centered; unit variance scaling was applied to rows. Rows were clustered using correlation distance and average linkage.
Figure 6
(a) Top figure: The variable bifidogenic effect observed in vivo was reproduced in vitro in the organoid platform (data from 16S rRNA amplicon sequencing). Bottom figure: The genus Bifidobacterium was quantified by high throughput qPCR in stools from mice and organoid contents. (b) Shannon diversity and species richness within microbiota-colonized organoids declined over time. The continuous lines represent data from organoids injected with stools from young mice. Dash lines are results from organoids injected with stools from old mice. (c) Unweighted Unifrac PCoA plots revealed differences between old and young microbiota upon colonization in organoids, which converged to a single grouping over 72-hours. (e) Taxonomy plots of microbiota-colonized organoids showed changes over time in communities derived from both old and young animals.

**Supplementary Files**

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