257: Effect of Probiotics on Gut Microbiota and PET/MRI Activity in HIV-infected Persons

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Objective

The aim of this study was to investigate the effects of the probiotic strain *Lactobacillus rhamnosus GG (LGG)* on the gut microbiota composition, microbial translocation and intestinal inflammation.

Background

- Despite combination antiretroviral therapy (cART) with suppressed viral replication, gut microbiota alterations, microbial translocation and low-grade inflammation persist in several HIV-infected individuals. [1]
- Microbial translocation is the translocation of microbial products including lipopolysaccharide (LPS) produced by gram negative bacteria, such as Enterobacteriaceae, from the intestinal lumen into the portal and systemic circulation. The Enterobacteriaceae family contains several known pathogenic strains with pro-inflammatory properties and ability to locally invade the gut mucosa.
- Recently, PET/MRI was introduced as a novel non-invasive imaging tool combining the morphological patterns obtained by Magnetic Resonance Imaging (MRI) with the metabolic activity given by Position Emission Tomography (PET), in a single test. [2]
- *Lactobacillus rhamnosus GG (LGG)* supplementation is believed to alter the intestinal microbiome, displace pathogenic bacteria and increase the number of intestinal cells. [3]

References:
1. Nowak P, Troseid M, Avershina E, et al. Gut microbiota diversity predicts immune status in HIV-1 infection. AIDS. 2015 Nov 28;29(18):2409-18.
2. Pichler BJ, Kolb A, Nagele T, et al. PET/MRI: paving the way for the next generation of clinical multimodality imaging applications. J Nucl Med. 2010 Mar;51(3):333-6.
3. Banasaz M, Norin E, Holma R, et al. Increased enterocyte production in gnotobiotic rats mono-associated with Lactobacillus rhamnosus GG. Appl Environ Microbiol. 2002 Jun;68(6):3031-4.

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Results

After probiotic intervention, no overall change in microbial alpha diversity (Fig. 1A) or microbial translocation (LPS) (Fig. 1B) could be detected in the study group. However, reduction in the bacterial families Enterobacteriaceae (p=0.018) and Erysipelotrichaceae (p=0.037) were found after probiotic intervention, along with increased abundance of an uncultured genus from the Lachnospiraceae family as well as *Ruminiclostridum* (Fig.2). Overall, intestinal inflammation decreased significantly (-0.3 mean difference in combined activity grade score from six regions, p=0.006) (Fig. 4 and 5). Comparing individuals with decreased ^{18}F-FDG-uptake on PET/MRI (responders) with individuals with no change in uptake after intervention (non-responders), the relative abundance of Enterobacteriaceae (p=0.048) was significantly reduced in in the gut microbiome of the responders (Fig. 3).

Figure 1:

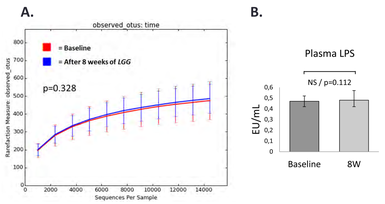


Figure 1. A) Microbial alpha (within-individual) diversity measured as mean change in observed operational taxonomic units (OTUs). B) Marker of microbial translocation , lipopolysaccharide (LPS) measured by level of endotoxin units (EU) per mL in blood plasma.

Figure 2:

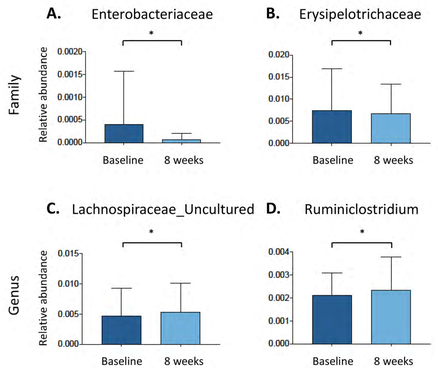


Figure 2. A-D) Significantly changed bacterial taxa after eight weeks of probiotic intervention (n=40). Relative abundances are shown as median values with interquartile ranges (IQR). BL=Baseline, 8W=Eight weeks after intervention. *p<0.05.

Figure 3:

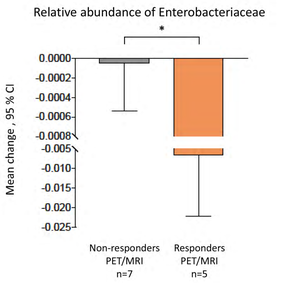


Figure 3. A) Less abundance of Enterobacteriaceae in individuals with decreased ^{18}F-FDG-uptake on PET/MRI (responders, n=5) than individuals with no change in uptake (non-responders, n=7). Results are shown as mean change with 95% confidence intervals (CI). *p<0.05.

Figure 4:

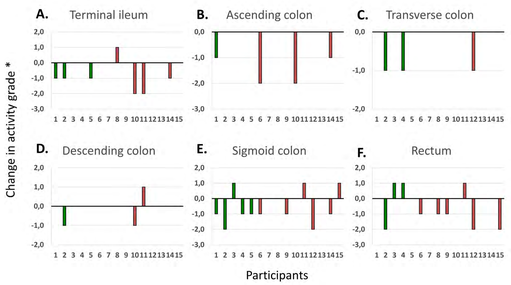


Figure 4. A-F) Change in ^{18}F-FDG-uptake in fifteen consecutively selected individuals after eight weeks of probiotics, as measured by change in activity grade in each of the six regions. The green bars show cART-naïve participants, while red bars show cART-treated participants. An activity grade of 0, 1, 2 or 3 was assigned to each region comparing the area with the highest ^{18}F-FDG-uptake in the relevant bowel segment to the ^{18}F-FDG-uptake in the liver. *Mean difference of 0.3, p=0.006, as computed by bootstrapping (10,000 random permutations).

Figure 5:

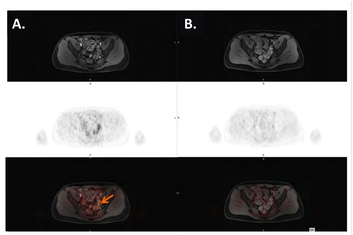


Figure 5. A) Representative ^{18}F-FDG PET/MRI scan (axial T1 VIBE fat saturated) on a cART-treated participant before and 8) after ingestion of probiotics for eight weeks. The arrow is pointing at ^{18}F-FDG uptake in the sigmoid colon which decreases after probiotics.

Study Design

The study was a prospective, clinical interventional trial including 45 individuals (15 cART naïve and 30 cART-treated) during the period of January to September 2015 from the outpatient clinic at the Department of Infectious Diseases, Copenhagen University Hospital, Rigshospitalet (see consort diagram and Table 1). Participants ingested *LGG* twice daily at a dosage of 6 x10^9 colony-forming units per capsule for a period of eight weeks.

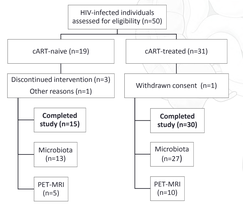


Table 1. Demographics

	All participants	cART-treated	cART-naïve
	n=45	n=30	n=15
Age, yrs *	47 (39-53)	47 (39-52)	40 (36-53)
Male, n (%)	38 (84)	26 (87)	12 (80)
Ethnicity (caucasian), n (%)	44 (98)	30 (100)	14 (93)

Data are presented as mean and standard deviation (SD) or * median and interquartile range (IQR) as appropriate.

Methods

The gut microbiota composition (sequencing of the V3-V4 region of the 16s rRNA gene using the MiSeq platform by Illumina) and level of microbial translocation (circulating lipopolysaccharide, LPS) were determined at baseline and after probiotic intervention. Gut microbial profiles were compared with measurements of intestinal inflammation by ^{18}F-2-fluoro-2-deoxy-D-glucose positron emission tomography/magnetic resonance imaging (^{18}FDG PET/MRI) scans in 15 consecutively selected individuals.

Conclusions

- **Reduced abundance of Enterobacteriaceae after probiotic intervention could potentially explain the local anti-inflammatory effect in the gut measured by PET/MRI activity.**
- **Future studies should focus on the potential effects on mucosal immunology and HIV pathogenesis, with PET/MRI as a non-invasive alternative to regionally explore the distribution of inflammation in the gut.**