# IMPAIRMENT OF HUMAN COLONIC SMOOTH MUSCLE CONTRACTILITY: ROLE OF MICROBIOTA MODULATION

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## BACKGROUNDS

Several gastrointestinal (GI) tract disorders, such as acute enteritis, inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), are associated with non-specific alterations of GI motility. We recently demonstrated that Lipopolysaccharide (LPS), an endotoxin present in the bacterial cell wall, is able to induce an immune/inflammatory host response. Many disorders such as intestinal motility disturbances and oxidative stress production have been attributed to LPS.

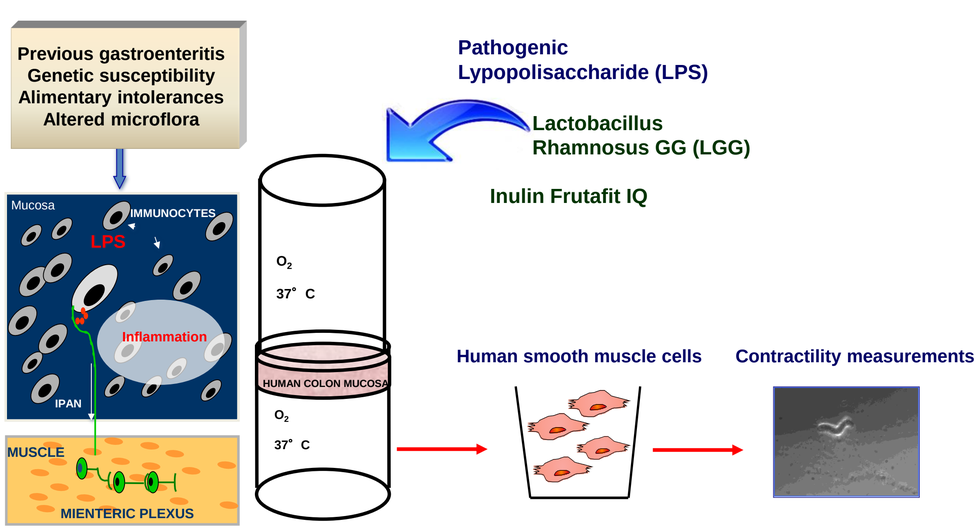
Clinical studies support the use of probiotics and prebiotics in the treatment of acute and chronic intestinal disorders and diarrhea; even if anti-inflammatory and immuno-modulatory action of these products, at the mucosal layer, might be involved, the exact mechanisms are not completely elucidated.

## METHODS

### I PHASE: Human colonic muscle cell impairment induced by Lipopolysaccharide mucosal exposure and protective effect of Inulin

Human colonic mucosa and submucosa, obtained from disease-free margins of resected segments for cancer, were sealed between two chambers, with the mucosal side facing upwards and covered with 5 mL of Krebs solution with or without purified LPS from a pathogenic strain of Escherichia coli (O111:B4) and Inulin (Frutaft IQ®), and the submucosal side face downwards into 25 mL of Krebs solution. The solution on the submucosal side was collected after 30 minutes of mucosal exposure to Krebs in the absence (N-Undernatant) or presence of LPS (LPS-Undernatant) and in presence of LPS and Inulin (LPS-INU Undernatant). Undernatants were tested for antioxidant activity and for their effects on isolated smooth muscle cells (SMCs) (**Fig.1**). The inulin protective effect on the mucosa and submucosa were assessed measuring the protein oxidation level in all the experimental conditions analyzed, as well as its ability to revert the LPS-induced impairment of SMC contraction.

**Figure 1**




### II PHASE: Lactobacillus Rhamnosus GG (LGG) protects human colonic muscle from pathogenic Lypopolysaccharide-induced damage

The effects of LGG (ATCC 53103 strain) and of supernatants have been tested both on human colonic smooth muscle strips and isolated cells alone or in the presence of LPS obtained from a pathogenic strain of Escherichia coli (**Fig. 2**).

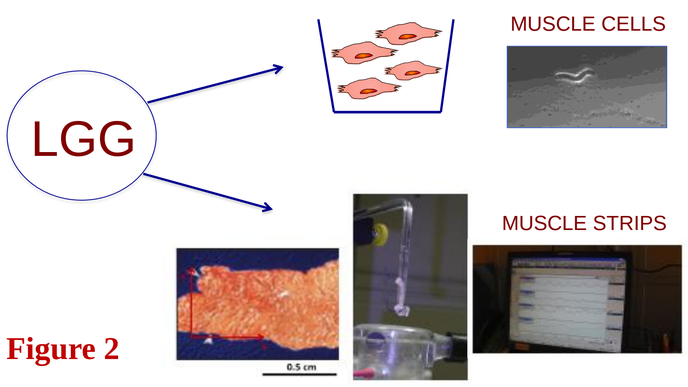



**Figure 2**

Their effects have been evaluated on myogenic morpho-functional properties and on LPS-induced NFκB activation and cytokines production. TLRs expression has been evaluated by qPCR and flow cytometry.

## RESULTS

*Human colonic myogenic dysfunction induced by mucosal Lipopolysaccharide translocation and oxidative stress.*
*Guarino MP, Sessa R, Altomare A et al. Dig Liver Dis. 2013 Dec;45(12):1011-6*

**Figure 3.** Thirty-minute exposure to LPS-undernatant did not decrease the resting cell length, but significantly reduced the maximal Ach-induced contraction.

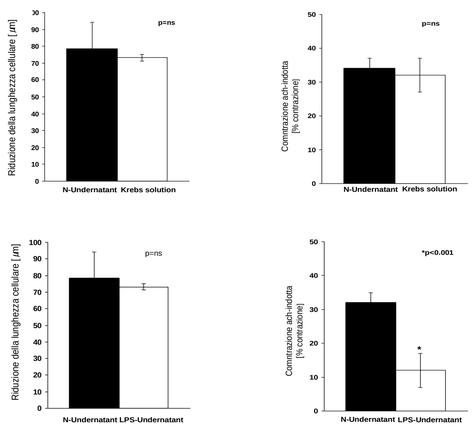



**Figure 4.** the contraction induced by LPS-undernatant was significantly reversed by pre-incubation of the undernatants with catalase, indomethacin and MG132.

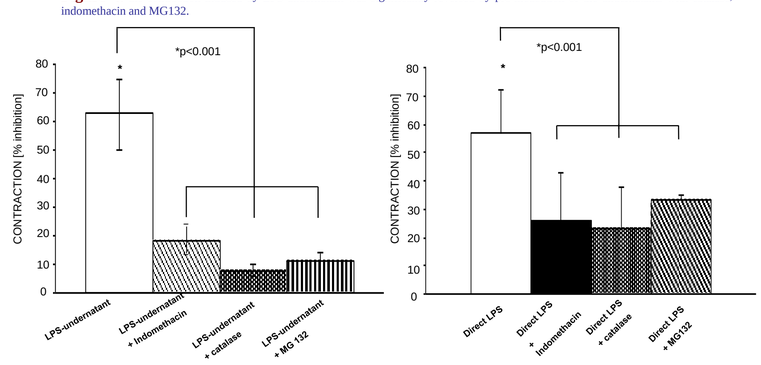



**Figure 5.** Translocation of LPS from E. coli (0111:B4) across human colonic mucosa assessed using Kinetic-turbidimetric LAL assay

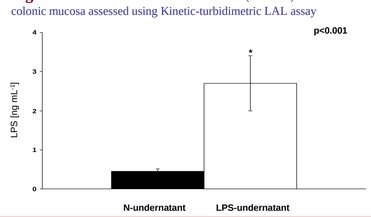
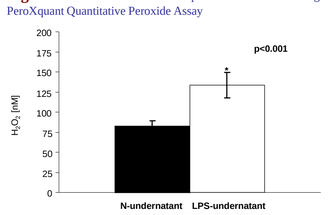



**Figure 6.** Effect of LPS on H2O2 production assessed using PeroXquant Quantitative Peroxide Assay




*Antioxidant activity of Inulin and its role in the prevention of human colonic muscle cell impairment induced by Lipopolysaccharide mucosal exposure. Pasqualetti V, Altomare A, Guarino MP, et al. PLoS One 2014 .16;9(5):e98031*

**Figure 7.** Effect of the exposure to N, LPS and LPS+INU-undernatants on resting length and Ach-induced contraction of colonic smooth muscle cells (SMCs).

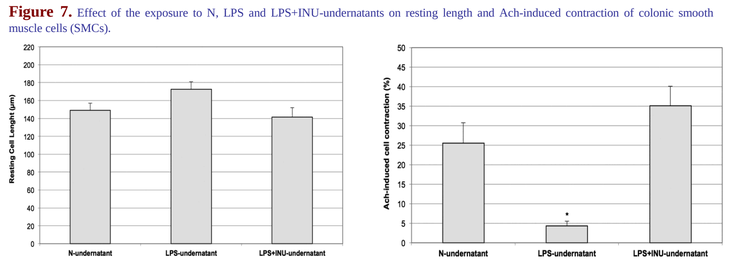
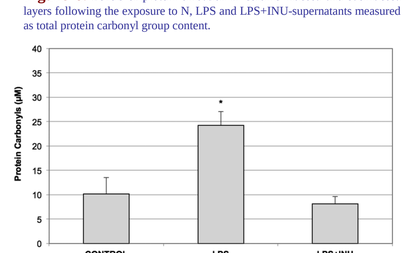



**Figure 8.** Levels of protein oxidation in colonic mucosa and submucosa layers following the exposure to N, LPS and LPS+INU-supernatants measured as total protein carbonyl group content.




*Lactobacillus Rhamnosus GG protects human colonic muscle against Lipopolysaccharide-induced damage.*
*Ammoscato F, Scirocco A, Altomare A, et al. Neurogastroenterol Motil. 2013 Dec;25(12):984-e777*

**Figure 9. A)** LGG dose-dependent increase of muscle strips "spontaneous" contraction and smooth muscle cells shortening. **B)** LGG dose-dependent decrease of contraction induced by muscarinic agonist acetylcholine (Ach) 1µM of muscle strips.

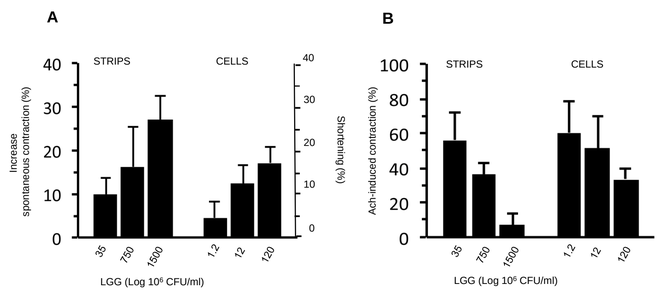



**Figure 10.**
**A and B)** Phosphorylation of NFκB subunits Ser468 (A) and Ser536 (B). * p<0.01 vs LGG+LPS.
**C and D)** Quantitative ELISA of IL-6 (C) and IL-10 (D) release. * p<0.05 vs LGG+LPS.
**E and F)** Evaluation of SMC length and contraction. * p<0.01 vs LGG+LPS.

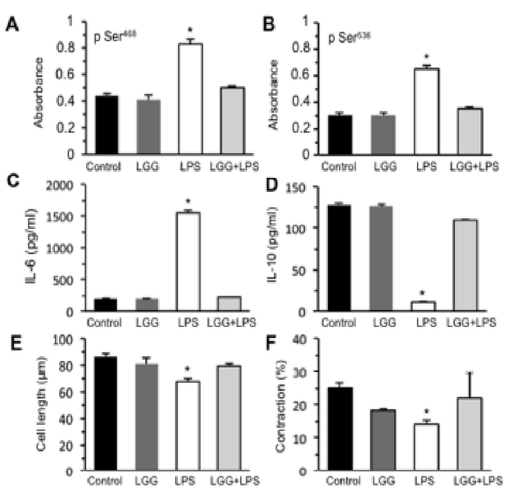



**Figure 11.** Spontaneous (Basal) and Ach-induced contraction (1 µM) of muscle strips before and after LGG washout.

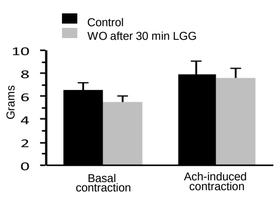
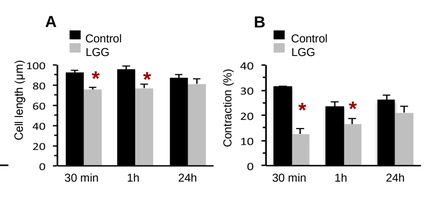



**Figure 12.** LGG effects on smooth muscle cells length **(A)** and acetylcholine-induced contractile response **(B)**. Cells were incubated for increasing time with LGG (120x106 CFU ml-1).




## CONCLUSIONS

Acute exposure of colonic mucosa to pathogenic LPS impairs muscle cell contractility due both to LPS mucosal translocation and to mucosal production of free radicals. Pre-treatment with a probiotic (LGG) or a prebiotic (Inulin) prevent the LPS contractile damage, probably counteracting the oxidative stress.