Microbially Derived Branched-Chain Fatty Acids Regulate Colonic Permeability In Protein Malnutrition

Lauren E. Lynch[1], Krishnakant G. Soni[1], Jennifer K. Spinler[2], Stephanie W. Fowler[3,4], Margaret E. Conner[3,5], Xi-Lei Zeng[3], Mary K. Estes[3], Sarah E. Blutt[3], Geoffrey A. Preidis[1]

[1]Pediatric Gastroenterology, Hepatology & Nutrition, Baylor College of Medicine and Texas Children's Hospital, Houston TX, USA. [2]Pathology and Immunology, Baylor College of Medicine, Houston TX, USA. [3]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston TX, USA. [4]Center for Comparative Medicine, Baylor College of Medicine, Houston TX, USA. [5]Department of Education, Innovation, and Technology, Baylor College of Medicine, Houston TX, USA.

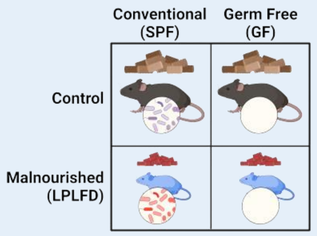
Introduction

- Malnutrition contributes to 45% of global child deaths.
- Malnutrition alters the gut microbiome and gut barrier permeability.
- The gut barrier is regulated by microbial metabolites including branched-chain fatty acids (BCFAs).
- BCFAs are produced by bacterial fermentation of dietary protein.
- Increased intestinal permeability can be caused by a depleted mucus layer.
- MUC2 is the predominant mucin in the intestinal mucus layer and is regulated by serotonin.

Objective

In the present study, we investigate whether changes in gut microbiota function contribute to heightened gut barrier permeability in malnutrition.

Methods

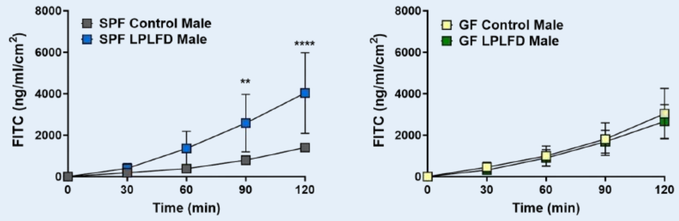


- Stool from 8 week old SPF mice was analyzed by metagenomic sequencing and mass spectrometry targeting BCFAs.
- Colonic permeability was assessed *ex vivo* with the Ussing chamber system.
- Livers were sterilely harvested and plated on Brain Heart Infusion agar plates and incubated aerobically and anaerobically at 37°C.
- Colonic tissue was analyzed via qPCR, immunofluorescence, and FISH.
- BCFAs were applied at doses spanning the physiologic range (1 - 10mM) to human-derived colonoid monolayers on Transwells.
- Leucine and alanine were administered via oral gavage 2X/day for 2.5d.

Results

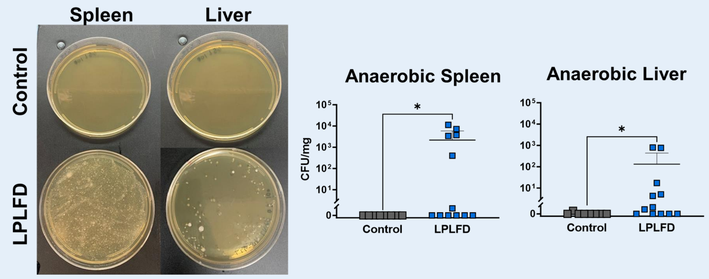
Question 1: Does malnutrition affect colonic permeability?

Malnutrition increases permeability in SPF, but not GF mice.



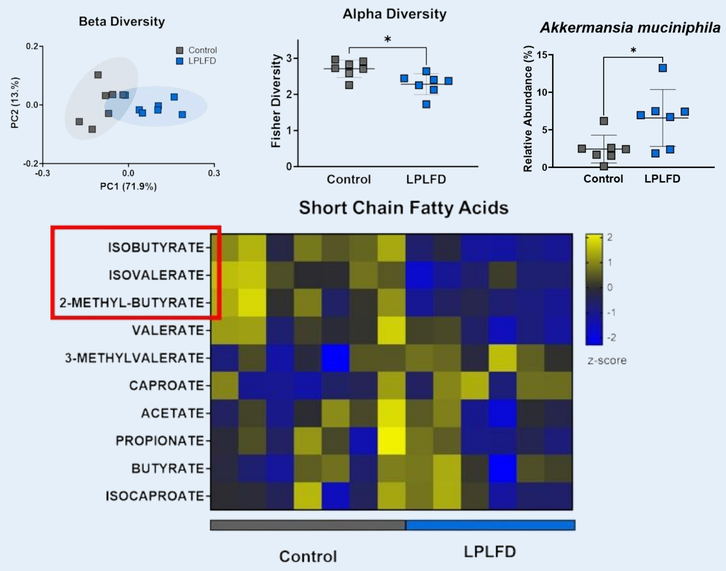
Question 2: Do malnourished mice have increased bacterial translocation?

Malnourished mice have increased bacterial translocation to the spleen and liver.



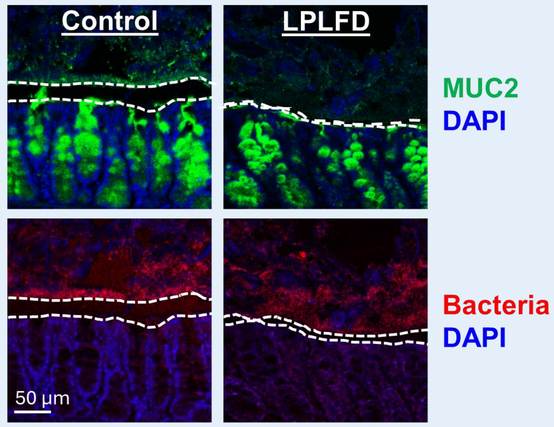
Question 3: How does malnutrition alter the microbiome?

Malnourished mice have decreased alpha diversity and profoundly decreased microbially derived BCFAs. Abundance of mucin-degrading A. muciniphila *is increased.*



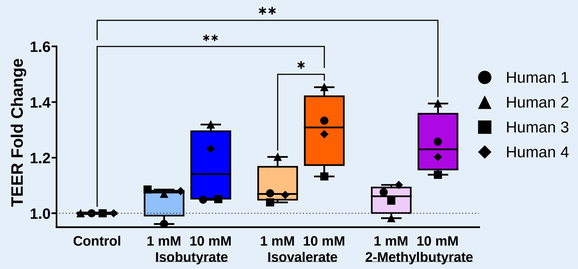
Question 4: Does malnutrition alter colonic mucus?

Malnourished mice have decreased MUC2 transcript (not shown) and fluorescence. Bacteria encroaching upon the epithelium. Serotonin homeostasis is altered (not shown).



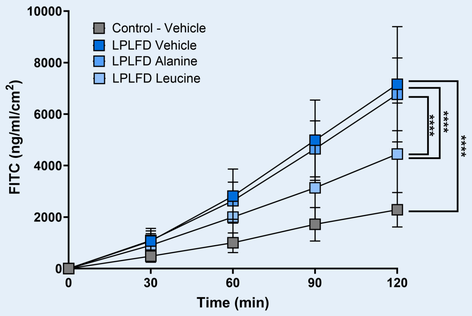
Question 5: Do microbial BCFAs affect barrier function *in vitro*?

Isovalerate and 2-methylbutyrate improve colonic barrier function in a dose-dependent manner in human-derived colonoids.



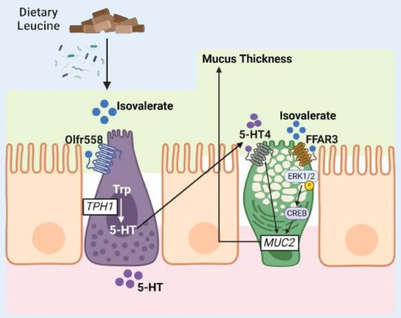
Question 6: Can enteral leucine rescue increased colonic permeability in malnourished mice?

The precursor to isovalerate, leucine, improves colonic permeability in malnutrition. The non-BCFA forming amino acid, alanine, has no effect.



Conclusions

- Malnutrition-induced gut barrier dysfunction requires gut bacteria.
- Malnutrition results in increased bacterial translocation to the spleen and liver.
- Microbially-derived BCFAs are reduced and serotonin is altered in malnourished mice.
- MUC2 mRNA and protein is reduced in the malnourished colonic mucus layer.
- Isovalerate improves colonic barrier function in a dose-dependent manner.
- Oral gavage of leucine, a branched-chain amino acid precursor to isovalerate, improved colonic permeability in malnourished mice, while alanine, a non BCFA forming amino acid, had no effect.
- This knowledge may facilitate novel microbiota-targeting therapies reduce the risk of sepsis and mortality in child malnutrition.



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Baylor College of Medicine

Texas Children's Hospital