SCIENTIFIC ARCHIVES.COM

Arch Gastroenterol Res. 2024;5(1):42-46.

Archives of Gastroenterology Research Mini Review

Obesity and Beyond: The Novel Technical Standard to Study the Small Bowel Microbiome

Daniel Brimberry[1], Gabriela Leite[1], Mark Pimentel[1,2], Gillian M Barlow[1], Ruchi Mathur[1,3,*]

[1]Medically Associated Science and Technology (MAST) Program, Cedars-Sinai, Los Angeles, CA, USA

[2]Karsh Division of Gastroenterology and Hepatology, Department of Medicine, Cedars-Sinai, Los Angeles, CA, USA

[3]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Cedars-Sinai, Los Angeles, CA, USA

[*]Correspondence should be addressed to Ruchi Mathur, ruchi.mathur@cshs.org

Received date: September 13, 2024, **Accepted date:** September 22, 2024

Citation: Brimberry D, Leite G, Pimentel M, Barlow GM, Mathur R. Obesity and Beyond: The Novel Technical Standard to Study the Small Bowel Microbiome. Arch Gastroenterol Res. 2024;5(1):42-46.

Copyright: © 2024 Brimberry D, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The small intestinal microbiome (SIM) is colonized by a variety of bacteria, archaea, viruses, and eukarya which contribute to the overall health of the human host. Commensal bacteria of the small intestine are crucial suppliers of essential nutrients, aid in the metabolism of indigestible carbohydrates, provide defenses against pathogenic bacteria, and are critical components of the mucosal architecture. Initial work in this field focused on characterization using stool as a surrogate for the whole gut microbiome due to ease and frequency of collection. Most of the literature continues to use stool as the vehicle for gut microbial analysis. However, studying only stool is limited due to significant variances of structure, environment, and ecology which exist along the length of the gastrointestinal tract. These variances are significant enough that studying only stool does not reflect the microbial composition of the small intestine, requiring a new approach to accurately characterize the relationship between the SIM and host physiology. Our most recent work characterizes the SIM in people with normal and elevated body weights (overweight and obesity); we identified key genera associated with elevated body weight, as well as escalation factors, de-escalation factors, and associated predicted pathways that may represent potential targets for future therapeutics.

Keywords: Metabolism, Obesity, Small bowel aspirates, Small intestinal microbiome

Introduction

A deep understanding of the microbial composition of the small intestine is crucial because the SIM contributes significantly to catabolism of carbohydrates, amino acids, lipids, and micronutrient metabolism [1]. Using a novel double-lumen catheter system sealed with bone wax to obtain small bowel aspirates (SBA), followed by mucolytic processing with dithiothreitol (DTT) under rigorous sterile technique [2], is yielding a growing body of evidence which supports the establishment of a new technical standard for SIM research. As a result, we propose that studying the SIM through direct collection of SBA during endoscopy with careful attention paid to rigorous sterile technique, should become the gold standard on which conclusions in this field are interpreted.

Our method has yielded significant insights into the SIMs of artificial sweetener consumers [3], current and former smokers [4], women taking hormone therapy [5], subjects taking proton pump inhibitors [6], as well as the effects COVID lockdowns [7] on the SIM. Most recently, our group characterized the SIM in subjects with normal weights versus subjects who were overweight or had obesity [8]. Using these validated techniques, we found specific genera exclusively associated with overweight or obesity, as well as associated escalation and de-escalation features representing potential therapeutic targets.

Sim Profiles in Normal Weight Individuals Versus Individuals with Elevated Body Weights Significantly Differ between Groups and from Stool-Only Analyses

Previous work used stool samples as a surrogate to characterize the gut microbiome in subjects with elevated body weights undertaking treatment with a high-protein, low

carbohydrate diet [9]. Similarly, other work examined stool specimens from adult, female mono- and dizygotic twin pairs in parallel with their mothers [10], to quantify the differences in gut microbiota and short-chain fatty acid concentrations in people with normal or elevated body weight [11], to quantify the varying bacterial relative abundances in people with normal weight versus obesity on a weight-maintenance plan compared to those on a reduced-carbohydrate diet [12]. Previously, we compared the SIM to the stool microbiome in a large cohort of subjects enrolled in the REIMAGINE Study at Cedars-Sinai Medical Center using 16S rRNA sequencing. We found the stool microbiome is dominated by Bacteroidetes and Firmicutes phyla which is consistent with previous findings in the human gut [13] and across the human lifespan [14]. Conversely, the SIM differs from stool with respect to its microbial composition, being dominated by the phyla Proteobacteria and Firmicutes; we also detected substantial differences in the classes, families, and even genera in the SIM across the length of the small intestine when compared to the stool microbiome [15].

With respect to obesity, at the genus level, *Lactobacillus* [16] and *Bifidobacterium* [17,18] were identified as important contributors to both the stool microbiome and, in our most recent work, also in the SIM [3]. However, utilizing shotgun sequencing of duodenal SBA of subjects with normal weight and those who were overweight or had obesity, we also successfully elucidated species and strain level data. We identified significant differences in the SIM of normal weight subjects compared to subjects with elevated body weights, specifically identifying bacterial species with varying abundances across the normal weight, overweight, and obesity groups under study. In our work, we categorize the SIM differences into four different "features", depending upon the changes observed between groups: obesity-specific, overweight-specific, escalatory, and de-escalatory.

Obesity-specific features

Alterations of the SIM unique to individuals with obesity when compared to those who were overweight or of normal weight. These include decreased relative abundances of *Leptotricia* sp., *Alloprevoltella rava*, and *Lactobacillus reuteri*, and an increased relative abundance of *Lactobacillus gasseri*. With respect to biochemical pathways, predicted reductions were identified for biogenic amine metabolism and sulfoacetaldehyde degradation pathways.

Overweight-specific features

Alterations of the SIM unique to individuals who were overweight when compared to those of normal weight or with obesity. These include decreased relative abundances of *Paraprevotella*, *Escherichia coli K12*, *Pseudomonas* spp., and *Bifidobacterium* spp., and an increased relative abundance of *Lactobacillus intestinalis*, *Lactobacillus johnsoni*, and *Prevotella loescheli*. With respect to biochemical pathways, predicted reductions were identified for polymyxin resistance, sulfoquinovose degradation I, ursodeoxycholate biosynthesis (bacteria), bile acid epimerization, and biogenic amine metabolism pathways.

Escalation features

Alterations of the SIM going in the same direction from normal weight to obesity. These include decreased relative abundances of *Faecalibacterium* sp., *Bacteroides pyogenes*, and *Staphylococcus hominis*, and an increased relative abundance of *Mycobacterium* sp. and *Lactobacillus* sp.

De-escalation features

Alterations of the SIM for which the direction of change going from normal weight to overweight *reverses* going from overweight to obesity. These included increased relative abundance of *Lactobacillus acidophilus*, *Lactobacillus hominis*, and *Prevotella* spp. in subjects who were overweight compared to normal weight subjects and subjects with obesity. Additionally, decreased relative abundances of *Lactobacillus iners* and *Bifidobacterium dentium* were identified in subjects who were overweight compared to those with normal weight and those with obesity.

In our work, we present the largest and most significant study that characterizes the significant SIM differences in normal weight subjects compared to subjects with elevated body weights. Using advanced shotgun sequencing techniques, we can more precisely identify escalatory or de-escalatory perturbations in specific bacteria. It is widely understood that obesity is a complex, progressive disease process which is impacted by genetics, diet, sedentary behavior, biopsychosocial factors. As gut microbes impact host metabolism [1,19], our work here highlights this understudied aspect of the complex obesity question by systematically probing the microbial population of the SIM to aid in the future development of therapeutics to treat obesity.

Directly Profiling Small Bowel Aspirates (SBA) Plays a Critical Role in Other Significant Findings

Beyond our recent work examining the impacts of obesity on the SIM, we have developed other significant findings using the technique of direct SBA collection and analysis of the SIM. Here, we briefly highlight some of our other previous findings to demonstrate the significance of this technique.

Hormone therapy (HT)

Postmenopausal women on HT have SIMs and metabolic profiles more like that of reproductive-age women than postmenopausal women who are not on HT [5]. Conversely, post-menopausal women who are not on HT have

decreased microbial diversity, notably increased levels of Proteobacteria that correspond to higher reported dysbiosis and increased levels of taxa associated with cardiovascular disease. Metabolically, post-menopausal women not on HT demonstrate increased fasting glucose levels and decreased testosterone levels. Women on HT have similar levels of microbial diversity, fasting glucose, decreased testosterone, relative abundance of phylum Bacteroidetes and genus *Prevotella* compared to reproductive-age women, which suggests a potential protective mechanism of the SIM with respect to the development of cardiovascular disease.

Smoking negatively impacts the SIM

We determined that smoking is associated with significant deleterious impacts on the SIM, particularly with increased relative abundances of the genera *Escherichia-Shigella*, *Lactobacillus*, and *Klebsiella*, and decreased relative abundances of genera associated with SIM diversity (*Prevotella* and *Neisseria*) in current smokers [4]. Interestingly, we found that the deleterious alterations in the SIM caused by smoking appear to be attenuated—but not fully reversed—in former smokers after 10 years or more of smoking cessation. This work is significant because there is increasing evidence to support the existence of the "gut-lung axis", which posits an influence of the SIM on lung function and lung immune response [20]. In the reverse direction, some of the microbes that we detected as altered in the SIM were also altered in the lung microbiome surveyed by other studies (i.e. decreased relative abundances of the genera *Neisseria* and *Porphyromonas* were lower in both the SIM and respiratory microbiome of smokers [21], and *Prevotella* relative abundance was lower in the SIM of smokers and the lung microbiome of lung cancer patients [22].

Artificial sweetener consumption impacts the SIM and the stool microbiome

Non-sugar artificial sweeteners differentially impact the SIM and stool [3], highlighting the importance of direct sampling of the SIM and not limiting the analysis to stool alone. In cases where a stool specimen was available for co-analysis in parallel with SIM, there were no significant differences in alpha and beta diversities across the three groups (control [CON] subjects; aspartame [ASP] users; and non-aspartame, non-sugar sweetener [NANS] users). When the stool microbiome was analyzed at the genus level, there were significant differences in the relative abundances of various microbes between both NANS/CON and ASP/CON groups. However, when the SIM alpha and beta diversities are analyzed, a more complex story emerges of the role non-sugar sweeteners have on the SIM. Of note, the microbial alpha diversity of the SIM is significantly lower in the NANS group compared to the CON group, but no other significant differences were detected, which differs from the stool analysis. Furthermore, when the SIM is analyzed at the genus level, we detected lower relative abundances of *Escherichia* and *Klebsiella* than was found in the stool analyses. These findings suggest that routine consumption of artificial sweeteners has an impact on the SIM, differences which are not captured in stool-only analysis.

Proposed Technical Standard to Accurately Study the SIM

We have and continue to amass a growing body of evidence that demonstrates significant differences between the stool microbiome and SIM as characterized in aspirates and biopsies collected during endoscopy. As we have discussed and wish to emphasize, most of the previous work done in the field of gut microbial analysis has used stool as a surrogate, since it is non-invasive, and can be collected in a longitudinal fashion in great numbers. While there are valuable insights to be gained from analysis of the stool microbiome, stool alone is *not* a suitable means of making accurate conclusions about the entire gut microbiome. We have shown that using a sterile double lumen catheter significantly attenuates the level of contamination of the small intestinal aspirate by microflora present in the mouth and on the non-sterile duodenoscope by protecting the inner lumen until introduction into the small intestinal segment which is being sampled. Proper sterile technique is essential during sampling due to the high risk for contamination when this is not accounted for [23]. The aspirated fluid is then subjected to mucolysis with the reducing agent DTT, permitting significant increase in the final library concentration/DNA yield for 16S and shotgun sequencing without negatively impacting the bacteria themselves.

Finally, to ensure that we are obtaining the most comprehensive picture of the SIM, collection of biopsies during endoscopy under appropriate sterile technique is also part of our protocol, as well as the protocol of our Australian colleagues [24,25]. Collection of small intestinal biopsies is extremely valuable to better understand the mucosal microbiome because many bacteria are incapable of penetrating the dense mucous layer that lines the small intestinal—as well as colonic—mucosa [26]. The important contributions of mucosal biopsy sampling by other researchers [24,25] as well as our sterile aspiration and processing techniques for analyzing the luminal microbiome highlight the importance of obtaining a complete set of specimens to maximize understanding of the entire SIM.

Conclusion

Properly studying the human SIM requires establishing a new technical standard in the scientific community that relies on direct sampling of aspirate and mucosal biopsies during endoscopy with appropriate attention paid to sterile technique. Other methods, such as analyses of stool or small intestinal biopsies, provide different and valuable data, but they are not reflective of the full luminal and mucosal microbial compositions within the small intestine and their effects on the host organism. The layered approach we incorporate

into our growing sample collection provides critical insights to improve understanding of the associations between microbial elements within the SIM and host physiology. With clearer insights into these physiological associations, we hope to conduct the necessary metabolomic and transcriptomic studies to elucidate potential causation, which would aid in the development of targeted therapeutics.

Acknowledgements

The authors thank Frank Lee, Joel Levine, the Monica Lester Charitable Trust, and the Elias, Genevieve, and Georgianna Atol Charitable Trust for their generous support of the MAST program.

Conflicts of Interest

M.P. is a consultant for Bausch Health, Ferring Pharmaceuticals Inc., Salvo Health, Dieta Health, and Cylinder Health Inc. M.P. has received grant support from Bausch Health and Synthetic Biologics. R.M. has received grant support from Bausch Health. Cedars-Sinai has a licensing agreement with Gemelli Biotech and Hobbs Medical. M.P. and R.M. have equity in Gemelli Biotech and GoodLFE. MP has equity in Cylinder Health, Salvo Health, and Dieta Health. All other authors report no conflicts of interest.

Author Contributions

DB wrote the manuscript. GL, MP, GMB, RM revised the manuscript.

References

1. Yersin S, Vonaesch P. Small intestinal microbiota: from taxonomic composition to metabolism. Trends in Microbiology. 18 Mar 2024;18:S0966-842X(24)00056-8.

2. Leite G, Morales W, Weitsman S, Celly S, Parodi G, Mathur R, et al. Optimizing microbiome sequencing for small intestinal aspirates: validation of novel techniques through the REIMAGINE study. BMC Microbiology. 2019 Oct 20;19(239):239-252.

3. Hosseini A, Barlow GM, Leite G, Rashid M, Parodi G, Wang J, et al. Consuming artificial sweeteners may alter the structure and function of duodenal microbial communities. iScience. 2023 Nov 20; 26(12):108530.

4. Leite G, Barlow GM, Hosseini A, Parodi G, Pimentel ML, Wang J, et al. Smoking has disruptive effects on the small bowel liminal microbiome. Sci Rep. 2022 Mar 16;12:6231.

5. Leite G, Barlow GM, Parodi G, Pimentel ML, Chang C, Hosseini A, et al. Duodenal microbiome changes in postmenopausal women: effects of hormone therapy and implications for cardiovascular risk. Menopause. 2021 Oct 21;29(3), 264-275.

6. Weitsman S, Celly S, Leite G, Mathur R, Sedighi R, Barlow GM, et al. Effects of proton pump inhibitors on the small bowel and stool microbiomes. Dig Dis Sci. 2021 Jan 17;67:224-232.

7. Hosseini A, Rashid M, Leite G, Barlow GM, Parodi G, Sanchez M, et al. Coronavirus Disease 2019 (COVID-19) pandemic lifestyle changes may have influenced small bowel microbial composition and microbial resistance. Dig Dis Sci. 2023 Jul 26;68:3902-3912.

8. Leite G, Barlow GM, Rashid M, Hosseini A, Cohrs D, Parodi G, et al. Characterization of the small bowel microbiome reveals different profiles in human subjects who are overweight or have obesity. Am J Gastroenterol. 2024 Mar 14;119(6):1141.

9. Alili R, Belda E, Fabre O, Pelloux V, Giordano N, Legrand R, et al. Characterization of the gut microbiota in individuals with overweight or obesity during a real-world weight loss dietary program: a focus on Bacteroides 2 enterotype. Biomedicines. 2021 Dec 17;10(1):16.

10. Turnbaugh PJ, Hamaday M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 2008 Nov 30;457:480-484.

11. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, et al. Microbiota and SCFA in lean and overweight healthy subjects. Obesity. 2012 Dec 31;18:190-195.

12. Duncan S, Lobley G, Holtrop G, Ince J, Johnstone AM, Louis P, et al. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obesity. 2008 Aug 10;32:1720-1724.

13. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity. Nature. 21 Dec 2006;444:1027-1031.

14. Mariat D, Firmesse O, Levenez F, Gimaraes VD, Sokol H, Dore J, et al. The Firmicutes/Bacteriodetes ratio of the human microbiota changes with age. BMC Microbiology. 2009 Jun 09;9:123.

15. Leite GGS, Weitsman S, Parodi G, Celly C, Sedighi R, Sanchez M, et al. Mapping the segmental microbiomes in the human small bowel in comparison with stool: a REIMAGINE study. Dig Dis Sci. 2020 Feb 24;2020(65):2595-2604.

16. Crovesy L, Ostrowski M, Ferreira DMTP, Rosado EL, Soares-Mota M. Effect of Lactobacillus on body weight and body fat in overweight subjects: A systematic review of randomized controlled clinical trials. Int J Obes. 2017;41(11):1607-1614.

17. Kim G, Yoon Y, Park JH, Park JW, Noh M-G, Kim H, et al. Bifidobacterial carbohydrate/nucleoside metabolism enhances oxidative phosphorylation in white adipose tissue to protect against diet-induced obesity. Microbiome. 2022;10(1):188.

18. Ma L, Zheng A, Ni L, Wu L, Hu L, Zhao Y, et al. Bifidobacterium animalis subsp. lactis lkm512 attenuates obesity-associated inflammation and insulin resistance through the modification of gut microbiota in high-fat diet-induced obese mice. Mol Nutr Food Res. 2022;66(3):e2100639.

19. Barlow GM, Mathur R. Type 2 Diabetes and the Microbiome. Journal of the Endocrine Society. 2022;7(2):bvac184.

20. Budden KF, Gellatly SL, Wood DLA, Cooper MA, Morrison

M, Hugenholtz P, et al. Emerging pathogenic links between microbiota and the gut-lung axis. Nature Rev Micro. 2016 Oct 03;15(1):55-63.

21. Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Am J Resp Crit Care Med. 2013 Feb 22;187(10):1067-1075.

22. Zheng X, Sun X, Liu Qm Huang Y, Yuan Y. The composition alteration of respiratory microbiota in lung cancer. Cancer Invest. 2020 Feb 17;38(3):158-168.

23. Cangemi DJ, Lacy BE, Wise J. Diagnosing small intestinal bacterial overgrowth: a comparison of lactulose breath tests to small bowel aspirates. Dig Dis Sci. 2021 Jun;66(6):2042-2050.

24. Shanahan ER, Shah A, Koloski N, Walker MM, Talley NJ, Morrison M, et al. Influence of cigarette smoking on the human duodenal mucosa-associated microbiota. Microbiome. 2019 Aug 29;6(1):150.

25. Shanahan ER, Kang S, Staudacher H, Shah A, Do A, Burns G, et al. Alterations to the duodenal microbiota are linked to gastric emptying and symptoms in functional dyspepsia. Gut. 2023 Jul 12;72(5):929-938.

26. Schroeder BO. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. Gastro Report. 2019 Feb 13;7(1):3-12.