START CENTER | STRATEGIC ANALYSIS, RESEARCH & TRAINING CENTER
Department of Global Health | University of Washington

GUT HEALTH DIGEST

UNIVERSITY OF WASHINGTON STRATEGIC ANALYSIS, RESEARCH & TRAINING (START) CENTER

REPORT TO THE BILL & MELINDA GATES FOUNDATION

JANUARY 9, 2018

PRODUCED BY: HERGOTT, D; ARNDT, M.

Want the Gut Health Digest delivered directly to your inbox?
Subscribe on the Digest website: http://uwstartcenter.org/what-we-do/gut-health-digest/

LIST OF ARTICLES

1. **Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria.** {Abstract & START Commentary} {Full article}
    - Series of mouse experiments to evaluate immune response pre and post-weaning.
2. **Dysbiosis-Associated Change in Host Metabolism Generates Lactate to Support *Salmonella* Growth.** {Abstract & START Commentary} {Full article}
    - Mouse experiments evaluating the biological response in the gut during *Salmonella* infection.
3. **Recurrent infection progressively disables host protection against intestinal inflammation.** {Abstract & START Commentary} {Full article}
    - Series of mouse experiments to explore immunological response to low titer *Salmonella* infections.
4. **Enteropathogens and Gut Inflammation in Asymptomatic Infants and Children in Different Environments in Southern India.** {Abstract & START Commentary} {Full article}
    - Analysis of gut microbiomes in infants and young children from a high and low SES area in Vellore.
5. **Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants.** {Abstract & START Commentary} {Full article}
    - Analysis and comparison of infant gut microbiome during first 6 weeks of life from mothers who did and did not receive intrapartum antibiotics during birth.
6. **Galactooligosaccharide supplementation provides protection against *Citrobacter rodentium*-induced colitis without limiting pathogen burden.** {Abstract & START Commentary} {Full article}
    - *In vitro* and *in vivo* mouse experiments to explore mechanism of the protective effect of GOS on colitis following *C. rodentium* infection.
7. **Breastfeeding-associated microbiota in human milk following supplementation with *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La-5, and *Bifidobacterium animalis* subspecies *lactis* Bb-12.** {Abstract & START Commentary} {Full article}
    - Analysis of microbiota in breastmilk from mothers in the ProPACT trial.
8. ***Bifidobacteria* or Fiber Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration.** {Abstract & START Commentary} {Full article}
    - Investigation of the impact of Western style diet on the gut mucus composition of mice using an *ex vivo* transplant method.
9. **Enterotypes in the landscape of gut microbial community composition.** {Abstract & START Commentary} {Full article}
    - Review and reanalysis of three large databases to test different enterotype hypotheses.
10. **Persistence of Supplemented *Bifidobacterium longum* subsp. *infantis* EVC001 in Breastfed Infants.** {Abstract & START Commentary} {Full article}
    - Comparison of microbiota composition and lactate production in infants who received supplemental *B. longum* along with breastmilk during first month of life.

DETAILS OF ARTICLES

1. Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria.
   Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Coughin PE, et al.
   Science Immunology. 2(18). 2017 December 15.
   PubMed ID: 29246946

ABSTRACT
We have a mutually beneficial relationship with the trillions of microorganisms inhabiting our gastrointestinal tract. However, maintaining this relationship requires recognizing these organisms as affable and restraining inflammatory responses to these organisms when encountered in hostile settings. How and when the immune system develops tolerance to our gut microbial members is not well understood. We identify a specific preweaning interval in which gut microbial antigens are encountered by the immune system to induce antigen-specific tolerance to gut bacteria. For some bacterial taxa, physiologic encounters with the immune system are restricted to this interval, despite abundance of these taxa in the gut lumen at later times outside this interval. Antigen-specific tolerance to gut bacteria induced during this preweaning interval is stable and maintained even if these taxa are encountered later in life in an inflammatory setting. However, inhibiting microbial antigen encounter during this interval or extending these encounters beyond the normal interval results in a failure to induce tolerance and robust antigen-specific effector responses to gut bacteria upon reencounter in an inflammatory setting. Thus, we have identified a defined preweaning interval critical for developing tolerance to gut bacteria and maintaining the mutually beneficial relationship with our gut microbiota.

DOI: 10.1126/sciimmunol.aao1314
IMPACT FACTOR: *NA*
CITED HALF-LIFE: *NA*

START COMMENTARY: The authors ran a series of experiments in mice to evaluate the immune responses to microorganisms during three distinct early life phases: the neonatal phase (Day 0-10), day 11 to weaning, and post-weaning. Initial experiments revealed that the formation of colonic Goblet Cell –associated antigen passages (GAPs) was necessary for the immune system's encounter with gut microbial antigens and the development of peripheral Foxp3+ regulatory T cells (pT_{regs}). The authors then investigated how GAP inhibition is controlled during the three life phases. Figure 8 presents a graphical summary of the immunological events determined by the investigators during these three stages of life. In brief, preweaning, breastmilk introduces a high level of luminal EGF into the immune system, which inhibits the ability of GAPs. As the mice wean and the luminal EGF decreases, GAPs are able to form and the colonic immune system is introduced to bacterial antigens. Post-weaning, GAPs are once again inhibited by the high microbial load, and the population of pTregs that was developed during the weaning stage is maintained. Figure 2D shows that the cecal microbial load increased logarithmically during the post-weaning phase. Figure 2E demonstrates the shift in microbiota composition and load that occurred during the first 28 days of life. Preweaning, the gut was dominated by low levels of *Clostridia* and *Gammaproteobacteria*. During the weaning phase, Clostridia continued to increase in abundance, along with bacteroidia and bacilli. These results are consistent with several human studies that show a shift in the gut microbiota during weaning.

{Return to List of Articles}

2. Dysbiosis-Associated Change in Host Metabolism Generates Lactate to Support Salmonella Growth.
   Gillis CC, Hughes ER, Spiga L, Winter MG, Zhu W, Furtado de Carvalho T, *et al.*
   Cell Host & Microbe. 23. 2017 December 21.
   PubMed ID: 29276172

ABSTRACT:
During *Salmonella*-induced gastroenteritis, mucosal inflammation creates a niche that favors the expansion of the pathogen population over the microbiota. Here, we show that *Salmonella* Typhimurium infection was accompanied by dysbiosis, decreased butyrate levels, and substantially elevated lactate levels in the gut lumen. Administration of a lactate dehydrogenase inhibitor blunted lactate production in germ-free mice, suggesting that lactate was predominantly of host origin. Depletion of butyrateproducing Clostridia, either through oral antibiotic treatment or as part of the pathogen-induced dysbiosis, triggered a switch in host cells from oxidative metabolism to lactate fermentation, increasing both lactate levels and *Salmonella* lactate utilization. Administration of tributyrin or a PPARγ agonist diminished host lactate production and abrogated the fitness advantage conferred on Salmonella by lactate utilization. We conclude that alterations of the gut microbiota, specifically a depletion of Clostridia, reprogram host metabolism to perform lactate fermentation, thus supporting Salmonella infection.

DOI: http://dx.doi.org/10.1016/j.chom.2017.11.006
IMPACT FACTOR: 14.946
CITED HALF-LIFE: 4.3

START COMMENTARY: The authors used an untargeted semi-quantitative analysis to profile the extracellular metabolites present in the gut lumen during *S.* Typhimurium (Tm) infection. After lactate was discovered to be highly elevated, the authors performed a series of experiments in mice to better understand the source and purpose of the increased lactate levels during infection. One experiment investigated changes in the host microbiota after infection. Swiss Webster mice were intragastrically inoculated with *S.* Tm infection or mock treated. After 8 days, the cecal contents of the mice were analyzed by rDNA sequencing. Figure 4A shows that *S.* Tm infection shifted the microbiota composition of the host. Figure 4B shows that mock treated mice had high levels of *Bacteroidetes* and *Firmicutes*, while the microbiota following *S.* Tm infection was dominated by *Proteobacteria* with reduced abundance of *Bacteroidetes* and *Firmicutes.* Figure 4C confirms that there were significantly higher levels of Clostridia in mock treated mice, and an increase of Gammaproteobacteria in *S.* Tm infected mice. Clostridia produce butyrate. Butyrate is consumed through β-oxidation. As shown in Figures 3B and C, when butyrate concentration decreased, lactate production increased, as cells shifted to fermentation. Through additional experimentation, the authors tested the hypothesis that lactate production from fermentation could be prevented by activating PAPRγ signaling, which controls the switch from fermentation to β-oxidation, as shown in Figure 5A. Figures 5B and C show the butyrate and lactate concentrations in streptomycin-treated mice given a vehicle control versus rosiglitazone (a PPARγ agonist). As predicted, lactate concentrations were significantly lower in rosiglitazone mice, and the fitness advantage provided by L-lactate utilization during *S.* Tm infection was lost in these mice, supporting the hypothesis that PAPRγ controls the switch responsible for lactate utilization during *S.* Tm infection. The authors concluded that *S. Tm* infection reduces the butyrate-producing Clostridia, which increases the availability of lactate that promotes *S.* Tm growth.

{Return to List of Articles}

3. Recurrent infection progressively disables host protection against intestinal inflammation.
Yang WH, Heithoff DM, Aziz PV, Sperandio M, Nizet V, Mahan MJ, Marth JD.
Science. 358. 2017 December 22.
PubMed ID: 29269445

ABSTRACT
Intestinal inflammation is the central pathological feature of colitis and the inflammatory bowel diseases. These syndromes arise from unidentified environmental factors. We found that recurrent nonlethal gastric infections of Gram-negative *Salmonella enterica* Typhimurium (ST), a major source of human food poisoning, caused inflammation of murine intestinal tissue, predominantly the colon, which persisted after pathogen clearance and irreversibly escalated in severity with repeated infections. ST progressively disabled a host mechanism of protection by inducing endogenous neuraminidase activity, which accelerated the molecular aging and clearance of intestinal alkaline phosphatase (IAP). Disease was linked to a Toll-like receptor 4 (TLR4)–dependent mechanism of IAP desialylation with accumulation of the IAP substrate and TLR4 ligand, lipopolysaccharide-phosphate. The administration of IAP or the antiviral neuraminidase inhibitor zanamivir was therapeutic by maintaining IAP abundance and function.

DOI: 10.1126/science.aao5610
IMPACT FACTOR: 37.205
CITED HALF-LIFE: >10.0

START COMMENTARY: The authors conducted a series of experiments in mice to explore the effect of low titer ST infection on the colon and the mechanism by which the effect occurred. Initial experiments involved injecting mice with low level doses of ST infection every 4 weeks for six months. All mice did not show disease phenotypes (diarrhea, fecal blood, poor stool consistency) until subsequent infections occurred, most commonly presenting following the fourth infection, as shown in Figure 1C, D and E. Concordantly, investigators observed that IAP abundance and AP activity decreased in infected mice at the time that symptoms worsened (Figure 1G and H). Additionally, intestinal LPS abundance was significantly higher in ST treated mice than in WT mice. When calf IAP (cIAP) therapy was administered to ST infected mice, LPS levels were not significantly different from WT mice (Figure 1K), suggesting that IAP is necessary to dephosphorylate and detoxify the LPS endotoxins produced by the commensal microbiota. The same reduction in AP activity and IAP abundance, and subsequent increase in LPS abundance, was seen in mice who lacked a functional *St3gal6* gene, as shown in Figures 3A, B, and C. The addition of cIAP therapy to *St3gal6* knockout mice and those who received low level infections of ST restored the levels of IAP, increased AP activity and maintained low levels of LPS. The corresponding disease phenotypes were not present once cIAP treatment was given. Analysis of the microbiota of WT mice and those lacking *St3gal6* showed that *St3gal6*$^{\Delta/\Delta}$ had significantly higher levels of *Enterobacteriaceae* than WT mice, which is consistent with human studies that show an abundance of this family in individuals with IBS. In addition to an increase in LPS, ST infected mice also showed an increase in Neuraminidase (Neu) activity. Figure 5B shows that Neu3 activity was significantly increased following ST infection. When investigators treated mice with Zanamivir, a Neu inhibitor, inflammation following ST infection was significantly reduced, suggesting the Neu3 is necessary in the inflammatory response induced by ST, and that Neu inhibitors could be a possible treatment to prevent IBS and similar diseases. A graphical summary of the proposed mechanism of intestinal inflammation due to ST infection is presented in Figure 8.

{Return to List of Articles}

4. Enteropathogens and Gut Inflammation in Asymptomatic Infants and Children in Different Environments in Southern India.
   Praharaj I, Revathy R, Bandyopadhyay R, Benny B, Azharuddin M, Lie J, et al.
   American Journal of Tropical Medicine and Hygiene. 2017 December 11. [Epub ahead of print]
   PubMed ID: 29231154

ABSTRACT
Children in poor environmental conditions are exposed early and often to enteric pathogens, but within developing countries, heterogeneity in enteropathogen exposure in different settings and communities is rarely addressed. We tested fecal samples from healthy infants and children from two different environments in the same Indian town for gut enteropathogens and biomarkers of gut inflammation. A significantly higher proportion of infants and children from a poor semi-urban neighborhood (93%) had one or more enteropathogens than those from a medical college campus (71.7%). Infants and children from the poor neighborhood had an average of 3.3 (95% confidence interval [CI]: 2.9–3.7) enteropathogens compared with an average of 1.4 (95% CI: 1.0–1.7) enteropathogens in campus infants/children. Viral and bacterial infections, including enteroviruses, adenoviruses, Campylobacter spp., and diarrhegenic *Escherichia coli* were more common and fecal biomarkers of inflammation were higher in the poor neighborhood. The findings demonstrate significant difference in the asymptomatic carriage of gut enteropathogens and gut inflammatory biomarkers in infants and children from two different environments within the same town in south India.

DOI: 10.4269/ajtmh.17-0324
IMPACT FACTOR: 2.485
CITED HALF-LIFE: >10.0

START COMMENTARY: Infants and children up to 4 years of age were recruited from two different settings in Vellore, southern India. There were 53 samples collected from children living in a college medical campus, where most families have high SES and access to piped chlorinated drinking water and closed water drainage. There are no livestock living on the campus. 86 samples were collected from children living in Chinnallapuram, a semi-urban area on the outskirts of Vellore that in contrast has intermittent access to water, an open drainage system, high levels of contaminants in drinking water, and 18% of households with domestic cattle residing in our near their household. While gut enteropathogens were detected in samples from both localities in high numbers, there were significantly more infected children and more pathogens per child on average in Chinnallapuram compared to the medical campus. Figure 1 shows the percentage of samples from each locality that were infected with different viruses, bacterial enteropathogens and parasites. No parasitic infections were observed in children from the medical campus, while almost 30% of children from Chinnallapuram had *Giardia intestinalis*. In all age groups, fecal MPO concentrations were significantly higher in Chinnallapuram infants/children compared with those living on the hospital campus. Fecal MPO was also associated with enteropathogen burden. The study is limited by the fact that individual household level data on access and quality of water was not collected which would allow further investigation of the driving factors of enteropathogen infection. The study suggests that there can be significant heterogeneity in pediatric enteropathogen burden within the same city that should be further explored to best target interventions and preventative measures.

{Return to List of Articles}

5. Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants.
   Stearns JC, Simioni J, Gunn E, McDonald H, Holloway AC, Thabane L, *et al*.
   Scientific Reports. 7. 2017 November 28.
   PubMed ID: 29184093

ABSTRACT
Early life microbial colonization and succession is critically important to healthy development with impacts on metabolic and immunologic processes throughout life. A longitudinal prospective cohort was recruited from midwifery practices to include infants born at full term gestation to women with uncomplicated pregnancies. Here we compare bacterial community succession in infants born vaginally, with no exposure to antibiotics (n = 53), with infants who were exposed to intrapartum antibiotic prophylaxis (IAP) for Group B *Streptococcus* (GBS; n = 14), and infants born by C-section (n = 7). Molecular profiles of the 16 S rRNA genes indicate that there is a delay in the expansion of *Bifidobacterium*, which was the dominate infant gut colonizer, over the first 12 weeks and a persistence of Escherichia when IAP for GBS exposure is present during vaginal labour. Longer duration of IAP exposure increased the magnitude of the effect on *Bifidobacterium* populations, suggesting a longer delay in microbial community maturation. As with prior studies, we found altered gut colonization following C-section that included a notable lack of Bacteroidetes. This study found that exposure of infants to IAP for GBS during vaginal birth affected aspects of gut microbial ecology that, although dramatic at early time points, disappeared by 12 weeks of age in most infants.

DOI: 10.1038/s41598-017-16606-9
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 2.0

START COMMENTARY: Baby-mother pairs were recruited from the Baby & Mi pilot cohort study in Ontario, Canada and requested to collect infant stool samples on 3 days, 10 days, 6 weeks and 12 weeks postpartum. Figure 1 shows the relative differences in abundance of microbial communities between the different delivery methods and IAP administration groups at these time points. In all three groups, *Proteobacteria* was the dominate microbial community at 3 days, while the presence of *Actinobacteria* increased over time. Babies delivered vaginally without IAP had the greatest microbial diversity at day 3, but the authors note that by week 12, there were no differences between groups. Figure 2B compares the change in abundance of different bacterial genera over time in vaginally delivered infants whose mothers did or did not receive IAP. This figure further shows that there were larger differences in gut microbiota in early infancy, with IAP exposed infants having higher levels of *Escherichia and Parabacteroides* and lower diversity at week 2, but by week 14, the composition between the two groups was fairly similar. However, as seen in Table 4, microbiota compositional change was greater and more durable in infants who were exposed to IAP for longer amounts of time. The generalizability of the study is limited by the small number of infants who were born to mothers who received IAP.

{Return to List of Articles}

6. Galactooligosaccharide supplementation provides protection against Citrobacter rodentium-induced colitis without limiting pathogen burden.
   Kittana H, Quintero-Villegas M, Bindels LB, Gomes-Neto JC, Schmaltz RJ, Segura Munoz RR, *et al. Microbiology.* 2017 December 19. [Epub ahead of print]
   PubMed ID: 29256851

ABSTRACT
Many enteric pathogens, including *Salmonella* and enteropathogenic and enterohemorrhagic *Escherichia coli*, express adhesins that recognize and bind to carbohydrate moieties expressed on epithelial cells. An attractive strategy for inhibiting bacterial adherence employs molecules that mimic these epithelial binding sites. Prebiotic oligosaccharides are non-digestible, fermentable fibres capable of modulating the gut microbiota. Moreover, they may act as molecular decoys that competitively inhibit adherence of pathogens to host cells. In particular, galactooligosaccharides (GOS) and other prebiotic fibres have been shown to inhibit pathogen adherence to epithelial cells *in vitro*. In the present study, we determined the ability of prophylactic GOS administration to reduce enteric pathogen adherence both *in vitro* and *in vivo* as well as protect against intestinal inflammation. GOS supplementation significantly reduced the adherence of the epithelial-adherent murine bacterial pathogen *Citrobacter rodentium* in a dose-dependent manner to the surface of epithelial cells *in vitro*. A 1- to 2-log reduction in bacterial adherence was observed at the lowest and highest doses tested, respectively. However, mouse studies revealed that treatment with GOS neither reduced the adherence of *C. rodentium* to the distal colon nor decreased its dissemination to systemic organs. Despite the absence of adherence inhibition, colonic disease scores for GOS-treated*, C. rodentium*-infected mice were significantly lower than those of untreated *C. rodentium*-infected animals (P=0.028). Together, these data suggest that GOS has a direct protective effect in ameliorating disease severity following *C. rodentium* infection through an anti-adherence-independent mechanism.

DOI: 10.1099/mic.0.000593
IMPACT FACTOR: 0.856
CITED HALF-LIFE: 10.0

START COMMENTARY: *In vitro* experiments were performed using a HEp-2 cell line, incubated with *C. rodentium* either with or without GOS. While not tested, the authors suggest that the reduced adherence seen *in vitro* but not *in vivo* may be due to differential gene expression in the *in vivo* model that only allows for the expression of GOS-sensitive adhesins. While GOS did not reduce the adherence of *C. rodentium* in infected mice, it did reduce colonic tissue damage in infected mice, as shown in Figure 3. The authors conducted a series of experiments to explore the possible mechanisms by which this occurs. Addition of GOS did not alter expression of colonic IL-1B nor IL-6, as shown in Figure 4. Investigators examined the abundance of *C. rodentium* in the spleen and liver of infected mice with and without GOS administration, and found significantly higher levels of *C. rodentium* in the spleens of mice who received GOS. Based on these findings, the authors suggested that GOS may reduce colonic tissue damage by altering intestinal permeability and promoting bacterial translocation. However, there are contrasting findings in the literature related to intestinal permeability and prebiotics, and further investigation is needed. The authors also suggested that GOS may alter the composition of the gut microbiota, which therefore provides a protective environment, however this hypothesis was not tested by the investigators.

{Return to List of Articles}

7. Breastfeeding-associated microbiota in human milk following supplementation with *Lactobacillus rhamnosus GG, Lactobacillus acidophilus* La-5, and *Bifidobacterium animalis* subspecies *lactis* Bb-12.
   Simpson MR, Avershina E, Storro O, Johnsen R, Rudi K, and Oien T.
   Journal of Dairy Science. 2017 December 13. [Epub ahead of print]
   PubMed ID: 29248229

ABSTRACT
Breastfeeding is one of the major factors affecting the early development of the infant gut microbiota, and weaning is associated with a shift in the gut microbiota toward a more adult composition. Through breastfeeding, infants receive bioactive components that shape their microbiota while also being exposed to the breast milk and breast surface microbial communities. Recent studies have suggested the possibility of an enteromammary route of microbial transfer, opening the possibility of infant gut microbiota modulation through maternal probiotic supplementation. In this study, we have analyzed breast milk samples collected at 10 d and 3 mo postpartum from women participating in the Probiotics in the Prevention of Allergy among Children in Trondheim (ProPACT) placebo controlled trial. Women who were randomized to the probiotic arm of the Probiotics in the Prevention of Allergy among Children in Trondheim trial received a fermented milk supplemented with *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La-5, and *Bifidobacterium animalis* ssp. *lactis* Bb-12, consuming this daily from 4 wk before their expected due date until 3 mo after birth. In total, 472 breast milk samples were assessed for the administered bacteria using quantitative real-time PCR and the microbiota transferred during breastfeeding was analyzed using 16S ribosomal RNA gene sequencing of 142 samples. We found that breastfeeding is unlikely to be a significant source of *L. rhamnosus* GG, *L. acidophilus* La-5, and *B. animalis* ssp*. lactis* Bb-12 for infants in the probiotic arm of the trial. Furthermore, maternal supplementation did not significantly affect the overall composition of the breast milk microbiota transferred during breastfeeding. We also present a descriptive analysis of this microbiota, which was largely dominated by *Streptococcus* and *Staphylococcus* genera at both 10 d and 3 mo postpartum. Samples collected at 3 mo postpartum had a statistically significant lower presence and relative abundance of the Staphylococcus genus. These samples also had a greater number of observed species and diversity, including more operational taxonomic units from the *Rothia, Veillonella, Granulicatella,* and *Methylbacterium* genera.

DOI: 10.3168/jds.2017-13411
IMPACT FACTOR: 1.564
CITED HALF-LIFE: 9.9

START COMMENTARY: Authors analyzed 472 breastmilk samples from 252 women who participated in the in the ProPACT trial. Previous analysis of stool samples collected from mothers and babies in this trial showed that women who received probiotic supplementation and their children both had higher abundance of all 3 supplemental probiotic strains in their samples than women and infants in the placebo arm. Analysis of the breastmilk samples in this study suggest that the transfer of these probiotics does not occur through the breast milk or the breastfeeding process. Breast milk samples were collected by the women themselves at home, without sterilization procedures before collection. Analysis of the microbiota at the genus and phyla level in the breastmilk samples revealed high variability between all mothers. Only 11 genera were detected by qPCR in at least 10% of samples at either time point, and are presented in Figure 2 and Table 3. There were relatively few differences between placebo and probiotic group.

{Return to List of Articles}

8. Bifidobacteria or Fiber Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration.
   Schroeder BO, Birchenough GMH, Ståhlman M, Arike L, Johansson MEV, Hansson GC, Bäckhed F.
   Cell Host & Microbe. 2017 December 16. [Epub ahead of print]
   PubMed ID: 29276171

ABSTRACT
Diet strongly affects gut microbiota composition, and gut bacteria can influence the colonic mucus layer, a physical barrier that separates trillions of gut bacteria from the host. However, the interplay between a Western style diet (WSD), gut microbiota composition, and the intestinal mucus layer is less clear. Here we show that mice fed a WSD have an altered colonic microbiota composition that causes increased penetrability and a reduced growth rate of the inner mucus layer. Both barrier defects can be prevented by transplanting microbiota from chow-fed mice. In addition, we found that administration of *Bifidobacterium longum* was sufficient to restore mucus growth, whereas administration of the fiber inulin prevented increased mucus penetrability in WSD-fed mice. We hypothesize that the presence of distinct bacteria is crucial for proper mucus function. If confirmed in humans, these findings may help to better understand diseases with an affected mucus layer, such as ulcerative colitis.

DOI: 10.1016/j.chom.2017.11.004
IMPACT FACTOR: 14.946
CITED HALF-LIFE: 4.3

START COMMENTARY: The authors investigated the impact of WSD on the gut mucus composition of mice using an *ex vivo* transplant method. Briefly, at study conclusion, the mouse intestine was flushed with Krebs buffer to remove luminal content and unattached mucus to allow for the measurement of the inner mucus layer. Initial experiments demonstrated that compared to chow-fed mice, a WSD resulted in increased glucose and insulin levels, as well as increased penetrability and decreased mucus growth rates (Figure 1). Authors investigated penetrability and mucus growth rates in genetically obese mice fed a chow diet and observed no significant changes compared to lean chow fed mice, suggesting that the WSD itself, not the obesity that results from it, causes the changes in gut composition. The authors performed further experiments to evaluate differences in gut microbiota composition between the two different diets. Figures 4E and F show the relative abundance of luminal colonic bacteria at the phylum and genus level as well as phylogenetic diversity between day 0 and 28, in mice fed a WSD. Taken together, these two figures demonstrate that WSD alters the gut microbiota, decreasing diversity and increasing the presence of Firmicutes while decreasing the abundance of Bacteroidetes. To investigate whether the diet itself was impacting the microbiota, the authors transplanted microbiota from chow-fed mice to WSD mice, while continuing WSD feeding. Figure 5 shows that mice with chow transplanted microbiota had higher levels of Bacteroidetes and greater phylogenetic diversity than non-chow transplant mice. While there were no differences in metabolic parameters between the two groups of mice, penetrability of the mucus layer was significantly lower in WSD->Chow mice and mucus growth was fully restored. The authors hypothesized that the presence of distinct bacteria, including Bacteroidetes, is necessary for proper mucus function. Finally, the impact of inulin or Bifidobacteria supplementation on WSD mice was examined in Figure 6, where investigators observed increased mucus growth in the former and decreased colonic penetrability in the latter.

{Return to List of Articles}

9. Enterotypes in the landscape of gut microbial community composition.
   Costea PI, Hildebrand F, Manimozhiyan A, Bäckhed F, Blaser MJ, Bushman FD, et al.
   Nature microbiology. 3(1). 2017 December 17. [Epub ahead of print]
   PubMed ID: 29255284

ABSTRACT
Population stratification is a useful approach for a better understanding of complex biological problems in human health and wellbeing. The proposal that such stratification applies to the human gut microbiome, in the form of distinct community composition types termed enterotypes, has been met with both excitement and controversy. In view of accumulated data and re-analyses since the original work, we revisit the concept of enterotypes, discuss different methods of dividing up the landscape of possible microbiome configurations, and put these concepts into functional, ecological and medical contexts. As enterotypes are of use in describing the gut microbial community landscape and may become relevant in clinical practice, we aim to reconcile differing views and encourage a balanced application of the concept.

DOI: 10.1038/s41564-017-0072-8
IMPACT FACTOR: NA
CITED HALF-LIFE: NA

START COMMENTARY: The authors of this study reviewed current literature and suggested enterotype compositions and then tested the hypotheses in three large metagenomics datasets: (1) the US National Institute of Health Human Microbiome Project (HMP) (2) the European Metagenomics of the Human Intestinal Tract (MetaHIT) project and (3) a Chinese type II diabetes study. A limitation of the analysis, noted by the authors, is that samples are not consistently collected and analyzed across study populations, so it is difficult to compare enterotypes between studies or define meaningful and robust boundaries to define enterotypes when not comparing between the same population. However, despite the limitations, the authors found that in both a two and three-cluster enterotype model, a *Prevotella*-dominated microbiome could always be separated out, and a three cluster-model can also identify *Bacteroides* and Firmicutes dominated clusters as well. As can be seen in Figure 2D, microbiomes dominated by *Prevotella* (ET P) were easily separated from other enterotypes, while it was more difficult to definitively separate enterotypes with predominate *Bacteroides,* Firmicutes or mixed. The HMP dataset contains metagenomics time series data. Analysis of the longitudinal data showed that there was significant stability in enterotypes of individuals over a 6-month period, supporting the hypothesis that the gut microbiome remains relatively stable over time. However, about 16% of individuals shifted enterotypes in this time period, suggesting there are factors that influence changes. The dataset was limited in that it did not contain more detailed individual-level data on diet, underlying health conditions, or other factors that would allow authors to further investigate what might drive enterotype changes.

{Return to List of Articles}

10. Persistence of Supplemented *Bifidobacterium longum* subsp. *infantis* EVC001 in Breastfed Infants.
    Frese SA, Hutton AA, Contreras LN, Shaw CA, Palumbo MC, Casaburi G, *et al.*
    mSphere. 2(6). 2017 December 6.
    PubMed ID: 29242832

ABSTRACT
Attempts to alter intestinal dysbiosis via administration of probiotics have consistently shown that colonization with the administered microbes is transient. This study sought to determine whether provision of an initial course of *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) would lead to persistent colonization of the probiotic organism in breastfed infants. Mothers intending to breastfeed were recruited and provided with lactation support. One group of mothers fed *B. infantis* EVC001 to their infants from day 7 to day 28 of life (n = 34), and the second group did not administer any probiotic (n = 32). Fecal samples were collected during the first 60 postnatal days in both groups. Fecal samples were assessed by 16S rRNA gene sequencing, quantitative PCR, mass spectrometry, and endotoxin measurement. *B. infantis*-fed infants had significantly higher populations of fecal *Bifidobacteriaceae*, in particular *B. infantis*, while EVC001 was fed, and this difference persisted more than 30 days after EVC001 supplementation ceased. Fecal milk oligosaccharides were significantly lower in *B. infantis* EVC001-fed infants, demonstrating higher consumption of human milk oligosaccharides by *B. infantis* EVC001. Concentrations of acetate and lactate were significantly higher and fecal pH was significantly lower in infants fed EVC001, demonstrating alterations in intestinal fermentation. Infants colonized by Bifidobacteriaceae at high levels had 4-fold-lower fecal endotoxin levels, consistent with observed lower levels of Gram-negative *Proteobacteria* and *Bacteroidetes*. IMPORTANCE The gut microbiome in early life plays an important role for long-term health and is shaped in large part by diet. Probiotics may contribute to improvements in health, but they have not been shown to alter the community composition of the gut microbiome. Here, we found that breastfed infants could be stably colonized at high levels by provision of *B. infantis* EVC001, with significant changes to the overall microbiome composition persisting more than a month later, whether the infants were born vaginally or by caesarean section. This observation is consistent with previous studies demonstrating the capacity of this subspecies to utilize human milk glycans as a nutrient and underscores the importance of pairing a probiotic organism with a specific substrate. Colonization by *B. infantis* EVC001 resulted in significant changes to fecal microbiome composition and was associated with improvements in fecal biochemistry. The combination of human milk and an infant-associated Bifidobacterium sp. shows, for the first time, that durable changes to the human gut microbiome are possible and are associated with improved gut function.

DOI: 10.1128/mSphere.00501-17
IMPACT FACTOR: NA
CITED HALF-LIFE: NA

START COMMENTARY: In addition to the primary comparison of infants who received *B. infantis* and those who did not, the authors compared the relative abundance of bacteria in control infants delivered vaginally (DV) to those delivered via caesarean section (CS). Consistent with other studies, figure 1A shows that DV infants had higher levels of *Bacteroidaceae* and lower levels of several other bacteria. These differences persisted over the first 60 days of life, even though all infants were exclusively breastfed (Figure 2). In EVC001-fed infants, the within-group UniFrac distances decreased significantly between day 6 and day 10 of life regardless of delivery mode (Figure 3), indicating that the microbiome of supplemented individuals became more similar over this time. This phenomenon was not observed in

control participants. The investigators observed higher microbial community stability through the first 60 days of life in the EVC001-fed participants, compared with CS or VD control infants (Figure 4). Investigators also compared fecal lactate and short chain fatty acid profiles at day 6 and day 29 of the study, and observed significant increases in lactate and acetate in the EVC001-fed participants (Table 4). Authors noted this was not surprising, given that the central metabolic pathways for the supplemented *B. infantis* species produces both of these compounds as products of fermentation. Figure 4 shows that fecal HMO concentration decreased in the EVC001 group between days 6 and 29, suggesting increased oligosaccharide consumption by *B. infantis*, and reflected by the increased concentration of organic acid in feces.

{Return to List of Articles}

ADDITIONAL ARTICLES OF INTEREST

Impact of prematurity and nutrition on the developing gut microbiome and preterm infant growth.

Intergenerational transfer of antibiotic-perturbed microbiota enhances colitis in susceptible mice.

Bacteriological and Immunological Profiling of Meconium and Fecal Samples from Preterm Infants: A Two-Year Follow-Up Study

Gut epithelial vitamin D receptor regulates microbiota-dependent mucosal inflammation by suppressing intestinal epithelial cell apoptosis.

Development of iron homeostasis in infants and young children.