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GUT HEALTH DIGEST

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

REPORT TO THE BILL & MELINDA GATES FOUNDATION

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DETAILS OF ARTICLES

1. Complementary feeding with cowpea reduces growth faltering in rural Malawian infants: a blind, randomized controlled clinical trial.
   Stephenson K, Agapova S, Divala O, Kaimila Y, Maleta K, Thakwalakwa C, *et al*.
   American Journal of Clinical Nutrition. 2017 November 1. [Epub ahead of print]
   PubMed ID: 29092882

ABSTRACT
BACKGROUND: Growth faltering is common in rural African children and is attributed to inadequate dietary intake and environmental enteric dysfunction (EED).
OBJECTIVE: We tested the hypothesis that complementary feeding with cowpea or common bean flour would reduce growth faltering and EED in 6-mo-old rural Malawians compared with the control group receiving a corn-soy blend.
DESIGN: A prospective, double-blind, randomized controlled clinical trial was conducted in which children received daily feeding for 6 mo (200 kcal/d when 6–9 mo old and 300 kcal/d when 10–12 mo old). The primary outcomes were change in length-for-age z score (LAZ) and improvements in EED, as measured by percentage of lactulose excretion (%L). %L <0.2% was considered normal. Anthropometric measurements and %L through urine were compared between each legume group and the control group with Student's t test.
RESULTS: Of the 355 infants enrolled, 291 infants completed the trial, and 288 were breastfed throughout the duration of the study. Cowpea and common bean added 4.6–5.2 g protein/d and 4–5 g indigestible carbohydrate/d to the diet. LAZ and weight-for-height z score were reduced in all 3 groups from 6 to 12 mo of age. The changes in LAZ [mean (95% CI)] for the cowpea, common bean, and control groups from 6 to 9 mo were 20.14 (20.24, 20.04), 20.27 (20.38, 20.16), and 20.27 (20.35, 20.19), respectively. LAZ was reduced less in infants receiving cowpea than in those receiving control food from 6 to 9 mo (P = 0.048). The absolute value of %L did not differ between the dietary groups at 9 mo of age (mean 6 SD: 0.30 6 0.43, 0.23 6 0.21, and 0.26 6 0.31 for cowpea, common bean, and control, respectively), nor did the change in %L from 6 to 9 mo.
CONCLUSION: Addition of cowpea to complementary feeding in Malawian infants resulted in less linear growth faltering. This trial was registered at clinicaltrials.gov as NCT02472262

DOI: 10.3945/ajcn.117.160986
IMPACT FACTOR: 6.567
CITED HALF-LIFE: >10.0

START COMMENTARY: The study was carried out in 17 villages in Southern Malawi, where corn porridge is the primary complementary food given during the first year of life. After recruitment, children aged 6 months were randomly assigned to receive daily complementary food with cowpea, common beans, or a corn-soy blend flour (control group) for 24 weeks. All infants were breastfed at recruitment, and only 1% had stopped breastfeeding by 12 months. All groups received the same amount of energy from complementary feeding, but the intervention flours contained 10-13% more protein than the control. While there was an impact of the intervention feedings on linear growth faltering (Figure 2), there were no apparent differences in %L or L:M ratios between groups (Figure 3), suggesting that reduced gut inflammation may not be the mechanism by which the intervention improved LAZ. The study did not collect stool samples from participants, so comparative analyzes of intestinal inflammation and possible changes to the gut microbiota composition were not possible.

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2. Cereal products derived from wheat, sorghum, rice and oats alter the infant gut microbiota *in vitro.*
   Gamage H, Tetu S, Chong R, Ashton J, Packer N, and Paulsen I.
   Scientific Reports. 7(1). 2017 October 30.
   PubMed ID: 29085002

ABSTRACT
The introduction of different nutrient and energy sources during weaning leads to significant changes in the infant gut microbiota. We used an in vitro infant digestive and gut microbiota model system to investigate the effect of four commercially available cereal products based on either wheat, sorghum, rice or oats, on the gut microbiota of six infants. Our results indicated cereal additions induced numerous changes in the gut microbiota composition. The relative abundance of bacterial families associated with fibre degradation, *Bacteroidaceae*, *Bifidobacteriaceae*, *Lactobacillaceae*, *Prevotellaceae*, *Ruminococcaceae* and *Veillonellaceae* increased, whilst the abundance of *Enterobacteriaceae* decreased with cereal additions. Corresponding changes in the production of SCFAs showed higher concentrations of acetate following all cereal additions, whilst, propionate and butyrate varied between specific cereal additions. These cereal-specific variations in the concentrations of SCFAs showed a moderate correlation with the relative abundance of potential SCFA-producing bacterial families. Overall, our results demonstrated clear shifts in the abundance of bacterial groups associated with weaning and an increase in the production of SCFAs following cereal additions.

DOI: 10.1038/s41598-017-14707-z
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 2

START COMMENTARY: The authors examined biological samples from six different infants between the ages of 5 and 11 months. Samples were cultured on a basal medium designed to simulate the conditions of the large intestine, and then different cereal products were introduced into the in *vitro* model for analysis. Two infants were strictly breast fed, two were strictly formula fed, and two were mixed-fed. Before the introduction of cereal products into the model, breast-fed infants had higher levels of *Veillonellaceae* bacteria compared to formula fed infants. In addition, the composition of the gut microbiota differed by age: children over 6 months of age had significantly higher relative abundance of *Lachnospiraceae* than younger infants. However, despite the differences in the gut microbiota before introduction of cereals, all 6 samples showed significant shifts in gut composition following the addition of these products, as shown in Figure 1. Figure 2 highlights the differences in initial gut composition and changes in gut composition between the six different samples. In addition to bacterial composition, the authors evaluated the pH levels of the cultures before addition of cereals and 48 hours after. Supplementary Figure S4 shows that compared to no added cereal, all four cereal products significantly reduced the pH levels in the samples, and rice produced the most dramatic decrease. The authors hypothesized that the reduced pH may be a result of the increase in SCFA producing bacterial families previously shown to reduce pH and the lower abundance of *Enterobacteriaceae*.

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3. Gut microbiome of mothers delivering prematurely shows reduced diversity and lower relative abundance of *Bifidobacterium* and *Streptococcus*.
   Dahl C, Stanislawski M, Iszatt N ,Mandal S, Lozupone C, Clemente J, et al.
   PLoS One. 12(1). 2017 October 25.
   PubMed ID: PMC5656300

ABSTRACT
OBJECTIVE: Preterm birth is the main reason for neonatal deaths worldwide. We investigate whether maternal gut microbiota may play a previously overlooked role.
METHODS: The Norwegian Microbiota Study (NoMIC) is a case control study on preterm birth (<259 days of gestation, calculated primarily based on the last menstrual period), including two consecutively born term infants per infant born prematurely. Eligible mothers were fluent in Norwegian and recruited from the maternity ward at a county hospital in Eastern Norway in the period 2002–2005. Fecal samples were collected at day 4 postpartum, and analyzed using 16S ribosomal RNA gene sequencing. We used samples from 121 mothers giving birth vaginally. Measures of alpha diversity (Shannon, Phylogenetic Diversity and Observed Operational Taxonomic Units) and microbiome composition were combined with information from the Medical Birth Registry, pregnancy journals, and questionnaires.
RESULTS: The association between maternal gut diversity and preterm delivery was examined using logistic regression. One IQR increase in Shannon diversity was significantly associated with 38% lower odds of spontaneous preterm birth, (95% confident interval (CI): 1%, 61%), and the association was stronger when adjusting for maternal age, marital status, ethnicity, parity, BMI, education, antibiotic use, pets in the household, income and smoking (48% lower odds, 95% CI: 4.2%, 72%). Mothers delivering prematurely also had lower abundance of OTUs belonging to *Bifidobacterium* and *Streptococcus*, and of the Clostridiales order.
CONCLUSION: Analysis of maternal gut microbiota using next-generation sequencing shows that low gut diversity, with a distinct microbial composition, is associated with spontaneous preterm delivery.

DOI: https://doi.org/10.1371/journal.pone.0184336
IMPACT FACTOR: 2.806
CITED HALF-LIFE: 3.7

START COMMENTARY: The authors analyzed stool from 19 women who delivered preterm and 102 control women who delivered at term. Women who had taken antibiotics within four days after delivery were excluded from the analysis in an attempt to ensure samples taken 4-days postpartum were a good representation of the microbiota at delivery. Random forest analysis of the OTUs showed that low levels of four OTUs were predictive of preterm birth in the sample. Table 4 shows all OTUs that were examined, as well as the 4 predictive of preterm birth. Additionally, women who spontaneously delivered prematurely had lower bacterial diversity than women delivering at term, shown in Figure 1. Gestational age has been linked to changing gut diversity. To evaluate whether the lower bacterial diversity in preterm deliveries was an artifact of earlier gestational age, the authors compared the gut diversity of preterm mothers who ultimately delivered by caesarian section with term mothers who delivered vaginally. As can be seen in Supplemental Figure 5, preterm caesarian mothers did not have lower diversity than term vaginal mothers, suggesting that the differences in gut microbiota in preterm vaginal mothers contributed to the preterm delivery. The authors noted several limitations to their study, including the small sample size of preterm deliveries, as well as collecting fecal sample 4 days post-partum instead of prior to, or at delivery.

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4. Quantitative microbiome profiling links gut community variation to microbial load.
Vandeputte D, Kathagen G, D'hoe K, Vieira-Silva S, Valles-Colomer M, Sabino J, *et al.*
Nature. 551. 2017 November 23.
PubMed ID: 29143816

ABSTRACT
Current sequencing-based analyses of faecal microbiota quantify microbial taxa and metabolic pathways as fractions of the sample sequence library generated by each analysis. Although these relative approaches permit detection of disease-associated microbiome variation, they are limited in their ability to reveal the interplay between microbiota and host health. Comparative analyses of relative microbiome data cannot provide information about the extent or directionality of changes in taxa abundance or metabolic potential. If microbial load varies substantially between samples, relative profiling will hamper attempts to link microbiome features to quantitative data such as physiological parameters or metabolite concentrations. Saliently, relative approaches ignore the possibility that altered overall microbiota abundance itself could be a key identifier of a disease-associated ecosystem configuration. To enable genuine characterization of host–microbiota interactions, microbiome research must exchange ratios for counts. Here we build a workflow for the quantitative microbiome profiling of faecal material, through parallelization of amplicon sequencing and flow cytometric enumeration of microbial cells. We observe up to tenfold differences in the microbial loads of healthy individuals and relate this variation to enterotype differentiation. We show how microbial abundances underpin both microbiota variation between individuals and covariation with host phenotype. Quantitative profiling bypasses compositionality effects in the reconstruction of gut microbiota interaction networks and reveals that the taxonomic trade-off between *Bacteroides* and *Prevotella* is an artefact of relative microbiome analyses. Finally, we identify microbial load as a key driver of observed microbiota alterations in a cohort of patients with Crohn's disease, here associated with a low-cell-count *Bacteroides* enterotype (as defined through relative profiling).

DOI: 10.1038/nature24460
IMPACT FACTOR: 40.137
CITED HALF-LIFE: >10.0

START COMMENTARY: The authors ran a variety of experiments which highlighted limitations of current methods for fecal microbiome profiling and made a number of recommendations for future study designs and analyses. This study incorporated flow cytometry into the laboratory work flow, which assessed bacterial cell count and moisture content in the fecal samples. Analysis of daily samples from 20 patients collected over the span of a week showed substantial microbial variation among individuals throughout the week, emphasizing the importance of incorporating longitudinal sampling in microbial studies to get accurate representations of gut microbiome composition. The authors suggested that the wide variation in microbial loads between individuals and sampling techniques necessitated the need to generate quantitative microbiome profiles (QMP), calculated as 16S rRNA gene-copy-number-corrected sequencing depth divided by sample cell count. Figure 2 shows the differences in genus-level microbial variation in 40 participants when using relative microbiota profiles (RMP) and QMP. As shown in Figure 4, flow cytometry enabled comparison of cell counts between healthy and diseased individuals. RMP indicated that Crohn's patients had significantly higher abundance of *Bacteroides* than healthy controls, but non-significant differences in *Prevotella* abundance. When samples were analyzed with QMP, the opposite was observed- as the respective bacterial cell counts had been incorporated. These data suggest that just using RMP could mislead researchers into making inaccurate conclusions about microbe abundance in disease states.

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5. Direct Biomarkers of Microbial Translocation Correlate with Immune Activation in Adult Zambians with Environmental Enteropathy and Hepatosplenic Schistosomiasis.
Kaonga P, Kaimoyo E, Besa E, Zyambo K, Sinkala E, and Kelly P.
The American Journal of Tropical Medicine and Hygiene. 97(5). 2017 November.
PubMed ID: 29140241

ABSTRACT
Microbial translocation is a poorly understood consequence of several disorders such as environmental enteropathy (EE) and hepatosplenic schistosomiasis (HSS). Herein, we compared biomarkers of microbial origin and immune activation in adults with these disorders and in healthy controls. A cross-sectional study was conducted in participants with EE recruited from Misisi compound, Lusaka, Zambia; HSS patients and healthy controls from the University Teaching Hospital, Lusaka. Plasma lipopolysaccharides (LPSs) was measured by limulus amoebocyte lysate assay, plasma 16S ribosomal RNA (16S rRNA) gene copy number was quantified by quantitative real-time polymerase chain reaction, Toll-like receptor ligand (TLRL) activity by QUANTI-Blue detection medium, and cytokines from cell culture supernatant by Cytometric Bead Array. In univariate analysis LPS, 16S rRNA gene copy number, and TLR activity were all high and correlated with each other and with cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-10, and IL-4 secreted by the RAW-Blue cells. After controlling for baseline characteristic, biomarkers of microbial translocation in blood were predictors of TNF-α, IL-6, and IL-10 activation in cell culture supernatant from EE participants and HSS patients but not in healthy controls. TLR activity showed the strongest correlation with TNF-α. These data provide correlative evidence that microbial translocation contributes to systemic cytokine activation in two disorders common in the tropics, with total TLR ligand estimation showing the strongest correlation with TNF-α ($r = 0.66$, $P < 0.001$).

DOI: https://doi.org/10.4269/ajtmh.17-0365
IMPACT FACTOR: 2.549
CITED HALF-LIFE: >10.0

START COMMENTARY: This study looked at blood samples from 66 participants with EE, 86 with HSS and 40 healthy controls from the study area. All three groups were similar, however the EE group had a lower average age and a higher proportion of HIV seropositive individuals than the other groups. Figure 1 presents the results of the comparison of three direct microbial translocation (MT) markers LPS, 16S rRNA and TLRL activity between the three groups. All three direct biomarkers were significantly higher in EE patients than both HSS patients and healthy controls, while 16S rRNA and TLRL activity was also significantly higher in HSS patients compared to healthy controls. RAW-Blue cell culture supernatants were incubated with mouse macrophages, and cytokine activity was measured 24 hours post incubation to assess immune activity in each group. Immune activation was considered if the cytokine ratio was 2 or more compared to control conditions. Activity of all inflammatory cytokines evaluated in cell-culture supernatant were higher in both the EE and HHS groups compared to the controls, and the EE group had significantly higher levels of cytokines (except for IL-2) than the HHS group (Figure 2). Similar results were observed in plasma immune response biomarkers (Figure 3). The results suggest that an increase in MT biomarkers is associated with increased immune responses, and that this response is greater in EE patients than in HHS patients. These data support the hypothesis that microbial components move across the intestinal barrier in EE and HHS patients and induce a systemic immune response. The study was limited by the high proportion of HIV positive patients in the EE group, which may have altered immune responses not attributed to EE.

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6. Influence of vitamin D on key bacterial taxa in infant microbiota in the KOALA Birth Cohort Study.
   Talsness C, Penders J, Jansen E, Damoiseaux J, Thijs C, and Mommers M.
   PLoS One. 12(11). 2017 November 9.
   PubMed ID: 29121673

ABSTRACT
Vitamin D has immunomodulatory properties giving it the potential to affect microbial colonization of the intestinal tract. We investigated whether maternal vitamin D supplementation, maternal plasma 25-hydroxyvitamin D concentration, or direct supplementation of the infant influences key bacterial taxa within microbiota of one month old infants. Infant and maternal vitamin D supplement use was ascertained via questionnaires. Maternal plasma 25-hydroxyvitamin D was determined at approximately the 36th week of pregnancy. In 913 one month old infants in the prospective KOALA Birth Cohort Study, fecal *Bifidobacterium* spp*., Escherichia coli*, *Clostridium difficile, Bacteroides fragilis* group, *Lactobacillus* spp. and total bacteria were quantified with real-time polymerase chain reaction assays targeting 16S rRNA gene sequences. The association between vitamin D exposure and prevalence or abundance of a specific bacterial group or species was analyzed using logistic or linear regression, respectively. There was a statistically significant negative linear trend between counts of *Bifidobacterium* spp. and levels of maternal vitamin D supplementation and maternal 25-hydroxyvitamin D quintiles, respectively. In addition, a positive linear trend between quintile groups and *B. fragilis* group counts was observed. Lower counts of *C. difficile* were associated with vitamin D supplementation of breast fed infants whose mothers were more likely to adhere to an alternative lifestyle in terms of, e.g., dietary habits. These data suggest that vitamin D influences the abundance of several key bacterial taxa within the infant microbiota. Given that intestinal microbiotic homeostasis may be an important factor in the prevention of immune mediated diseases and that vitamin D status is a modifiable factor, further investigation of the impact of postnatal vitamin D supplementation should be conducted in older infants.

DOI: 10.1371/journal.pone.0188011
IMPACT FACTOR: 2.806
CITED HALF-LIFE: 3.7

START COMMENTARY: In this study, mother-child pairs were analyzed based on self-administered Vitamin D supplementation during pregnancy and/or during the first month of the infant's life. The authors observed an association between both maternal Vitamin D supplementation and circulating maternal 25(OH)D levels with *Bifidobacterium* spp. levels in infant stool samples (Table 3 and Table 4). In contrast, no association was observed between infant Vitamin D supplementation and bacterial levels (Table 5). As the half-life of circulating 25(OH)D is approximately three weeks, the authors suggested that infants maintain the Vitamin D from their mother in the first month of life, which is why supplementation of the infant during this time period showed no association with gut microbiota composition. The authors suggested repeating the study in older infants to see if vitamin D supplementation in infants has a greater impact once the maternal Vitamin D has waned. The generalizability of the study is limited because vitamin D supplementation was not randomly assigned to infants or their mothers, and the authors restricted their microbiota analyses to 5 bacterial groups. Randomization would eliminate the effects of any unmeasured confounders that differ between women who elect to take Vitamin D supplementation during pregnancy and those who do not.

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7. Enteric Infections in Young Children are Associated with Environmental Enteropathy and Impaired Growth.
   George C, Burrowes V, Perin J, Oldja L, Biswas S, Sack D, *et al.*
   Tropical Medicine & International Health. 2017 November 9. [Epub ahead of print]
   PubMed ID: 29121442

ABSTRACT
OBJECTIVE: To investigate the relationship between fecal contamination in child play spaces, enteric infections, environmental enteropathy (EE), and impaired growth among young children.
METHODS: Prospective cohort study of 203 children 6-30 months of age in rural Bangladesh. Stool samples were analyzed by quantitative PCR for *Shigella*, *Enterotoxigenic Escherichia coli* (ETEC), *Campylobacter jejuni*, *Giardia* spp, and *Cryptosporidium* spp. Four fecal markers of intestinal inflammation were also measured: alpha-1-antitrypsin, myeloperoxidase, neopterin, and calprotectin. Child growth was measured at baseline and 9 months after enrollment. E. coli was measured in soil in the child's play spaces.
RESULTS: 47% of study children had three or more enteric pathogens in their stool. 35% (71/203) of children had *Shigella*, 30%(61/203) had ETEC, 73% (148/203) had *C. jejuni*, 79% (160/203) had *Giardia*, and none had *Cryptosporidium*. Children with ETEC had significantly higher calprotectin concentrations (Coefficient: 1.35, 95% Confidence Interval [CI]: 1.005, 1.82). Children with *Shigella* had a significantly higher odds of being stunted at our 9-month follow-up (OR: 2.01 (95% CI: 1.02, 3.93). Children with *Giardia* in their stool played in spaces with significantly higher *E.coli* counts in the soil (OR: 1.23, 95% CI: 1.02, 1.48).
CONCLUSION: The presence of enteric pathogens in stool was significantly associated with EE and impaired growth in rural Bangladesh. These findings provide further evidence to support the hypothesis that contaminated soil in child play spaces can lead to enteric infections, many of which are likely subclinical, resulting in EE and impaired growth in young children.

DOI: 10.1111/tmi.13002
IMPACT FACTOR: 2.85
CITED HALF-LIFE: 7.9

START COMMENTARY: Authors collected stool samples at enrollment, and measured height and weight of the children once at baseline and once after 9-months. Authors restricted their cohort to children between 6 and 30 months of age to focus on the time period when child growth faltering is greatest, and when child mouthing behaviors increase exposure to pathogens. Significant associations were found between *Shigella* infection at enrollment and stunting status, as well as risk of stunting at month 9 of follow up. The study used a threshold of 1 copy per 100 ng of DNA to define a *Shigella* positive sample, which is lower than the threshold used to diagnose diarrhea due to *Shigella*. This suggests that even low intensity *Shigella* infections may contribute to stunting, even in the absence of diarrhea, but may not be associated with concurrent levels of fecal biomarkers commonly used to assess EE. The study has several limitations. Most notably, authors did not analyze the association between the pathogens and three of the four fecal markers for intestinal inflammation that were measured (they were only used to calculate an EE score, which was treated as the dependent variable in the analysis). Authors also neglected to evaluate the association between EE score and linear growth in the cohort.

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8. A role for bacterial urease in gut dysbiosis and Crohn's disease.
   Ni J, Shen T, Chen E, Bittinger K, Bailey A, Roggiani M, *et al.*
   Science Translational Medicine. 9(416). 2017 November 15.
   PubMed ID: 29141885

ABSTRACT
Gut dysbiosis during inflammatory bowel disease involves alterations in the gut microbiota associated with inflammation of the host gut. We used a combination of shotgun metagenomic sequencing and metabolomics to analyze fecal samples from pediatric patients with Crohn's disease and found an association between disease severity, gut dysbiosis, and bacterial production of free amino acids. Nitrogen flux studies using ^{15}N in mice showed that activity of bacterial urease, an enzyme that releases ammonia by hydrolysis of host urea, led to the transfer of murine host-derived nitrogen to the gut microbiota where it was used for amino acid synthesis. Inoculation of a conventional murine host (pretreated with antibiotics and polyethylene glycol) with commensal *Escherichia coli* engineered to express urease led to dysbiosis of the gut microbiota, resulting in a predominance of Proteobacteria species. This was associated with a worsening of immune-mediated colitis in these animals. A potential role for altered urease expression and nitrogen flux in the development of gut dysbiosis suggests that bacterial urease may be a potential therapeutic target for inflammatory bowel diseases.

DOI: 10.1126/scitranslmed.aah6888
IMPACT FACTOR: 16.761
CITED HALF-LIFE: 3.6

START COMMENTARY: This study analyzed fecal samples from 90 pediatric patients from the Pediatric Longitudinal Study of Elemental Diet and Stool Microbiome Composition (PLEASE) study and 26 healthy controls. Untargeted fecal analysis using liquid chromatography-mass spectrometry identified 341 small molecules among all samples. Figure 1A shows the proportion of small molecules in each broad category that were or were not significantly associated with Crohn's disease. Figure 1B presents a heat map of the relative abundance of fecal amino acids between the two groups, further demonstrating that almost all amino acids were significantly elevated in patients with Crohn's. The authors hypothesized that bacterial urease was important in microbial amino acid synthesis, and they tested this hypothesis using ^{15}N-labeled urea in three different groups of mice: (i) controls; (ii) mice treated with antibiotics (ABX) and PEG to deplete endogenous gut microbiota; and (iii) mice treated with ABX/PEG and then inoculated with altered Schaedler flora (ASF) to restore commensal gut bacteria with minimal urease content. Figure 3B demonstrates that control mice produced significantly greater quantities of fecal [^{15}N]lysine than either of the treated mice groups, further supporting the importance of urease in providing nitrogen needed for amino acid synthesis in the gut. Figure 6 shows the results of a set of experiments performed to evaluate the effect of *E.coli* urease on colitis in a mouse model. $Rag^{-/-}$ mice were inoculated with urease negative or urease positive *E. coli* MP1, and after 30 days transferred $CD45Rb^{high}$ naïve T cells (cells capable of inducing chronic small bowel and colonic inflammation) into the mice. Ure^{+} mice lost a significant amount of body weight post transfer of $CD45Rb^{high}$ naïve T cells compared to Ure^{-} mice (Fig 6B), but the weight of their colon was significantly higher (Fig 6C). These results suggest that overabundance of bacterial urease may have a direct effect on establishing gut dysbiosis, and therefore could be a potential target in diseases where dysbiosis is implicated.

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9. Impact of Lactobacillus reuteri colonization on gut microbiota, inflammation, and crying time in infant colic.
   Nation M, Dunne E, Joseph S, Mensah F, Sung V, Satzke C, Tang M.
   Scientific Reports. 7(1). 2017 November 8.
   PubMed ID: 29118383

ABSTRACT
Infant colic is a distressing condition of unknown etiology. An aberrant gastrointestinal microbiota has been associated, and *Lactobacillus reuteri* supplementation has been shown to reduce crying and/or fussing time ('crying time') in some infants with colic. The relationship between *L. reuteri* gut colonization and crying time has not been examined. We investigated the relationship between *L. reuteri* colonization and fecal microbiota (microbial diversity and *Escherichia coli*), intestinal inflammation, and crying time in infants with colic, using a subset of 65 infants from the Baby Biotics trial, which randomized healthy term infants aged <13 weeks with infant colic to receive probiotic *L. reuteri* DSM 17938 (1 × 108 colony forming units) or placebo daily for 28 days. We observed an overall reduction in median crying time, regardless of *L. reuteri* colonization status (n = 14 colonized). There were no differences in *E. coli* colonization rates or densities, microbial diversity or intestinal inflammation by *L. reuteri* colonization status. We found that *L. reuteri* density positively correlated with crying time, and *E. coli* density negatively correlated with microbial diversity. As density of *L. reuteri* was associated with increased crying time, *L. reuteri* supplementation may not be an appropriate treatment for all infants with colic.

DOI: 10.1038/s41598-017-15404-7
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 7.1

START COMMENTARY: Contrary to several other studies, the Baby Biotics trial suggested that there was no relationship between *L. reuteri* probiotic supplementation and reduction in infant crying time. The authors hypothesized that it was perhaps due to the fact that a low percentage of infants who received the probiotic actually had *L. reuteri* in their stool samples at the end of the trial. However, as shown in Figure 3, further analysis of a subset a subset of stool samples from the original study chosen for this paper showed that there was a significant decrease in crying time independent of *L. reuteri* colonization. There was also no difference in median fecal calprotectin levels between infants with colonized *L. reuteri* and those without, suggesting that the supplemented bacteria does not have an effect on intestinal inflammation. The authors suggested several possible explanations as to why their results were contrary to previous studies of similar interventions. Only 35% of the infants in this analysis were exclusively breastfed, compared to almost exclusive breastfeeding in all subjects in other similar studies. Breastfeeding has been shown to influence the microbiota, so *L. reuteri* may interact differently with the gut in children who are not breastfed. Additionally, stool samples were only collected on day 28 post-intervention in this study, during a time when most infants' crying was reduced without an intervention. The small sample size limited the authors' ability to explore other relationships which might explain the contrary results observed in this study.

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10. Determinant Variables, Enteric Pathogen Burden, Gut Function and Immune-related Inflammatory Biomarkers Associated With Childhood Malnutrition: A Prospective Case-Control Study in Northeastern Brazil.
Lima A, Leite Á, Di Moura A, Lima N, Soares A, Abreu CB, *et al.*
The Pediatric Infectious Disease Journal. 36(12). 2017 December 1.
PubMed ID: 28230705

ABSTRACT
Malnutrition results in serious consequences for growth and cognitive development in children. We studied select child and maternal biologic factors, socioeconomic factors, enteric pathogenic burden and gut function biomarkers in 402 children 6–24 months of age in Northeastern Brazil. In this prospective case–control study, not being fed colostrum [odds ratio (OR): 3.29, 95% confidence interval (CI): 1.73–6.26], maternal age ≥18 years (OR: 1.88, 95% CI: 1.10–3.22) and no electric fan (OR: 2.46, 95% CI: 1.22–4.96) or bicycle (OR: 1.80, 95% CI: 1.10–2.95) in the household were positively associated, and higher birth weight (OR: 0.27, 95% CI: 0.19–0.38), larger head circumference (OR: 0.74, 95% CI: 0.66–0.82) and shortness of breath in the last 2 weeks (OR: 0.49, 95% CI: 0.27–0.90) were negatively associated with malnutrition. Subclinical enteric pathogen infections were common, and enteroaggregative *Escherichia coli* infections were more prevalent in malnourished children (P = 0.045). Biomarkers such as the lactulose–mannitol test, myeloperoxidase, neopterin and calprotectin were highly elevated in both malnourished and nourished children. Nourished children had a better systemic immune response than the malnourished children, as detected by elevated serum amyloid A-1 and soluble cluster of differentiation protein 14 biomarkers (P < 0.001). Serum amyloid A-1 and soluble cluster of differentiation protein 14 were also associated with better nutritional Z scores. Neonatal, maternal and socioeconomic factors were associated with malnutrition in children. There was a substantial subclinical enteric pathogen burden, particularly with enteroaggregative *E. coli*, in malnourished children.

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START COMMENTARY: This study compared nourished and malnourished children in Northeastern Brazil who participated in the Malnutrition and Enteric Disease (MAL-ED) case-control study. Analysis of stool samples from the study group showed high levels of enteric pathogen subclinical infections in both nourished and malnourished children, with the highest prevalence attributed to atypical enteropathogenic *E. coli* in both groups (Figure 2). However, despite similarities in enteric pathogen infections between the two groups, nourished children had better systemic immune responses than malnourished children based on elevated sCD14 and SAA (Table 5), while malnourished children with EAEC infections had elevated levels of LPS IgA and IgG compared to nourished children with the same infection (Figure 3). Children in the lowest quartile for LAZ had significantly more small intestinal damage (I-FABP) and reduced systemic immune responses (based on sCD14 and SAA) compared with children in the highest quartile for LAZ (Table 6). The authors concluded that well-nourished children have a better immune response against LPS/Gram-negative bacteria, while malnourished children display reduced systemic immune responses and experience damage to the intestinal barrier function with bacterial translocation of LPS.

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ADDITIONAL ARTICLES OF INTEREST

Probiotic, Prebiotic, and Brain Development.

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The Gut Microbiota Facilitates Drifts in the Genetic Diversity and Infectivity of Bacterial Viruses

Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use.

Severely inadequate micronutrient intake among children 9–24 months in Nepal—The MAL-ED birth cohort study.

Position paper: The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction.