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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. Commensal Microbes Induce Serum IgA Responses that Protect against Polymicrobial Sepsis.
   Wilmore JR, Gaudette BT, Gomez Atria D, Hashemi T, Jones DD, Gardner CA, *et al.*
   Cell Host & Microbe. 23(3). 2018 March 14.
   PubMed ID: 29478774

ABSTRACT: Serum immunoglobulin A (IgA) antibodies are readily detected in mice and people, but the mechanisms underlying the induction of serum IgA and its role in host protection remain uncertain. We report that select commensal bacteria induce several facets of systemic IgA-mediated immunity. Exposing conventional mice to a unique but natural microflora that included several members of the Proteobacteria phylum led to T cell-dependent increases in serum IgA levels and the induction of large numbers of IgA-secreting plasma cells in the bone marrow. The resulting serum IgA bound to a restricted collection of bacterial taxa, and antigen-specific serum IgA antibodies were readily induced after intestinal colonization with the commensal bacterium *Helicobacter muridarum*. Finally, movement to a Proteobacteria-rich microbiota led to serum IgA-mediated resistance to polymicrobial sepsis. We conclude that commensal microbes overtly influence the serum IgA repertoire, resulting in constitutive protection against bacterial sepsis.

DOI: 10.1016/j.chom.2018.01.005
IMPACT FACTOR: 14.946
CITED HALF-LIFE: 4.3

START COMMENTARY: In this publication, investigators ran a series of experiments in mice to demonstrate that commensal bacteria in the gut induces the production of serum IgA which confers protection against sepsis. Figure 1 shows results of initial experiments in which different measures of serum IgA were compared between mice reared in the author's laboratory (PENN-SPF) and those housed at a Jackson laboratory (JAX-SPF). Despite similar levels of IgA-secreting plasma cells in the small intestine (Figure 1B), the serum IgA concentrations were significantly higher in the PENN-SPF mice (Figure 1A). Cohousing the JAX-SPF mice with the PENN-SPF mice resulted in increased numbers of IgA secreting bone marrow (BM) plasma cells (PCs) in JAX-SPF mice (Figure 1C). When JAX-SPF mice were cohoused with PENN-SPF treated with an antibiotic cocktail (vancomycin, neomycin, ampicillin, and metronidazole), no significant change was observed in the percentage of BM PCs that secreted IgA in the cohoused mice (Figure 1F). These experiments suggested that there were unique bacterial taxa in the PENN-SPF mice that increased IgA levels. To further explore this hypothesis, the authors performed 16S rRNA sequencing on stool samples from JAX-SPF, PENN-SPF and cohoused JAX-SPF mice (Figure 2 and supplemental Figure 2). Compared to PENN-SPF or cohoused mice, JAX-SPF mice had lower levels of Bacteroidetes and Proteobacteria, and higher levels of *Firmicutes* in their stool samples. The increase in relative abundance of Bacteroidetes and Proteobacteria in the cohoused mice, along with the observed increase in serum IgA in these mice suggest that certain bacterial microbiota induce the production of systemic IgA responses. Figure 4B and 4C show bacterial genera that were highly enriched with IgA binding in PENN-SPF mice. This study highlights the possible role of systemic IgA in protecting against certain enteric pathogens and suggests that modulating the gut microbiota to boost the IgA immune response may be an efficient way to decrease the burden of sepsis caused by the passage of enteropathogenic bacteria into systemic circulation.

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2. Understanding the prebiotic potential of different dietary fibers using an in vitro continuous adult fermentation model (PolyFermS).
   Poeker SA, Geirnaert A, Berchtold L, Greppi A, Krych L, Steinert RE, *et al.*
   Scientific Reports. 8(12). 2018 March 12.
   PubMed ID: 29531228

ABSTRACT: Consumption of fermentable dietary fibers (DFs), which can induce growth and/or activity of specific beneficial populations, is suggested a promising strategy to modulate the gut microbiota and restore health in microbiota-linked diseases. Until today, inulin and fructo-oligosaccharides (FOS) are the best studied DFs, while little is known about the gut microbiota-modulating effects of β-glucan, α-galactooligosaccharide (α-GOS) and xylo-oligosaccharide (XOS). Here, we used three continuous *in vitro* fermentation PolyFermS model to study the modulating effect of these DFs on two distinct human adult proximal colon microbiota, independently from the host. Supplementation of DFs, equivalent to a 9 g daily intake, induced a consistent metabolic response depending on the donor microbiota. Irrespective to the DF supplemented, the Bacteroidaceae-Ruminococcaceae dominated microbiota produced more butyrate (up to 96%), while the Prevotellaceae-Ruminococcaceae dominated microbiota produced more propionate (up to 40%). Changes in abundance of specific bacterial taxa upon DF supplementation explained the observed changes in short-chain fatty acid profiles. Our data suggest that the metabolic profile of SCFA profile may be the most suitable and robust read-out to characterize microbiota-modulating effects of a DF and highlights importance to understand the inter-individual response to a prebiotic treatment for mechanistic understanding and human application.

DOI: 10.1038/s41598-018-22438-y
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 2.0

START COMMENTARY: This study used an *in vitro* model of the colon to investigate the impact of different dietary fibers on the SCFA profile and microbiota composition. Fecal samples from two different human donors were inoculated on the PolyFermS continuous intestinal fermentation model. Both donors had high levels of *Firmicutes* and *Bacteroidetes*, but their family level microbiota composition differed greatly. The first donor had higher levels of *Bacteroidaceae*, *Verrucomicrobiaceae* and *Methanobacteriaceae*, while the second donor had higher levels of *Prevotellaceae* and *Lachnospiraceae.* Both donors had similar levels of microbiota diversity. Before introduction of dietary fibers, the microbiota of donor one (*Bacteroidaceae*-dominated) had high butyrate production, while the microbiota of donor two (*Prevotellaceae*-dominated) had high propionate production. When dietary fibers were introduced into both *in vitro* models, the production of these respective dominant byproducts was increased, as shown in Figure 2. As also seen in Figure 2, inulin increased butyrate concentrations in both donors, while acetate levels were mostly unchanged, suggesting cross-feeding of butyrate-producers on acetate. This study is novel because the *in vitro* model allowed investigators to observe effects of dietary fibers in the absence of competing factors, and better understand the complex cross-feeding mechanisms that occur. The study showed that responses to dietary fibers were dependent on the microbial composition, and not the choice of fiber. Additional studies should be done to examine effects in additional donor fecal samples with different dominating microbiota, particularly those from diseased individuals.

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3. Characterizing the metabolic phenotype of intestinal villus blunting in Zambian children with severe acute malnutrition and persistent diarrhea.
   Farràs M, Chandwe K, Mayneris-Perxachs J, Amadi B, Louis-Auguste J, Besa E, *et al.*
   PLoS One. 13(3). 2018 March 02.
   PubMed ID: 29499047

ABSTRACT:
BACKGROUND: Environmental enteric dysfunction (EED) is widespread throughout the tropics and in children is associated with stunting and other adverse health outcomes. One of the hallmarks of EED is villus damage. In children with severe acute malnutrition (SAM) the severity of enteropathy is greater and short term mortality is high, but the metabolic consequences of enteropathy are unknown. Here, we characterize the urinary metabolic alterations associated with villus health, classic enteropathy biomarkers and anthropometric measurements in severely malnourished children in Zambia.
METHODS/PRINCIPAL FINDINGS: We analysed 20 hospitalised children with acute malnutrition aged 6 to 23 months in Zambia. Small intestinal biopsies were assessed histologically (n = 15), anthropometric and gut function measurements were collected and the metabolic phenotypes were characterized by 1H nuclear magnetic resonance (NMR) spectroscopy. Endoscopy could not be performed on community controls children. Growth parameters were inversely correlated with enteropathy biomarkers (p = 0.011) and parameters of villus health were inversely correlated with translocation and permeability biomarkers (p = 0.000 and p = 0.015). Shorter villus height was associated with reduced abundance of metabolites related to gut microbial metabolism, energy and muscle metabolism (p = 0.034). Villus blunting was also related to increased sucrose excretion (p = 0.013).
CONCLUSIONS/SIGNIFICANCE: Intestinal villus blunting is associated with several metabolic perturbations in hospitalized children with severe undernutrition. Such alterations include altered muscle metabolism, reinforcing the link between EED and growth faltering, and a disruption in the biochemical exchange between the gut microbiota and host. These findings extend our understanding on the downstream consequences of villus blunting and provide novel non-invasive biomarkers of enteropathy dysfunction. The major limitations of this study are the lack of comparative control group and gut microbiota characterization.

DOI: 10.1371/journal.pone.0192092
IMPACT FACTOR: 2.806
CITED HALF-LIFE: 3.7

START COMMENTARY: This study had the unique ability to collect endoscopies from a subset of 15 children and performed three small intestinal biopsies on each child. In addition, urine and blood samples were collected from all 20 children in the study. All samples were collected after children had been stabilized in the hospital, and all had received F100 milk-based formula in the days prior to sample collection. As seen in Figure 1, there were several significant correlations between growth measures, serum biomarkers, urine biomarkers, and villus measurements from endoscopies. Interestingly, there was a strong negative correlation between villus height and lactulose-rhamnose, and serum intestinal fatty acid binding protein (FABP) was negatively correlated with MUAC. Additional tests of metabolites found in the urine showed positive correlation between several metabolites related to gut microbial metabolism with villus height (VH). Individuals with shorter VH excreted lower amounts of these gut microbial metabolites as well as metabolites related to energy and muscle metabolism, and higher levels of sucrose. The authors noted that while the increased sucrose observation was highly driven by two children with extreme villus blunting, they suggested that this finding could be used to support using sucrose excretion as a simple diagnostic test to evaluate mucosal barrier function, without the need for

consumption of test sugars (i.e., lactulose and rhamnose/mannitol). The results of this study suggest that several urine-based biomarkers measured from non-invasive procedures may serve as good proxy measures for villus status.

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4. Additional Common Bean in the Diet of Malawian Children Does Not Affect Linear Growth, but Reduces Intestinal Permeability.
   Agapova SE, Stephenson KB, Divala O, Kaimila Y, Maleta KM, Thakwalakwa C, *et al.*
   The Journal of Nutrition. 148(2). 2018 February 01.
   PubMed ID: 29490090

ABSTRACT:
BACKGROUND: Chronic malnutrition, as manifested by linear growth faltering, is pervasive among rural African children. Improvements in complementary feeding may decrease the burden of environmental enteric dysfunction (EED) and thus improve growth in children during the critical first 1000 d of development.
OBJECTIVE: We tested the hypothesis that systematically including common bean or cowpea into complementary feeding would reduce EED and growth faltering among children in rural Malawi.
METHODS: This was a double-blind clinical trial in which children 12-23 mo of age were randomly assigned to receive complementary feeding with 1 of 3 foods: roasted cowpea or common bean flour, or an isoenergetic amount of corn-soy blend as a control food for 48 wk. Children aged 12-23 mo received 155 kcal/d and thereafter until 35 mo received 200 kcal/d. The primary outcomes were change in length-for-age z score (LAZ) and improvements in a biomarker of EED, the percentage of lactulose (%L) excreted as part of the lactulose:mannitol dual-sugar absorption test. Anthropometric measurements and urinary %L excretion were compared between the 2 intervention groups and the control group separately with the use of linear mixed model analyses for repeated measures.
RESULTS: A total of 331 children completed the clinical trial. Compliance with the study interventions was excellent, with >90% of the intervention flour consumed as intended. No significant effects on LAZ, change in LAZ, or weight-for-length z score were observed due to either intervention legume, compared to the control. %L was reduced with common bean consumption (effect estimate was -0.07 percentage points of lactulose, P = 0.0007). The lactulose:mannitol test was not affected by the legume intervention.
CONCLUSION: The addition of common bean to complementary feeding of rural Malawian children during the second year of life led to an improvement in a biomarker of gut health, although this did not directly translate into improved linear growth. This trial was registered at clinicaltrials.gov as NCT02472301.

DOI: 10.1093/jn/nxx013
IMPACT FACTOR: 4.145
CITED HALF-LIFE: >10.0

START COMMENTARY: This study entailed a randomized controlled trial of complementary foods readily available in the community. The typical diet of children in the trial community was corn based. The control flour, which was corn-soy based, contained 12% protein and 8% fiber. In contrast, cowpea flour had 26% protein and 21% fiber, while common bean flour contained 23% protein and 28% fiber. While both intervention foods had higher protein and fiber content than the control, only the common bean supplementation was shown to decrease urinary %L excretion, indicating improved gut (decreased) permeability. The authors suggested that the fiber found in common bean is rich in α-galactosides, which may alter the microbiota and reduce inflammation. Laboratory work on the intestinal microbiota composition of stool samples collected throughout this study is ongoing and may help elucidate why only common bean had an effect of %L. A limitation of the study is that authors conducted a per protocol analysis, as opposed to an intent to treat; 52 children were excluded from the study after

randomization due to acute malnutrition (stopping rule) or loss to follow up, and therefore were not included in the analysis.

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5. Environment dominates over host genetics in shaping human gut microbiota.
   Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, *et al.*
   Nature. 555(7695). 2018 March 08.
   PubMed ID: 29489753

ABSTRACT:
Human gut microbiome composition is shaped by multiple factors but the relative contribution of host genetics remains elusive. Here we examine genotype and microbiome data from 1,046 healthy individuals with several distinct ancestral origins who share a relatively common environment, and demonstrate that the gut microbiome is not significantly associated with genetic ancestry, and that host genetics have a minor role in determining microbiome composition. We show that, by contrast, there are significant similarities in the compositions of the microbiomes of genetically unrelated individuals who share a household, and that over 20% of the inter-person microbiome variability is associated with factors related to diet, drugs and anthropometric measurements. We further demonstrate that microbiome data significantly improve the prediction accuracy for many human traits, such as glucose and obesity measures, compared to models that use only host genetic and environmental data. These results suggest that microbiome alterations aimed at improving clinical outcomes may be carried out across diverse genetic backgrounds.

DOI: 10.1038/nature25973
IMPACT FACTOR: 40.137
CITED HALF-LIFE: >10.0

START COMMENTARY: This study used a large cohort with varying ancestries but many shared environmental factors to evaluate the impact of heredity and environment on the gut microbiota composition. The study sample included individuals who self-reported as Ashkenazi, North African, Middle Eastern, Sephardi, Yemenite, and “admixed/other” ancestries. The microbiota composition of individuals was determined by metagenome-sequencing and 16S rRNA gene-sequencing. In addition, investigators used a dataset from a previous study of 2252 twins and another from 836 Dutch individuals to replicate and validate all of the associations and statistical models determined from the original cohort. From these validation cohorts, the authors observed only weak associations between ancestry and microbiome. This finding was strengthened when investigators analyzed microbiome composition of 24 pairs of related individuals in their study who had no history of sharing a household and found no evidence of a shared microbiome between family members. Contrary to some previous studies, this investigation found limited evidence for associations between microbiomes and specific single nucleotide polymorphisms (SNPs), as shown in Figure 3. Figure 4 shows the added value of including microbiome data in a linear prediction model used to predict human phenotypes. The prediction accuracy for 10 of the 12 traits investigated was improved when microbiome data was included in the model. While the microbiome is thought to remain relatively stable throughout adulthood, a limitation of this study is that stool samples were collected at only a single time-point for each participant and included in microbiome analyses.

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6. Immunomodulation by food: impact on gut immunity and immune cell function.
Hachimura S, Totsuka M, & Hosono A.
Bioscience, Biotechnology, and Biochemistry. 2018 February 26. [Epub ahead of print]
PubMed ID: 29448897

ABSTRACT:
Recent studies have revealed that various food components affect the immune response. These components act on various immune cells, and their effects are mediated through the intestinal immune system and, in some cases, the intestinal microbiota. In this review, we describe the immunomodulating effects of various food components, including probiotics, prebiotics, polysaccharides, vitamins, minerals, fatty acids, peptides, amino acids and polyphenols. Some of these components enhance immune responses, leading to host defense against infection, whereas others inhibit immune responses, thus suppressing allergy and inflammation.

DOI: 10.1080/09168451.2018.1433017
IMPACT FACTOR: 1.295
CITED HALF-LIFE: >10.0

START COMMENTARY: This is a comprehensive review article that summarizes human and mice studies conducted on the immunomodulation effects of food products on the intestinal immune system, including microbiota composition, growth, and allergies. Table 1 provides a summary of all food components discussed in the review, including their function in the immune response and the mechanism by which they act. The review highlights several enhancements to the immune response by food. Probiotics, especially lactic acid bacteria (LAB) and *Bifidobacterium* have been shown to enhance IgG and IgA immune responses, which the authors noted may be important in preventing pathogens from invading at mucosal surfaces. LAB have also been shown to increase IL-12 production, which in turn augments the natural killer (NK) response that is important for the elimination of virus-infected cells. Prebiotics have been shown to increase the proliferation of several beneficial gut bacteria, including *Bifidobacterium* and *Bacteroides*. The short-chain fatty acids (SCFAs) produced by these bacteria activate G-protein coupled receptors GPR41 and GPR43 in the intestinal tract, which increases the immune response through production of chemokines and cytokines. Several digestible and non-digestible polysaccharides have been shown to have direct effects on immune responses, most commonly through a mechanism that promotes NK activity. An interesting example of microbiota-sourced peptides are polyamines, which are important for enhancing the intestinal barrier function. Gut microbiota naturally produce polyamines, but these are also produced by the catabolism of the amino acid arginine. Several of the food components covered in this review have not been studied as possible food supplementations to improve gut health and may warrant use in future clinical trials.

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7. Competitively Selected Donor Fecal Microbiota Transplantation: Butyrate Concentration and Diversity as Measures of Donor Quality.
   Barnes D, Ng K, Smits S, Sonnenburg J, Kassam Z, & Park KT.
   Journal of Pediatric Gastroenterology and Nutrition. 2018 February 21. [Epub ahead of print]
   PubMed ID: 29470297

ABSTRACT:
In this prospective cohort study, we examine the feasibility of a protocol to optimize microbiota for fecal microbiota transplantation (FMT). Donor stool metrics generally accepted as markers of gut health were used to select a stool donor based on superior microbial diversity, balanced constitution of *Bacteroidetes* versus *Firmicutes* and high concentration of fecal butyrate. Selected donor microbiota was then administered via FMT. A total of 10 patients with median age of 12 years with recurrent Clostridium difficile infection (rCDI) received the intervention. The rate of recurrence-free resolution with 1-2 FMTs was 100% at Week 10. With a single FMT, 80% of patients cleared CDI without recurrence, while 20% of patients required a single re-treatment. No serious adverse events occurred. Microbiota sequencing revealed that recipients' gut microbiota phylogenic diversity increased by 72-hours post-transplantation, with sustainment over 10-week follow-up. This study highlights the feasibility of purposefully selecting the most ideal microbiota for transplantation.

DOI: 10.1097/MPG.0000000000001940
IMPACT FACTOR: 2.799
CITED HALF-LIFE: 7.3

START COMMENTARY: The authors noted that this is the first known study to conduct FMT donor selection for use in pediatrics. A single donor was selected from a pool of healthy adults recruited from Stanford University (following vegetarian or vegan diets) or from the OpenBiome stool bank based on a combination of clinical, microbiome composition, and metabolomic characteristics. Figure 1C shows the changes in alpha diversity observed in the recipients' microbiota in the 10 weeks of follow-up. As has been observed in adult FMT studies, the microbiota of the children quickly evolved and became more diverse following fecal transplantation. The study is limited by the lack of a pediatric control group that received FMT from a donor (or donors) who did not undergo the selective screening process. Furthermore, it would be useful to clarify the importance of specific microbiome characteristics (e.g. butyrate-producing bacteria) in the success of FMT in reducing recurrence of *C. difficile* infection so that a microbial consortia approach could be tested. Including results from a group like this would strengthen the authors' conclusion that this selective screening process is necessary and beneficial when choosing FMT donors.

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8. Validation of endotoxin-core antibodies in dried blood spots as a measure of environmental enteropathy and intestinal permeability.
   Hoke MK, McCabe KA, Miller AA, & McDade TW.
   American Journal of Human Biology. 2018 March 13. [Epub ahead of print].
   PubMed ID: 29532544

ABSTRACT:
OBJECTIVE: To validate a method for measuring endotoxin-core antibodies (EndoCAb) from dried blood spots (DBS)-drops of capillary whole blood collected and dried on filter paper-as an indicator of environmental enteropathy (EE) in infancy and early childhood.
METHODS: A commercially available enzyme immunoassay kit was adapted for use with DBS, with optimized steps for sample elution. Technical validation included analysis of linearity/recovery, precision and reliability, lower limit of detection, and correspondence between matched plasma and DBS samples. Validation in a field-based setting was implemented with samples from Peruvian infants (n = 82; age = 2-33 months) collected at two time points six months apart.
RESULTS: A high correspondence between plasma and DBS levels of EndoCAb was observed (R^2 = 0.93, P < .001). The lower limit of detection was found to be 0.01 GMU/mL. Interassay coefficient of variation (CV) was 10.9% and 8.06% for low and high controls, respectively. Mean intra-assay CVs were 3.22% and 1.83%, respectively. In a sample of Peruvian infants, EndoCAb levels increased with age as expected (P < .001). Age explained nearly 34.6% of the variance in EndoCAb across the sample.
CONCLUSION: These findings demonstrate the validity and feasibility of measuring EndoCAb in remote field settings using minimally invasive DBS sampling.

DOI: 10.1002/ajhb.23120
IMPACT FACTOR: 1.780
CITED HALF-LIFE: 7.5

START COMMENTARY: This study investigated the feasibility of using a minimally invasive sampling technique for detecting EE in children. Currently, EndoCAb levels are measured in plasma or serum spun down from whole blood. The authors first sought to determine if EndoCAb could be detected in blood from DBS. They initially tested their protocol with plasma blood and DBS from 3 healthy adults to determine a combination of punch size and dilution that yielded the best results. After determining a single 3.2 mm punch produced detectable EndoCAb levels in the adult samples, they characterized DBS assay performance (linearity, recovery, and lower limit of detection) through dilution of 18 adult samples, and then compared EndoCAb levels in DBS (with assay diluent) to that of matched serum. Authors determined the appropriate dilution levels for infants using samples from five infants collected in the Peru field study. The infant blood samples used in this study were collected from a cohort of 84 infants participating in a larger project of early growth and nutrition in Peru. Two DBS samples, taken 6 months apart, were collected from each infant for analysis. The DBS were run according to the protocols previously determined, and were compared to controls determined to have low, medium and high EndoCAb levels. This study provides promising evidence that DBS can be used to detect biomarkers of EE in children. A limitation of the study is a lack of additional EE markers with which to compare results. Future studies using DBS should include additional EE markers to strengthen the case for the use of DBS sample collection for the purpose of EE assessment in low-resource settings.

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9. Serum anti-flagellin and anti-lipopolysaccharide immunoglobulins as predictors of linear growth faltering in Pakistani infants at risk for environmental enteric dysfunction.
Syed S, Iqbal NT, Sadiq K, Ma JZ, Akhund T, Xin W.
PLoS One. 13(3). 2018 March 06.
PubMed ID: 29509790

ABSTRACT:
BACKGROUND: Environmental Enteric Dysfunction (EED) in children from low-income countries has been linked to linear growth declines. There is a critical need to identify sensitive and early EED biomarkers.
OBJECTIVE: Determine whether levels of antibodies against bacterial components flagellin (flic) and lipopolysaccharide (LPS) predict poor growth.
DESIGN/METHODS: In a prospective birth cohort of 380 children in rural Pakistan blood and stool samples were obtained at ages 6 and 9 months. Linear mixed effects models were used to examine longitudinal associations between quartiles of anti-flic and anti-LPS antibodies and changes in LAZ, WAZ and WLZ scores. Spearman's correlations were measured between anti-flic and anti-LPS immunoglobulins with measures of systemic/enteric inflammation and intestinal regeneration.
RESULTS: Anti-LPS IgA correlated significantly with CRP, AGP and Reg1 serum at 6mo and with MPO at 9mo. In multivariate analysis at 6mo of age, higher anti-LPS IgA levels predicted greater declines in LAZ scores over subsequent 18mo (comparing highest to lowest quartile, β (SE) change in LAZ score/year = -0.313 (0.125), p-value = 0.013). Anti-flic Ig A in the two highest quartiles measured at 9mo of age had declines in LAZ of -0.269 (0.126), p = 0.033; and -0.306 (0.129), p = 0.018 respectively, during the subsequent 18mo of life, compared to those in the lowest quartile of anti-flic IgA.
CONCLUSIONS AND RELEVANCE: Elevated anti-flic IgA and anti-LPS IgA antibodies at 6 and 9mo, predict declines in linear growth. Systemic and enteric inflammation correlated with anti-LPS IgA provides mechanistic considerations for potential future interventions.

DOI: 10.1371/journal.pone.0193768
IMPACT FACTOR: 2.806
CITED HALF-LIFE: 3.7

START COMMENTARY: The authors of this study used data collected from children in a Pakistani birth cohort. Children were recruited at birth and followed for 18 months with weekly home visits and monthly anthropometric measurements. In addition, blood samples were collected from enrolled children at 6 and 9 months of age. The blood markers from these infants were compared to 36 healthy infants seen at Boston Children's Hospital who had an excess blood sample in the Hospital's laboratory, with mean age of 9.5 months. As shown in Figure 2, the mean anti-flic and anti-LPS IgA concentrations were significantly lower in Pakistani infants compared to the healthy controls, while anti-flic and anti-LPS IgG concentrations were significantly higher. The authors used linear mixed effects models to evaluate possible relationships between monthly Z-scores and anti-flic and anti-LPS IgA and IgG concentrations (categorized in quartiles) over the length of this study. Each model included an interaction term between the biomarker and time, which was the independent variable of greatest interest (Table 3). The authors noted that the results of their study somewhat contradict those of a recent study done in Tanzania, which reported no significant association between anti-flic and anti-LPS IgA and IgG concentrations and stunting, but did observe negative associations with WAZ and WLZ. The authors noted that the baseline IgA levels of the Tanzanian children were higher than that of the healthy Boston controls, suggesting a possible IgA deficiency in Pakistani children that should be further explored.

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10. Environmental enteric dysfunction and systemic inflammation predict reduced weight but not length gain in rural Bangladeshi children.
Campbell RK, Schulze KJ, Shaikh S, Raqib R, Wu LSF, Ali H, *et al.*
British Journal of Nutrition. 119(4). 2018 February 28.
PubMed ID: 29498344

ABSTRACT: Environmental enteric dysfunction (EED) and systemic inflammation (SI) are common in developing countries and may cause stunting. In Bangladesh, >40 % of preschool children are stunted, but EED and SI contributions are unknown. We aimed to determine the impact of EED and SI (assessed with multiple indicators) on growth in children (n 539) enrolled in a community-based randomised food supplementation trial in rural Bangladesh. EED was defined with faecal myeloperoxidase, α-1 antitrypsin and neopterin and serum endotoxin core antibody and glucagon-like peptide-2, consolidated into gut inflammation (GI) and permeability (GP) scores, and urinary lactulose:mannitol α-1 acid glycoprotein (AGP) characterised SI. Biomarker associations with anthropometry (15-, 18- and 24-month length-for-age (LAZ), weight-for-length (WLZ) and weight-for-age (WAZ) z scores) were examined in pairwise correlations and adjusted mixed-effects regressions. Stunting, wasting and underweight prevalence at 18 months were 45, 15 and 37 %, respectively, with elevated EED and SI markers common. EED and SI were not associated with 15–24-month length trajectory. Elevated (worse) GI and GP scores predicted reduced 18–24-month WLZ change (β −0·01 (se 0·00) z score/month for both). Elevated GP was also associated with reduced 15–18-month WLZ change (β −0·03 (se 0·01) z score/month) and greater 15-month WLZ (β 0·16 (se 0·05)). Higher AGP was associated with reduced prior and increased subsequent WLZ change (β −0·04 (se 0·01) and β 0·02 (se 0·00) z score/month for 15–18 and 18–24 months). The hypothesised link from EED to stunting was not observed in this sample of Bangladeshi 18-month-olds, but the effects of EED on constrained weight gain may have consequences for later linear growth or for other health and development outcomes.

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IMPACT FACTOR: 3.706
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START COMMENTARY: This study used longitudinal anthropometric data collected from an RCT to examine possible associations between EED biomarkers and weight and length growth trajectories. Anthropometry was measured at 15, 18, and 24 months of age in all participants. In addition, a stool sample was collected at 18 months to test for EED biomarkers. The authors noted that a major strength of this study is the longitudinal data that allowed comparisons of individual growth history in each child in relation to EED biomarkers. Gut inflammation (GI) and gut permeability (GP) scores were devised using a principal component analysis on log-transformed biomarkers, a method was previously described in Campbell *et al* in a 2017 study in the same cohort of Bangladeshi children. Linear mixed effects regression models were then created to analyze the associations between GI, GP and other biomarkers with anthropometric measures. A spline with a knot at 18 months was used to allow for examination of the relationship between growth from 15 to 18 months with scores at month 18 (retrospective), and separately allowed for examination of the relationship between scores at 18 months of age with growth from months 18-24 (prospective). The associations from the model can be seen in Table 3. Contrary to several other published studies, this investigation found no association between EED measured at 18 months and linear growth trajectories. A major limitation of the study is that serum and stool samples were collected at a single time point and used to assess EED status in a model which broke the 15 to 24 months age period into two periods (months 15-18 and 18-24). It has been suggested that the state(s) reflected by EED biomarkers, especially in young children, are transient,

and therefore EED biomarker measurement at age 18 months may not be reflective of the EED burden that was experienced during a period of early life. The transient property of systemic inflammation biomarkers used in this study was illustrated in the case of AGP, an acute phase inflammatory protein. Children with elevated AGP (suggestive of the end of an infection) at age 18 months, showed less weight gain between 15 to 18 months, but then from 18 to 24 months, AGP was positively associated with change in WAZ. This suggests that while systemic inflammation is negatively associated with growth during acute infection, catchup growth may be observed following resolution of infection. In contrast to systemic inflammation markers, negative associations were observed between GP and GI scores at age 18 months with change in WAZ and WLZ in the latter period (18 to 24 months), but the magnitude of the effect was relatively small.

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