Title: A Study to Evaluate Efficacy and Tolerability of Partial Enteral Nutrition with Exclusion Diet and its effect on microbiome change in Patients with Mild to Moderate Ulcerative Colitis: A Quasi-Experimental Study.

Introduction:

Inflammatory bowel disease (IBD) is a complex immune mediated inflammatory disease which results from interaction of environment, host and microbiome[1]. Role of microbiome in pathogenesis has recently got attention and many studies have shown association of specific microbiota with IBD[2]. Many therapeutic modalities have been developed to modify the composition of microbiota and diet is one of them. There is increasing evidence to suggest role of diet in influencing gut microbiota. Most of the data on effectiveness of diet as a therapy in IBD comes from pediatric Crohn's disease (CD). Exclusive enteral nutrition has been shown to be effective than steroids in children with CD and is recommend as treatment of choice by international societies[3]. There are many observational studies to suggest effectiveness of EEN in adults as well[4]. However, tolerability of EEN is poor and its effects are short term. Partial enteral nutrition might improve tolerability but it has not been shown to be as effective as EEN, indicating requirement for exclusion of certain dietary components[5]. Hence, various elimination diets have been developed. These include the Specific Carbohydrate Diet (SCD), the Crohn's Disease Exclusion Diet (CDED), the Anti-inflammatory diet (IBD-AID), Allergen elimination diet (IgG), and the Semi-vegetarian diet (SVD). The CDED excludes those dietary components which impair innate immunity, increase intestinal permeability, cause microbial dysbiosis, or allow bacteria to adhere and translocate through the intestine epithelium in animal models. A recent RCT by same group compared CDED+PEN with EEN and found that both these were effective in inducing remission and microbiome changes induced by both these were similar[6]. There are very limited studies on enteral nutrition in UC. A Study in 1993, showed significant improvement of serum albumin and less adverse events with total enteral nutrition compared with parenteral nutrition in acute UC. However, this study did not demonstrate clinical benefit[7]. A recent study from our institute has shown significant decrease in steroid failure rate in patients with EEN compared to standard of care in patients with acute severe UC (unpublished). However, there are limited data on microbiome change with diet and enteral nutrition.

Review of literature:

Role of enteral nutrition and diet in UC:

Role of microbiome in the pathogenesis of IBD has becoming more evident and therapeutic approaches like FMT and specific diets, to manipulate microbiome have been investigated. The microbiota first time colonizes following birth and matures in next three years and after that it becomes relative stable through adulthood[8]. However, in later life it is influenced by various factors like diet, aging, medications leading dysbiosis and predisposition to IBD[9,10]. Effect of diet on microbiome is exemplified by study by De Filippo et al., where they compared microbiome of children from European and African countries. They found a significant enrichment in Bacteroidetes and depletion in Firmicutes, with a unique abundance of bacteria from the genus Prevotella and Xylanibacter which were lacking in European children. Diet also has influence on incidence of IBD[11]. A meta-analysis of 14 cohort studies shown that intake of fruits and vegetables is associated with low incidence of UC. In the same way intake of omega-3 polyunsaturated fatty acids is associated with low incidence of UC[9]. First randomized controlled trial of diet in UC was done be Wright et al., where they looked at effect of milk free diet in acute exacerbation of UC and showed low relapse rate with milk

free diet[12]. In another study of symptom guided diet in UC authors showed higher clinical remission rate at 6 weeks with avoidance of specific products during relapse compared to regular diet (36.3% vs. 0.0%). However, there was no difference in endoscopic and histological parameters. Similarly, other dietary interventions like low FODMAP diet, no-carrageenan and cow milk protein elimination diet have also been tried in UC.

Enteral nutrition is an established primary therapeutic modality in children with CD and it promotes mucosal healing, restores bone mineral density and improves growth. The European Society of Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Crohn's Colitis Organization (ECCO) recommend EEN as a first-line therapy in children with active CD and emphasize the use of EEN over CS in all children with active inflammatory intestinal luminal disease, including colonic involvement[3]. EEN can be elemental or polymeric and there is no difference in clinical efficacy. Role of EEN in UC has not been clear. One prospective randomized trial compared the efficacy of total EN and total parenteral nutrition as an adjunct therapy in patients with acute severe UC on intensive corticosteroid therapy. After a 48hr steroid therapy, patients were randomized to receive polymeric total EN (n = 22) or TPN (n = 20)[7]. Remission rates and the need for colectomy were similar in the two groups. No significant change in anthropometric parameters was observed in either group. However, the 16.7% median increase in serum albumin in the EN group was significantly greater than 4.6% in the TPN group. In another study by Klaassen J et al., there was no significant improvement in albumin levels with EEN compared to TPN. However, the sample size was small[13]. Partial enteral nutrition was shown to be less efficacious than EEN. In 2006, Jones et al. have published the first RCT on partial enteral nutrition (PEN). Fifty children with active CD (PCDAI >20) were randomly assigned in PEN or EEN group. Children in PEN group had obtained 50% of their caloric requirements from elemental formula and 50% from 80 New Concepts in Inflammatory Bowel Disease unrestricted diet, while children in EEN had received 100% of their energy requirements from elemental formula for 6 weeks. This study showed that the conventional treatment with EEN was associated with a significantly higher remission rate in comparison with PEN (42 versus 15%)[5]. Recently, Sigall-Boneh et al. treated 47 patients (34 children and 13 young adults) with early mild-to-moderate luminal CD with PEN. Their approach allowed patients to consume 50% of dietary calories from a polymeric formula and remaining calories from a special Crohn's disease exclusion diet (CDED). In the study, a clinical response and remission were achieved in 78.7 and 70.2% patients, respectively[14]. CDED is a structured diet that excludes animal fats, milk and dairy, gluten, and all processed and canned foods, which contain additives. A recent RCT by same group compared CDED+PEN with EEN and found that both these were effective in inducing remission and microbiome changes induced by both these were similar[6]. Combination of PEN and exclusion diet improves tolerability, compliance and is equally efficacious to EEN.

Effect of enteral nutrition on microbiome:

Microbiome modulates gut barrier function, helps in maturation of gut immune system and produce short chain fatty acids (SCFA) which are important source of energy for colonocytes. SCFA are produced by fermentation of undigestible carbohydrates by microbiota and western diet is characterized by high sugars and less fibers are associated with low SCFA[15]. The gut microbiome, considered as the 'second metabolic organ' of human body, performs several important functions that are beneficial for the host, and change in the quality (change in bacterial composition) and quantity (altered diversity and abundance) of the gut flora, defined as dysbiosis, has been associated with IBD[16]. The dysbiosis in IBD is characterized by decreased microbial diversity, abundance and decline in the

population of useful bacteria such as Ruminococcus, Faecalibacteriumprausnitzii, Lachanospiracae, Bifidobacteria and increased population of pathogenic bacteria such as Enterobacteriacae[17]. Diet is one of the most important determinants of gut microbiome, and both short and long term dietary patterns as well as individual dietary constituents have been correlated with the gut microbiome[18]. Several epidemiological studies have found both positive and negative associations between various dietary constituents and IBD[19]. Among them higher consumption of soluble fiber (derived from fresh fruits and vegetables), vitamin D, zinc, and potassium had a protective effect for CD. There was an inconsistent negative association of ω -3 fatty acid consumption, and inconsistent positive association of ω -6 fatty acid and animal protein consumption with UC. EEN has also been shown to influence microbiome Lionetti et al. observed marked compositional changes over an eight-week period of EEN treatment in nine patients, while samples collected from healthy children over the same period appeared relatively stable[20]. Gerasimidis et al. reported a significant decrease in F. prausnitzii with EEN treatment, which appears to contradict previous associations of this bacterium with positive clinical outcomes in adult CD[21]. Reduced abundance of F. prausnitzii (specifically, two subgroups of F. prausnitzii) was also reported to correlate with clinical improvement with EEN treatment in an adult CD study by Jia et al[22]. The paradoxical decrease of F. prausnitzii in correlation with improvement in clinical symptoms indicates that the therapeutic effect of EEN is not mediated by F. prausnitzii. However, the physiological significance of F. prausnitzii levels in CD has not been fully established. Recent study by Levine et al. where they compared EEN with PEN + CDED in patients with paediatric CD demonstrated change in microbiome in patients who achieved remission by 6 weeks[6]. These changes were retained in patients who were continued on PEN+ CDED but not in patients in whom EEN was stopped. There are no studies in UC to demonstrate change in microbiome.

Methodology:

Primary objective:

1. To evaluate efficacy and tolerability of partial enteral nutrition (PEN) and exclusion diet in inducing clinical and biochemical remission in patients with mild to moderate UC

Secondary objective:

1. To evaluate effect of partial enteral nutrition (PEN) and exclusion diet on microbiome in patients with mild to moderate UC

Materials and Methods

Study location: The study will be conducted in Inflammatory Bowel Disease clinic, Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi.

Study design: Single center, open label, quasi experimental trial of partial enteral nutrition (PEN) and exclusion diet in comparison to standard medical therapy in adult patients with mild to moderate ulcerative colitis

Study Population

All patients suffering from mild to moderate Ulcerative Colitis (defined by standard criteria) and following up at Inflammatory Bowel Disease clinic at All India Institute of Medical Sciences, New Delhi will be screened for inclusion in this study.

Inclusion Criteria:

1. Patients who are confirmed cases of mild to moderate ulcerative colitis
2. Age: 18 – 70 years of either gender
3. Patients on stable dose of mesalamine for past 4 weeks
4. Patients on stable dose of azathioprine/6-mercaptopurine for past 6 months
5. Patients on stable doses of topical therapy for past 2 weeks
6. Patients who are willing to participate

Exclusion Criteria

1. Received oral steroids in the past 4 weeks
2. Received oral antibiotics within past 2 weeks
3. Patients with severe disease activity
4. Patients who have had colectomy
5. Patients with significant hepatic, renal, endocrine, respiratory, neurologic, or cardiovascular diseases will also be excluded
6. Pregnant, lactating females

Baseline assessment

Included patients will undergo a uniform baseline evaluation which will include a clinical evaluation, and laboratory assessment.

Clinical evaluation

Detailed history will be taken regarding demographic information, onset and duration of symptoms, and clinical features. Disease activity will be assessed at baseline using SCCAI and partial Mayo score.

Laboratory investigation

Hemogram, liver function test, renal function test, and serum C-reactive protein will be done in all patients. Stool samples will also be collected in a sterile airtight container for microbiota assessment and fecal calprotectin.

Colonoscopy: Because of ongoing pandemic and difficulty in performing invasive endoscopic procedures, colonoscopic assessment of disease activity will not be done. Instead, non-invasive

measures of disease activity which include stool fecal calprotectin and clinical disease activity scores will be measured.

Blinding

Neither the patient nor the investigator can be blinded to the treatment. However, the investigator analyzing the data will be blinded to the treatment details.

Intervention and follow up

Patients with active disease will either receive PEN and exclusion diet (experimental arm) or standard medical therapy (SMT, control arm). Patients in both groups will continue the ongoing treatment prior to inclusion in study and local therapy will be optimized before upgrading to higher immunosuppresants.

Experimental arm: Patients will be asked to strictly adhere to the PEN along with exclusion diet for 4 weeks. PEN is a soya based powdered polymeric nutrition supplement formula (REMATIN, Waterley) which contains nutrients like peptides, triglycerides, carbohydrates along with vitamins and minerals which provides 1kcal/ml energy. Patients will be administered 50% of caloric requirement in the form of PEN and exclusion diet (table 1), which would be rich in dietary constituents that expand T-regulatory cells, promote healthy microbiota and improve the intestinal barrier, and will be poor in dietary constituents that cause dysbiosis or have negative effect on intestinal barrier. Patients will be given a diet chart accordingly and will be counselled to adhere to the diet protocol. Regular telephonic interviews will be done to ensure compliance with the diet. Exclusion diet is continued till 4 weeks.

Control arm: Patients will be given SMT (continuation of baseline medications), along with optimizing the dose of 5-ASA and/or addition of topical therapy.

Follow-up

Patients will be followed at weeks 2 and 4. Clinical disease activity (SCCAI) will be assessed at all visits, and dietary adherence in the experimental arm will be assessed at weeks 2 and 4. Stool sample for microbiome analysis and fecal calprotectin will be collected at baseline and 4 weeks. The stool samples will be stored at -80℃ for microbiome analysis. Microbiome analysis will be done only in intervention arm and compared with baseline analysis. Dietary assessment with the help of IBD Nutricare mobile application will be done.

Microbiome analysis

The fecal samples will be kept at −80°C before extraction of genomic DNA. Around 200 mg frozen samples will be used for DNA extraction by manually (Bag et al., 2016 and THSTI methods) with some modification modifications. The quality and quantity of DNA will be assessed using Biospectrometer (Eppendorf, Germany) and 0.8% agarose gel electrophoresis. Variable regions V3-V5 of the 16S rRNA genes will be amplified in 50µl reaction volume using 0.1 ng of template DNA and 27 F(C1) and 926 R(C5) primers. The 950-bp long PCR products will be gel purified using QIAquick gel elution Kit (Qiagen, Germany). Equimolar concentration amplicon libraries will be mixed and sequenced by using Illumina platform. 16S rRNA gene sequencing of the gut microbial genomic DNA will be done for species and strain-level microbiome analysis. Sequence reads obtained in FASTQ format will be evaluated by FASTQC, using default parameters.

Data processing, OTU clustering and taxonomic profiling

The samples will be quality filtered and multiplexed using the Next Generation Sequencing (NGS) tag cleaner software. Operational Taxonomic Units (OTUs) will be predicted by clustering the sequences with identities greater than 97% using UCLUST software package. The genus and phylum affiliations of the representative sequences corresponding to each OTU will be predicted using the Naïve-Bayesian based RDP classifier. The species level affiliations of the representative sequences will be

obtained by performing a BLAST search of the representative sequences with an in-house database. Based on the taxonomic affiliations of the representative sequences, the number of sequences belonging to each taxonomic group will then be cumulated. Abundances of the various taxa in a given sample will be calculated as the total number of sequences assigned to a given taxa divided by the total number of sequences in that sample. Scaled abundances (with values 0 to 1) will be obtained by comparing the abundances of each taxa across samples.

Fecal Calprotectin Assay

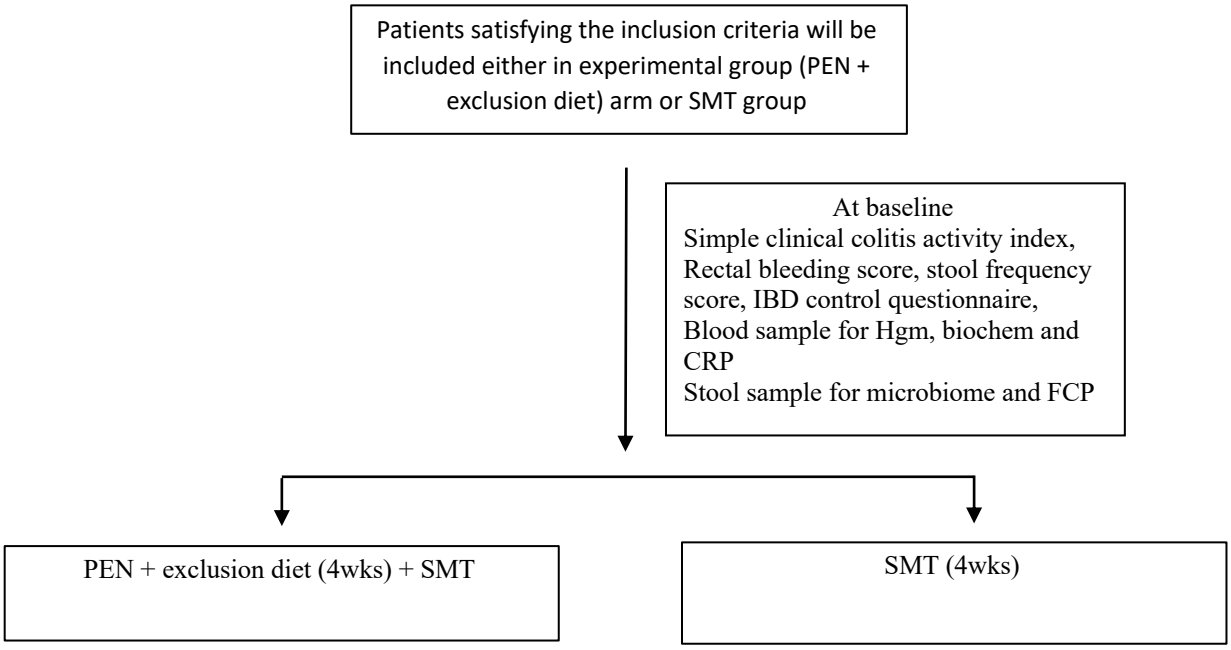
Quantitative Fecal Calprotectin ELISA Kit is intended for use in the quantitative determination of human calprotectin (neutrophil cytoplasmic protein S100A8/A9) levels in stool samples. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human calprotectin.

Statistical analysis:

The baseline data will be recorded as number (%) or mean ± SD or median (range) as appropriate. To compare the baseline parameters between the two groups of patients, Chi square test will be used for categorical variables, Student's t-test will be used for continuous variable with normal distribution and Wilcoxon-Mann-Whitney U test will be used for continuous variables without normal distribution. A 2-sided p-value <0.05 will be considered to be statistically significant**.** Data will be analyzed using IBM SPSS Statistics software (version 21.0, Chicago, IL, USA). Multivariate analysis done to find out predictors of treatment failure.

Sample size: The sample size was based on 81% response to intravenous steroids in patients of acute severe ulcerative colitis (ASUC) with exclusive enteral nutrition (EEN) compared to 57% in standard of care, as reported in a previous study from our centre. Assuming 25% higher remission rate in the PEN + exclusion diet, with 80% power, alpha of 0.05 and drop-out rate of 10%, total of 114 patients will be required for the desired outcome.

Figure 1: Study flow for patient recruitment and follow-up till 4 weeks (induction phase)



CRP: C-reactive protein; FCP: Faecal calprotectin; PEN: Partial enteral nutrition; SMT: Standard medical therapy; SCCAI: Simple clinical colitis activity index;
Investigations to be done during follow up of patients after inclusion in study

Weeks	SCCAI score	Partial Mayo score	IBD control questionnaire	Blood investigations	Fecal microbiome analysis	Fecal calprotectin
Baseline	√	√	√	√	√	√
Week 2	√	√	√	-	-	-
Week 4	√	√	√	√	√	√

Outcome measures

Primary outcome:

1. Composite of clinical remission (SCCAI≤2) and faecal calprotectin <150ug/gm at 4 weeks

Secondary outcome:

1. Change in microbiome at 4 weeks after partial enteral nutrition (PEN)
2. Proportion of patients with rectal bleeding score of 0 and stool frequency score of ≤1 at 4 weeks
3. Tolerability of PEN + exclusion diet at 4 weeks

Definitions

Clinical remission: SCCAI score ≤2

Stool Frequency score	0: 1-2 stools more than normal/ day 1: 3-4 stools more than normal/ day 2: >4 stools more than normal/ day
Rectal bleeding score	0: none 1: visible blood with stool less than half times the day 2: visible blood with stool half times the day or more 3: passing blood alone

Table 1: Dietary items that are allowed and that are not allowed in the exclusion diet

FOOD GROUP	AVOID	RECOMMENDED	CHARACTERISTICS	REASONING
Cereal and Grains	Gluten-based grains – Wheat, Maida, sooji	Rice, Maize, Ragi, kuttu, singhara, samak, bajra, jawar	Preferably in fermented, germinated,soaked or soft form e.g. Idli, cheela, dosa, appam, utappam, dhokla etc.	Help restore anti-inflammatory environment
Nuts, seeds, legumes	Whole seeds and nuts	Flaxseeds, walnuts, almonds etc All seeds such as cumin, fenugreek ,flax	Legumes in soaked and germinated form. Seeds in powdered form. Nuts in soaked	Intact fiber can be problematic for highly active mucosal inflammation.

		seeds etc in powdered form Washed dals and legumes		
Dairy	Dairy products of any kind and margarine	Fresh curd, Lactose free milk	Live bacteria	Friendly gut bacteria
Non Veg.	Processed, canned, irradiated, smoked or red meat	Fresh chicken (breast) and Fish, egg	Soft, well cooked and fish preferably of omega 3 rich variety	Omega 3 rich and less consumption of red meat
Fruits	Fruits with seeds	All fruits with soft texture and strained out seeds can be included	Soft, pureed, blenderized in the form of smoothies can be included. Apples, Bananas	Antioxidant, Soluble fiber and pre biotic properties
Vegetables (600 gms.)	Tough texture, with seed and in salad form	All vegetables with soft texture Garlic, Onion, Leek, Asparagus Cruciferous Veg e.g. Cauliflower, cabbage, boroccoli, radish, turnip, Kohlrabi.	Soft, cooked, pureed or fermented form	Soluble fibre, Antioxidants, Prebiotics
Oil	Sunflower Safflower oil	Mustard, Soyabean, Olive, Canola and Rice bran oil	Omega 3 rich	Anti inflammatory properties
Sugar	Refined sugars	Honey	2- 3 tsp table sugar can be taken	
Cooking Methods	Frying, shallow frying, charred/ burnt, too much tadka/ too much churning or food overcooked at high temperatures.	Sautéed, steamed, boiled, baked, cooked on tawa		To avoid oxidation and hard texture.

Table 2: Simple Clinical Colitis Activity Index

Parameter	**Variable**

Stool Frequency day	0: 1 - 3 1: 4 - 6 2: 7 - 9 3: >9
Stool frequency night	1: 1 – 3 2: 4 – 6
Urgency	1: Hurry 2: Immediately 3: Incontinence
Rectal bleeding	1: Trace 2: Occasionally frank 3: Usually frank
General well being	0: very well 1: Slightly below par 2: Poor 3: Very poor 4: Terrible
Extracolonic	1/ every
TOTAL	

References:

1. Abraham C, Cho JH. Inflammatory Bowel Disease. N Engl J Med. 2009;361(21):2066-2078. doi:10.1056/NEJMra0804647
2. Knights D, Lassen KG, Xavier RJ. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. Gut. 2013;62(10):1505-1510. doi:10.1136/gutjnl-2012-303954
3. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis. 2014;8(10):1179-1207. doi:10.1016/j.crohns.2014.04.005
4. Sharma S, Gupta A, Kedia S, et al. Efficacy and tolerability of exclusive enteral nutrition in adult patients with complicated Crohn's disease. *Intest Res*. Published online May 26, 2020. doi:10.5217/ir.2019.09172
5. Johnson T, Macdonald S, Hill SM, Thomas A, Murphy MS. Treatment of active Crohn's disease in children using partial enteral nutrition with liquid formula: a randomised controlled trial. *Gut*. 2006;55(3):356-361. doi:10.1136/gut.2004.062554
6. Levine A, Wine E, Assa A, et al. Crohn's Disease Exclusion Diet Plus Partial Enteral Nutrition Induces Sustained Remission in a Randomized Controlled Trial. Gastroenterology. 2019;157(2):440-450.e8. doi:10.1053/j.gastro.2019.04.021

7. González-Huix F, Fernández-Bañares F, Esteve-Comas M, et al. Enteral versus parenteral nutrition as adjunct therapy in acute ulcerative colitis. *Am J Gastroenterol*. 1993;88(2):227-232.
8. Robertson RC, Manges AR, Finlay BB, Prendergast AJ. The Human Microbiome and Child Growth – First 1000 Days and Beyond. Trends in Microbiology. 2019;27(2):131-147. doi:10.1016/j.tim.2018.09.008
9. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. Long-term Intake of Dietary Fat and Risk of Ulcerative Colitis and Crohn's Disease. Gut. 2014;63(5):776-784. doi:10.1136/gutjnl-2013-305304
10. Shanahan F, Ghosh TS, O'Toole PW. The Healthy Microbiome (What is the definition of a healthy gut microbiome?). *Gastroenterology*. 2020;0(0). doi:10.1053/j.gastro.2020.09.057
11. Filippo CD, Cavalieri D, Paola MD, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. PNAS. 2010;107(33):14691-14696. doi:10.1073/pnas.1005963107
12. Wright R, Truelove SC. A Controlled Therapeutic Trial of Various Diets in Ulcerative Colitis. Br Med J. 1965;2(5454):138-141.
13. J K, R Z, Jg M, et al. [Enteral nutrition in severe ulcerative colitis. Digestive tolerance and nutritional efficiency]. *Rev Med Chil*. 1998;126(8):899-904.
14. Sigall-Boneh R, Pfeffer-Gik T, Segal I, Zangen T, Boaz M, Levine A. Partial Enteral Nutrition with a Crohn's Disease Exclusion Diet Is Effective for Induction of Remission in Children and Young Adults with Crohn's Disease. *Inflamm Bowel Dis*. 2014;20(8):1353-1360. doi:10.1097/MIB.0000000000000110
15. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology*. 2013;145(5):970-977. doi:10.1053/j.gastro.2013.07.050
16. Bäckhed F, Fraser CM, Ringel Y, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe*. 2012;12(5):611-622. doi:10.1016/j.chom.2012.10.012
17. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014;15(3):382-392. doi:10.1016/j.chom.2014.02.005
18. Cotillard A, Kennedy SP, Kong LC, et al. Dietary intervention impact on gut microbial gene richness. *Nature*. 2013;500(7464):585-588. doi:10.1038/nature12480
19. Pfeffer-Gik T, Levine A. Dietary clues to the pathogenesis of Crohn's disease. *Dig Dis*. 2014;32(4):389-394. doi:10.1159/000358143

20. Lionetti P, Callegari ML, Ferrari S, et al. Enteral nutrition and microflora in pediatric Crohn's disease. *JPEN J Parenter Enteral Nutr*. 2005;29(4 Suppl):S173-175; discussion S175-178, S184-188. doi:10.1177/01486071050290S4S173
21. Gerasimidis K, Bertz M, Hanske L, et al. Decline in presumptively protective gut bacterial species and metabolites are paradoxically associated with disease improvement in pediatric Crohn's disease during enteral nutrition. *Inflamm Bowel Dis*. 2014;20(5):861-871. doi:10.1097/MIB.0000000000000023
22. Jia W, Whitehead RN, Griffiths L, et al. Is the abundance of Faecalibacterium prausnitzii relevant to Crohn's disease? *FEMS Microbiol Lett*. 2010;310(2):138-144. doi:10.1111/j.1574-6968.2010.02057.x