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Comparison of clinical effectiveness and microbiome impact between specific polymeric and monomeric diet for exclusive enteral nutrition in pediatric Crohn's disease: a prospective cohort study

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ABSTRACT

Background: According to the ECCO-ESPGHN guideline for the treatment of newly diagnosed pediatric Crohn's disease (CD), exclusive enteral nutrition (EEN) treatment is recommended for inducing remission. This study aimed to compare the clinical effectiveness and changes in the microbiome between the monomeric diet and a specific polymeric diet.

Methods: This prospective study included 38 healthy children and 48 pediatric patients with moderate to severe CD. The patients divided into two groups according to the type of EEN: monomeric diet (group A, $n = 24$) and polymeric diet (group B, n = 24).

Results: All patients achieved clinical remission. Baseline alpha diversity was lower in CD patients than in healthy controls ($p < 0.001$). Shannon diversity was statistically associated with ESR ($p = 0.037$). *Faecalibacterium, Streptococcus sanguinis* group, *Enterococcus* had decreased after EEN treatment ($p = 0.003$, $p = 0.003$, $p = 0.026$, respectively). There were differences in bacterial taxa between the two groups before starting EEN, but these differences disappeared after EEN. *Bacteroides acidifaciens* group and *Butyricicoccus faecihominis* group were associated with CRP ($p = 0.016$, $p = 0.027$, respectively) and ESR ($p = 0.004$, $p = 0.012$, respectively).

Conclusions: The polymeric diet, like the monomeric diet, can also induce clinical remission and is therefore a viable option for EEN treatment. An 8-week course of exclusive enteral nutrition is sufficient to achieve significant improvements in inflammation and weight gain. The microbiome was correlated with clinical indicators, but no significant differences were observed between the two groups.

1. Introduction

Crohn's disease (CD) is a chronic progressive and destructive inflammatory condition known to arise from a combination of genetic, environmental, and immunological factors. In patients who do not receive appropriate treatment, intestinal complications such as strictures or fistulae can develop within 10 years of diagnosis and often require surgery within 20 years. The primary target in treating Crohn's disease is the healing of the inflamed mucosa. At the time of diagnosis, the inflammatory burden is typically high; thus, induction therapy is used to rapidly and effectively reduce inflammation. In pediatric patients with CD, the types of induction therapy currently used include systemic steroids, exclusive enteral nutrition (EEN), and biologics (anti–tumor necrosis factor [TNF]α) such as infliximab and adalimumab (Van Rheenen et al., 2021). However, in the case of biologics, insurance restrictions may prevent their use from the time of diagnosis in some countries. In some countries, biologics are covered by insurance only for patients with luminal CD who have failed conventional treatments. Consequently, the available options for induction therapy include EEN and systemic steroids.

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The advantage of EEN is that it does not have the side effects typically associated with drugs as it is nutritional therapy. However, the downside is that patients may have an aversion to consuming a single liquid formula, which leads to a higher likelihood of withdrawing from the allocated treatment (Pahsini et al., 2016). Some patients may experience nausea and vomiting due to this aversion. The benefit of steroids is their ability to rapidly reduce high inflammatory burden. However, steroids are associated with a higher incidence of complications compared to other medicines. Two meta-analyses have evaluated the effectiveness of EEN with systemic steroid induction therapy in pediatric patients with CD and found no statistically significant difference in clinical remission rates (Narula et al., 2018; Swaminath et al., 2017). Some studies have found EEN to be superior to systemic steroids in achieving mucosal healing (Borrelli et al., 2006; Pigneur et al., 2019; Swaminath et al., 2017).

The high dropout rate during the 8-week EEN course prompted us to plan this study with the aim of reducing dropout rates by providing patients with more options. The first objective of this study was to evaluate the clinical efficacy of a specific polymeric diet compared to that of a monomeric diet. Given the growing medical interest in the relationship between the microbiome and CD—particularly its role as a causative and aggravating factor—further research on this relationship in children is needed. Additionally, while many studies have evaluated the clinical efficacy of monomeric and polymeric diets, research assessing the changes and differences in the microbiome between these two EEN methods is limited. Thus, the second objective of this study was to assess the changes and differences in the microbiome between polymeric and monomeric diets.

2. Methods

2.1. Patients and study design

This prospective study included 48 pediatric patients under the age of 19 who were newly diagnosed with moderate to severe luminal CD between June 2020 to March 2021. The diagnosis of CD was made according to the Porto criteria established by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (IBD Working Group of the European Society for Pediatric Gastroenterology & Nutrition, 2005). The diagnostic process for CD involved conducting laboratory examination to assess nutritional and inflammatory status, along with a fecal calprotectin test, endoscopic examinations (which included esophagogastroduodenoscopy and colonoscopy), and magnetic resonance enteroclysis. Additional stool samples were also collected for microbial analysis. All registered patients were initially divided into two groups and started EEN treatment for initial induction method. One group (group A, $n = 24$) consumed Elemental 028 Extra (monomeric formula), while the other group (group B, $n = 24$) consumed Encover with a corn flavor (polymeric formula). The division into these groups was based on the patients' preferences. The nutritional composition of the two formulas is shown in **Supplementary table 1**. Patients were instructed not to take any other supplements, such as probiotics or vitamins, during the EEN treatment. Owing to variations in age and weight among the patients, the total EEN dosage was adjusted individually for each patient, as outlined in **Supplementary table 2** (Thompson, 2024; Torun, 2005). Patients were hospitalized initially at the time of diagnosis and discharged only after they had reached the target total EEN dosage. Unlike the ready-to-drink Encover, the Elemental 028 Extra formula is a powdered product that must be mixed with water to achieve a concentration of 1 kcal/ml. Patients were evaluated in an outpatient clinic 2 weeks after discharge to assess whether they were adhering to the EEN regimen and to check for any side effects. The total EEN treatment was conducted over an 8-week period, at which point laboratory examination, fecal calprotectin tests, and microbiome analysis were reassessed. At the 8-week mark, the disease status of CD was evaluated by assessing whether each patient had achieved clinical remission (CR) or biochemical remission (BR). The CR was defined as PCDAI $\leq$10 and ER was defined as a SES-CD score of 0–2. Biochemical remission (BR) evaluated as outcome was defined as C-reactive protein (CRP) $<$ 0.5 mg/dl and fecal calprotectin $<$200 μg/g. These patients received treatment with mesalazine and immunomodulators (azathioprine or methotrexate) alongside EEN therapy; however, none of them received systemic steroid treatment. Biologics treatment was also not administered until EEN therapy had been completed.

The primary objective of this study was to evaluate the clinical effectiveness of the previously commonly used monomeric diet and a specific polymeric diet in EEN therapy. This evaluation aims to reduce dropout rates and provide flavor variety for pediatric patients, despite the challenges and limitations in recruiting for prospective studies in pediatric CD. The second aim of this study was to evaluate the changes in microbiome composition following the administration of a monomeric diet and a polymeric diet to determine whether one product has any superior aspect over the other. All procedures in this study were conducted in accordance with relevant guidelines and regulations and received approval from the Clinical Research Ethics Committee of Samsung Medical Center (IRB File No.: SMC 2020–05-075). Informed written consent was appropriately obtained for the analysis of clinical data.

2.2. Microbiome analysis

2.2.1. Genomic DNA extraction and 16S rRNA gene amplicon sequencing

The total bacterial genomic DNA extraction from fecal samples was conducted using a Maxwell® RSC PureFood GMO and Authentication Kit (Promega, Madison, WI, USA), according to the manufacturer's instructions. The concentration of the DNA was determined by means of a UV–vis spectrophotometer (NanoDrop 2000c, USA), while the quantification of DNA was performed using a QuantiFluor® ONE dsDNA System (Promega, USA).

The V3-V4 variable region of the 16S rRNA gene was amplified from DNA extracts using the 16S metagenomic sequencing library protocol (Illumina). Two PCR reactions were completed on the template DNA. Initially, the DNA was amplified with primers specific to the V3-V4 region of the 16S rRNA gene. PCR products were visualized using gel electrophoresis (1× TAE buffer, 2 % agarose, 100 V). Successful PCR products were cleaned using AMPure XP magnetic bead-based purification (Beckman Coulter, UK) and run on the Agilent Bioanalyzer for quality analysis. A second PCR reaction was completed on the purified DNA (5 μl) to index each of the samples. Two indexing primers (Illumina Nextera XT indexing primers, Illumina, Sweden) were used per sample. Successful PCR products were cleaned using AMPure XP magnetic bead-based purification (Beckman Coulter, UK). The purified products were quantified using a QuantiFluor® ONE dsDNA System (Promega, USA) and run on the Agilent Bioanalyzer. Samples were sequenced on the MiSeq sequencing platform, using a 2 × 300 cycle V3 kit, following standard Illumina sequencing protocols.

2.2.2. Sequencing data processing

The raw sequence reads of 16S rRNA gene data were processed with a pipeline for taxonomic profiling based on an algorithm using amplicon sequence variants (ASV), which was implemented in QIIME2 (updated in 2022.11). The sequence reads were processed through demux and cutadapt plugins. Filtering steps based on sequence quality, denoising, trimming, and merging processes were performed, and chimera removal was conducted with dada2 plugin. The EzBioCloud database was used for the taxonomic profiling with 80 % coverage and 97 % identity thresholds by using a feature-classifier plugin. A total of 114 samples were successfully profiled and used in the downstream analysis.

2.3. Statistical analysis

For the clinical results' analysis, the R package "moonBook" was

used with default options. The *t*-test and chi-square test were used for the continuous and categorical variables, respectivelyAdditionally, to compare clinical variables with the adjustment of age, sex, and BMI, logistic regression was performed. The changes of clinical variables before and after EEN treatment were assessed using paired Wilcoxon signed rank tests. Further, to compare the longitudinal change of each clinical variable between EEN types with the consideration of interaction effect, a mixed model analysis of variance (mixed ANOVA) test was conducted.

For microbiome alpha- and beta-diversity analysis, R packages "microbiome" and "vegan" were used. As diversity analysis is prone to total read counts, a species-level count table randomly subsampled to 19,844 reads was used, where the target read was decided based on the minimum read sum across samples. To compare whether the beta diversity was significantly different based on each of explanatory variable, permutational analysis of variance (PERMANOVA) tests were performed for clinical variables with 1000 permutations, respectively. Additionally, the distances of pairwise Bray-Curtis dissimilarity within individual were compared between two EEN types using a Wilcoxon rank-sum test. For the assessment of gut microbial signatures of EEN treatment, the R package "SIAMCAT" was implemented with a paired test condition for the longitudinal comparison and without paired test option for the two group comparison, with prevalence 10 %, Benjamini-Hochberg (BH)-based multiple testing correction options with a significance cutoff at 0.25 for profiles of each taxon level (phylum, class, order, family, genus, and species, respectively). The results were concatenated and summarized with a volcano plot, where the x axis represented fold change and the y axis represented minus log p-adjusted values, using the R package "ggplot."

For the association analysis between microbiome features and clinical variables, Spearman's correlation test was used for the baseline clinical variables and species features using R. For further examining robust taxonomic signatures, only taxa with more than 20 % prevalence across patient samples and at least two-times significant with clinical variables were selected for the heatmap and scatter plots using Python matplotlib and seaborn libraries. On the other hand, for the species with significant associations with fecal calprotectin, the relationship was visualized with scatter plot.

3. Results

3.1. Initial clinical characteristics

The demographic information and clinical characteristics at the time of diagnosis for the two groups of patients are shown in Table 1. No statistically significant differences were observed between the two groups in terms of patient age, gender, body mass index (BMI), or hematological markers assessed in Crohn's disease. Although the differences were not statistically significant, examining the individual mean values revealed a tendency for higher ESR and CRP levels, which reflect the inflammatory state of CD, in patients from group B. The mean fecal calprotectin level was also higher in group B compared to group A, but this difference was not considered statistically significant. When the two groups were evaluated using the Paris classification, similar distributions were observed in terms of age, location, behavior, and perianal involvement. Most patients fell into the A1b category, which corresponded to ages 10 to <17 years. For location, many patients were classified as L3 (ileocolonic type) and L4 (with upper gastrointestinal tract involvement). In terms of behavior, most patients in both groups (87.5 %) were categorized as B1, which indicated inflammation without complications such as stricturing or penetrating disease. Additionally, perianal involvement was observed in more than 75 % of patients. However, one variable that differed between the two groups was growth. In group B, 58.3 % of patients exhibited growth delay, while only 20.8 % of patients in group A showed growth delay. This difference was considered statistically significant with a *p*-value of 0.017. No difference between the two groups in terms of CD severity was assessed endoscopically using the SES-CD score. Additionally, no differences were observed in the treatment regimens, including the use of mesalazine and immunomodulators, which were initiated alongside EEN therapy.

Table 1
Baseline clinical characteristics and initial treatment of the two groups divided by EEN types (Group A: Elemental group, Group B: Encover) at diagnosis of Crohn's disease.
EEN; Exclusive enteral nutrition.

	Group A $N = 24$	Group B N = 24	P-value
Characteristics			
Age, years	13.58 ± 3.02	13.54 ± 3.36	0.964
Sex, M/F	19 (79.2)/5 (20.8)	18 (75.0)/6 (25.0)	0.731
BMI, kg/m^2	19.27 ± 3.46	17.53 ± 2.56	0.054
PCDAI[a]	32.92 ± 12.28	35.00 ± 9.41	0.513
Hematocrit, %	36.66 ± 4.76	36.82 ± 4.48	0.906
Albumin, g/dL	3.95 ± 0.48	3.68 ± 0.48	0.056
ESR, mm/h	49.08 ± 29.24	64.71 ± 29.99	0.074
CRP, mg/dL	1.90 ± 2.18	3.21 ± 3.33	0.115
Fecal calprotectin, mg/kg	2298.42 ± 1986.06	2878.64 ± 2136.45	0.376
Paris classification at diagnosis			
Age at diagnosis A1a	2 (8.3)	4 (16.7)	0.668
A1b	17 (70.8)	16 (66.7)	
A2	5 (20.8)	4 (16.7)	
Location L1	1 (4.2)	1 (4.2)	0.600
L2	1 (4.2)	0	
L3	22 (73.3)	23 (95.8)	
L4a	21 (87.5)	15 (62.5)	
L4b	14 (58.3)	11 (45.8)	
Behavior B1	21 (87.5)	21 (87.5)	0.549
B2	3 (12.5)	2 (8.3)	
B3	0	1 (4.2)	
B2B3	0	0	
p	19 (79.2)	18 (75.0)	0.731
Growth G0	19 (79.2)	10 (41.7)	**0.017**
G1	5 (20.8)	14 (58.3)	
SES-CD[b] score	18.67 ± 7.98	20.46 ± 9.28	0.477
Initial treatment with EEN			
Mesalazine	24 (100.0)	24 (100.0)	1
Steroids	0	0	1
Immunomodulators	19 (79.2)	16 (66.7)	0.517
Azathioprine	5 (20.8)	8 (33.3)	
Methotrexate			

Variables are represented by mean (SD) or frequency.
[a] The pediatric Crohn's disease activity index (PCDAI) score can range from 0 to 100, with higher scores signifying more active disease. A score of <10 is consistent with inactive disease; 11–30 indicates mild disease, and > 30 is a moderate-to-severe disease.
[b] The simple endoscopic score for Crohn's disease (SES-CD) assesses the size of mucosal ulcers, ulcerated surface, endoscopic extension, and presence of stenosis.

3.2. The clinical effects after exclusive enteral nutrition

Fig. 1 illustrates how the clinical variables at week 0 changed by week 8. As shown in the figure, all patients who underwent EEN treatment exhibited clinical improvements. The PCDAI score, ESR, CRP, and fecal calprotectin levels significantly decreased with a *p*-value of <0.0001, while albumin and hematocrit levels significantly increased, also with a p-value of <0.0001. This trend remained consistent when the two groups were evaluated separately, which confirmed that both groups experienced clinical benefits following EEN treatment. As both groups showed clinical improvements, we evaluated whether clinical and biochemical remissions were achieved (**Supplementary Fig. 1**). All patients in both groups achieved 100 % clinical remission. In the case of

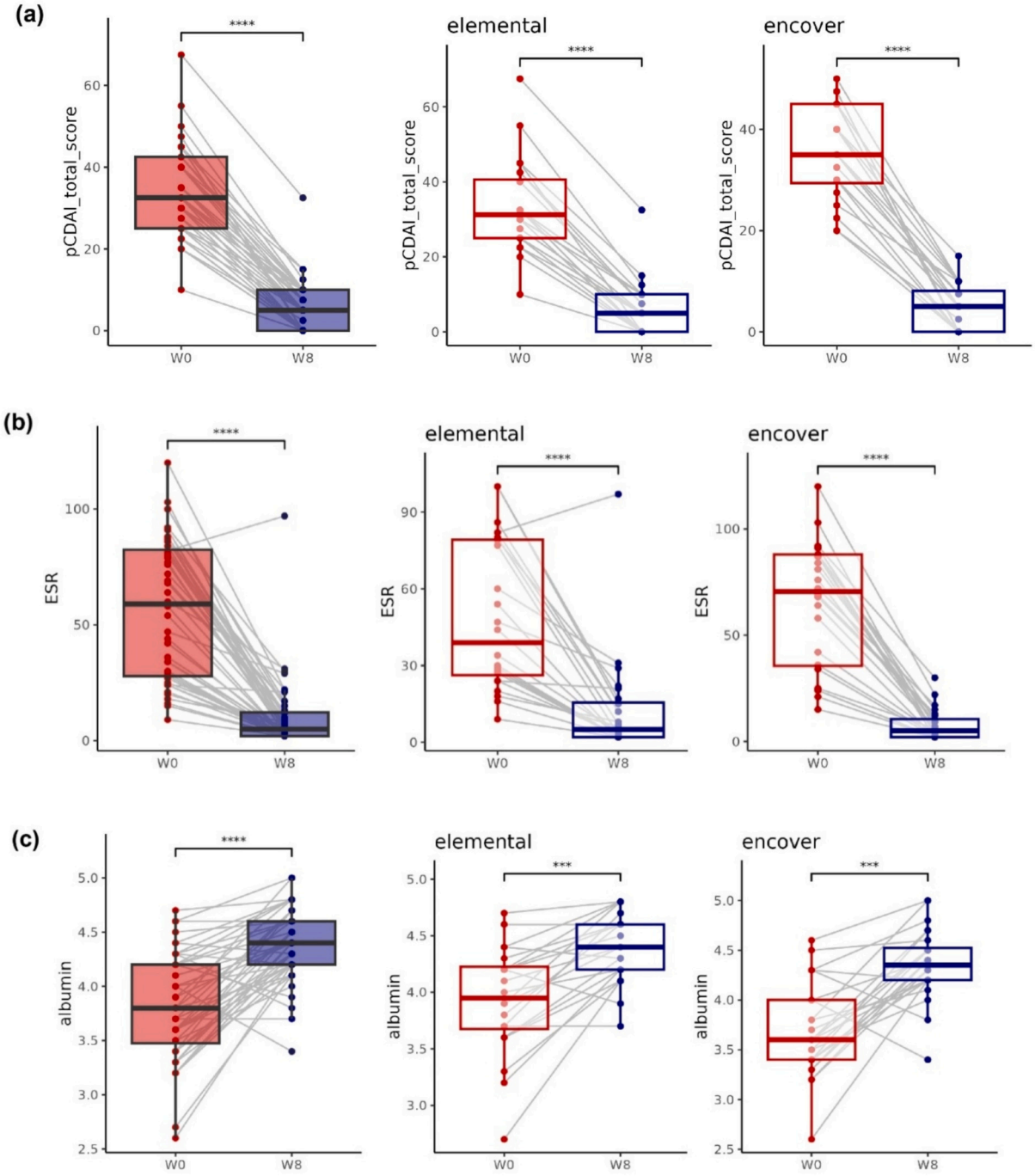


Fig. 1. Change of clinical variables before and after EEN treatment with or without stratification by EEN types. (a) PCDAI, (b) ESR, (c) albumin, (d) Hematocrit, (e) CRP, (f) fecal calprotectin (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, Wilcoxon signed-rank test).

biochemical remission, 53.3 % of patients in group A and 45.8 % of patients in group B achieved it, with no significant difference between the two groups (p-value = 0.564).

The comparison of clinical effects of 8 weeks of EEN treatment between the two groups, evaluated using a two group t-test, are shown in **Supplementary Table 3**. The 8-week data of PCDAI, laboratory results, and fecal calprotectin did not show any significant differences between the groups. However, when weight gain was evaluated over the 8-week period, patients in group B showed an average increase of 3.01 kg, which was significantly higher compared to the average increase of 1.53 kg in group A. Given the time variable of 8 weeks, we went beyond simply comparing the data at the 8-week time point between the two groups. A mixed ANOVA test was conducted using EEN type, time point, and the interaction term as explanatory variables (Table 2). Even the time point was accounted for, no differences were observed between the two groups for PCDAI, hematocrit, albumin, CRP, and calprotectin. However, ESR levels decreased significantly more in group B compared to group A over the 8-week period, demonstrating a statistically significant difference of the interaction term of the group and time point (p-value = 0.012).

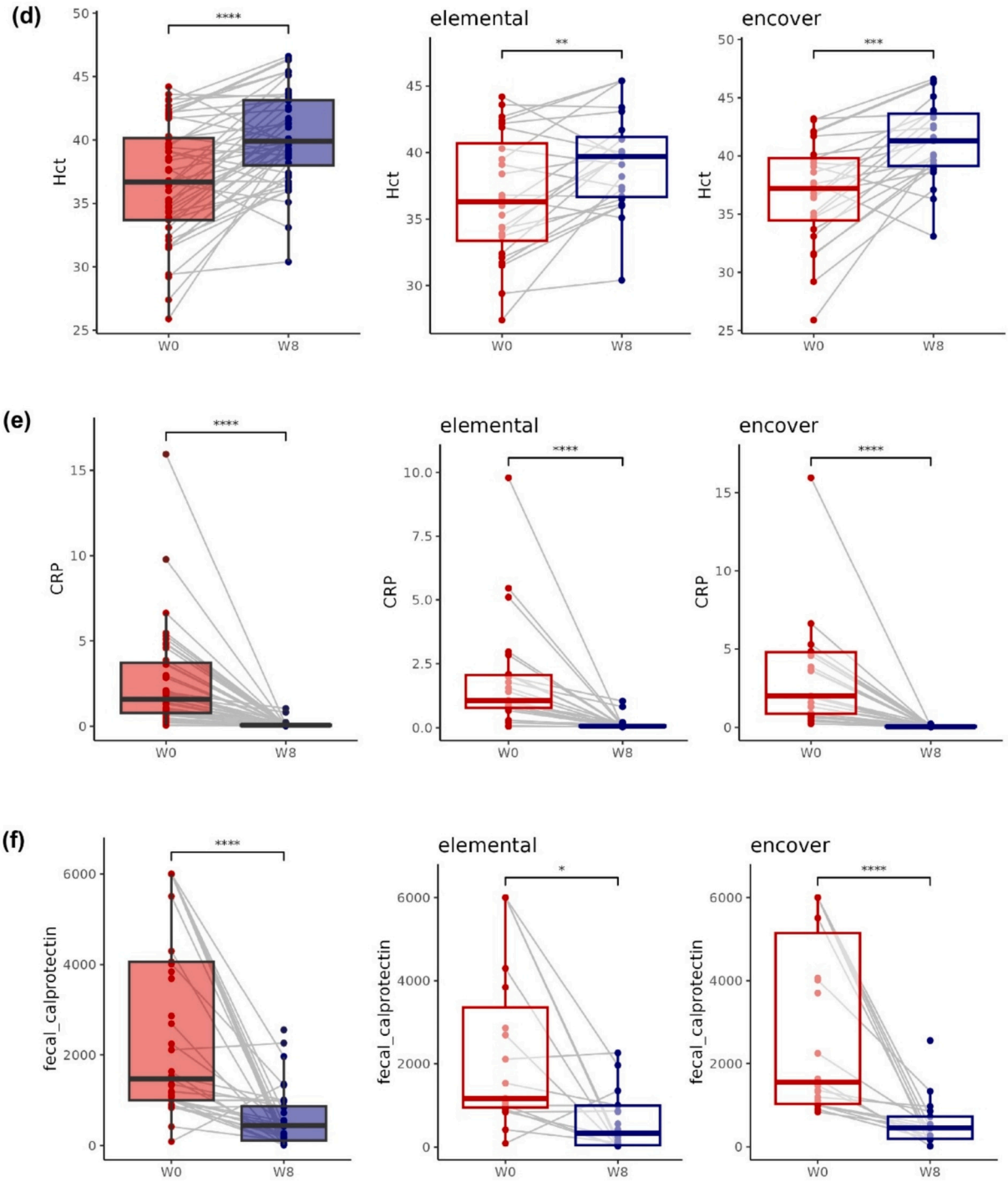


Fig. 1. (*continued*).

Table 2
Mixed ANOVA test results for each clinical variable with EEN type, time point, and interaction term as explanatory variables.

Characteristics	Group			Sampling_point			Group:sampling_point		
	ANOVA.ges	ANOVA.F	ANOVA.p	ANOVA.ges	ANOVA.F	ANOVA.p	ANOVA.ges	ANOVA.F	ANOVA.p
PCDAI	0	0.005	0.944	0.731	481.973	**<0.001**	0.012	2.189	0.146
Hematocrit, %	0.016	0.992	0.324	0.161	38.154	**<0.001**	0.012	2.371	0.13
Albumin, g/dL	0.037	2.752	0.104	0.331	64.578	**<0.001**	0.019	2.545	0.117
ESR, mm/h	0.013	0.934	0.339	0.507	139.764	**<0.001**	0.048	6.798	**0.012**
CRP, mg/dL	0.025	2.336	0.133	0.283	36.45	**<0.001**	0.03	2.843	0.099
Fecal calprotectin, mg/kg	0.003	0.19	0.666	0.301	24.581	**<0.001**	0.016	0.903	0.35

3.3. Changes in microbiome after exclusive enteral nutrition

When assessing the microbiome alpha diversity in patients after EEN treatment ($n = 36$ for before EEN, $n = 40$ for after EEN, and $n = 38$ for control samples), although the results were not statistically significant, the median values indicated a trend toward a decrease in diversity (**Supplementary Fig. 2 (a)**). However, when the microbiome diversity of 38 healthy individuals' stool samples was compared, the baseline diversity at week 0 in patients with CD was significantly lower than that of healthy controls. This trend was similarly observed when the two groups were analyzed separately and compared (**Supplementary Fig. 2 (b)**). Both groups showed significantly lower microbiome alpha diversity at weeks 0 and 8 compared to the control group.

The evaluation of microbiome beta diversity before and after EEN treatment is shown in Fig. 2. In beta-diversity analysis, a group-wide comparison across baseline, after EEN groups as well as the control group, showed significant difference (p-value = 0.001, PERMANOVA test). The PCoA coordinates of axis1 and axis2 showed significant difference between control and baseline or after EEN groups (p-value <0.001 and p-value <0.001 for axis1, respectively, and p-value = 0.012 and p-value <0.001 for axis2, respectively, Wilcoxon rank-sum test) but not significant between baseline and after EEN groups (p-value = 0.235 for axis1 and p-value = 0.059 for axis2).

Fig. 2 **(b)** illustrates the changes in each individual's beta-diversity by connecting the week-0 and week-8 data points with lines, demonstrating observable microbiome changes in individual patients following EEN treatment. However, when the microbiome shifts within individuals were quantified by Bray-Curtis dissimilarity and compared between EEN types, no statistically significant differences were observed (Fig. 2 **(c)**). The two-time point comparisons (at the time of diagnosis and at the 8-week time point) between groups divided by EEN type were not significant (Fig. 2 **(d)** and **(e)**).

We evaluated whether taxonomic changes occurred in the microbiome after EEN treatment. Fig. 3 shows the differentially abundant taxonomic signatures between before and after EEN treatment based on SIAMCAT (Statistical Inference of Associations between Microbial Communities And host phenoTypes) tool results. Fig. 3 **(a)** is a volcano plot; taxa with an adjusted p-value <0.25 were considered as significantly different, and those with an absolute fold change >0.5 were further represented as texts. Based on this SIAMCAT results, significant taxa with a prevalence ≥0.5 in the case samples regarding changes before and after EEN were shown in boxplots as examples (Fig. 3 **(b)**). At the Species level, the relative abundances of the *Faecalibacterium prausnitzii* group, *Streptococcus salivarius* group, and *Streptococcus*

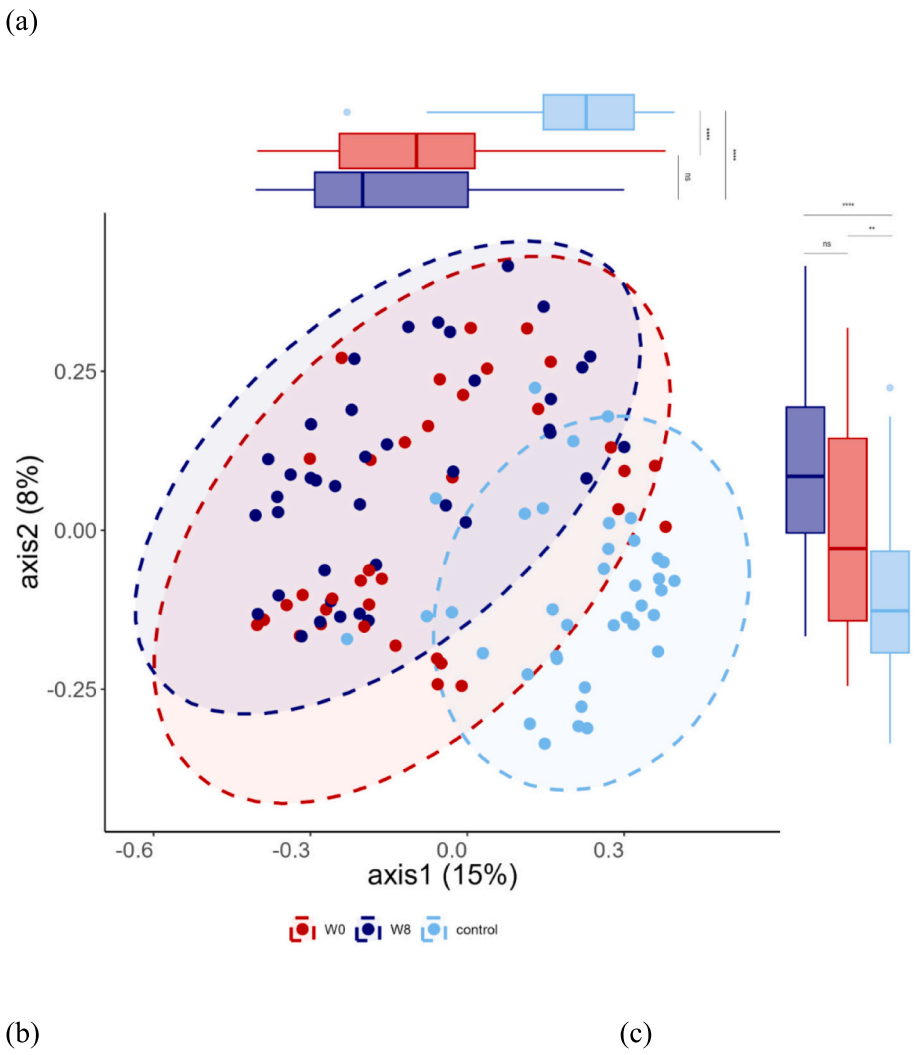
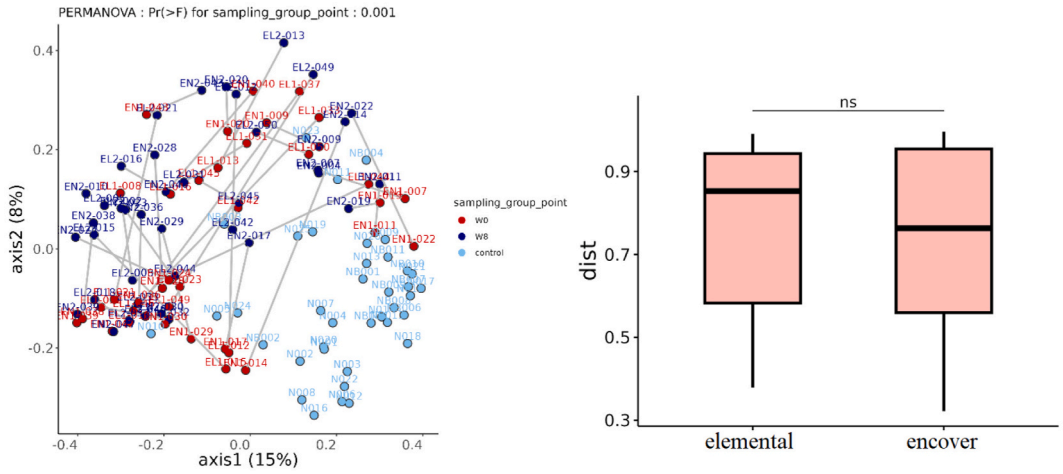


Fig. 2. Beta-diversity of species profiles before and after EEN treatment and the control group. (a) PCoA plot based on Bray-Curtis dissimilarity grouped by before and after EEN treatment and the control group, (b) PCoA plot with lines connected by the same individuals, and (c) comparison of intra-individual change of gut microbiome between EEN types. (d) PCoA plot based on Bray-Curtis dissimilarity grouped by EEN types at the time of diagnosis (e) PCoA plot based on Bray-Curtis dissimilarity grouped by EEN types at the 8-week time point (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, Wilcoxon rank-sum test).



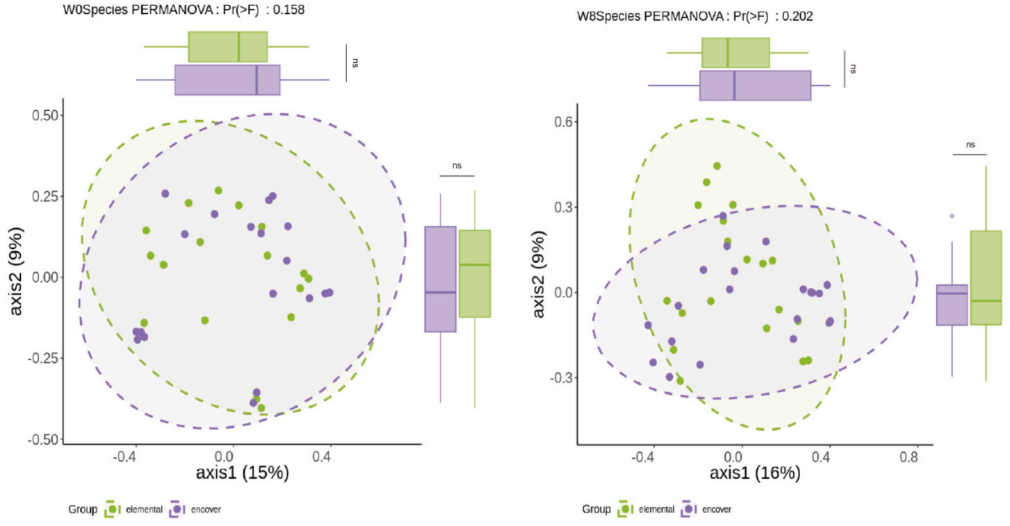


Fig. 2. (*continued*).

sanguinis group were found to decrease (p-values = 0.003, p-values = 0.002 and 0.003, respectively). At the Genus level, the relative abundance of *Pseudoflavonifractor* increased after EEN (p-values = 0.002), while *Enterococcus* and *Faecalibacterium* decreased (p-values = 0.026 and 0.003, respectively).

3.4. Difference in the microbiome between the two groups

Previously, we assessed the overall changes in the microbiome before and after EEN treatment by combining the two groups. In this analysis, we evaluated the differences of taxa between the two groups divided by EEN types before and after EEN treatment (Fig. 4) and analyzed the data using two methods: the SIAMCAT test (Fig. 4 **(a)** and **(b)**) and Linear Discriminant Analysis Effect Size (LEfSe) (Fig. 4 **(c)**).

In the SIAMCAT analysis, at week 0, we compared the relative abundance of the microbiome between the Elemental (group A) and Encover (group B) groups, identifying taxa with differences at the Phylum and Class levels. At the Phylum level, *Firmicutes* had a significantly higher relative abundance in the Elemental group compared to the Encover group. At the Class level, *Erysipelotrichia* and *Tissierellia* were more abundant in the Elemental group. Both classes were within the phylum *Firmicutes*. While *Betaproteobacteria* was more abundant in the Encover group. However, at the 8-week mark following the completion of EEN treatment, no differences in the relative abundance of specific microbiome taxa were observed between the two groups.

As differential abundance analysis tools can provide heterogeneous results depending on the algorithms (Nearing et al., 2022), and the SIAMCAT analysis did not show any significant differences after EEN treatment, we decided to reassess the same data using LEfSe analysis to evaluate potential variations at week 8 (Fig. 4 **(c)**). At the Order level, *Tissierellales*, which belongs to the *Tissierellia* class, and *Mycobacteriales* were identified as taxa with higher abundance in the Elemental group. At the family level, *Peptoniphilaceae,* which belongs to the *Tissierellia* class was significantly more abundant in the Elemental group compared to the Encover group. At the Genus level, more taxa were relatively abundant in the Encover group. Specifically, *Acutalibacter, Flintibacter* and one phylotype genus (PAC000671_g) belonging to *Lachnospiraceae* were more abundant in the Encover group, while *Mycosynbacter* was relatively more abundant in the Elemental group. At the Species level, unclassified *Anaerotignum* species was relatively more abundant in the Encover group. Otherwise, the *Peptoniphilus harei* group, belonging to the *Peptoniphilaceae* family mentioned earlier, was relatively abundant

(a)

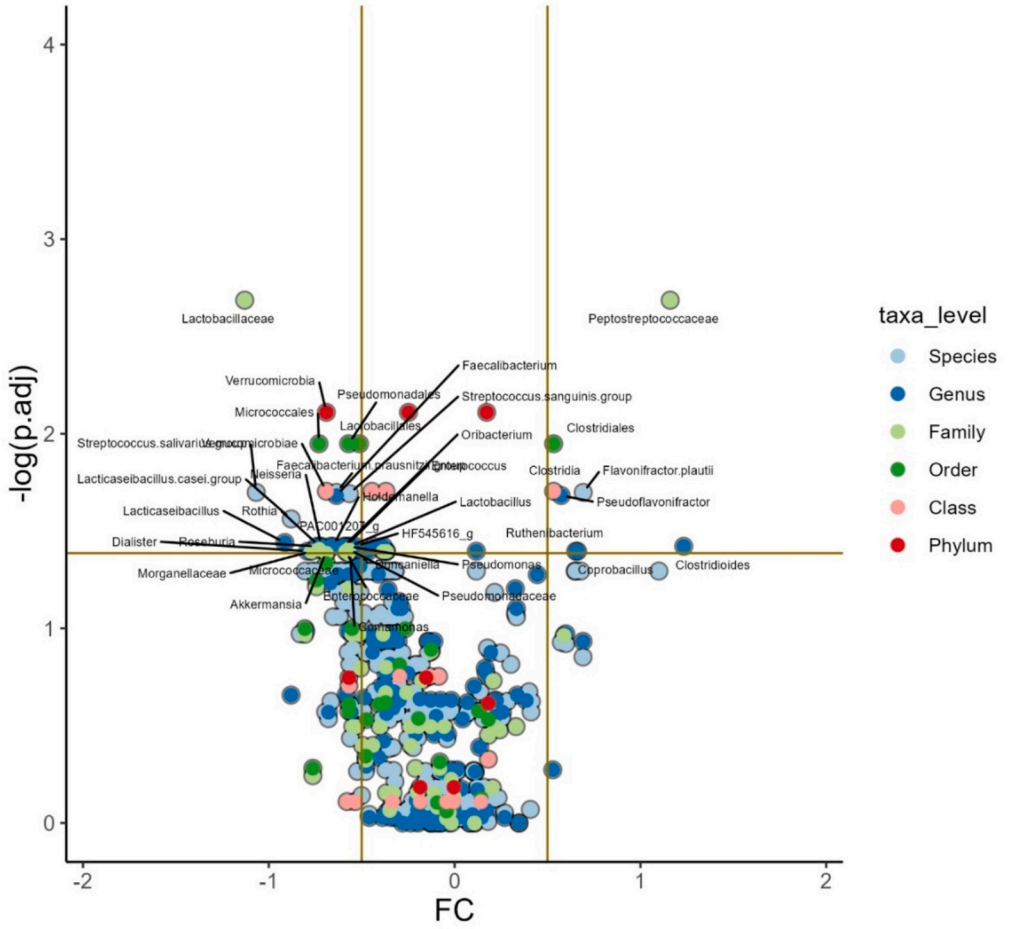


Fig. 3. Differentially abundant taxonomic signatures between before and after EEN treatment based on SIAMCAT (Statistical Inference of Associations between Microbial Communities And host phenoTypes) results. (a) volcano plot of x-axis as fold change (FC) and y-axis as −1 * log (adjusted *p*-value). Taxa with absolute FC > 0.5 and adjusted p-value <0.25 are further represented by texts. (b) boxplot of each signature's percent abundances which had absolute FC > 0.5 and adjusted p-value <0.25 based on the SIAMCAT results and prevalence ≥0.5 in the case samples.

in the Elemental group. These results may reflect that the baseline characteristics were weakly detectable at week 8 with the more relaxed statistical approach. However, given the algorithmic limitations of the current nonparametric method that does not directly consider prevalence and amount of relative abundance, we additionally examined descriptive statistics and observed descriptive differences between groups.

3.5. Association between the microbiome and clinical markers

We evaluated whether the state of the microbiome and clinical variables showed an association (**Supplementary Table 4** and Fig. 5). First, using clinical data at the time of diagnosis (PCDAI, hematocrit, albumin, ESR, CRP, and fecal calprotectin) and the microbiome, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) test, which revealed that among the clinical indicators, ESR and CRP were associated with beta diversity (*p*-value = 0.018 and 0.030, respectively) (**Supplementary Table 4**).

We evaluated Spearman's correlation analysis of species-level Shannon diversity and clinical data, and only ESR showed statistically significant correlation with *p*-value of 0.037 (Fig. 5 **(a)**). The results showed a negative correlation, indicating that patients with higher ESR tend to have reduced microbiome diversity. Furthermore, we evaluated which specific taxa at the species level are statistically significant in relation to clinical variables (Fig. 5 **(c)**). *Bacteroides acidifaciens* group showed a significant negative correlation with both ESR and CRP, indicating that patients with higher ESR and CRP levels had lower levels of *Bacteroides acidifaciens* group with p-value of 0.004 and 0.016, respectively. *Butyricicoccus faecihominis* group showed similar results, with a significant negative correlation to ESR and CRP. This indicates that patients with higher ESR and CRP levels had lower levels of *Butyricicoccus faecihominis* group with p-value of 0.012 and 0.027, respectively. Otherwise, The *Blautia luti* group showed a statistically significant negative correlation with ESR and a positive correlation with hematocrit (p-value = 0.04 and 0.022, respectively). In other words, patients with higher ESR had lower levels of the *Blautia luti* group, whereas patients with higher hematocrit levels had a greater abundance of the *Blautia luti* group.

4. Discussion

This is a prospective study evaluating the differences in clinical

(b)

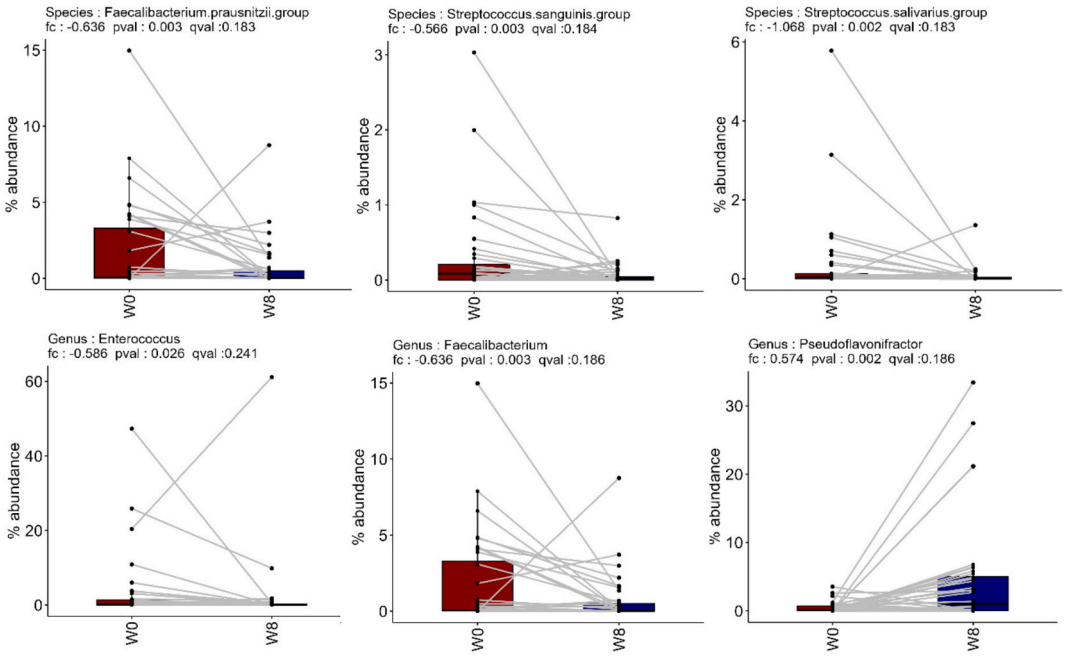


Fig. 3. (*continued*).

effectiveness and the changes in microbiome environment between the previously commonly used monomeric diet and a specific polymeric diet in EEN therapy for pediatric CD. Nutritional research is crucial for achieving one of the treatment goals in pediatric CD, which is promoting normal growth. Additionally, the microbiome environment is considered one of the key factors in the analysis of the causes of CD, and it has been approached from various angles. Therefore, evaluating the changes in the microbiome based on different types of nutritional treatments is considered a highly important area of research.

First, we found no difference in the effectiveness between monomeric and polymeric formulas in achieving clinical and biochemical remission in pediatric CD. All patients who underwent EEN treatment achieved clinical remission with a PCDAI score of 10 or below, and approximately 50 % of patients in both groups achieved biochemical remission. Most patients who did not achieve biochemical remission showed improvement in ESR and CRP levels, although they were excluded for biochemical remission as their fecal calprotectin levels remained above 200 μg/g (**Supplementary Fig. 1**). Through Fig. 1, the improvement in each clinical variable could be clearly observed. This finding aligns with previous studies that have examined the clinical effectiveness of monomeric and polymeric formulas. In our study, two aspects explain the differences between the two formulations in terms of clinical outcomes. First, when weight gain for weight recovery after EEN treatment was evaluated, the group consuming the polymeric formula experienced greater weight gain compared to the group consuming the monomeric formula (**Supplementary Table 3**). This can be attributed to the severity of CD at the time of diagnosis, before starting EEN treatment. As seen in **Table 1**, although no statistically significant differences were observed in the clinical indicators of disease severity between the two groups, a closer look at the mean values of each indicator shows that the polymeric formula group had lower mean values for BMI and albumin and higher mean values for ESR, CRP, and fecal calprotectin. This suggests that the polymeric formula group had a more severe degree of inflammation and greater weight loss due to nutritional deficiencies. Owing to the greater pre-existing nutritional deficits, the improvement in inflammation following EEN treatment might have led to better recovery of nutritional status, resulting in more substantial weight gain. The fact that patients in the polymeric group were in a more severe state of malnutrition can also be confirmed by **Table 1**, which shows statistically significantly more patients in category G1. While the 8-week period is too short to evaluate changes in height, further assessment is necessary to determine whether catch-up growth occurs in terms of height improvement in the future. The level of inflammation at the time of CD diagnosis varies among individuals, and how quickly EEN treatment reduces inflammation is an important factor. Considering the time variable of 8 weeks, a statistically significant difference existed between the two groups in terms of the rate of ESR reduction, with the polymeric formula group showing a more rapid decrease in ESR (Table 2). As previously mentioned, the polymeric group had higher average ESR levels at the time of diagnosis, but both groups' inflammation levels converged toward resolution with EEN treatment. This indicates that, despite the initial higher inflammation, the 8-week period is sufficient for EEN treatment to effectively reduce the inflammatory response in both groups. Although no consensus has been reached on the optimal duration for EEN treatment (Day & Burgess, 2013), this study demonstrated that even an 8-week period is sufficient to achieve substantial improvements in inflammation and weight gain. From a nutritional perspective extending beyond weight gain, although the nitrogen sources differ between the two products, the absolute protein content is higher in the polymeric formula (Supplementary Table 1), which may have contributed to the faster albumin recovery observed in group B patients. Although the monomeric formula contained lower levels of folate and pyridoxine compared to the polymeric formula—both of which are involved in erythropoiesis—there was no significant difference in hemoglobin levels after 8 weeks of exclusive enteral nutrition, suggesting that the difference in vitamin and mineral content may not be clinically meaningful. Finally, in evaluating the lipid composition, researchers have emphasized the importance of the omega-3 to omega-6 ratio, as omega-3 fatty acids act as precursors to anti-inflammatory prostaglandins and leukotriene B5, exert protective effects, and inhibit arachidonic acid metabolism, thereby reducing the production of pro-inflammatory eicosanoids (Gorard, 2003; Lohoues et al., 1992; Verma

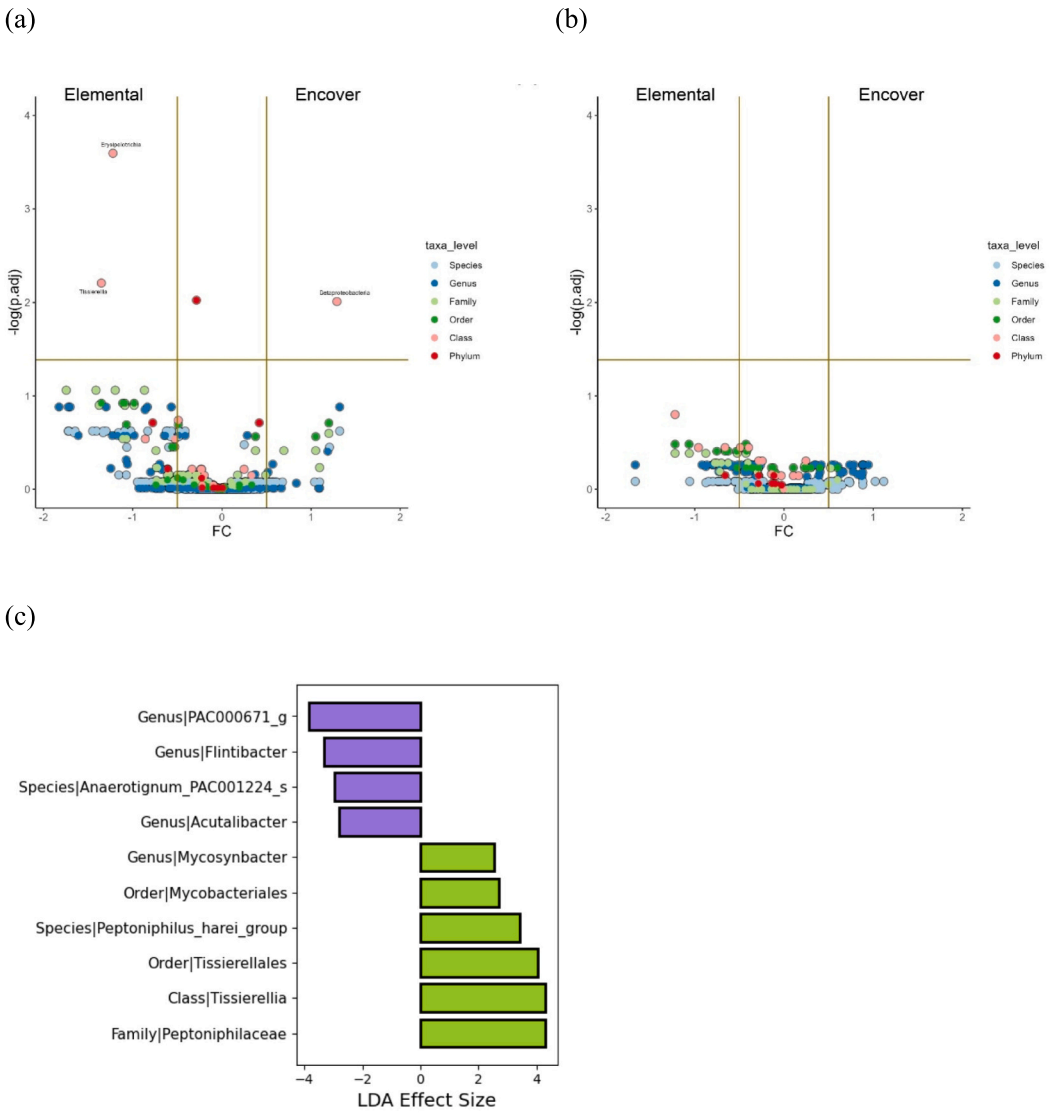


Fig. 4. Comparison of gut microbiome profiles by EEN types at each time point. (a) Volcano plot of SIAMCAT results at the time of diagnosis with absolute fold change (FC) > 0.5 and adjusted p-value <0.25, (b) Volcano plot of SIAMCAT results after EEN treatment with absolute fold change (FC) > 0.5 and adjusted p-value <0.25. (c) Linear Discriminant Analysis Effect Size (LEfSe) analysis results comparing the two groups after EEN treatment (with an LDA cut-off of 2.5) Green: Elemental group (group A), Purple: Encover group (group B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2000). Although the two formulas differ in fat content and fat sources, the identical omega-6 to omega-3 ratio may have contributed to the similar therapeutic effects observed in Crohn's disease.

Our second finding is that, following EEN treatment, microbiome diversity decreased compared to the control group, though the change of the alpha diversity was not significant but showed a decreasing tendency, and individual-level shifts were observed. However, the microbiome did not shift toward the typical composition seen in healthy individuals. The fact that microbiome diversity still showed a decreasing trend after EEN treatment has been confirmed in numerous previous studies. Many studies have suggested that EEN leads to a reduction in microbial diversity, possibly owing to the restricted nature of the diet—especially fiber—and changes in nutrient availability within the gut. Although the carbohydrate, fat, protein ratios, and nitrogen sources differ, both monomeric and polymeric formulas limit the amount of fiber available to the microbiome. Additionally, as both products promote nutrient absorption by the host, the nutrients available to the microbiome after host absorption are limited. Therefore, even when evaluating the diversity before and after EEN treatment in both groups, we observe a similar decrease in diversity. As dysbiosis with lower diversity is already present at the time of diagnosis compared to healthy controls, this suggests that examining individual changes and taxa variations following EEN treatment is more important than merely focusing on changes in overall diversity. In addition, this study confirmed individual changes in beta diversity, and the distance of change between monomeric and polymeric formulas showed no significant difference. Moreover, we also compared changes in each taxon before and after the EEN treatment. As shown in Fig. 3 **(b)**, we evaluated the taxa that significantly changed after EEN treatment. A key point to note when interpreting Fig. 3 **(b)** is that it evaluates the relative abundance of bacteria, not their absolute amounts. Considering that the absolute abundance of bacteria may decrease after EEN, bacteria that appear to have increased might not have actually increased but may instead seem relatively higher owing to the reduction of other bacteria. Meanwhile, the interpretation of the observations that significantly different bacteria that have relatively decreased were more than those that have increased may suggest more consistent change across individuals in decreased bacteria. At the species and gene level, we observed a decrease in *Faecalibacterium*

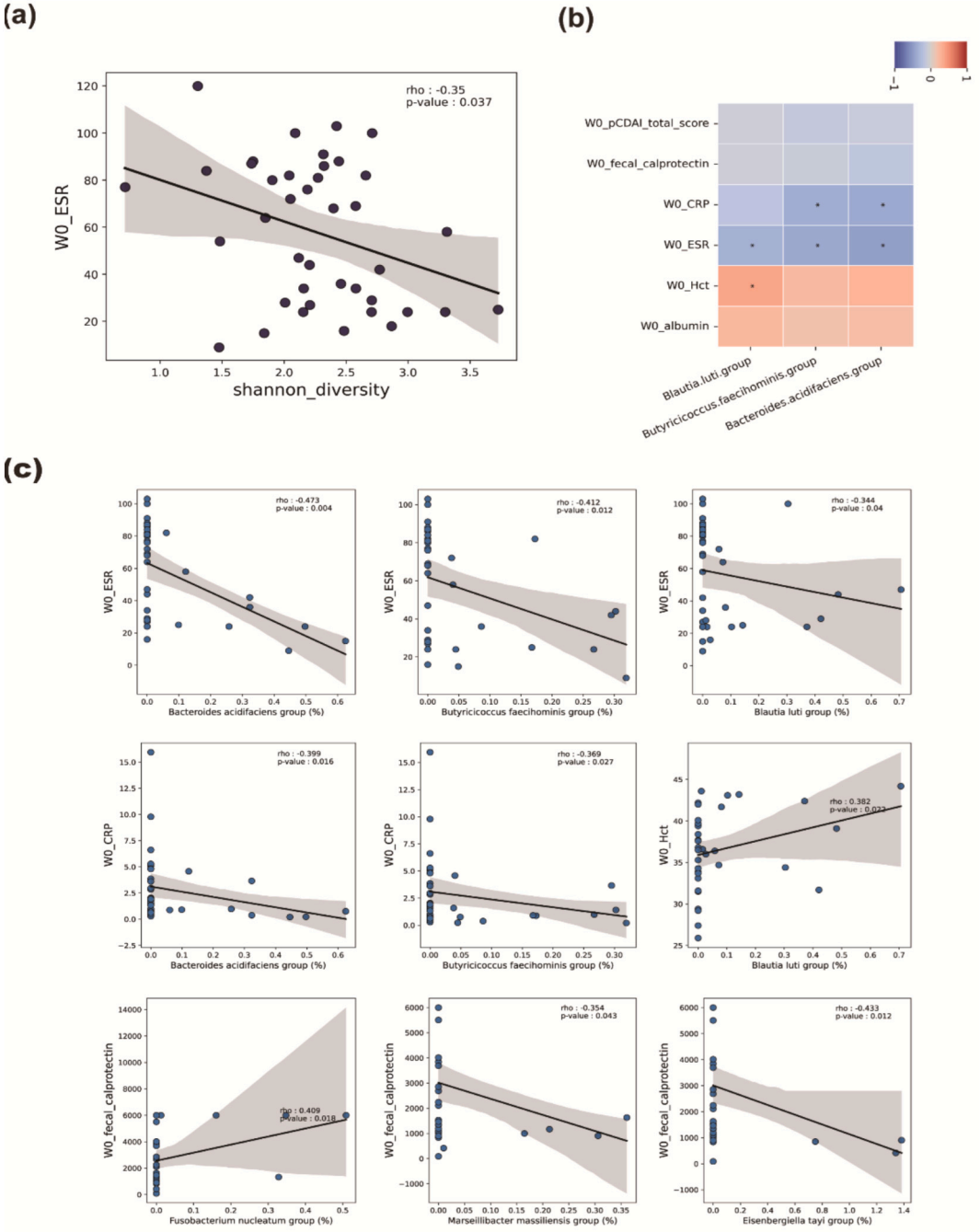


Fig. 5. Association between clinical variables and gut microbial profiles at baseline time point. (a) scatter plot of species-level Shannon diversity and ESR with Spearman's correlation results (b) heatmap of Spearman's correlation results for taxa with two or more times significant and prevalence ≥0.2 in the case samples. Significant results (p-value <0.05) are further represented by asterisk (*). (c) scatter plot of each taxon (species) and clinical variable for the significant results in (b) as well as species that are associated with fecal calprotectin.

prausnitzii, *Streptococcus* group, and *Enterococcus*.

Faecalibacterium prausnitzii is regarded as an important bacterium for gut health, with reduced levels contributing to the dysbiosis and inflammation seen in CD (Lopez-Siles et al., 2017; Miquel et al., 2013; Sokol et al., 2008). *F. prausnitzii* is a key producer of SCFAs—particularly butyrate, which plays a vital role in maintaining the health of the intestinal barrier, as mentioned in the introduction part. Lower levels of *F. prausnitzii* have been associated with increased inflammation and a higher risk of postoperative recurrence of CD. **Supplementary Fig. 3** shows that *Faecalibacterium* has significantly reduced in both patient groups compared to healthy individuals. As a result of this study, the decrease in *Faecalibacterium* following EEN treatment is worth noting; other studies have also observed this phenomenon (Gerasimidis et al., 2014; MacLellan et al., 2017; Quince et al., 2015). The fiber depletion diet resulting from EEN treatment leads to a decrease in the abundance of the fiber-degrading bacteria, *Faecalibacterium*. On the other hand, *Enterococcus* and *Streptococcus* are known as harmful pathogenic bacterial genera and have increased in studies on patients with IBD (Fyderek et al., 2009; Kang et al., 2010; Ran et al., 2013; Takaishi et al., 2008). **Supplementary Fig. 3** shows that *Enterococcus* and *Streptococcus* have increased in both patient groups compared to healthy individuals. In line with our findings, a few recent studies have demonstrated the reduction of these bacteria following EEN treatment (Kerbiriou et al., 2024; Li et al., 2022). In our study, we observed that both beneficial and

pathogenic bacteria decrease after EEN treatment, which is related to the temporary "reset" or "wash out" of the microbiome, reducing both the beneficial and harmful bacteria, allowing the gut environment to stabilize and potentially reduce inflammation. *Pseudoflavonifractor*, which has relatively increased in Fig. 3 **(b)**, is not a well-known bacterium, and no direct studies have conclusively defined its role in CD. However, *Pseudoflavonifractor* is associated with gut health through its involvement in butyrate production (Carlier et al., 2010). Further research is required to understand the full impact of *Pseudoflavonifractor* in CD, but its role in butyrate production suggests that its presence or absence could influence the course of gut inflammation and IBD.

Our third finding is that, although a difference was observed in the microbiome between the two groups before EEN treatment, no significant differences were found after treatment. In other words, monomeric and polymeric formulas showed no differences, not only in clinical outcomes but also in the microbiome environment. The classes *Erysipelotrichia*, *Tissierellales*, and *Betaproteobacteria*, which showed differences between the two groups before EEN treatment in Fig. 4 **(c)**, contain many pathogenic bacteria. Specifically, *Erysipelotrichia* is associated with inflammatory conditions. One study suggests that *Erysipelotrichia* is associated with fat intake and may also have a connection to ankylosing spondylitis; additionally, it has been evaluated for its correlation with serum levels of pro-inflammatory cytokines such as IFN-α (Zhang et al., 2019). Certain species within the *Tissierellales* and *Betaproteobacteria* order can cause opportunistic infections, particularly under conditions such as immunosuppression (Jardine et al., 2017; Lu et al., 2022; Schmidt & Neumann, 2023). Although both groups showed differences in the bacteria, the presence of harmful bacteria before EEN treatment was relatively high. However, the fact that this difference disappeared after EEN treatment in both groups is an encouraging sign of the effectiveness of EEN therapy. After EEN treatment was completed, when the differences between the two groups were evaluated, no significant differences due to the overall reduction in bacterial abundance as a result of EEN treatment were observed. Nonetheless, we conducted the LEfSe analysis as a means of more relaxed statistical approach and included the results as no standardized protocol currently exists in microbiome analysis, and the identified bacteria could be utilized in future studies' databases.

Our fourth finding is that microbiome diversity and certain bacteria are associated with clinical markers. As dysbiosis in CD reduces microbial diversity, confirming the negative correlation between inflammation-related markers (ESR, CRP) and diversity, as well as evaluating specific bacteria associated with these markers, is important. This evaluation provides valuable insight into the factors contributing to the onset, relapse, and exacerbation of CD. When the relationship between beta diversity and clinical markers was evaluated, both ESR and CRP showed a negative correlation. This suggests that patients with higher disease activity in CD have fewer opportunities to maintain or increase microbial diversity. When assessing the relationship between Shannon diversity and clinical markers, only ESR exhibited a statistically significant negative correlation. This finding likely reflects the own inflammation of CD at the time of diagnosis for all patients. From a clinical perspective, only ESR showing a significant association may be more appropriate as ESR (a marker of chronic inflammation) better reflects the disease trend in CD than CRP, which is a marker of acute inflammation. Therefore, ESR may more accurately represent the disease's chronic nature in CD, hence alpha diversity was clearly associated with it than other clinical variables. In our results, *Bacteroides acidifaciens* and *Butyricicoccus faecihominis* groups showed a significant negative correlation to ESR and CRP, and the *Blautia luti* group showed a statistically significant negative correlation with ESR and a positive correlation with hematocrit.

Not many studies on *Bacteroides acidifaciens* are available, but recent research using mice has shown that *Bacteroides acidifaciens* can reduce the severity of colitis symptoms by modulating the immune response and gut microbial environment (Staley et al., 2023; Zheng et al., 2023). According to these studies, *Bacteroides acidifaciens* also produces SCFAs, such as butyrate. Although evidence regarding *Bacteroides acidifaciens* in clinical studies related to IBD is not conclusive, studies have suggested its potential as a therapeutic candidate for treating IBD. Our study is the first to identify a possible association between *Bacteroides acidifaciens* and clinical indicators in patients with CD. *Butyricicoccus faecihominis*, discovered in 2016, is a newly identified bacterium isolated from human fecal samples and is known for its ability to produce butyrate (Takada et al., 2016). Owing to its production of butyrate, it is recognized for its role in reducing inflammation and maintaining gut integrity. Clinical studies related to *Butyricicoccus faecihominis* are limited, with one from Japan suggesting its positive effects in Alzheimer's disease (Nguyen et al., 2018). In our study, we found that patients with higher inflammatory markers, such as elevated ESR and CRP levels, had lower levels of this bacterium, which aligns with the current hypothesis about the bacterium's anti-inflammatory properties. This is the first study to clinically investigate the status of this bacterium in IBD, and it supports the potential of *Butyricicoccus faecihominis* as a therapeutic target for treating CD. Although *Blautia luti* is not a widely studied bacterium, a review paper evaluates its potential probiotic properties (Liu et al., 2021). *Blautia luti* is known for its ability to produce butyrate, which plays a crucial role in anti-inflammatory effects and maintaining gut barrier function. One clinical study related to *Blautia luti* showed that it helps reduce mucosal injury in patients undergoing chemotherapy (da Silva Ferreira et al., 2022). While no direct research has linked *Blautia luti* to IBD, studies have found a decrease in the abundance of the *Blautia* group in CD (Davrandi et al., 2022; Takahashi et al., 2016). Our study is the first to confirm a direct association between *Blautia luti* and clinical markers such as ESR and hematocrit, supporting the bacterium's anti-inflammatory properties. For a microorganism to be associated with an increase in hematocrit, it would need to support iron absorption, contribute to the production of B vitamins, and reduce inflammation. While no direct research has shown that *Blautia* species increase iron absorption, studies have linked SCFA to the biosynthesis of B vitamins (Peterson et al., 2022). Additionally, the anti-inflammatory properties of *Blautia* could support overall metabolic health, indirectly influencing iron utilization and blood health. This suggests that *Blautia luti* could serve as an important probiotic in the treatment of IBD. *Fusobacterium nucleatum*, the only species found to have a positive correlation with fecal calprotectin, has been reported in the literature as an opportunistic pathogen and has been implicated in colorectal cancer (Brennan & Garrett, 2019). Moreover, it has been demonstrated to influence the expression of proinflammatory cytokines and activate monocytes within human colorectal tumors (Ye et al., 2017). The *Merdibacter massiliensis* and *Eisenbergiella tayi* groups have been reported only as newly identified bacterial genera isolated from the human ileum, with no known association with any specific disease. In our study, its negative correlation with the inflammatory marker calprotectin suggests a potential positive or protective role.

The limitation of this study is the small number of patients. Future research should focus on involving a larger number of pediatric patients. Additionally, for microbiome analysis, it would be preferable to use the method which evaluates absolute quantities, rather than the method which assesses relative abundance. However, despite the small sample size, this study has a significant impact as a prospective study on pediatric patients. Moreover, it is the first analysis comparing the microbiome between monomeric formula and polymeric formula as a method of EEN.

5. Conclusion

This prospective study demonstrated that EEN with a polymeric formula shows no significant differences in clinical effectiveness or gut microbiome changes compared to a monomeric formula, and thus can be considered an equally effective therapeutic option for pediatric patients with CD. This study additionally demonstrated that an 8-week course of

exclusive enteral nutrition is sufficient to achieve significant improvements in inflammation and weight gain. Finally, the microbiome was correlated with clinical markers indicating inflammation.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon request.

CRediT authorship contribution statement

Yiyoung Kwon: Writing – original draft, Visualization, Validation, Software, Formal analysis, Data curation, Conceptualization. **Yeonjae Jung:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis. **Hyun-Seok Oh:** Software, Methodology, Formal analysis. **Eun Sil Kim:** Resources, Methodology. **Yoon Zi kim:** Data curation. **Yon Ho Choe:** Writing – review & editing, Supervision, Conceptualization. **Mi Jin Kim:** Writing – review & editing, Supervision.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The medical records of the patient were reviewed retrospectively with the approval of the Clinical Research Ethics Committee, and the requirement for a consent form was waived (IRB File No.: SMC 2020–05-075).

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2025.106997.

Data availability

Data will be made available on request.

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