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Exclusive Enteral Nutrition versus Corticosteroids: Immunometabolic Profiling in Children with Newly Diagnosed Crohn's Disease

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Article

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Abstract

Background

Exclusive Enteral Nutrition (EEN) is the first-line therapy for inducing remission in pediatric Crohn's disease (CD), with fewer adverse effects than corticosteroids (CCS). However, its immunometabolic mechanisms and the impact of gluten reintroduction remain poorly understood. We investigated the effects of EEN on immune metabolism, cytokine profiles, regulatory T cells, and the influence of gluten reintroduction.

Methods

Peripheral immune phenotyping, serum cytokines, and immune cell metabolism were assessed at diagnosis (T0), after 8 weeks of EEN or CCS (T1), and after 8 weeks of food reintroduction (T2). Following EEN, patients were assigned to either a gluten-containing or gluten-free diet.

Results

Thirty-nine newly diagnosed pediatric CD patients were enrolled (31 EEN, 8 CCS). At baseline, CCS patients showed higher inflammatory markers, increased naïve T cells, reduced central memory T cells, and lower oxidative phosphorylation. At T1, EEN was associated with fewer $CD3^+CD16^+CD56^+$ cells and increased Foxp3 expression. At T2, EEN patients had higher numbers of $CD19^+$ B cells and naïve $CD4^+$ T cells, whereas CCS patients exhibited increased NK cells, effector memory and TEMRA $CD4^+$ T-cell subsets, and higher IL-17A levels. Leptin and $Foxp3^+$ regulatory T cells increased over time in both groups. Following food reintroduction, patients consuming gluten showed higher lymphocyte, $CD3^+$, $CD4^+$, $CD19^+$, $CD4^+CD28^+$, and $CD4^+CD45RA^+$ cell counts, whereas the gluten-free group displayed higher $CD4^+HLA\text{-}DR^+$ and $CD8^+HLA\text{-}DR^+$ cell percentages.

Conclusions

EEN promotes a more regulatory and less activated immune profile than CCS in pediatric CD. Gluten reintroduction influences immune activation, supporting personalized dietary strategies after EEN.

Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic gastrointestinal disorders caused by interactions between genetic susceptibility, immune dysregulation, and environmental factors [1]. Among environmental influences, diet plays a pivotal role. Western dietary patterns, characterized by high intake of fats and animal proteins and low consumption

of fruits and vegetables, have been associated with increased IBD risk [2, 3]. Both adult and pediatric studies suggest that specific nutrients may influence disease onset and progression [4].

In pediatric CD, Exclusive Enteral Nutrition (EEN) is recommended as first-line therapy for remission induction because of its efficacy and favorable safety profile compared with corticosteroids (CCS). EEN induces clinical remission, promotes mucosal healing, improves nutritional status, reduces inflammatory markers, and supports growth [7]. Although CCS are effective for induction, their adverse effects limit their long-term use [6, 7]. Gluten, a wheat-derived protein, has been investigated as a potential contributor to intestinal inflammation. Gliadin and amylase-trypsin inhibitors may promote pro-inflammatory immune responses, alter the intestinal microbiota, and impair epithelial integrity [8].

Specifically, gliadin peptides may increase intestinal permeability through activation of the zonulin pathway and trigger immune responses in genetically predisposed individuals [9]. IBD and celiac disease also share several immunological features, including overlapping cytokine profiles, suggesting partially common pathogenic mechanisms [8].

This study aimed to evaluate the effects of EEN on immunometabolic pathways, cytokine expression, and regulatory T cells (Tregs) in pediatric CD. We also investigated immunophenotypic changes during food reintroduction, comparing diets with and without gluten, to explore whether gluten may act as a dietary trigger of intestinal inflammation.

Materials and methods

Study population

This prospective observational study was conducted at the Department of Translational and Medical Science, Section of Paediatrics, University of Naples “Federico II”. Children younger than 18 years with newly diagnosed CD between May 2017 and February 2020 were enrolled. Diagnosis was based on clinical, radiological, endoscopic, and histological findings according to the revised Porto criteria [10]. Patients with IBD unclassified (IBD-U) or other comorbidities were excluded. According to induction therapy, patients were divided into an EEN group or a CCS group.

Data collection and therapeutic approach

Demographic and clinical data were collected at diagnosis (T0), after 8 weeks of induction therapy (T1), and after 8 weeks of food reintroduction (T2). Disease activity was assessed using the Pediatric Crohn’s Disease Activity Index (PCDAI) [11], and disease phenotype was classified according to the Paris classification [12]. Laboratory assessments included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fecal calprotectin (FC). Weight, height, and body mass index were also recorded.

EEN consisted of a polymeric formula administered for 6–8 weeks, followed by gradual food reintroduction. Oral methylprednisolone (1 mg/kg/day, maximum 40 mg/day) was administered for 4 weeks, followed by tapering through week 11, in patients who refused EEN or when EEN was considered insufficient by the treating physician. After induction, patients in clinical remission received maintenance therapy with mesalazine or immunomodulators according to clinical indication.

During food reintroduction, EEN-treated patients were divided into a gluten-containing diet group (Group A) or a strict gluten-free diet group (Group B).

Immunophenotypic and flow cytometry analysis

Peripheral blood immunophenotyping was performed by multiparametric flow cytometry to characterize lymphocyte subsets, including T cells, B cells, natural killer cells, activated T cells, and memory/naïve populations.

Regulatory T cells were further characterized using CD4, CD45RA, CCR7, and FOXP3 expression, including the FOXP3 Exon 2 (FOXP3-E2) isoform. Cells were analyzed using Cytomics FC500 and FACSCanto II flow cytometers with dedicated analysis software.

Bioenergetic and cytokine analyses

Oxygen consumption rate (OCR), as an indicator of oxidative phosphorylation, was measured in stimulated peripheral blood mononuclear cells (PBMCs) using an XFe96 Analyzer. Basal and maximal respiration were calculated from the OCR profile following sequential exposure to oligomycin, FCCP, and antimycin/rotenone.

Plasma cytokines were quantified using a Human Custom ProcartaPlex 13-plex assay, including GM-CSF, IFN-α, IFN-γ, IL-10, IL-17A, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, MIP-1β, and TNF-α. Plasma leptin was measured using a commercial ELISA kit.

Ethical considerations

The study was conducted in accordance with the Helsinki Declaration and Italian national legislation. The Ethics Committee of the University of Naples “Federico II” approved the protocol (registration number 225/16). Written informed consent was obtained from parents, with assent from children when appropriate.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR) and compared using the Mann–Whitney U test. Categorical variables were analyzed using chi-square or Fisher’s exact test.

Longitudinal changes were assessed using linear mixed-effects models with random intercepts for subjects and fixed effects for time, treatment group, and their interaction. Estimated marginal means were used for post-hoc comparisons. Analyses were performed using R, with statistical significance set at $\alpha = 0.05$.

Results

Baseline characteristics

Thirty-nine children with CD were enrolled: 31 received EEN and 8 received CCS as induction therapy. Median age was 14 years (IQR 11–15), and 71.8% were male. No significant baseline differences were observed in age, sex, anthropometric parameters, or disease activity. Median PCDAI was 12.5 in the EEN group (5–15.6) and 13.8 in the CCS group (6.9–16.9), p = 0.639. Clinical characteristics of the study population at baseline and at all time points are summarized in Table 1.

CRP was significantly higher in the CCS group than in the EEN group (5.0 [3.3–7.7] vs. 2.1 [1.4–4.3] mg/dL; p = 0.024). Peripheral blood immunophenotyping revealed a higher percentage of CD19 + B cells in EEN group (16.8 [12.2–22.1] vs. 9.7 [7.9–13.7]; p = 0.026).

We next evaluated the percentage of regulatory T cells expressing the FOXP3 Exon2 splicing variants and we observed a higher percentage of naïve ($CD45RA^{+}CCR7^{+}$: 45.9 [44.7–47.0] vs 32.2 [24.5–41.0], p = 0.046) and a lower percentage of central memory ($CD45RA^{-}CCR7^{+}$: 26.8 [25.5–33.1] vs 19.5 [13.1–23.1], p = 0.008) FOXP3-$E2^{+}$ Treg cells in the CCS group compared with the EEN group At baseline, stimulated PBMCs from EEN-treated patients showed greater mitochondrial respiration, with higher basal (33.7 [20.5–43.0] vs. 12.2 [9.8–23.5]; p = 0.028) and maximal respiration (82.0 [33.2–127.7] vs. 27.5 [15.9–45.5]; p = 0.048).

The CCS group also showed higher plasma IFN-γ (4.5 [4.0–7.8] vs. 4.0 [3.7–4.0] pg/mL; p = 0.05) and IL-5 (15.8 [10.0–30.0] vs. 3.3 [0–9.3] pg/mL; p = 0.022), with a non-significant trend toward higher IL-6 levels. Finally, regarding cytokine correlations, no significant associations were detected between IFN-γ levels and ESR, CRP, fecal calprotectin, or PCDAI values. Cytokine expression and immunometabolic status of the 39 patients enrolled at baseline were summarized in Supplementary Table 1.

T1 outcomes

At T1, 34 patients were evaluable: 26 in the EEN group and 8 in the CCS group. After 8 weeks of treatment, both groups showed significant reductions in PCDAI and inflammatory parameters, with no significant differences between treatments. No significant anthropometric differences were observed (Table 1).

FC tended to be higher in the EEN group than in the CCS group (251 [161–500] vs. 98.5 [58.5–215.8] mg/kg; p = 0.07).

Immunophenotyping revealed a lower absolute number of CD3 + CD16+CD56 + cells in the EEN group (61.0 [44.8–90.4] vs. 104.6 [85.2–149.4] cells/mm³; p = 0.042). Moreover, Foxp3 mean fluorescence intensity in Tregs was higher following EEN (1592 [1363–1800.5] vs. 1197 [1165.8–1413.5]; p = 0.05), suggesting enhanced regulatory activity. No significant differences were observed in metabolic parameters or cytokine levels. All data observed after 8 weeks of induction therapy with EEN or CCS were summarized in Supplementary Table 2.

T2 outcomes

At T2, 32 patients were evaluable. PCDAI and most inflammatory parameters did not differ between groups. FC remained significantly higher in the EEN group than in the CCS group (148 mg/kg [62.5–368.7] vs. 59 mg/kg; p = 0.041) (Table 1).

The EEN group showed significantly higher absolute and percentage of CD19 + B cells (306.0 [235.8–415.8] vs. 146.5 [116–186.8] cells/mm³, p = 0.001; and 14.6% [11.0–17.3] vs. 7.2% [5.3–8.9], p = 0.001). Conversely, the CCS group had a higher percentage of CD16 + CD56+ natural killer cells (17.9% [10.7–22.5] vs. 9.4% [7.0–14.1]; p = 0.015).

The EEN group also showed a higher proportion of naïve CD4 + T cells (61.0% [53.0–68.1] vs. 52.9% [41.5–54.0]; p = 0.024), whereas effector memory CD4 + T cells were more frequent in the CCS group (21.3% [19.4–26.9] vs. 16.0% [10.3–18.4]; p = 0.031). Within the FOXP3-E2 + compartment, TEMRA cells were significantly more frequent in CCS-treated patients (6.5% [4.5–10.6] vs. 1.3% [0–2.8]; p = 0.002). IL-17A levels were also significantly higher in the CCS group (3.6 [1.8–3.6] vs. 1.2 [1.2–1.2] pg/mL; p = 0.03). No other differences in cytokines expression in this time point. All data observed after 8 weeks of dietary reintroduction were summarized in Supplementary Table 3.

Mixed-effects models were employed to assess treatment-related changes in biomarkers over time. For leptin, the time-by-treatment interaction was not significant (p = 0.471); therefore, the final model included only time and treatment effects. Mean leptin concentrations increased significantly from baseline at both T1 (estimate = 0.67 ± 0.23 ng/ml; p = 0.005) and T2 (estimate = 0.56 ± 0.23 ng/ml; p = 0.020), with no significant difference between CCS and EEN groups (p = 0.523) (Fig. 1A).

For IFN-γ, a significant time-by-treatment interaction was observed (p = 0.025). At baseline, IFN-γ concentrations were higher in the CCS than in the EEN group (5.80 ± 0.55 vs. 3.95 ± 0.29 pg/ml; p = 0.005), whereas no differences were observed at T1 or T2 (p = 0.65 and p = 0.29, respectively). IFN-γ increased slightly from T0 to T1 in the EEN group (+ 0.46 ± 0.27 pg/ml; p = 0.099) and remained stable thereafter, while it decreased significantly in the CCS group from T0 to T1 and T2 (−1.10 ± 0.51 pg/ml; p = 0.035) (Fig. 1B).

Similarly, IL-4 showed a significant time-by-treatment interaction (p = 0.011). Baseline levels were higher in the CCS group than in the EEN group (2.71 ± 0.33 vs. 1.90 ± 0.18 pg/ml; p = 0.035), with no differences at T1 or T2. IL-4 increased from T0 to T1 in the EEN group (+ 0.35 ± 0.15 pg/ml; p = 0.028), whereas it decreased in the CCS group (−0.66 ± 0.29 pg/ml; p = 0.027) (Fig. 1C).

For CD4 + Foxp3+ cells, the interaction was not significant (p = 0.811). Their percentage increased over time independently of treatment (p = 0.045), with no between-group difference (p = 0.869), increasing significantly from T0 to T1 (p = 0.021) and remaining elevated at T2 (p = 0.044) (Fig. 1D). Finally, MIP-1β showed no significant effects of time or treatment (p = 0.199 for both), remaining relatively stable throughout follow-up (Fig. 1E).

Gluten versus gluten-free diet

After EEN induction, 24 patients were evaluable during food reintroduction: 12 consumed gluten-containing foods (Group A) and 12 followed a strict gluten-free diet (Group B).

No significant differences were observed in age, sex, anthropometric parameters, PCDAI, or inflammatory markers. However, patients consuming gluten showed higher total lymphocyte counts (2841.5 [2130.8–3089.8] vs. 2003.5 [1760.8–2271.0] cells/mm³; p = 0.018), as well as higher absolute numbers of CD3 + T cells (1994.0 [1493–2399] vs. 1497.0 [1141.5–1648.5]; p = 0.038), CD4 + T cells (1099.5 [828–1440] vs. 775 [607.5–887.2]; p = 0.028), CD19 + B cells (418.5 [283.8–563.2] vs. 267.5 [202.8–309.2]; p = 0.015), CD4 + CD28+ cells (1059.6 [810.9–1282.2] vs. 742.2 [564.8–873.2]; p = 0.021), and CD4 + CD45RA+ naïve T cells (641.4 [544.9–804.5] vs. 411.4 [281.2–537.4]; p = 0.007).

Conversely, the gluten-free group showed higher percentages of CD4 + HLA-DR + and CD8 + HLA-DR+ activated T cells. A trend toward a higher percentage of CD4 + CD25+ cells was also observed in the gluten-free group (p = 0.055). All data observed were summarized in Supplementary Table 4.

Discussion

This study provides an immunological characterization of children with newly diagnosed CD treated with EEN or CCS and evaluates immune changes during subsequent food reintroduction. Although both treatments achieved comparable clinical improvement, they were associated with different immunological profiles.

At diagnosis, the CCS group had higher CRP, IFN-γ, and IL-5 levels and a trend toward higher IL-6, suggesting greater systemic inflammatory activity. Differences in Treg subsets and mitochondrial respiration were also observed. These baseline differences likely reflect heterogeneity in disease and immune activation rather than treatment effects, as treatment had not yet been administered [13].

After 8 weeks, both EEN and CCS induced significant clinical and biochemical improvement, but the two treatments appeared to modulate the immune system through distinct mechanisms.

However, EEN was associated with increased Foxp3 expression in Tregs and a reduction in CD3 + CD16+CD56 + cells. The increased Foxp3 expression may indicate improved functional competence of Tregs rather than simply an increase in their number. Foxp3 is essential for Treg development and suppressive activity and contributes to the maintenance of immune tolerance [14]. Previous studies in pediatric IBD have shown that circulating Tregs may increase during active disease as a compensatory

response to inflammation [15]. In this context, our observation of increased FoxP3 expression following EEN may suggest not only preservation of the regulatory T-cell compartment but also enhancement of its functional competence, supporting the hypothesis that EEN promotes restoration of immune homeostasis beyond simple expansion of Treg numbers.

These findings are consistent with experimental evidence showing that EEN can promote Foxp3 + Treg expansion and reduce intestinal inflammation [16]. Similar enhancement of Treg number and function has also been reported following anti-TNFα treatment, suggesting that restoration of regulatory pathways may be a common feature of effective IBD therapies [17].

An important observation concerns the period following EEN. Food reintroduction is considered a vulnerable phase because inflammatory markers may increase after return to a habitual diet [18]. In our cohort, clinical remission was maintained and FC did not show a rebound from T1 to T2, although values remained higher than in the CCS group. Thus, the anti-inflammatory effects of EEN appeared to persist during the short-term food reintroduction period. Food reintroduction applies specifically to the EEN group, since patients in the CCS group continued their habitual diet throughout induction; by T2, however, both groups were on an unrestricted diet, thus allowing the immunological differences observed at this time point to be interpreted primarily as a consequence of the induction treatment rather than of the current diet. Interestingly, these sustained clinical findings were accompanied by persistent differences in immune cell composition, suggesting that EEN and CCS induce distinct patterns of immune reconstitution. At T2, EEN and CCS were associated with distinct immune profiles despite comparable clinical disease activity. EEN-treated patients had higher CD19 + B-cell numbers and a greater proportion of naïve CD4 + T cells, whereas CCS-treated patients showed more natural killer cells, effector memory T cells, and higher IL-17A levels. These findings suggest that EEN may favor a more homeostatic adaptive immune phenotype, characterized by greater representation of naïve lymphocytes and reduced effector differentiation.

The higher IL-17A levels observed in the CCS group are particularly relevant because IL-17A is a key cytokine of the IL-23/Th17 axis involved in CD pathogenesis. EEN may therefore restrain Th17-associated inflammation. In support of this hypothesis, Geesala et al. [19] showed in a rodent model that EEN attenuated Th17 responses and reduced IL-6, a cytokine involved in Th17 polarization. Although these findings are preclinical, they provide a possible mechanistic explanation for our observations. Moreover, the predominance of naïve T cells together with the expansion of the $CD19^{+}$ B-cell compartment suggests that EEN may favour restoration of a more homeostatic adaptive immune profile. Although the functional phenotype of these B cells was not investigated, preservation of a larger naïve T-cell pool and reduced frequencies of effector memory cells may reflect lower chronic antigenic stimulation and a more physiological reconstitution of adaptive immunity following nutritional therapy

Whereas immune cell remodelling differed substantially between treatment groups, leptin concentrations increased similarly in both the EEN and CCS groups following induction therapy and remained stable after food reintroduction, independently of the induction strategy. Leptin links energy

metabolism and immune regulation and is frequently altered in active CD [20–24]. The similar increase following EEN and CCS suggests that metabolic recovery is largely independent of the induction strategy, whereas the persistent differences in immune phenotype appear more closely related to treatment.

We also investigated whether gluten reintroduction influenced systemic immune parameters. Gliadin and other wheat components can stimulate inflammatory pathways and increase intestinal permeability [8, 25–28]. In our cohort, gluten consumption was associated with higher absolute numbers of total lymphocytes, CD3+, CD4+, CD19+, CD4 + CD28+, and naïve CD4 + CD45RA+ cells. Conversely, the gluten-free group showed higher percentages of HLA-DR+ activated CD4 + and CD8 + T cells. The biological significance of this latter finding remains unclear.

Importantly, these immunological differences occurred in the absence of significant differences in clinical disease activity or inflammatory biomarkers, suggesting that gluten reintroduction may influence systemic immune cell composition without translating into overt clinical inflammation during the relatively short follow-up period.

Previous studies support a possible role for gluten-containing foods in IBD. Non-celiac gluten sensitivity has been associated with IBD [29], while Gkikas et al. [30] reported higher FC levels in children with CD consuming greater amounts of gluten-containing cereals during food reintroduction after EEN. Our findings complement these observations by suggesting that gluten exposure may also influence systemic immune-cell composition. More broadly, dietary interventions such as EEN, gluten-free diets, and other exclusion diets may affect intestinal inflammation through changes in the gut microbiota and its metabolites [31], although these mechanisms were not directly evaluated in our study.

Several limitations should be considered. The sample size was small, particularly in the CCS group, and the follow-up period may have been insufficient to detect long-term clinical or immunological effects. Some baseline immunological differences were present between groups, although mixed-effects models were used to account for longitudinal variability. The study was exploratory and not designed to test predefined hypotheses, while the limited sample size prevented robust multivariable adjustment for potential confounders. In addition, the study did not directly assess changes in gut microbiota or intestinal tissue.

Despite these limitations, the study provides novel evidence that EEN and CCS may achieve similar clinical remission while promoting different patterns of immune recovery in pediatric CD. EEN was associated with enhanced regulatory pathways, greater Foxp3 expression, increased naïve T-cell representation, and lower IL-17A levels during follow-up, suggesting a more homeostatic immune profile. In contrast, CCS was associated with a more activated and differentiated immune phenotype.

Furthermore, gluten reintroduction after EEN was associated with changes in systemic immune-cell composition without evidence of short-term clinical relapse. These findings support the concept that dietary factors may continue to influence immune regulation beyond the induction phase. Larger prospective studies integrating immune, microbiome, metabolic, and clinical parameters are needed to

determine whether these changes predict long-term disease activity and whether personalized dietary strategies could improve outcomes in pediatric CD.

Abbreviations

IBD: Inflammatory Bowel Disease; **CD**: Crohn's Disease; **EEN**: Exclusive Enteral Nutrition; **CCS**: Corticosteroid Therapy; **Tregs**: Regulatory T Cells; **PCDAI**: Pediatric Crohn's Disease Activity Index; **CRP**: C-reactive Protein; **IFN-γ**: Interferon gamma; **IL**: Interleukin (e.g., IL-5, IL-6, IL-4, IL-17A); **MIP-1β**: Macrophage Inflammatory Protein-1beta and **NK**: Natural Killer.

Declarations

Data Availability Statement: Our data are entered into a database and are available by agreement with authors.

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References

1. Fisher, S.A., et al., Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. Nat Genet, 2008. 40(6): p. 710–2.
2. Hou, J.K., B. Abraham, and H. El-Serag, Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. Am J Gastroenterol, 2011. 106(4): p. 563–73.
3. Thia, K.T., et al., An update on the epidemiology of inflammatory bowel disease in Asia. Am J Gastroenterol, 2008. 103(12): p. 3167–82.
4. Ananthakrishnan, A.N., et al., Long-term intake of dietary fat and risk of ulcerative colitis and Crohn's disease. Gut, 2014. 63(5): p. 776–84.

5. van Rheenen, P.F., et al., The Medical Management of Paediatric Crohn's Disease: an ECCO-ESPGHAN Guideline Update. J Crohns Colitis, 2021. 15(2).
6. Ruemmele, F.M., et al., Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis, 2014. 8(10): p. 1179–207.
7. Penagini, F., et al., Nutrition in Pediatric Inflammatory Bowel Disease: From Etiology to Treatment. A Systematic Review. Nutrients, 2016. 8(6).
8. Cenni, S., et al., The Role of Gluten in Gastrointestinal Disorders: A Review. Nutrients, 2023. 15(7).
9. Yan, J., et al., Dietary Patterns and Gut Microbiota Changes in Inflammatory Bowel Disease: Current Insights and Future Challenges. Nutrients, 2022. 14(19).
10. Levine, A., et al., ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. J Pediatr Gastroenterol Nutr, 2014. 58(6): p. 795–806.
11. Turner, D., et al., Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. Inflamm Bowel Dis, 2012. 18(1): p. 55–62.
12. Levine, A., et al., Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis, 2011. 17(6): p. 1314–21.
13. Scarpato, E., et al., Exclusive enteral nutrition effect on the clinical course of pediatric Crohn's disease: a single center experience. Eur J Pediatr, 2020. 179(12): p. 1925–1934.
14. Hariyanto, A.D., T.B.M. Permata, and S.A. Gondhowiardjo, Role of CD4(+)CD25(+)FOXP3(+) T(Reg) cells on tumor immunity. Immunol Med, 2022. 45(2): p. 94–107
15. Vitale G., et al. Increased frequency of regulatory T cells in pediatric inflammatory bowel disease at diagnosis: a compensative role? Pediatr Res. 2020. PMID: 31715619.
16. Lunken, G.R., et al., Prebiotic Enriched Exclusive Enteral Nutrition Suppresses Colitis via Gut Microbiome Modulation and Expansion of Anti-inflammatory T Cells in a Mouse Model of Colitis. Cell Mol Gastroenterol Hepatol, 2021. 12(4): p. 1251–1266.
17. Boschetti, G., et al., Therapy with anti-TNFα antibody enhances number and function of Foxp3(+) regulatory T cells in inflammatory bowel diseases. Inflamm Bowel Dis, 2011. 17(1): p. 160–70.
18. Logan, M., et al., The reduction of faecal calprotectin during exclusive enteral nutrition is lost rapidly after food re-introduction. Aliment Pharmacol Ther, 2019. 50(6): p. 664–674
19. Geesala, R., et al., Exclusive enteral nutrition alleviates Th17-mediated inflammation via eliminating mechanical stress-induced Th17-polarizing cytokines in Crohn's-like colitis. Inflamm Bowel Dis, 2024. 30(3): p. 429–440
20. Otero, M., et al., Towards a pro-inflammatory and immunomodulatory emerging role of leptin. Rheumatology (Oxford), 2006. 45(8): p. 944–50.
21. Waluga, M., et al., Serum adipokines in inflammatory bowel disease. World J Gastroenterol, 2014. 20(22): p. 6912–7.
22. Franchimont, D., et al., Impact of infliximab on serum leptin levels in patients with Crohn's disease. J Clin Endocrinol Metab, 2005. 90(6): p. 3510–6.

23. La Cava A. & Matarese G., The weight of leptin in immunity Nature Reviews Immunology, 2004
24. Albella V et al. Leptin in the interplay of inflammation, metabolism and immune system disorders Nat Rev Rheumatol. 2017;13(2):100–109..
25. Roncoroni, L., et al., Nutrition in Patients with Inflammatory Bowel Diseases: A Narrative Review. Nutrients, 2022. 14(4).
26. Cinova, J., et al., Gliadin peptides activate blood monocytes from patients with celiac disease. J Clin Immunol, 2007. 27(2): p. 201–9.
27. Drago, S., et al., Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol, 2006. 41(4): p. 408–19.
28. Lammers, K.M., et al., Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology, 2008. 135(1): p. 194–204.e3.
29. Aziz, I., et al., A study evaluating the bidirectional relationship between inflammatory bowel disease and self-reported non-celiac gluten sensitivity. Inflamm Bowel Dis, 2015. 21(4): p. 847–53.
30. Gkikas, K., et al., Dietary triggers of gut inflammation following exclusive enteral nutrition in children with Crohn's disease: a pilot study. BMC Gastroenterology, 2021. 21(1):454)
31. Deas, J., et al., Dietary therapies for adult and pediatric inflammatory bowel disease. Nutr Clin Pract, 2024. 39(3): p. 530–545.

Tables

Table 1 not available with this version.

Figures

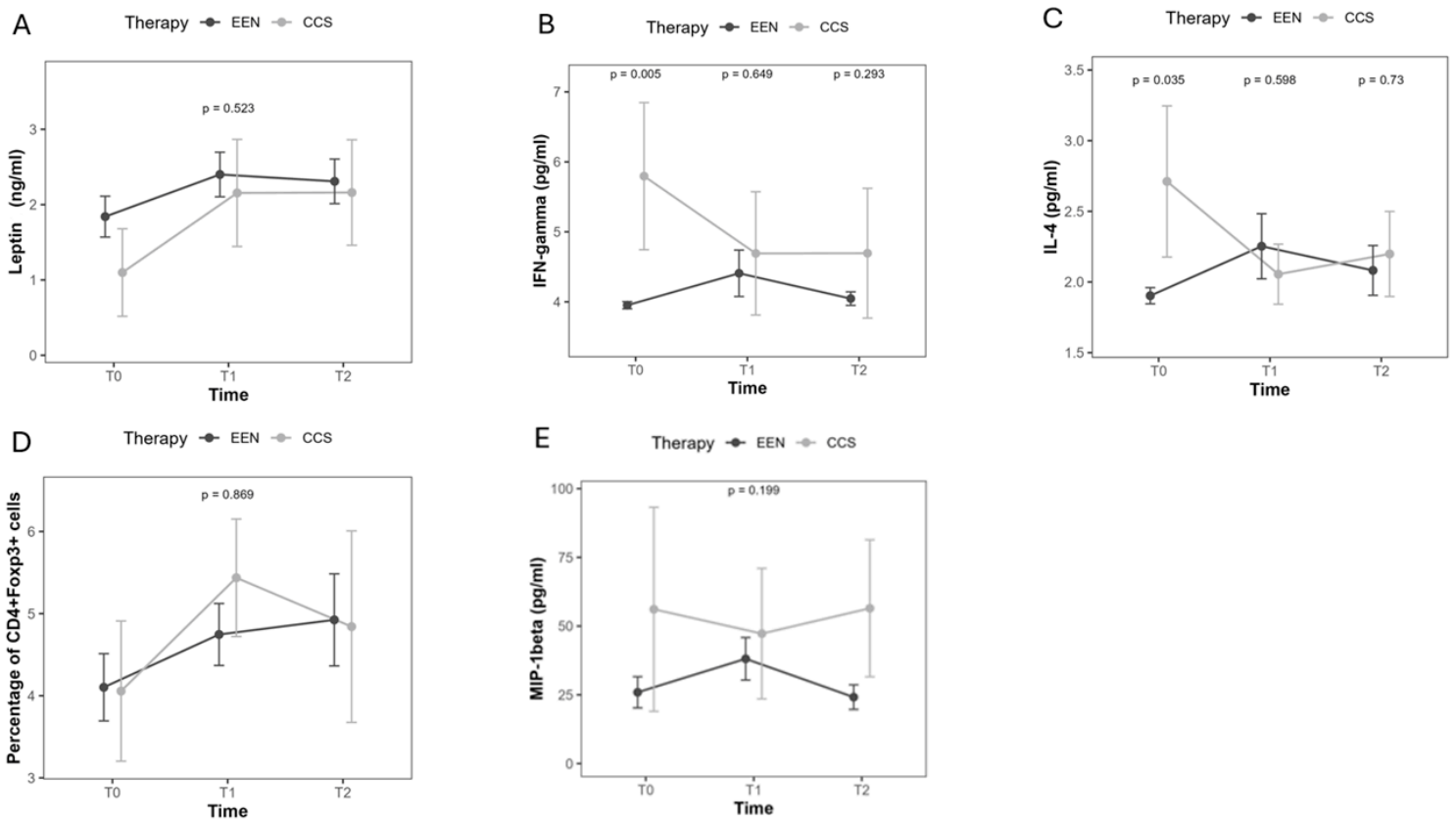

Figure 1

Temporal changes of Leptin (ng/ml) (1A), IFN-gamma (pg/ml) (1B), IL-4 (pg/ml) (1C), Percentage of $CD4^{+}Foxp3^{+}$ cells (1D), and MIP-1beta (pg/ml) (1E) in patients treated with EEN or CCS at baseline (T0), after 8 weeks of induction therapy (T1), and after 8 weeks of dietary reintroduction (T2). Lines represent model-adjusted means ± SE, with time × therapy interactions and within-group changes derived from the mixed-effects models.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable4.xlsx
- SupplementaryTable2.xlsx
- SupplementaryTable3.xlsx
- SupplementaryTable1.xlsx