TITLE: Effect of exclusive palatable elemental diet on systemic inflammation and gut microbiome in microbial overgrowth syndromes: ad hoc analysis of a prospective trial

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Abstract Body: Elemental diets (EDs) are allergen-free, nutritionally complete, and easily digestible formulations composed of free amino acids, simple carbohydrates, readily absorbable fats, vitamins, and minerals. EDs exhibit clinical benefits across several inflammatory disorders, including Crohn's disease and eosinophilic esophagitis. Although palatable ED has shown efficacy in treating small intestinal bacterial overgrowth (SIBO) and intestinal methanogen overgrowth (IMO), it remains unclear whether these effects are driven by anti-inflammatory properties and/or alterations in the gut microbiome. In this study, we investigated the impact of a palatable ED on gut microbiota and circulating inflammatory markers in subjects with SIBO or IMO.

Methods: Subjects with IMO and/or SIBO underwent two weeks of exclusive palatable ED (mBiota Elemental, USA), followed by 2 weeks of reintroduction of regular diet (NCT05978973). Samples were taken at baseline, immediately upon completion of ED (during ED), and post-ED. Fasting plasma was tested for biomarkers using a multiplex immunoassay (FLEXMAP 3D). Stool quantitative metagenomic sequencing (NovaSeqX, Illumina) was performed, and Kraken2 was used for de-hosting and microbial classification. Microbial diversities (Shannon index and Bray-Curtis) and differential absolute abundance (AA) were performed on CLC Microbial Module. Cytokines/chemokine analysis were performed on GraphPad Prism v.10.

Results: A total of 30 subjects (63.3% female, mean age 45.9±14.5 years) were analyzed. The stool microbial profile shifted during ED and partially recovered post-ED (Bray-Curtis; PERMANOVA, P=0.00001, Figure 1A). Shannon diversity followed a similar pattern during ED (Figure 1B), and there were changes in the AA of 208 bacterial species as compared to baseline (FDR P<0.05). The reintroduction of a regular diet restored most commensal species altered during ED, and only 37 species remained significantly different post-ED (FDR P<0.05). In contrast, the AA of microbial species associated with microbiome alterations remained decreased post-ED relative to baseline, including *Methanobrevibacter smithii* ($\log_2$ Fold Change (FC)=-9.03, FDR P=4.59E-3) and *Candida albicans* (FC=-3.3, FDR P=0.04). Of note, no major bacterial pathogens were detected post-ED. Overall, systemic cytokines and chemokines were within normal limits at the 3 timepoints and showed no changes, except for modest differences in IL-13 and CCL20 (Table 1).

Conclusion: While EDs exhibit anti-inflammatory benefits in inflammatory disorders, such effects were not evident in the context of microbial overgrowth, a noninflammatory condition. Instead, the significant shifts observed in the gut microbiome suggest that microbiome modulation is the more predominant mechanism underlying efficacy of ED in SIBO and IMO. These findings warrant confirmation in a trial with healthy controls.

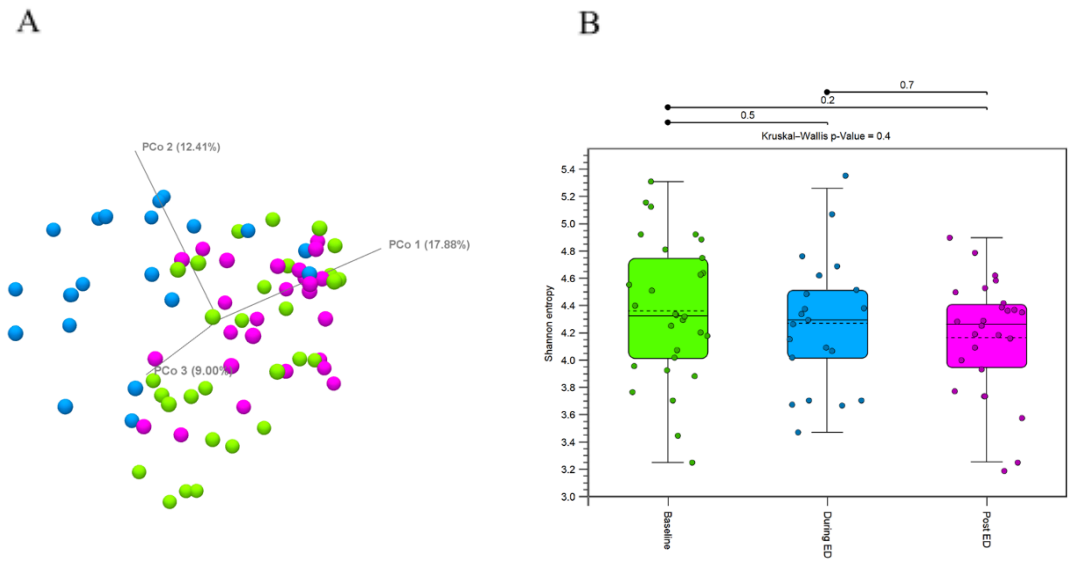


Figure 1. (**A**) Principal Coordinates Analysis (PCoA; PCo1, PCo2, PCo3) demonstrated distinct stool beta-diversity profiles across all groups (PERMANOVA P=0.00001). Green circles: baseline, blue circles: during ED, pink circles: post-ED (**B**) Stool alpha microbial diversity (Shannon index) across all time points.

Table 1. Plasma concentrations of cytokines and the chemokine CCL20 at baseline, immediately after 2 weeks of exclusive palatable elemental diet (during ED) and 2 weeks after reintroduction of regular diet (post-ED). All levels fell within normal limits showed no changes at the 3 times points except for modest differences in IL-13 and CCL20. Data are presented as mean ± SD.

	Baseline		During ED		Post-ED		
Analytes	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**p-value**
IL13 (pg/mL)	48.76	60.04	50.83	62.49	48.26	61.43	0.039
CCL20 (pg/mL)	35.78	13.27	36.41	16.42	37.06	20.52	0.049
IL10 (pg/mL)	7.25	6.27	7.4	6.18	7.17	6.23	ns
IL17F (ng/mL)	0.05	0.04	0.05	0.04	0.05	0.04	ns
GM_CSF (ng/mL)	0.3	0.22	0.3	0.2	0.28	0.24	ns
IL12p70 (pg/mL)	10.94	9.03	10.76	8.1	10.94	10.94	ns
IL15 (pg/mL)	16.7	8.84	17.22	9.27	16.29	9.15	ns
INFy (pg/mL)	14.56	16.97	16.25	21.06	13.4	19.26	ns
IL9 (pg/mL)	16.17	9.25	16.82	10.45	16.11	11.75	ns
IL22 (ng/mL)	0.76	0.9	0.78	0.9	0.8	1.03	ns
TNFa (pg/mL)	15.16	6.16	17.41	10.24	15.11	5.32	ns
IL4 (ng/mL)	0.82	1.37	0.88	1.35	0.81	1.4	ns
IL5 (pg/mL)	0.82	1.37	0.88	1.35	0.81	1.4	ns
IL6 (pg/mL)	31.88	48.34	34.35	48.59	32.35	48.22	ns
IL31 (ng/mL)	0.08	0.08	0.08	0.08	0.09	0.11	ns
IL17E_IL25 (ng/mL)	0.13	0.06	0.13	0.06	0.14	0.09	ns