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Short Communication

Dose-dependent effects of enteral nutrition on the faecal microbiota and short chain fatty acids

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SUMMARY

Introduction: Enteral nutrition (EN) involves replacing all or part of a person's habitual diet with a nutritional formula. The impact of varying doses of EN on the gut microbiome remains understudied.
Methods: Healthy adults replaced all (100% EN) or part (85% EN, 50% EN and 20% EN) of their energy requirements with EN for 7 days. Faecal samples were collected before and on day 7 of interventions. Faecal pH, short chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs) and 16S rRNA sequencing were performed. Dietary assessment was performed with 7-day food diaries.
Results: Sixty-one participants (31 females; median (IQR) age: 24.7 (23.0–27.8) years) were recruited. A dose-dependent impact of EN on faecal microbiota, SCFAs, BCFAs) and pH was observed, with changes detectable at EN intakes of at least 50% of energy requirements. 100% and 85% EN reduced the abundance of fibre-fermenting taxa such as *Agathobacter, Faecalibaterium, Succinivibrio and Acidaminococcus*. In parallel, potentially harmful organisms like *Eubacterium*, *Actinomyces, and Klebsiella* increased. In the 50% EN group, adherence to a diet high in fish, vegetables, potatoes, non-alcoholic beverages, and fat spreads, and low in cereal products, milk, and meat negatively correlated with changes in microbiota structure ($r = -0.75$, $P = 0.025$). This signal was not observed when using compositional tools for microbiota analysis.
Conclusions: EN detrimentally influences the faecal microbiota and diet-related bacterial metabolites in a dose-dependent manner, particularly at doses of at least 50%. The findings of this study have implications for the dietary management and counselling of patients receiving high volume EN.

1. Introduction

Enteral nutrition (EN) is a commonly used dietary treatment, which replaces either a portion (partial enteral nutrition, PEN) or the entirety (100% EN) of a person's diet with a nutritional formula. Such treatments are commonly used in nutritional rehabilitation and as efficacious disease-modifying therapies, for example in Crohn's disease (CD) [1] and eosinophilic oesophagitis [2]. Although the EN formulas vary in composition, most of them are nutritionally complete, gluten- and lactose-free ultra-processed foods containing food additives with no or little amount of dietary fibre [3]. As the gut microbiota is highly dependent on host diet, the impact EN composition may have on this, and by extension to host health is a topic of interest. Several metabolites produced through bacterial metabolism of diet components can have beneficial or deleterious effects for human health. As a prime example, bacteria ferment fibre to produce energy for their survival and growth, and the host uses the end-products of this anaerobic process, short-chain fatty acids (SCFAs), for whole body immunity, as energy substrate for colonocytes, regulation of appetite, and absorption of electrolytes in the colon [4]. Previous studies conducted in mice have demonstrated that fibre deprivation resulted in gut microbiota-mediated colonic barrier dysfunction, leading to increased intestinal permeability, subsequently altering host immune responses [5,6]. Human studies have shown that 100% EN reduced microbiota diversity, decreased concentrations of SCFAs,

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and lowered abundance of protective bacterial species. In parallel, it increased the concentrations of branched-chain fatty acids (BCFAs) and sulphide, bioproducts of protein fermentation, and the abundance of potentially harmful pro-inflammatory organisms [7]. What also remains unknown is the dosage of EN intake above which such effects on the microbiota are observed and whether concurrent habitual diet composition, in the case of PEN, is a modifying factor of these effects.

The present study explored the acute impact of varying dosages of EN on the faecal microbiota and diet-related bacterial metabolites, and the potential influence concurrent diet, during PEN, may have on these alterations.

2. Methods

2.1. Study design & participants

Healthy adults (>18 years old) with no underlying health conditions requiring regular medical consultations were recruited from the local community via advertisement. Exclusion criteria were change in weight (>2 kg) in the last month, gut surgery and use of antibiotics, and prebiotic/probiotic supplements in the past 3 months. Participants were asked to replace all (100%) or part (85%, 50%, 20%) of their daily energy requirements with a polymeric EN formula (Modulen IBD, Nestle©), which does not contain dietary fibre, lactose, and gluten, for 7 days. Participants were given the choice of group allocation to one of the four groups to maximise adherence to the dietary interventions. Participants were provided with EN formula and those on PEN with all their preferred meals free of charge to maximise compliance and facilitate dietary assessment. Participants' energy requirements were calculated using estimated energy requirements [8].

2.2. Dietary assessment and analysis

Participants recorded their diet during the intervention with 7-day estimated weight food diaries. Adherence to each intervention was assessed with dietary assessment and through counting left-over EN formula tins. Dietary analysis was performed as described in Supplementary Material Online.

2.3. Faecal sample collection and measurements

Fresh faecal samples were collected before and on day 7 of the interventions and processed for measurements of pH, water content, SCFAs/BCFAs, Bristol Stool Chart score and 16S rRNA amplicon sequencing as described in Supplementary Material Online. The whole bowel movement was collected in disposable tubs, stored under anaerobic conditions (Oxoid™ AnaeroGen™), and transferred to the laboratory in a cool bag with ice packs within 2 h of defecation. The whole sample was homogenized with mechanical kneading and aliquots were stored appropriately for downstream analysis.

2.4. Statistical analysis

Data analyses were performed in Minitab Version 20 (Minitab Ltd, Coventry, UK) and R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Between- and within-group comparisons were performed with general linear models with Box–Cox transformation and post-hoc pairwise Fisher's least significant difference tests, accounting for subject effect, or chi-square test, when appropriate. For microbiota data analysis, α-diversity indices (Chao1 index, Shannon α-diversity index and Pielou's evenness index) were calculated using the "vegan" package [9]. Overall community structure was visualised using non-metric multidimensional scaling (NMDS) analysis on the Aitchison distance. This distance metric was derived from Euclidean distances following centred log ratio (CLR) normalisation, which considers the compositional nature of microbial abundance data. In addition, a conventional NMDS approach using the Bray–Curtis dissimilarity matrix was used to visualise the data. Differences in community structure were assessed using permutation analysis of variance (ANOVA) for within-group comparisons and analysis of covariance (ANCOVA) and post-hoc Tukey honest significant difference test accounting for age, sex, and BMI for between-group comparisons. Quantification of differences in community structure involved calculating Aitchison and Euclidean distances from the combined baseline centroid coordinates derived from the Principal Coordinate Analyses (PCoA). For the case of Euclidean distances, the PCoA was first performed using the respective Bray–Curtis dissimilarity matrix, this method is an adaption of the PERMDISP2 procedure [10] for beta dispersion implemented in the "vegan" R package. The "maaslin2" package was used to identify the bacterial taxa that changed with each intervention. CLR normalisation was used to handle the compositional nature of microbial abundance data, while default total-sum scaling (TSS) normalisation was used for conventional analyses [11]. Correlations were analysed using Spearman rank correlation test. The significance was set at p-value <0.05 or adjusted p-value (q-value) < 0.10 after Benjamini-Hochberg corrections for multiple testing.

2.5. Ethical permissions and compensations

The study protocol was approved by the University of Glasgow Research Ethical Committee (Reference: 200130161). All participants provided informed consent and received £100 in shopping vouchers as participation compensation.

3. Results

3.1. Participants characteristics and dietary intake

Sixty-one participants (31 females and 30 males) were enrolled (Table 1). All participants completed the intervention, returned food diaries, and provided a total of 61 pairs of faecal samples (n = 122). No significant differences in baseline participant characteristics were observed among the four groups (Table 1). The intakes of EN formula across groups closely matched with prescribed intakes (Table 1). Differences in macronutrient and food group intakes between groups reflected the incremental increase of EN intake in diet (Table 1).

3.2. Faecal characteristics and metabolites

Faecal characteristics and metabolites were measured across all 122 samples collected. Faecal pH increased after 100%, 85% and 50% EN (Supplementary Table 1). Significant changes in the levels of faecal SCFAs and BCFAs were observed with 100% and 85% EN (Fig. 1). In these two groups, the concentrations of acetate, butyrate and caproate decreased whereas it was only in the 100% EN group that the concentrations of propionate and valeric acid reduced too, and that of isobutyrate and isovalerate increased (Fig. 1). A trend in increasing levels of BCFAs was also observed after 85% and 50% EN, but this did not reach statistical significance (p-values between 0.05 and 0.08) (Supplementary Table 1).

Table 1
Baseline participant characteristics, and dietary intakes assessed with food diaries during the interventions. Data presented as median (Q1-Q3) unless stated otherwise.

	100% EN (n = 25)	85% EN (n = 12)	50% EN (n = 12)	20% EN (n = 12)	p-value*
Age, years	23.3 (22.9–25.1)	24.8 (24.2–27.1)	27.5 (25.4–29.1)	26.7 (23.5–28.7)	0.158
Female, n (%)	13/25 (52%)	5/12 (42%)	6/12 (50%)	7/12 (58%)	0.875
BMI, kg/m^2	22.4 (20.5–24.3)	22.1 (20.1–25.8)	24.4 (21.9–26.5)	24.1 (21.6–24.9)	0.098
Height, cm	169.0 (166.0–178.0)	172.0 (168.0–178.3)	175.5 (170.5–182.5)	172.0 (168.5–175.0)	0.591
Body weight, kg	68.1 (56.3–74.4)	68.3 (57.5–76.4)	73.4 (63.6–85.0)	68.8 (62.5–75.3)	0.192
Estimated BMR, kcal/day	1578 (1331–1794)	1721 (1364–1823)	1685 (1458–1913)	1506 (1416–1780)	0.511
Estimated TEE, kcal/day	2209 (1872–2512)	2409 (2229–2644)	2567 (2090–2807)	2129 (2023–2603)	0.239
Total energy intake, kcal/day	2260 (1919–2628)	2608 (2276–2880)	2589 (2034–2854)	2178 (2004–2702)	0.248
Energy intake/EAR, %	92.3 (84.2–99.8)	99.8 (90.1–111.7)	102.7 (93.5–105.7)	95.6 (91.1–100.3)	0.111
EN intake/TEE, %	96.4 (95.5–98.3)^{C,F,I}	85.6 (84.7–86.9)^{F,I}	49.9 (48.9–51.0)^{I}	19.8 (19.6–20.6)	**<0.001**
Fat, g	99.7 (81.6–118.9)	115.4 (102.2–130.8)	110.0 (91.4–125.4)	87.9 (82.5–116.1)	0.105
Fat, %	39.9 (39.6–40.7)^{G}	40.3 (39.7–40.7)^{G}	39.8 (38.5–41.0)^{G}	38.6 (34.8–39.3)	**0.006**
Saturated fat, g	56.3 (46.1–67.2)^{I}	62.9 (55.4–69.8)^{I,D}	48.4 (41.1–55.8)^{G}	36.0 (31.4–43.7)	**<0.001**
Saturated fat, %	22.6 (22.4–23.0)^{F,I}	21.8 (21.6–21.9)^{F,I}	17.3 (16.8–18.0)^{I}	14.5 (13.1–15.9)	**<0.001**
Carbohydrate, g	252.1 (216.0–289.2)	285.5 (251.0–313.0)	244.7 (202.8–281.3)	232.4 (222.3–255.8)	0.388
Carbohydrate, %	45.0 (44.1–45.4)^{F,G}	43.7 (42.8–44.1)^{F}	39.1 (37.5–41.0)^{G}	43.7 (41.4–44.7)	**<0.001**
Total sugars, g	111.8 (98.3–122.8)	115.5 (97.0–122.4)	86.6 (77.2–103.4)	88.4 (81.3–100.1)	**0.022**
Total sugars, %	19.5 (18.1–20.2)^{A,F,I}	17.3 (16.3–18.4)^{E}	14.4 (14.0–15.0)	15.9 (15.3–17.7)	**<0.001**
Protein, g	78.9 (65.3–93.5)^{F,G}	97.5 (84.4–105.0)	104.9 (93.5–119.8)	90.7 (87.3–121.8)	**<0.001**
Protein, %	14.0 (13.9–14.2)^{A,F,I}	14.8 (14.2–15.6)^{F,H}	17.7 (16.6–18.1)	17.3 (16.4–17.7)	**<0.001**
Fibre, g	0.0 (0.0–0.0)^{B,F,I}	3.6 (2.9–4.3)^{F,I}	13.7 (11.5–16.0)^{I}	20.6 (15.0–24.4)	**<0.001**
Fibre, g/1000 kcal	0.0 (0.0–0.0)^{B,F,I}	1.5 (1.2–1.8)^{F,I}	5.3 (4.7–7.2)^{I}	8.1 (7.5–9.0)	**<0.001**
Cereals and Cereal Products, g/1000 kcal		106.0 (60.2)^{F,I}	377.8 (154.9)^{H}	663.5 (156.7)	**<0.001**
Milk and Milk Products, g/1000 kcal		32.1 (25.7)^{F,I}	141.4 (57.8)	190.2 (106.8)	**<0.001**
Eggs and Egg Dishes, g/1000 kcal		20.4 (19.5)	35.6 (21.4)	17.8 (0.0–39.3)	0.097
Fat Spreads, g/1000 kcal		10.6 (5.1–20.7)^{F,I}	152.9 (112.5–202.5)	182.4 (68.7)	**<0.001**
Meat and Meat Products, g/1000 kcal		48.8 (48.9)^{E,I}	145.8 (54.2)	225.2 (148.9)	**<0.001**
Fish and Fish Dishes, g/1000 kcal		8.1 (0.0–8.2)	34.8 (0.0–68.5)	48.8 (0.0–76.2)	**0.039**
Vegetables, Potatoes, g/1000 kcal		7.9 (0.8–62.4)^{E,I}	110.2 (58.8)	152.6 (79.8)	**<0.001**
Savoury Snacks, g/1000 kcal		0.0 (0.0–35.2)	0.0 (0.0–21.0)	0.0 (0.0–102.8)	0.133
Nuts and Seeds, g/1000 kcal		0.0 (0.0–39.3)	3.3 (0.0–75.7)	25.2 (0.0–120.4)	0.186
Fruit, g/1000 kcal		66.9 (49.1)^{I}	100.9 (41.7)^{H}	231.6 (115.2)	**<0.001**
Sugars, Preserves and Confectionery, g/1000 kcal		17.8 (0.0–81.0)	15.7 (0.0–77.8)	63.1 (59.7)	0.294
Non-Alcoholic Beverages, g/1000 kcal		0.0 (0.0–0.0)^{I}	3.7 (3.2)^{H}	31.0 (35.0)	**0.001**
Alcoholic Beverages, g/1000 kcal		0.0 (0.0–0.0)^{D}	19.8 (0.0–94.5)	0.0 (0.0–59.5)	**0.038**
Miscellaneous, g/1000 kcal		0.0 (0.0–0.0)^{E,G}	32.1 (21.4–61.2)	37.4 (40.7)	**0.003**

*P-values for between-group comparisons with general linear modelling with Box–Cox transformation. P-values for comparisons of macronutrients from food (habitual diet) and food groups are between 85%, 50% EN and 20% EN groups. P-values for comparisons of food groups are based on kilocalorie adjusted values (/1000 kcal).
[A] Significantly different than 85% EN group ($P < 0.05$); [B] Significantly different than 85% EN group ($P < 0.01$); [C] Significantly different than 85% EN group ($P < 0.001$); [D] Significantly different than 50% EN group ($P < 0.05$); [E] Significantly different than 50% EN group ($P < 0.01$); [F] Significantly different than 50% EN group ($P < 0.001$); [G] Significantly different than 20% EN group ($P < 0.05$); [H] Significantly different than 20% EN group ($P < 0.01$); [I] Significantly different than 20% EN group ($P < 0.001$) for Fisher pairwise comparisons after general linear modelling.
Abbreviations used: BMI: Body Mass Index; BMR: Basal Metabolic Rate; EAR: Estimated Average Requirement; EN: Enteral Nutrition; F: Female; M: Male; TEE: Total Energy Expenditure.

3.3. Faecal microbiota

Microbiota analysis was conducted on a total of 110 samples which passed the 10,000 reads quality control cut-off (Supplementary Table 2). Sequencing reads were annotated to 2971 unique amplicon sequence variants (ASVs) and 248 genera.

In all groups, apart from 20% EN, dietary interventions shifted microbiota structure (Fig. 2) towards the same direction on ordination plots, and in a dose-dependent manner (Fig. 3). After correcting for baseline values, using the Aitchison distances, which handle the compositional nature of microbial abundance data, we observed significant differences between the 100% EN and all other groups except for the 85% EN group (Fig. 3). Comparable effects in microbiota structure were observed using the conventional approach on the Bray–Curtis dissimilarity matrix (Supplementary Figs. 1 and 2).

Regarding α-diversity indices, an increase in the Chao1 index was observed in the 85% EN group ($P = 0.031$) only (Fig. 4); albeit samples from this group had lower baseline values compared to the 50% EN ($P = 0.002$) and 20% EN ($P = 0.023$) groups. Other estimates of α-diversity did not change in any of the groups (Fig. 4).

Among all four groups, the most significant changes in taxon relative abundance were evident after 100% and 85% EN with several of these changes overlapping between the two groups. Comparatively, fewer changes were observed after 50% and 20% EN, with the statistical significance of most changes lost when correcting for multiple testing especially for the 20% EN group. Compositional analysis with CLR normalisation revealed significant baseline changes in 26% (100/385) of analysed ASVs, 28% (32/114) genera, and 32% (12/37) families following 100% EN, and in 21% (61/290) ASVs, 29% (28/97) genera, and 22% (7/32) families following 85% EN (Fig. 5, Supplementary Figs. 3 and 4). At phylum level, we observed that 100% EN led to a decrease in *Bacteroidetes* abundance while increasing *Desulfobacterota* levels. 85% EN increased abundance of *Proteobacteria*, and 50% EN decreased both *Actinobacteriota* and *Bacteroidetes* (Supplementary Fig. 5). Consumption of 100% EN decreased the abundance of fibre-fermenting and SCFA-producing taxa such as members of *Succinivibrio, Acidaminococcus, Agathobacter, Faecalibaterium, Bifidobacterium* and Ruminonoccaceae, while in parallel increased the abundance of potentially harmful organisms like *Eubacterium*, *Actinomyces*, *Klebsiella, Ruminococcus torques* group, *Escherichia Shigella* and *Erysipelatoclostridium.* Many changes overlapped between the 85% EN and 100% EN groups (43% (12/28) genera) including reduced abundance of *Acidaminococcus, Agathobacter*, and *Bifidobacterium,* and increased abundance of the genus *R. torques* group. 50% EN induced

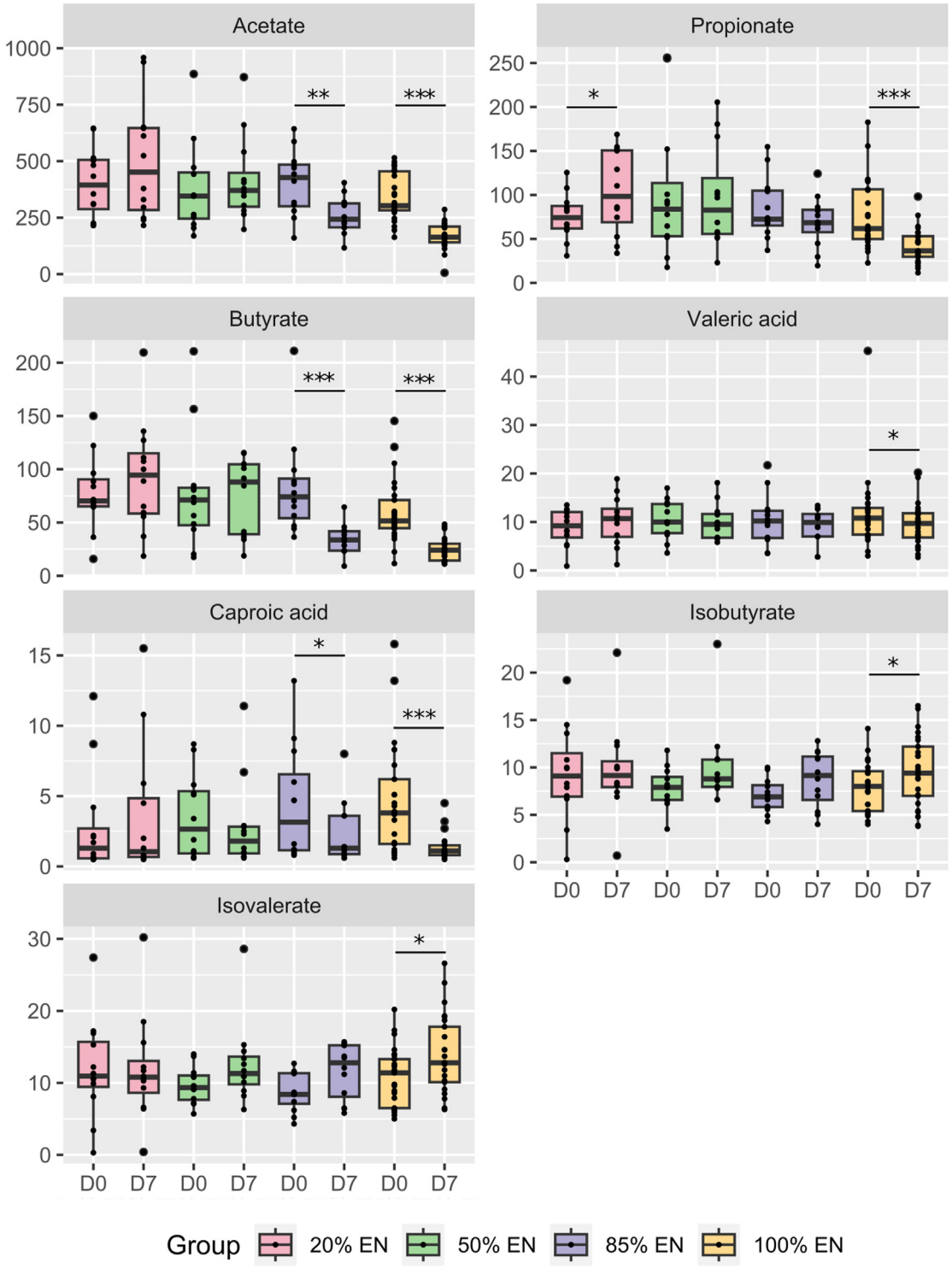

Fig. 1. Impact of 20% EN, 50% EN, 85% EN and 100% EN interventions on the faecal diet-related bacterial metabolites (μmol/g). *p-value<0.05, **p-value<0.01, ***p-value<0.001. Abbreviations used: D0: baseline (Day 0); D7: post-intervention (Day 7); EN: enteral nutrition.

changes to 10% (11/109) of analysed genera, of which 55% (6/11) also overlapped with the 100% EN group. Analysis with TSS normalisation revealed a broader spectrum of changes, particularly within the 85% and 100% EN groups; it is worth noting that many changes overlapped between the two approaches (100% EN group: 52/100 ASVs, 27/32 genera, and 11/12 families; 85% EN group: 25/61 ASVs, 18/28 genera, and 6/7 families) (Supplementary Figs. 6–9).

3.4. Correlations between concurrent diet with faecal microbiota changes

Last, we explored relationships between dietary intake and microbiota community structure (Aitchison and Euclidean distances from baseline centroid) in participants from the 50% EN group in which the dose of EN was sufficient to induce significant

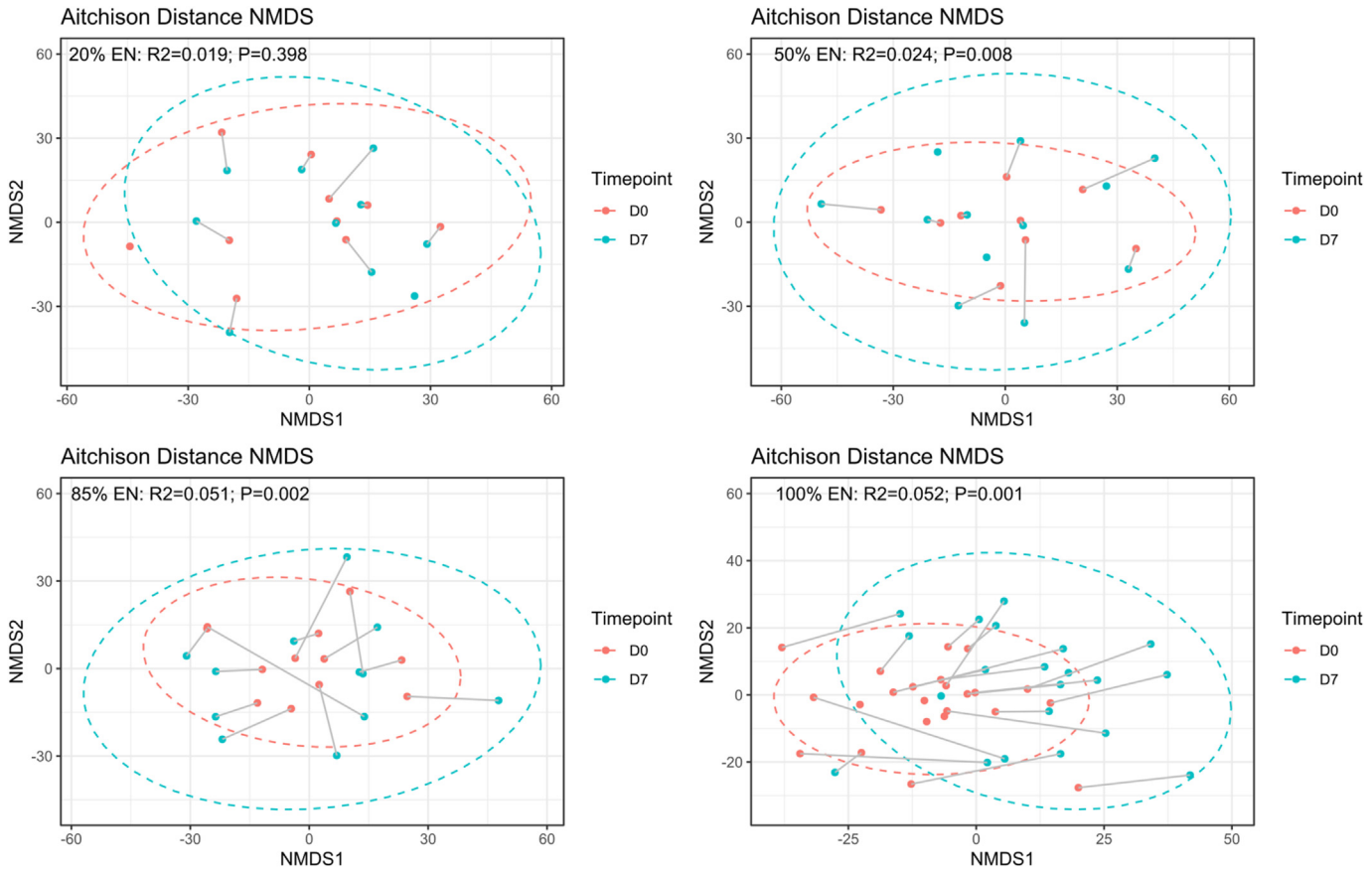

Fig. 2. Impact of 20% EN, 50% EN, 85% EN and 100% EN interventions on the faecal microbiota community structure with NMDS using the Aitchison distance and permutation ANOVA. Abbreviations used: ANOVA: analysis of variance; NMDS: nonmetric multidimensional scaling; D0: baseline (Day 0); D7: post-intervention (Day 7); EN: enteral nutrition.

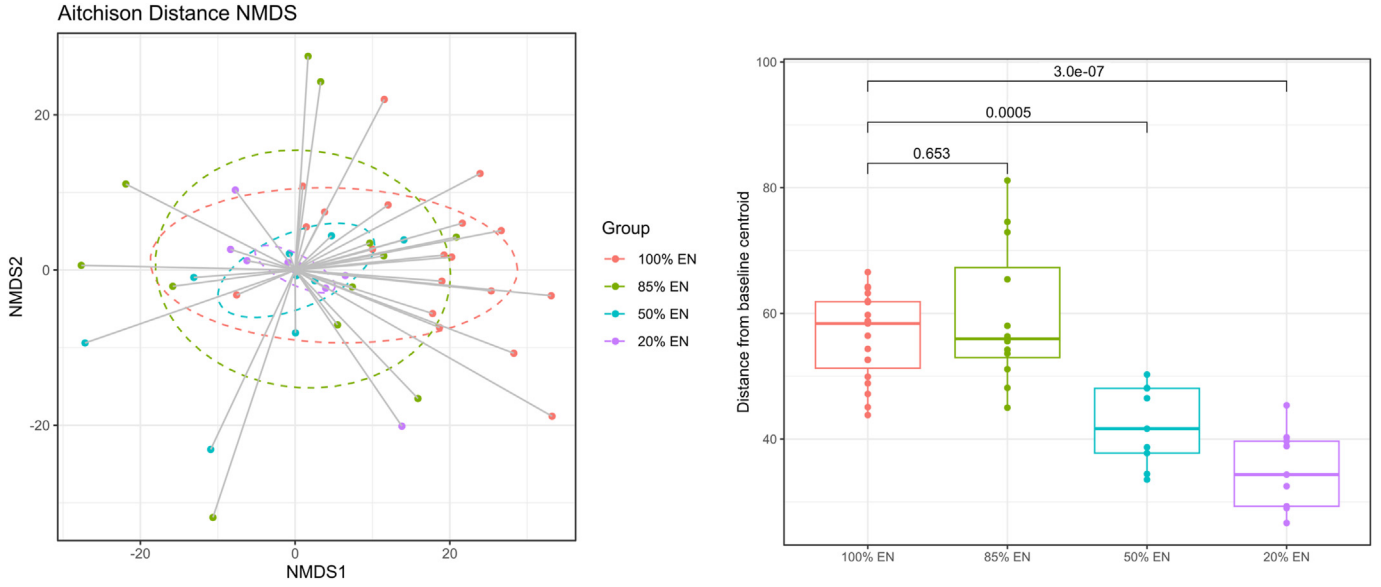

Fig. 3. Impact of 20% EN, 50% EN, 85% EN and 100% EN interventions on the faecal microbiota community structure with NMDS using the Aitchison distance with correction for baseline values, and differences between the groups in Aitchison distance from baseline centroid assessed with ANCOVA and post-hoc Tukey honestly significant difference test accounting for age, sex, and BMI. Abbreviations used: ANVOCA: analysis of covariance; NMDS: nonmetric multidimensional scaling; D0: baseline (Day 0); D7: post-intervention (Day 7); EN: enteral nutrition.

shifts in microbiota community and while concurrent diet was making up a significant fraction of their intake to potentially mitigate some of these shifts. Three dietary patterns were selected following PCA analysis which collectively explained 61% of data variance (Supplementary Figs. 10 and 11). While we found that changes in the community structure measured with the

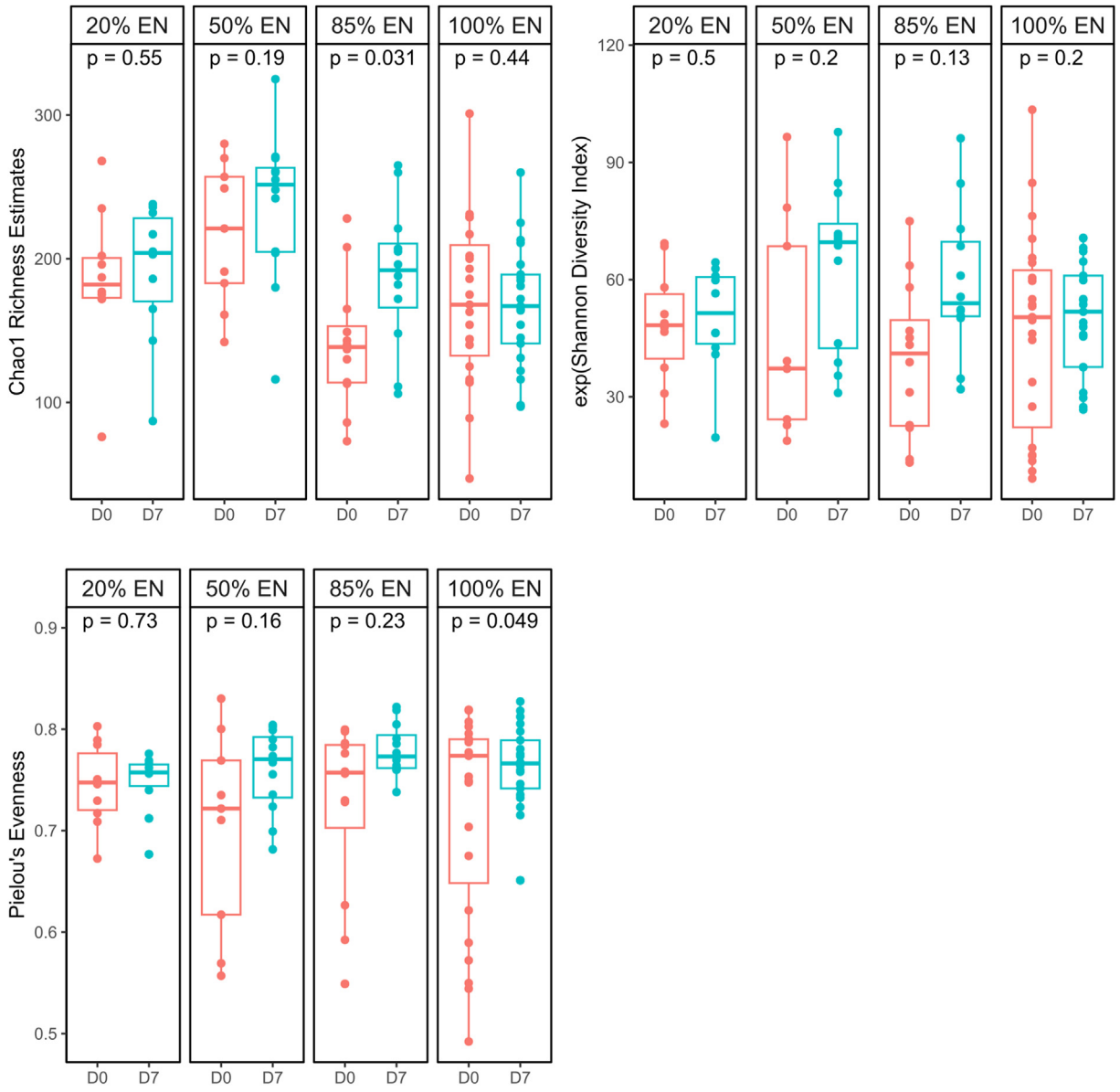

Fig. 4. Impact of 20% EN, 50% EN, 85% EN and 100% EN interventions on the Chao1 index, Shannon α-diversity index and Pielou's evenness index. Abbreviations used: D0: baseline (Day 0); D7: post-intervention (Day 7); EN: enteral nutrition.

Aitchison distances did not correlate with adherence to any of these dietary patterns, adherence to a pescetarian-like dietary pattern characterised by high consumption of fish and fish dishes, vegetables and potatoes, non-alcoholic beverages and fat spreads, and low consumption of cereal and cereal products, milk and milk products, and meat and meat products) was negatively correlated with changes in microbiota composition measured with the Euclidean distance ($r = -0.75$, $P = 0.025$).

4. Discussion

In the present study, we show that EN had significant effects on faecal microbiota and diet-related bacterial metabolites; in particular, demonstrating a dose-dependent relationship with changes detectable at EN intake of at least 50% of energy requirements. Certain microbial changes were observed solely after exclusive consumption of EN, including changes in the concentrations of BCFAs, whereas other alterations were shared between more than two groups such as the shifts in overall community structure. The increase in faecal pH has been observed in all four groups, indicating that even a small amount of EN (20%) may increase faecal pH levels. Such increments in pH levels with EN consumption may result from a concomitant decrease in fibre intake in diet and by extension less luminal fermentable substrate for bacterial production of SCFAs. The changes observed here are most likely the result of fibre deficit in the intestinal lumen for bacterial growth, a corresponding increase in gastrointestinal transit time and luminal pH when EN is used at doses of at least 85% of energy requirements. At such high volumes, it appears that EN influences the gut microbiota, and the effect of concurrent diet may be insignificant. However, when at least 50% of energy intake is replaced by EN, the composition of a concurrent diet, particularly a dietary pattern resembling a pescetarian diet, may mask the effect of EN alone on the gut microbiota, although these findings were not confirmed using compositional tools for microbiota analyses.

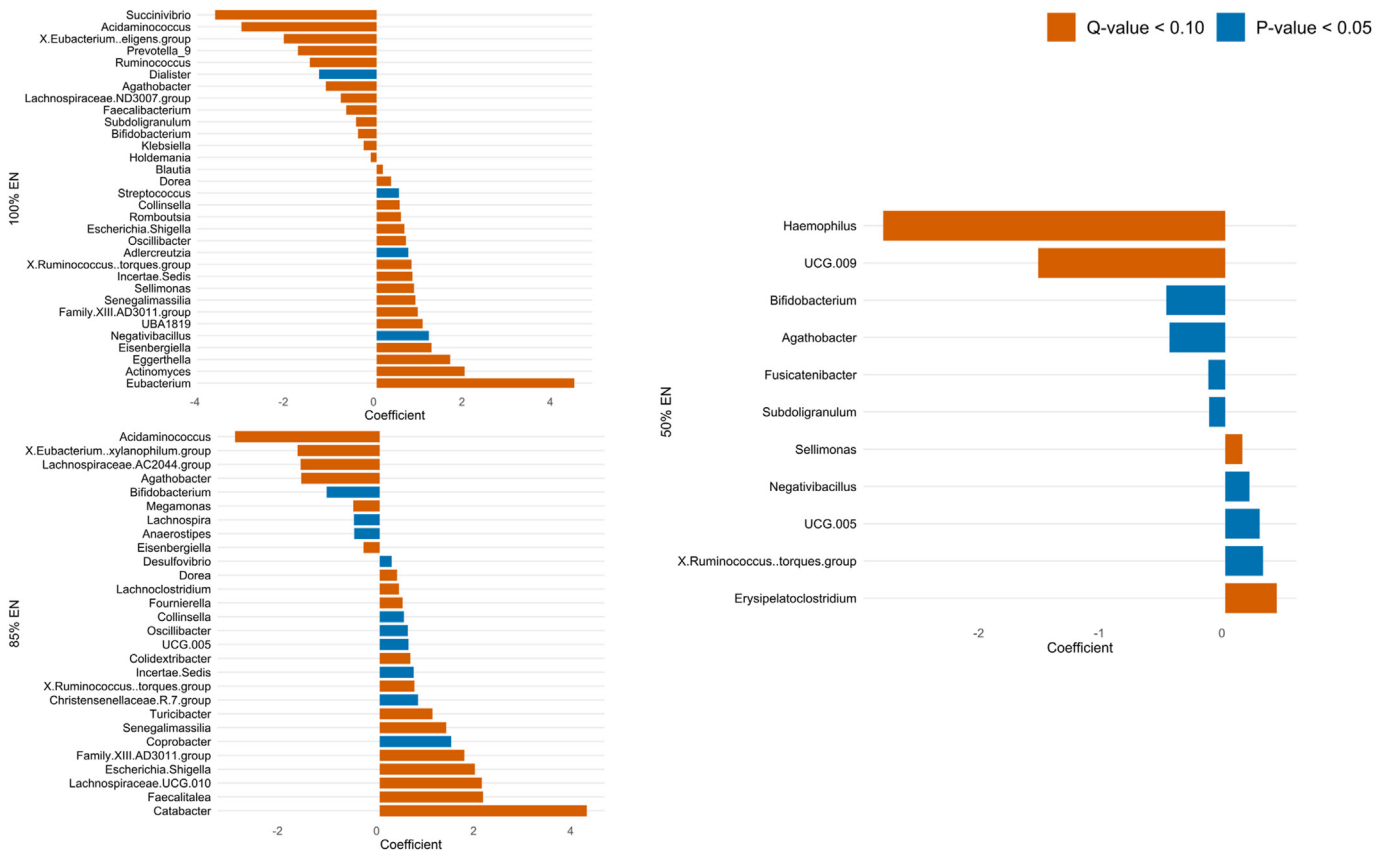

Fig. 5. Results from maaslin2 with centred log ratio normalisation showing significant changes to bacterial taxa at genus level following 20% EN, 50% EN, 85% EN and 100% EN interventions. The figure displays directions and magnitudes of these changes, accounting for correction for multiple testing (q-value <0.10) and without correction (p-value <0.05). No significant differences observed following 20% EN. Abbreviations used: EN: enteral nutrition.

There are implications from the findings of the current study for dietetic practice and research. In patients where dietary fibre is not contraindicated, use of EN formulas enriched with dietary fibres should be encouraged, particularly using blends of different dietary fibres. Alternatively healthcare professionals should provide advice to increase fibre-containing foods in the concurrent diet of patients alongside high-volume EN. Supplementing EN treatment with probiotics, including traditional and next-generation organisms such as *Bifidobacterium* and *Faecalibacterium*, respectively, may also aid in mitigating the potentially negative effects of high-volume EN on the gut microbiome.

Likewise, this study findings may also have indirect implications relevant to the mechanism of action of EN in the management of active CD. It has long been believed that the efficacy of 100% EN is mediated via modulation of the gut microbiota and the findings of this study align with this hypothesis [12]. In contrast, the inability of 20% EN to shift the microbiota and SCFAs may explain the ineffectiveness of low volume EN to maintain remission in CD [13]. Nonetheless, such suggestions need confirmation in clinical trials in patients with CD and against disease measures.

Another notable observation is that BCFAs increased only in participants on 100% EN and not in those on 85% EN and despite similar shifts to microbiota structure. This finding suggests that even small amounts of dietary fibre (e.g. average intake of 4 g per day in the case of the 85% EN) may be adequate to mitigate excessive protein fermentation and production of BCFAs by the gut microbiota.

This study has several limitations. We only assessed the short-term impact of EN, and further research is required to explore the long-term effects on the faecal microbiota. The consequences these microbial signals may have on host immune responses is also an important topic for further research. An important limitation of this study is the presence of baseline differences in the species richness among the study groups, with the 85% EN group demonstrating lower baseline values compared to other groups. This discrepancy may have introduced bias and the observed increase in species richness in the 85% EN group should be interpreted with caution. Furthermore, it is important to acknowledge that due to the exploratory nature of our study, formal power calculations were not carried out, which may have impacted the study ability to detect small or moderate effects. However, the paired study design we applied here, with most of the comparative analysis performed within a group, increased our statistical power to detect significant effects, despite a modest sample size.

In summary, EN modifies the faecal microbiota and diet-related bacterial metabolites in a dose-dependent manner, but marginal effects are to be expected in people consuming 20% EN or less.

Publication statement

We confirm that this manuscript including related data, figures and tables have not been previously published and is not under consideration elsewhere.

Funding statement

The formulas used in this study were a donation by Nestle Health Science.

Data availability statement

The raw sequencing data used for this project has been deposited in the European Nucleotide Archive (ENA) under accession number: PRJEB72881. Other datasets will be shared upon request.

Conflict of interest

The studentships of AJ and KGk are funded by Nestle Health Science and the University of Glasgow. KGe received research funding, speakers fee and travel expenses covered by Nestle Health Science, Nutricia-Danone, AbbVie, Janssen, Abbott. VS received consultancy fees from Chronicles Health. The rest of the authors have no conflicts of interest to disclose. The funders had no role in the conception, design, execution, interpretation, writing or submission of this manuscript.

Author contributions

KGk, KGe and VS contributed to the conception and design of the study. KGk, VS, VR, PK, JKG and EC contributed to delivery of the interventions and sample laboratory analysis. AJ, BN, and BS contributed to the data analysis. AJ produced the initial draft for publication. All authors were involved in revising the manuscript and have approved its final version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.04.010.

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