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

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1 Title

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

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21 Publication statement

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

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26 Data availability statement

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

29 Conflict of interest

30 The studentships of AJ and KGk are funded by Nestle Health Science and the University of Glasgow. KGe
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35 Author contributions

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

40 Abstract

41 **Introduction:** Enteral nutrition (EN) involves replacing all or part of a person's habitual diet with a
42 nutritional formula. The impact of varying doses of EN on the gut microbiome remains understudied.

43 **Methods:** Healthy adults replaced all (100% EN) or part (85% EN, 50% EN and 20% EN) of their energy
44 requirements with EN for 7 days. Faecal samples were collected before and on day 7 of interventions.
45 Faecal pH, short chain fatty acids (SCFAs), branched-chain fatty acids (BCFAs) and 16S rRNA sequencing
46 were performed. Dietary assessment was performed with 7-day food diaries.

47 **Results:** Sixty-one participants (31 females; median (IQR) age: 24.7 (23.0-27.8) years) were recruited. A
48 dose-dependent impact of EN on faecal microbiota, SCFAs, BCFAs) and pH was observed, with changes
49 detectable at EN intakes of at least 50% of energy requirements. 100% and 85% EN reduced the
50 abundance of fibre-fermenting taxa such as *Agathobacter*, *Faecalibacterium*, *Succinivibrio* and
51 *Acidaminococcus*. In parallel, potentially harmful organisms like *Eubacterium*, *Actinomyces*, and
52 *Klebsiella* increased. In the 50% EN group, adherence to a diet high in fish, vegetables, potatoes, non-
53 alcoholic beverages, and fat spreads, and low in cereal products, milk, and meat negatively correlated
54 with changes in microbiota structure ($r=-0.75$, $P=0.025$). This signal was not observed when using
55 compositional tools for microbiota analysis.

56 **Conclusions:** EN detrimentally influences the faecal microbiota and diet-related bacterial metabolites in
57 a dose-dependent manner, particularly at doses of at least 50%. The findings of this study have
58 implications for the dietary management and counselling of patients receiving high volume EN.

59 Introduction

60 Enteral nutrition (EN) is a commonly used dietary treatment, which replaces either a portion (partial
61 enteral nutrition, PEN) or the entirety (100% EN) of a person's diet with a nutritional formula. Such
62 treatments are commonly used in nutritional rehabilitation and as efficacious disease-modifying
63 therapies, for example in Crohn's disease (CD) [1] and eosinophilic oesophagitis [2]. Although the EN
64 formulas vary in composition, most of them are nutritionally complete, gluten- and lactose-free ultra-
65 processed foods containing food additives with no or little amount of dietary fibre [3]. As the gut
66 microbiota is highly dependent on host diet, the impact EN composition may have on this, and by
67 extension to host health is a topic of interest. Several metabolites produced through bacterial
68 metabolism of diet components can have beneficial or deleterious effects for human health. As a prime
69 example, bacteria ferment fibre to produce energy for their survival and growth, and the host uses the
70 end-products of this anaerobic process, short-chain fatty acids (SCFAs), for whole body immunity, as
71 energy substrate for colonocytes, regulation of appetite, and absorption of electrolytes in the colon [4].
72 Previous studies conducted in mice have demonstrated that fibre deprivation resulted in gut microbiota-
73 mediated colonic barrier dysfunction, leading to increased intestinal permeability, subsequently altering
74 host immune responses [5, 6]. Human studies have shown that 100% EN reduced microbiota diversity,
75 decreased concentrations of SCFAs, and lowered abundance of protective bacterial species. In parallel, it
76 increased the concentrations of branched-chain fatty acids (BCFAs) and sulphide, bioproducts of protein
77 fermentation, and the abundance of potentially harmful pro-inflammatory organisms [7]. What also
78 remains unknown is the dosage of EN intake above which such effects on the microbiota are observed
79 and whether concurrent habitual diet composition, in the case of PEN, is a modifying factor of these
80 effects.

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

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85 Methods

86 Study design & participants

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

97 Dietary assessment and analysis

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

101 Faecal sample collection and measurements

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

107 homogenized with mechanical kneading and aliquots were stored appropriately for downstream
108 analysis.

109 Statistical analysis

110 Data analyses were performed in Minitab Version 20 (Minitab Ltd, Coventry, UK) and R version 4.1.2 (R
111 Foundation for Statistical Computing, Vienna, Austria). Between- and within-group comparisons were
112 performed with general linear models with Box-Cox transformation and post-hoc pairwise Fisher's least
113 significant difference tests, accounting for subject effect, or chi-square test, when appropriate. For
114 microbiota data analysis, α -diversity indices (Chao1 index, Shannon α -diversity index and Pielou's
115 evenness index) were calculated using the "vegan" package [9]. Overall community structure was
116 visualised using non-metric multidimensional scaling (NMDS) analysis on the Aitchison distance. This
117 distance metric was derived from Euclidean distances following centred log ratio (CLR) normalisation,
118 which considers the compositional nature of microbial abundance data. In addition, a conventional
119 NMDS approach using the Bray-Curtis dissimilarity matrix was used to visualise the data. Differences in
120 community structure were assessed using permutation analysis of variance (ANOVA) for within-group
121 comparisons and analysis of covariance (ANCOVA) and post-hoc Tukey honest significant difference test
122 accounting for age, sex, and BMI for between-group comparisons. Quantification of differences in
123 community structure involved calculating Aitchison and Euclidean distances from the combined baseline
124 centroid coordinates derived from the Principal Coordinate Analyses (PCoA). For the case of Euclidean
125 distances, the PCoA was first performed using the respective Bray-Curtis dissimilarity matrix, this
126 method is an adaption of the PERMDISP2 procedure [10] for beta dispersion implemented in the
127 "vegan" R package. The "maaslin2" package was used to identify the bacterial taxa that changed with
128 each intervention. CLR normalisation was used to handle the compositional nature of microbial
129 abundance data, while default total-sum scaling (TSS) normalisation was used for conventional analyses
130 [11]. Correlations were analysed using Spearman rank correlation test. The significance was set at p-

131 value < 0.05 or adjusted p-value (q-value) < 0.10 after Benjamini-Hochberg corrections for multiple
132 testing.

133 Ethical permissions and compensations

134 The study protocol was approved by the University of Glasgow Research Ethical Committee (Reference:
135 200130161). All participants provided informed consent and received £100 in shopping vouchers as
136 participation compensation.

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138 Results

139 Participants characteristics and dietary intake

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

146 Faecal characteristics and metabolites

147 Faecal characteristics and metabolites were measured across all 122 samples collected. Faecal pH
148 increased after 100%, 85% and 50% EN (Supplementary Table 1). Significant changes in the levels of
149 faecal SCFAs and BCFAs were observed with 100% and 85% EN (Figure 1). In these two groups, the
150 concentrations of acetate, butyrate and caproate decreased whereas it was only in the 100% EN group
151 that the concentrations of propionate and valeric acid reduced too, and that of isobutyrate and
152 isovalerate increased (Figure 1). A trend in increasing levels of BCFAs was also observed after 85% and
153 50% EN, but this did not reach statistical significance (p-values between 0.05 to 0.08) (Supplementary
154 Table 1).

155 Faecal microbiota

156 Microbiota analysis was conducted on a total of 110 samples which passed the 10,000 reads quality
157 control cut-off (Supplementary Table 2). Sequencing reads were annotated to 2,971 unique amplicon
158 sequence variants (ASVs) and 248 genera.

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

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

169 Among all four groups, the most significant changes in taxon relative abundance were evident after
170 100% and 85% EN with several of these changes overlapping between the two groups. Comparatively,
171 fewer changes were observed after 50% and 20% EN, with the statistical significance of most changes
172 lost when correcting for multiple testing especially for the 20% EN group. Compositional analysis with
173 CLR normalisation revealed significant baseline changes in 26% (100/385) of analysed ASVs, 28%
174 (32/114) genera, and 32% (12/37) families following 100% EN, and in 21% (61/290) ASVs, 29% (28/97)
175 genera, and 22% (7/32) families following 85% EN (Figure 5, Supplementary Figures 3 and 4). At phylum
176 level, we observed that 100% EN led to a decrease in *Bacteroidetes* abundance while increasing
177 *Desulfobacterota* levels. 85% EN increased abundance of *Proteobacteria*, and 50% EN decreased both

178 *Actinobacteriota* and *Bacteroidetes* (Supplementary Figure 5). Consumption of 100% EN decreased the
179 abundance of fibre-fermenting and SCFA-producing taxa such as members of *Succinivibrio*,
180 *Acidaminococcus*, *Agathobacter*, *Faecalibacterium*, *Bifidobacterium* and Ruminonocaceae, while in
181 parallel increased the abundance of potentially harmful organisms like *Eubacterium*, *Actinomyces*,
182 *Klebsiella*, *Ruminococcus torques* group, *Escherichia Shigella* and *Erysipelatoclostridium*. Many changes
183 overlapped between the 85% EN and 100% EN groups (43% (12/28) genera) including reduced
184 abundance of *Acidaminococcus*, *Agathobacter*, and *Bifidobacterium*, and increased abundance of the
185 genus *Ruminococcus torques* group. 50% EN induced changes to 10% (11/109) of analysed genera, of
186 which 55% (6/11) also overlapped with the 100% EN group. Analysis with TSS normalisation revealed a
187 broader spectrum of changes, particularly within the 85% and 100% EN groups; it is worth noting that
188 many changes overlapped between the two approaches (100% EN group: 52/100 ASVs, 27/32 genera,
189 and 11/12 families; 85% EN group: 25/61 ASVs, 18/28 genera, and 6/7 families) (Supplementary Figures
190 6-9).

191 Correlations between concurrent diet with faecal microbiota changes

192 Last, we explored relationships between dietary intake and microbiota community structure (Aitchison
193 and Euclidean distances from baseline centroid) in participants from the 50% EN group in which the
194 dose of EN was sufficient to induce significant shifts in microbiota community and while concurrent diet
195 was making up a significant fraction of their intake to potentially mitigate some of these shifts. Three
196 dietary patterns were selected following PCA analysis which collectively explained 61% of data variance
197 (Supplementary Figures 10 and 11). While we found that changes in the community structure measured
198 with the Aitchison distances did not correlate with adherence to any of these dietary patterns,
199 adherence to a pescetarian-like dietary pattern characterised by high consumption of fish and fish
200 dishes, vegetables and potatoes, non-alcoholic beverages and fat spreads, and low consumption of
201 cereal and cereal products, milk and milk products, and meat and meat products) was negatively

202 correlated with changes in microbiota composition measured with the Euclidean distance ($r=-0.75$,
203 $P=0.025$).

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205 Discussion

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

222 There are implications from the findings of the current study for dietetic practice and research. In
223 patients where dietary fibre is not contraindicated, use of EN formulas enriched with dietary fibres
224 should be encouraged, particularly using blends of different dietary fibres. Alternatively healthcare
225 professionals should provide advice to increase fibre-containing foods in the concurrent diet of patients
226 alongside high-volume EN. Supplementing EN treatment with probiotics, including traditional and next-

227 generation organisms such as *Bifidobacterium* and *Faecalibacterium*, respectively, may also aid in
228 mitigating the potentially negative effects of high-volume EN on the gut microbiome.

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

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

239 This study has several limitations. We only assessed the short-term impact of EN, and further research is
240 required to explore the long-term effects on the faecal microbiota. The consequences these microbial
241 signals may have on host immune responses is also an important topic for further research. An
242 important limitation of this study is the presence of baseline differences in the species richness among
243 the study groups, with the 85% EN group demonstrating lower baseline values compared to other
244 groups. This discrepancy may have introduced bias and the observed increase in species richness in the
245 85% EN group should be interpreted with caution. Furthermore, it is important to acknowledge that due
246 to the exploratory nature of our study, formal power calculations were not carried out, which may have
247 impacted the study ability to detect small or moderate effects. However, the paired study design we
248 applied here, with most of the comparative analysis performed within a group, increased our statistical
249 power to detect significant effects, despite a modest sample size.

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

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253 References

- 254 [1] Caio G, Lungaro L, Caputo F, Zoli E, Giancola F, Chiarioni G, et al. Nutritional Treatment in Crohn's
255 Disease. *Nutrients*. 2021;13.
- 256 [2] Atwal K, Hubbard GP, Venter C, Stratton RJ. The use of amino acid-based nutritional feeds is effective
257 in the dietary management of pediatric eosinophilic oesophagitis. *Immun Inflamm Dis*. 2019;7:292-303.
- 258 [3] Logan M, Gkikas K, Svolos V, Nichols B, Milling S, Gaya DR, et al. Analysis of 61 exclusive enteral
259 nutrition formulas used in the management of active Crohn's disease-new insights into dietary disease
260 triggers. *Aliment Pharmacol Ther*. 2020;51:935-47.
- 261 [4] den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain
262 fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res*.
263 2013;54:2325-40.
- 264 [5] Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, et al. A Dietary Fiber-
265 Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility.
266 *Cell*. 2016;167:1339-53.e21.
- 267 [6] Parrish A, Boudaud M, Grant ET, Willieme S, Neumann M, Wolter M, et al. *Akkermansia muciniphila*
268 exacerbates food allergy in fibre-deprived mice. *Nat Microbiol*. 2023;8:1863-79.
- 269 [7] Gerasimidis K, Bertz M, Hanske L, Junick J, Biskou O, Aguilera M, et al. Decline in presumptively
270 protective gut bacterial species and metabolites are paradoxically associated with disease improvement
271 in pediatric Crohn's disease during enteral nutrition. *Inflamm Bowel Dis*. 2014;20:861-71.
- 272 [8] Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Hum*
273 *Nutr Clin Nutr*. 1985;39 Suppl 1:5-41.

- 274 [9] Oksanen J. CRAN – Package "vegan". 2022.
- 275 [10] Anderson MJ, Ellingsen KE, McArdle BH. Multivariate dispersion as a measure of beta diversity.
276 Ecology Letters. 2006;9:683-93.
- 277 [11] Mallick H, Rahnavard A, McIver L, Ma S, Zhang Y, Nguyen L, et al. Multivariable association
278 discovery in population-scale meta-omics studies. PLoS Computational Biology. 2021;17.
- 279 [12] Svolos V, Gkikas K, Gerasimidis K. Diet and gut microbiota manipulation for the management of
280 Crohn's disease and ulcerative colitis. Proceedings of the Nutrition Society. 2021;80:409-23.
- 281 [13] Gkikas K, Gerasimidis K, Milling S, Ijaz UZ, Hansen R, Russell RK. Dietary Strategies for Maintenance
282 of Clinical Remission in Inflammatory Bowel Diseases: Are We There Yet? Nutrients. 2020;12:2018.
- 283

284 Figure legends

285 **Figure 1:** Impact of 20% EN, 50% EN, 85% EN and 100% EN interventions on the faecal diet-related
286 bacterial metabolites ($\mu\text{mol/g}$). *p-value<0.05, **p-value<0.01, ***p-value<0.001. Abbreviations used:
287 D0: baseline (Day 0); D7: post-intervention (Day 7); EN: enteral nutrition.

288

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

293

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

300

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

304

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

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310 Tables

	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}	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

	100% EN (n=25)	85% EN (n=12)	50% EN (n=12)	20% EN (n=12)	p-value*
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

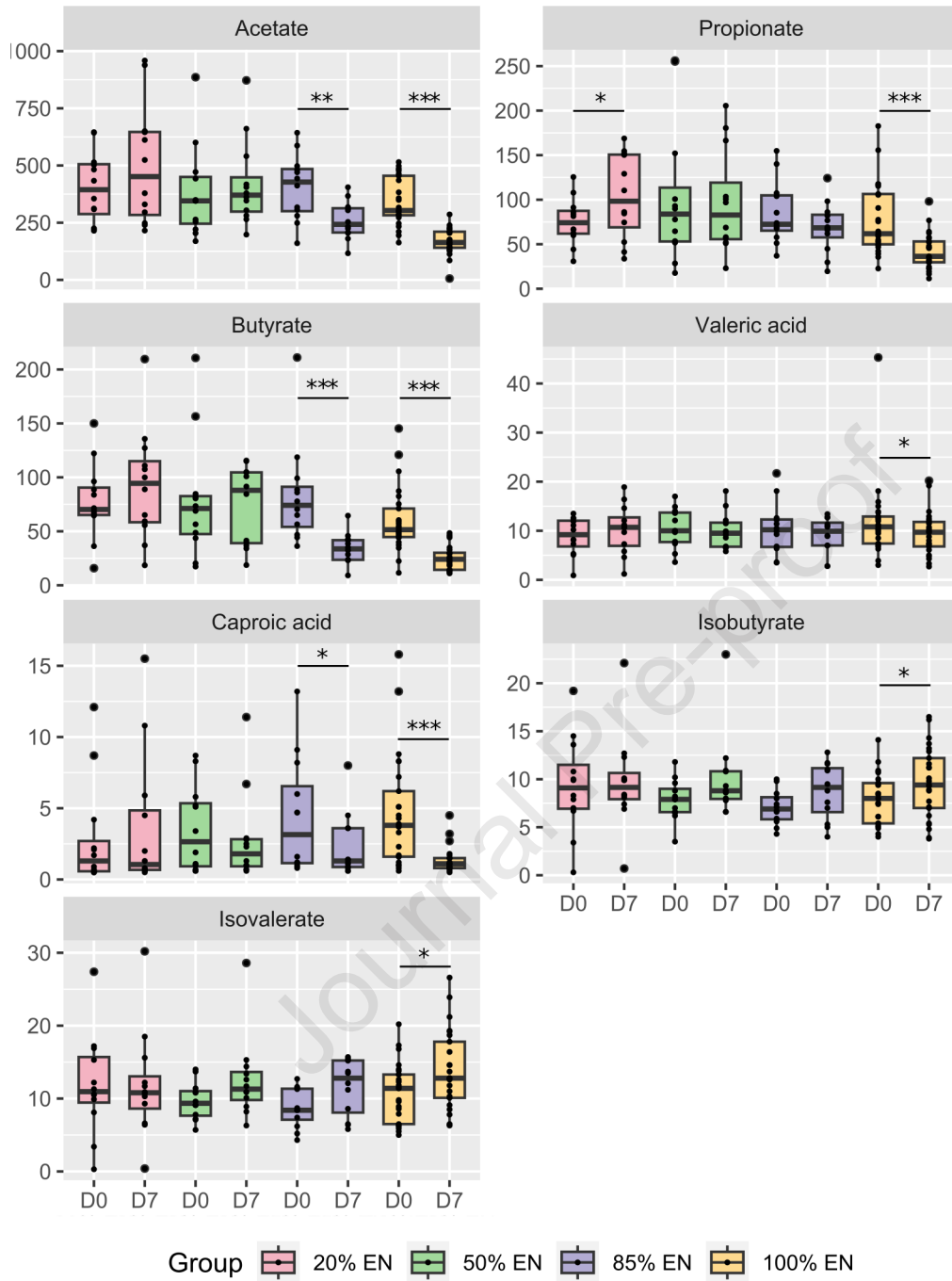
	100% EN (n=25)	85% EN (n=12)	50% EN (n=12)	20% EN (n=12)	p-value*
Fruit, g/1000 kcal		66.9 (49.1) ^I	100.9 (41.7) ^H	231.6 (115.2)	<0.001
Sugars, Preserves and Confectionery, g/1000 kcal		17.8 (0.0-81.0)	15.7 (0.0-77.8)	63.1 (59.7)	0.294
Non-Alcoholic Beverages, g/1000 kcal		0.0 (0.0-0.0) ^I	3.7 (3.2) ^H	31.0 (35.0)	0.001
Alcoholic Beverages, g/1000 kcal		0.0 (0.0-0.0) ^P	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

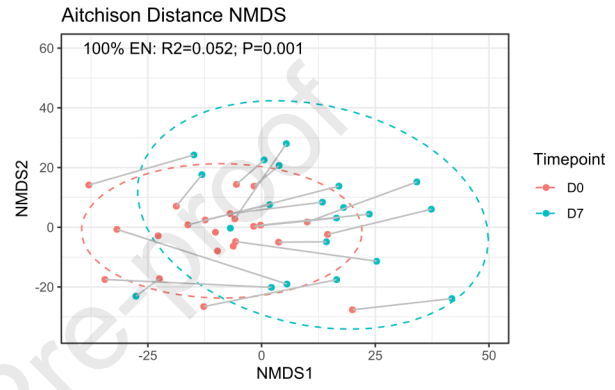
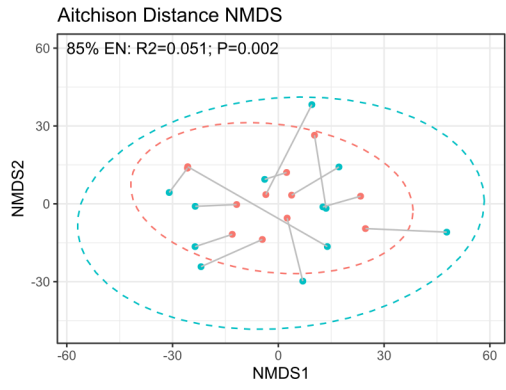
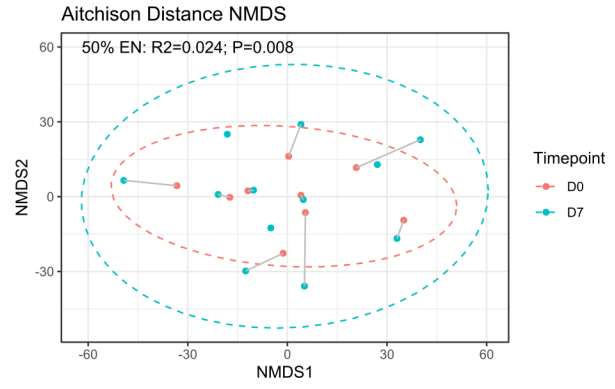
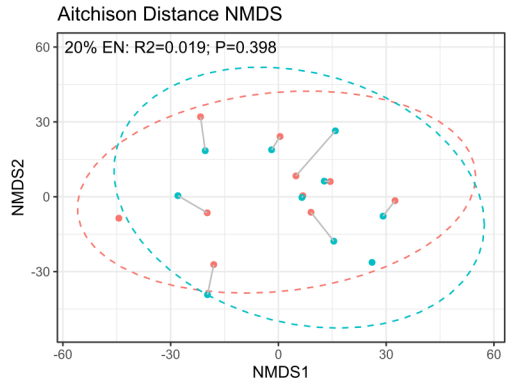
311 **Table 1:** Baseline participant characteristics, and dietary intakes assessed with food diaries during the
312 interventions. Data presented as median (Q1-Q3) unless stated otherwise. Abbreviations used: BMI:
313 Body Mass Index; BMR: Basal Metabolic Rate; EAR: Estimated Average Requirement; EN: Enteral
314 Nutrition; F: Female; M: Male; TEE: Total Energy Expenditure.

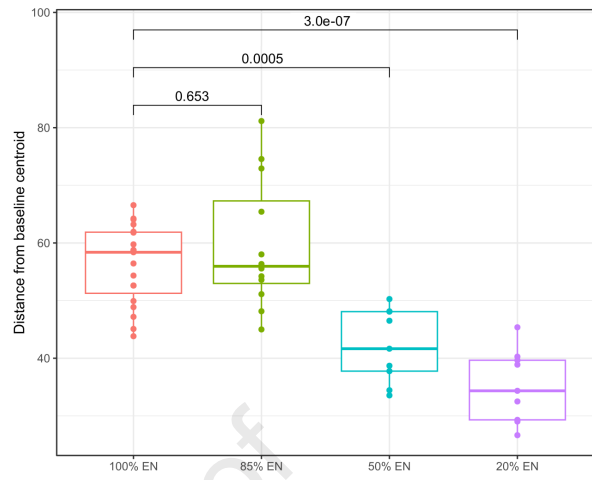
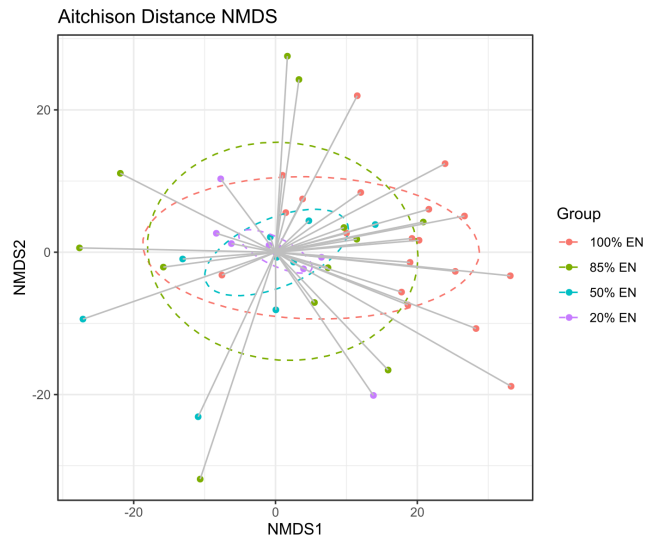
315 *P-values for between-group comparisons with general linear modelling with Box-Cox transformation. P-values for comparisons
316 of macronutrients from food (habitual diet) and food groups are between 85%, 50% EN and 20% EN groups. P-values for
317 comparisons of food groups are based on kilocalorie adjusted values (/1000 kcal).

318 ^A Significantly different than 85% EN group (P<0.05); ^B Significantly different than 85% EN group (P<0.01); ^C Significantly
319 different than 85% EN group (P<0.001); ^D Significantly different than 50% EN group (P<0.05); ^E Significantly different than 50%
320 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
321 Significantly different than 20% EN group (P<0.01); ^I Significantly different than 20% EN group (P<0.001) for Fisher pairwise
322 comparisons after general linear modelling.

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