Cohort Specific Effects of Cereal-bar Supplementation in Overweight Patients With or Without Type 2 Diabetes Mellitus

Short Title: Cohort specific effects of diet supplementation

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Abstract

The importance of gut microbes to metabolic health is becoming more evident and nutrition-based therapies to alter the composition of bacterial communities to manage metabolic disease are an attractive avenue to ameliorate some effects of Western diets. While the composition of gut microbial communities can vary significantly across disease states, it is not well known if these communities have common responses to nutritional interventions. To better understand diet-bacterial community interactions, we collected biological parameters and fecal samples of overweight non-diabetic (OND) and diabetic (OD) individuals before and after daily supplementation of 2.8 g β-glucan on their habitual diet for 30 days. Fecal bacterial communities in an age-matched cohort were measured by sequencing partial 16S rRNA genes and imputed metagenomic content. Unexpectedly, we observed disconnected responses of biological measurements and the bacterial community. Based on average effect size, biological measurements were greater in the OND group while effects on the bacterial community were greatest on the OD cohort, and we suspect these observations are due to the significantly lower alpha diversity in the OD cohort. Our data indicate that responses to cereal-bar supplementation are cohort specific and this should be considered when manipulating the microbiome via diet supplementation.

Introduction

As a result of a globalization of the economy, obesity and diabetes are becoming major issues not just for the developed but the developing world. The

50 global adoption of a Western lifestyle includes dietary modification to diets low
51 in fiber and high energy density. Deaths accounted for by non-communicable
52 diseases have now overtaken deaths due to communicable diseases virtually
53 world wide [1]. This statistic highlights the dilemma for providing high quality
54 foods and avoiding over nutrition for both the developed and developing world.
55 The gut bacterial community serves as one conduit by which the
56 consequences of nutrition and dietary choices integrate into human health, and
57 therefore must be considered in the context of any nutritional intervention.
58 Recent work shows abundances of dominant taxa in the distal gut vary widely
59 across individuals and habitual food choices [2-6]. As Firmicutes and
60 Bacteroidetes are by far the most prevalent taxa in the human gastro-intestinal
61 tract [7], their presence and response to diet can have a significant impact on
62 human health. For instance, members of the *Fecalbacterium* (a firmicute)
63 produce short-chain fatty acids such as butyrate [8] that serve as an energy
64 source for colonic epithelia. *Bacteroides and Ruminococcaceae* (both
65 Bacteroidetes) degrade polysaccharides found in foods containing fiber [9, 10].
66 Conversely, abundant taxa such as the Proteobacteria are typically associated
67 with dysbiosis observed in persons with inflammatory bowel disorders [11, 12]
68 or in the guts of those consuming a high fat or typical Western style diet [2].
69 Despite the inter- and intra-individual variability in the abundance of bacterial
70 taxa, these and other studies demonstrate the importance of the bacterial
71 communities to integrate diet and host metabolism. Dietary supplements aiming
72 to leverage the benefits of microbial function therefore may become practical
73 solutions to Western lifestyle associated diseases if their efficacy and the

molecular mechanisms underlying the bacteria-diet interactions could be demonstrated.

Diet supplemenation is one approach proposed to minimize the effects of Western life styles by potentially altering the composition and metabolism of the gut bacterial community [13, 14]. However it is not widely known if a diet supplement has the same effect on microbiomes in subjects of related but distinct disease states. For instance, there are numerous studies that document fiber induced alterations in the microbiomes of monotonic cohorts [15-19]. Hooda et al. [20] demonstrated the significant effect of corn derived fibers on abundances of genera of belonging to Firmicutes, Bacteroidetes and Verrucomicrobia in generally healthy subjects, while Benus et al. [21] further demonstrated that pea fiber and fructo-oligosaccharides supplementation was associated with increased abundances of butyrate producing bacteria in healthy subjects. Though these studies have given us great insight into the effect of fiber on the microbiome, each used unique diet amendments in cohorts that consisted almost exclusively of healthy, overweight, obese, or subjects with diagnosed metabolic disease. Given that bacterial community composition and diversity vary significantly across disease states, it is reasonable to assume that effects in one cohort may not be applicable to others. This lack of knowledge may be important when identifying patients whose microbiomes may be more or less responsive to diet supplementation and thus resistant to improvement of host metabolic markers potentially mediated by gut bacteria. Thus quantifying the responsiveness of the microbiome in similar but distinctive cohorts may help to define the limits of diet supplementation via changes in the microbiome.

Overweight diabetic (OD) and overweight non-diabetics (OND) represent two metabolic states that can be distinguished by the degree of insulin resistance. However, it is not known whether the two populations exhibit cohort specific effects to diet supplementation on the bacterial communities and host metabolism. To answer this question, we conducted a diet supplementation study in OD and OND subjects with a cereal-bar containing 1.4 g β-glucan. We investigated how the bacterial communities responded to consumption of two cereal-bars per day and determined whether there were unique features of the response in OND vs OD subjects. Specifically we predicted that cereal-bar supplementation would increase abundances of Bacteroidetes and Verrucomicrobia while decreasing Firmicutes and Proteobacteria in both cohorts with concomitant improvement in host metabolic markers.

Results

Biological Parameters

We recruited 46 overweight and obese individuals with and without diabetes to assess the effect of a β-glucan containing cereal-bar supplementation on biological parameters and the distal gut bacterial community. The subject characteristics are summarized in S1 Table. Subjects went through a diet normalization phase followed by consumption of 2 cereal-bars per day for 30 days to deliver 2.8g β-glucan fiber to the habitual diet. Twenty-six individuals were excluded for compliance issues, lack of sequencing success, or fell outside the age-range, leaving 10 individuals in each group for analysis (S5, S6). Non-parametric testing indicated no significant difference in any biological

measurement between the cohorts at the pre-supplementation time point. However, LDL and triglyceride concentrations were statistically different between the OND and OD post supplementation (Table 1, S2 Table), though these differences were not significant improvements from their pre-supplementation values. The remaining biological parameters were unaffected by consumption of the cereal-bar. Lastly, Kruskal-Wallis tests showed there were no differences within each cohort between pre- and post-supplementation time points (Table1).

Table 1. Biological parameters of age-matched cohorts at enrollment, pre-supplementation, and post-supplementation. The mean and standard deviation (in parentheses) are shown. Except for *Age and *Height, the rest of parameters were included in biological effect size calculation. Superscript "a" indicates significant difference between cohorts post-supplementation.

	OND Mean			OD Mean		
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation
*Age	48 (3.5)	48 (3.5)	48 (3.5)	47 (4.3)	47 (4.3)	47 (4.3)
*Height (cm)	167 (10.3)	167 (10.3)	167 (10.3)	171 (10)	171 (10)	171 (10)
Weight (kg)	95 (14.7)	95 (14.1)	94 (14.4)	98 (17)	96 (17)	96 (16)
BMI (kg m^2)	34 (5.2)	34 (5.2)	34 (5.3)	34 (4.66)	33 (4.5)	33 (4.4)
Waist Circumference (cm)	104 (11.4)	105 (11.3)	106 (11.3)	113 (10)	113 (10)	113 (12)
Hip Circumfrenece (cm)	117 (8.1)	117 (8.3)	116 (8.2)	116 (12)	114 (11)	115 (12)
Diastolic BP (mmHg)	95 (16)	87 (5.1)	89 (13)	87 (9.86)	85 (9.63)	88 (14)
Systolic BP (mmHg)	131 (15)	124 (13)	131 (21)	130 (17)	129 (22)	131 (17)
Cardiac Frequency (beats min^{-1})	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)
Total Cholesterol (mmol L^{-1})	5 (0.74)	4.9 (0.87)	5.2 (0.69)	4.9 (1.3)	4.6 (0.82)	4.7 (0.49)
Triglyceride (mmol L^{-1})	1.1 (0.42)	1.2 (0.51)	1.2 (0.43)	4.1 (5.8)	3.6 (4.3)	2.9 (2.1)
HDL cholesterol (mmol L^{-1})	1 (0.23)	1 (0.3)	1.1 (0.25)	0.9 (0.24)	0.8 (0.24)	0.8 (0.16)
LDL (mmol L^{-1})	3.5 (0.61)	3.4 (0.68)	3.6 (0.65)[a]	2.8 (0.74)	2.7 (0.7)	2.7 (0.67)[a]
Glucose (mmol L^{-1})	5 (0.68)	5 (0.63)	5.1 (0.46)[a]	6.4 (2)	6.5 (2.2)	6.3 (1.5)[a]
Insulin (uIU ml^{-1})	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)
FFA (mmol L^{-1})	0.5 (0.13)	0.5 (0.2)	0.6 (0.23)	0.6 (0.25)	0.5 (0.19)	0.5 (0.19)
HOMA_IR	3.9 (1.8)	4.4 (2.1)	4.3 (1.6)	6.2 (5.8)	5.1 (3.7)	5.5 (5)
LBP (ug ml^{-1})	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)

Taxon abundance and KEGG functions pre- and post-supplementation

141 Bacterial communities in each cohort were dominated by Firmicutes and
142 Bacteroidetes, followed by the Actinobacteria, Proteobacteria and
143 Verrucomicrobia (Fig 1A-1D, S3 Table). Abundances at the pre-supplementation
144 time point were, however, not statistically different between OND and OD groups
145 (Fig 1A-1D). Likewise the abundances of KEGG functions from predicted
146 metagenomes were not different between the cohorts pre-supplementation (S4
147 Table). However, we did observe a significant difference in alpha diversity
148 between OND and OD groups at enrollment and before the intervention (Fig 1E,
149 S3 Table). The OND cohorts had significantly greater community richness
150 compared to the OD group (Fig 1E, S3 Table).
151
152 Fig 1. **Proportional abundances of dominant taxa and Shannon diversity at**
153 **enrollment (EN), pre-supplementation (Pre) and post-supplementation**
154 **(Post).** Bar graphs for proportional abundances of dominant taxa are indicated
155 as (A) Firmicutes, (B) Bacteroidetes, (C) Proteobacteria, and (D)
156 Verrucomicrobia. (E) Shannon diversity. Filled bars represent the OND cohort,
157 open bars represent the OD group. Error bars depict the standard deviation for
158 the mean of 10 subjects. p-values for significant differences are shown.
159
160 Post-supplementation, we observed a decline in Firmicutes (Fig 1A) with
161 simultaneous increase in Bacteroidetes (Fig 1B) abundances in the OD cohort.
162 However, only the Firmicutes were statistically different between the cohorts
163 even though the Bacteroidetes increased by 12% over the pre-supplementation
164 abundance in the OD group (S3 Table). The remaining taxon abundances were
165 not significantly impacted by the supplementation but we did observe that the

166 Proteobacteria (Fig 1C) consistently decreased, while the Verrucomicrobia (Fig
167 1D) consistently increased in abundance after supplementation. The abundance
168 of individual KEGG functions were not significantly altered within or between
169 cohorts post-supplementation (S3 Table). Alpha diversity (Shannon Index)
170 remained significantly higher in the OND cohort at the last time point (Fig 1E).
171

172 Changes in effect size pre- and post-supplementation

173 We next examined the response to the β-glucan cereal-bar
174 supplementation by comparing average effect size across the biological
175 parameters, phyla, and predicted metagenomic content within each cohort (Fig
176 2, S2- S4 Tables). Firstly, effect size was not significantly different after
177 supplementation in the OND cohort when compared to pre-supplementation
178 abundances ($p > 0.3$ in all cases, Fig 2A-2C). Mean effect size was largest for the
179 phyla (mean 0.26, range 0.04-0.62, Fig 2B), followed by the biological (mean
180 0.20, range 0.04-0.42, Fig 2A) and predicted metagenome content (mean 0.11,
181 range 0.00-0.37, Fig 2C). In contrast, β-glucan supplementation had a significant
182 effect in the OD cohort for all data categories (Fig 2A-C). Effects for the phyla and
183 predicted metagenome content were significantly greater after supplementation
184 while ($p < 0.03$, Fig 2B) while physiology effect size was significantly greater pre-
185 supplementation for the OD ($p = 0.04$, Fig 2A, S3 Table).
186

187 Fig 2. **Effect size of cereal-bar supplementation on measured parameters.**
188 Box and whisker plots showing the mean, the minimum and maximum values for
189 (A) biological, (B) phyla, and (C) predicted metagenome. Kruskal-Wallis

comparisons with p-values are shown. EN-Pre = effect size for enrollment and pre-supplementation time points; Pre-Post = effect size for pre-supplementation and post-supplementation time points. Data can be found in S2-S4 Tables.

Finally, we compared the average effect size between cohorts using the Kruskal-Wallis test to determine which cohort was more responsive to the cereal-bar supplementation. Comparing the two treated groups illustrated significant differences in average effect size on the biological parameters, phyla, and predicted metagenomic content ($p < 0.02$, Fig 2A-C). Average effect size on biological parameters was higher in the OND compared to OD (0.19 and 0.09, respectively, Fig 2A, S2 Table), whereas average effects on the phyla and predicted metagenomic content were both higher in the OD cohorts (Fig 2B and 2C). Differences in effect sizes were most apparent for the predicted metagenomic content as there was a 4 fold greater effect in the OD versus the OND group (0.49 vs 0.11, respectively, $p = 4.3 \times 10^{-9}$, Fig 2C).

Discussion

We tested a cereal-bar supplementation containing β-glucan for 30 days in OD and OND subjects with the goal of quantifying the effect in two similar disease cohorts. We chose a cereal-bar with the goal of increasing fiber consumption and minimizing side effects of sudden increase of fiber intake. However, we found that consumption of 2 cereal-bars per day did not significantly affect blood glucose in either cohort nor did we observe improvement in any of the other individual biological parameters. The

214 ineffectiveness of the cereal-bar supplementation could result from several
215 possibilities. For instance, the amount of fiber delivered in this study was
216 relatively small even though it is near the FDA recommended amount for
217 cholesterol reduction (CFR 21 101.81, [22]). Martinez et al., [17] has showed
218 improvement in blood glucose with 60 g of additional oat fiber over four weeks
219 in healthy individuals while DeAngelis et al. [15] showed a similar result with
220 pasta containing β-glucan fiber in health individuals. In addition our subjects
221 were asked not to alter their normal dietary habit and the habitual fiber
222 consumption was not controlled, possibly confounding the effect of our cereal-
223 bar supplementation. We must also consider the possibility that our
224 observations are related to the medications taken by the OD subjects as we
225 asked the subjects not to change their drug treatment during the study.
226 Regardless of the reasons behind our observations, we found no significant effect
227 of cereal-bar supplementation on biological parameters in our cohorts.
228 As we had predicted, the abundance of Bacteroidetes responded
229 positively to the cereal-bar supplementation in the OD cohort while there was a
230 simultaneous decrease in the abundance of Firmicutes, suggesting small
231 additions of β-glucan in cereal-bars can alter taxonomic abundances in gut
232 bacterial communities. These observations are in line with studies highlighting
233 the saccharolytic nature of the Bacteroidetes in the human gut [23].
234 Interestingly, we did not observe any significant change in Bacteroidetes or
235 Firmicutes abundances in the OND participants. One possible explanation is that
236 the specific strains of Bacteroidetes and Firmicutes in the OND group are
237 somehow resistant to change with such a small infusion of nutrients. As
238 community analysis using 16S rRNA genes does not allow for strain level

239 resolution, an alternative approach may be necessary in order to identify specific
240 members of the microbial community that are resistant or susceptible to cereal-
241 bar supplementations. However, it is also possible that abundances of
242 Bacteroidetes and Firmicutes in the OND cohort are at levels where small diet
243 modifications can not overcome the inherent functional state of the bacterial
244 communities requiring more intense interventions to shift the abundance of
245 these taxa. As these two taxa represented more than 93% of the sequences in
246 this study, the possibility exists that consuming fiber containing cereal-bars may
247 be insufficient to garner consistent and significant changes of dominant taxa
248 across all patient cohorts.

249 Changes in abundance of Proteobacteria and Verrucomicrobia matched
250 our *a priori* predictions in both cohorts. The decline of Proteobacteria is
251 concomitant with studies indicating these bacteria are negatively associated with
252 diets high in fiber and positively associated with high fat diets [24, 25]. The
253 reduced abundance of the Proteobacteria has been linked to declines in
254 inflammatory markers and general host inflammation [26]. The reduction of
255 these organisms in human gut microbial communities is proposed to be
256 beneficial. Conversely, increases in Verrucomicrobia are associated with healthy
257 gut microbial communities [27] and diets high in fiber. The changes observed in
258 both cohorts suggest that small supplements of fiber delivered via cereal-bar can
259 alter abundances of these taxa. Though abundances of Proteobacteria and
260 Verrucomicrobia did not change significantly, our data nevertheless indicates
261 these taxa are responsive to diet modification across similar disease cohorts
262 possibly reflecting a general life history strategy of these organisms.

263 The alternative hypothesis that 2.8g β-glucan per day would have a
264 significant impact on average effect size in both cohorts was not supported.
265 Instead we observed significantly larger effects on the phyla and predicted
266 metagenomic content of the OD cohort compared to the OND individuals and
267 postulate that the lower species richness in the OD group is responsible for these
268 observations. Greater mean effect sizes in the OD group indicate these gut
269 communities were more susceptible to our cereal-bar supplementation than
270 were the OND group and coincide with recent report documenting richness as
271 factor in determining microbial responses to fiber supplementations [18]. Our
272 data build upon this observation and further suggest significantly changing
273 microbiomes with β-glucan containing cereal-bars may be limited to disease
274 cohorts harboring microbial communities that are relatively species poor as
275 these ecosystems have lost the functional flexibility needed to cope with a
276 changing environment. Unfortunately our experimental design did not allow us
277 to identify robust causal relationships between microbial communities and low
278 intensity diet supplementation (e.g. we used a single dosing level with small
279 cohort size). Nonetheless, our data suggest that small diet modifications
280 delivered by a cereal-bar have the potential to disproportionally affect low
281 diversity microbial communities and thus may be useful in studies addressing
282 ecological questions *in situ*, such as community stability, without altering host
283 physiology.
284 Given the significant effect on the OD bacterial communities, we would
285 have expected there to be a parallel effect on the biological parameters of these
286 participants as gut microbes and host phenotypes are clearly linked [7, 28, 29].
287 However, even though effect sizes were small in both cohorts, the physiology of

288 the OND cohort was more affected by the cereal-bar supplementation. The
289 intrinsic metabolic state of the host could explain this observation. In our
290 subjects it may be possible that insulin resistance, pancreatic beta cell functions
291 and even drug treatment dominate the biological set point of the diabetics and
292 small shifts in diet or bacterial communities had limited influence on the
293 metabolic outcomes. Unlike the diabetics, these same intrinsic factors in non-
294 diabetics may not be of equal intensity leaving a window for relatively small
295 dietary supplementations to have some degree of influence on the host. Low
296 dose fiber supplementation in healthy individuals reported by Martinez et al.
297 [17] would support this observation. Altogether, the results of this study suggest
298 bacterial community diversity in different cohorts may play a role in the
299 response of microbes to dietary supplementation aimed to manage host
300 metabolism.
301

302 Materials and Methods

303 Study population

304 A total of 46 overweight subjects with Body Mass Index (BMI) between 20
305 and 30 kg/m^2 were recruited in the Service of Therapeutic education for Chronic
306 Diseases of the University Hospitals of Geneva. After the inclusion and according
307 to the results of the initial screening, patients were assigned to either group 1 –
308 with type 2 diabetes mellitus (OD, n=21) or group 2 – without type 2 diabetes
309 mellitus (OND, n=25). We defined type 2 diabetes mellitus as fasting plasma
310 glucose > 7.0 mmol/l and/or HbA1c >7% and/or the presence of any glucose-
311 lowering treatment. OND subjects were matched for age, gender, BMI and ethnic

312 background of the OD subjects. Exclusion criteria were based on use of drugs
313 altering intestinal permeability (nonsteroidal anti-inflammatory drugs,
314 corticoids) or intestinal digestion and absorption (Orlistat, Colestipol,
315 anticoagulants, α-glucosidase inhibitors) and antibiotics administered in the 4
316 weeks preceding inclusion; previous abdominal surgery, gastro-intestinal
317 diseases interfering with intestinal absorption, cancer, bulimia, pregnancy;
318 parenteral nutrition or other ongoing dietary intervention, and diarrhea (>2
319 stools/day) within 7 days before enrolment. From this initial population, 26
320 were excluded for compliance with the supplementation protocol, they failed to
321 contribute samples or the sequencing effort was insufficient at any visit. The
322 resulting subset had a mean age of 42 yrs and 51 yrs for OND and OD,
323 respectively. Ten individuals for each cohort with overlapping age range were
324 selected for microbial analysis. Patient data is summarized in S1 Table. The study
325 is registered at ClinicalTrials.gov under the study number NCT02694172
326 (https://clinicaltrials.gov/ct2/results?term=NCT02694172).
327

328 # Study design and intervention

329 A case controlled, single center prospective clinical trial design was used.
330 The study consisted of 4 visits and 3 periods. The 3 periods were i. recruiting
331 period (10 to30 days), ii. Diet-normalization period (14 days), and iii.
332 intervention period (30 days). After their recruitment, subjects received
333 instructions by a nutritionist for dietary normalization. After the dietary
334 normalization period, subjects received instructions for taking Nutren Balance
335 cereal-bars per day (one between breakfast and lunch and the other one

336 between lunch and dinner), rich in viscous soluble fiber β-glucan. Each cereal-
337 bar contained 65 Kcal. The total carbohydrate is 10.3 g that includes 3.8 g of
338 sugars and 2.5 g of fructose. The total protein is 1.9 g per bar. There is 1.8 g of
339 total fat of which saturated fat is 0.7 g, monounsaturated fat is 0.7 g and
340 polyunsaturated fat is 0.3 g. Total dietary fiber is 4.4 g of which 1.4 g is β-glucan.
341 Sodium content is 33 mg in each bar. The cereal-bar was well tolerated by all
342 patients without clinically significant side effects. Subjects submitted blood and
343 fecal samples at the enrollment, pre- and post- cereal-bar supplementation to
344 monitor host physiology and bacterial community composition. The study
345 protocol (06.42NRC) was approved by the Geneva ethical committee.
346 Participants were informed about the aims of the study and gave their written
347 consent.
348

349 Fecal Sample Collection, DNA extraction, PCR and 350 sequencing

351 Stools were collected in sterile plastic 50 mL containers and frozen at -
352 80°C until processing. Frozen samples were partially thawed and from which
353 0.25 g of fecal matter was placed in the lysis tube and extracted according to the
354 manufacturers instructions. DNA was frozen at -20°C until its use in PCR
355 reactions to generate barcoded amplicons for sequencing on the MiSeq platform
356 [30]. Briefly, individual samples were amplified in triplicate, pooled then the
357 PCR products were quantified using PicoGreen dsDNS reagent. Equal amounts of
358 amplicon from each sample were then combined and sequenced on the MiSeq

359 platform. Sequencing was performed at the Nestlé Institute of Health Sciences
360 Functional Genomics Core facility.
361

362 Sequence analysis

363 Sequence data were quality filtered and demultiplexed in Qiime 1.8 [31]
364 using the default settings for the split_libraries_fastq.py command followed by
365 closed reference OTU picking at 97% sequence similarity against the gg_13_5
366 release (http://greengenes.secondgenome.com/downloads/database/13_5)
367 with the parallel_pick_otus_uclust_ref.py command. We additionally filtered out
368 Cyanobacteria to avoid chloroplast sequences and filtered out low abundance
369 OTUs according to Bokulich et al. 2013 [32] using the
370 filter_otus_from_otu_table.py command. Samples were then rarefied to 50000
371 sequences per sample (single_rarefaction.py) from which the relative abundance
372 of taxa (classified to the phylum level using summarize_taxa.py) was calculated
373 and used for downstream analysis. An estimate of bacterial richness was
374 performed using the Shannon index (alpha_diversity.py) on the rarefied data.
375 Predicted metagenomic content was also calculated using PiCRUST [33] and
376 summarized to KEGG level 2 for statistical evaluation. All bacterial community
377 data was expressed as the proportional abundance in each sample.
378

379 Data analysis

380 From the initial cohort we chose an age-matched sub set of patients to
381 assess changes in host metabolic measurements, taxonomic abundance, and
382 KEGG functions in response to the β-glucan cereal-bar supplementation. Using

Kruskal-Wallis tests implemented in Spotfire® (Göteborg, Sweden), we compared group means within (e.g. pre- vs post-supplementation in each cohort) and between cohorts (e.g. OND vs OD pre- supplementation and post-supplementation) with α = 0.05 for all individual measurements (e.g. blood glucose, taxon abundance, and individual KEGG functions, etc.) to identify changes in these measurements. Bonferroni multiple comparison correction was applied to the p-values for the taxonomic abundances and KEGG functions. We next evaluated the magnitude of response of a data category (biological, phyla, and predicted metagenome; S2-S4 Tables) by testing average effect size between cohorts before and after cereal-bar supplementation [34]. The absolute values of the effect size, calculated by Eq.1 where Es= effect size, m = mean, and σ = standard deviation, were compared using Kruskal-Wallis tests to determine of there were statistical differences in response.

Eq. 1

$$\mathbf{Es} = \frac{(m_1 - m_2)}{\sqrt{\frac{\sigma_1^2 + \sigma_2^2}{2}}}$$

Acknowledgements

We would like to thank Bernard Berger of the Nestlé Research Center Lausanne for his assistance, Patrick Descombes and Deborah Moine of the NIHS Functional Genomics core for their technical help with sequencing the 16S rRNA amplicons.

Supporting Information

S1 Table. Mean and standard deviation of biological parameters of 46 subjects at recruitment.

S2 Table. Means, standard deviations, and effect sizes for biological parameters for the age-matched cohort. *Age and *height are not included in the mean effect size calculation. Superscript "a" indicates significant difference between cohorts post-supplementation.

S3 Table. Means, standard deviations, and effect sizes for taxa and Shannon Diversity for the age-matched cohort.

S4 Table. Means, standard deviations, and effect sizes for the KEGG functions predicted by PiCRUSt for the age-matched cohort.

S5. Clinical Protocol

S6. TREND Flowchart

S7. TREND Checklist

429

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431

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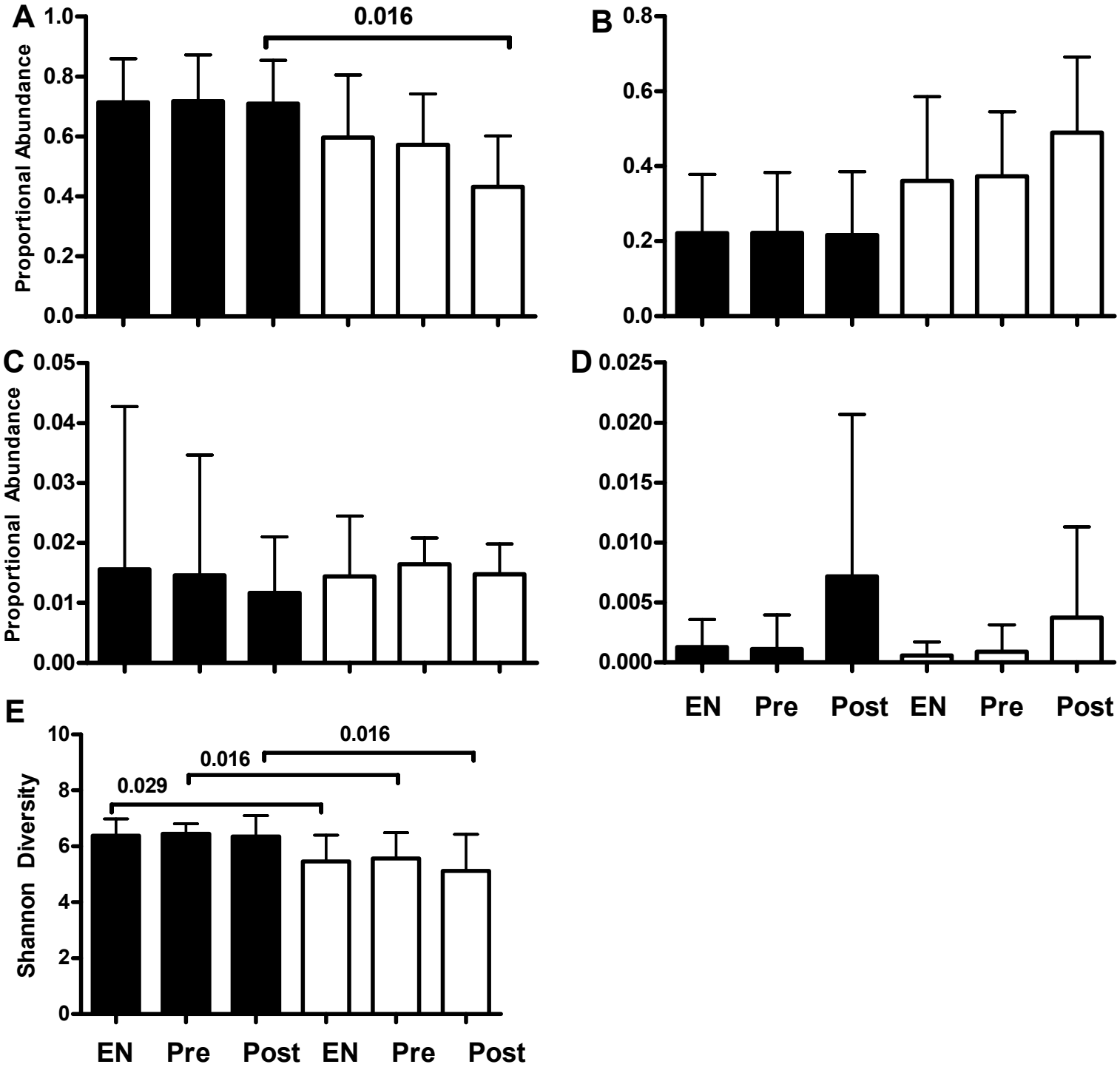
564

	OND Mean			OD Mean		
	Enrollment	Pre-supplementation	Post-supplementation	Enrollment	Pre-supplementation	Post-supplementation
*Age	48 (3.5)	48 (3.5)	48 (3.5)	47 (4.3)	47 (4.3)	47 (4.3)
*Height (cm)	167 (10.3)	167 (10.3)	167 (10.3)	171 (10)	171 (10)	171 (10)
Weight (kg)	95 (14.7)	95 (14.1)	94 (14.4)	98 (17)	96 (17)	96 (16)
BMI (kg m^2)	34 (5.2)	34 (5.2)	34 (5.3)	34 (4.66)	33 (4.5)	33 (4.4)
Waist Circumference (cm)	104 (11.4)	105 (11.3)	106 (11.3)	113 (10)	113 (10)	113 (12)
Hip Circumfrenece (cm)	117 (8.1)	117 (8.3)	116 (8.2)	116 (12)	114 (11)	115 (12)
Diastolic BP (mmHg)	95 (16)	87 (5.1)	89 (13)	87 (9.86)	85 (9.63)	88 (14)
Systolic BP (mmHg)	131 (15)	124 (13)	131 (21)	130 (17)	129 (22)	131 (17)
Cardiac Frequency (beats min^{-1})	67 (11)	68 (12)	72 (16)	79 (24)	78 (13)	77 (14)
Total Cholesterol (mmol L^{-1})	5 (0.74)	4.9 (0.87)	5.2 (0.69)	4.9 (1.3)	4.6 (0.82)	4.7 (0.49)
Triglyceride (mmol L^{-1})	1.1 (0.42)	1.2 (0.51)	1.2 (0.43)	4.1 (5.8)	3.6 (4.3)	2.9 (2.1)
HDL cholesterol (mmol L^{-1})	1 (0.23)	1 (0.3)	1.1 (0.25)	0.9 (0.24)	0.8 (0.24)	0.8 (0.16)
LDL (mmol L^{-1})	3.5 (0.61)	3.4 (0.68)	3.6 (0.65)[a]	2.8 (0.74)	2.7 (0.7)	2.7 (0.67)[a]
Glucose (mmol L^{-1})	5 (0.68)	5 (0.63)	5.1 (0.46)[a]	6.4 (2)	6.5 (2.2)	6.3 (1.5)[a]
Insulin (uIU ml^{-1})	17 (7.1)	19 (8.1)	19 (7.6)	20 (13)	17 (10)	18 (12)
FFA (mmol L^{-1})	0.5 (0.13)	0.5 (0.2)	0.6 (0.23)	0.6 (0.25)	0.5 (0.19)	0.5 (0.19)
HOMA_IR	3.9 (1.8)	4.4 (2.1)	4.3 (1.6)	6.2 (5.8)	5.1 (3.7)	5.5 (5)
LBP (ug ml^{-1})	28 (6)	27 (3.8)	29 (4.8)	31 (8.7)	27 (7.4)	29 (7.7)


A
B
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Proportional Abundance
Shannon Diversity
0.016
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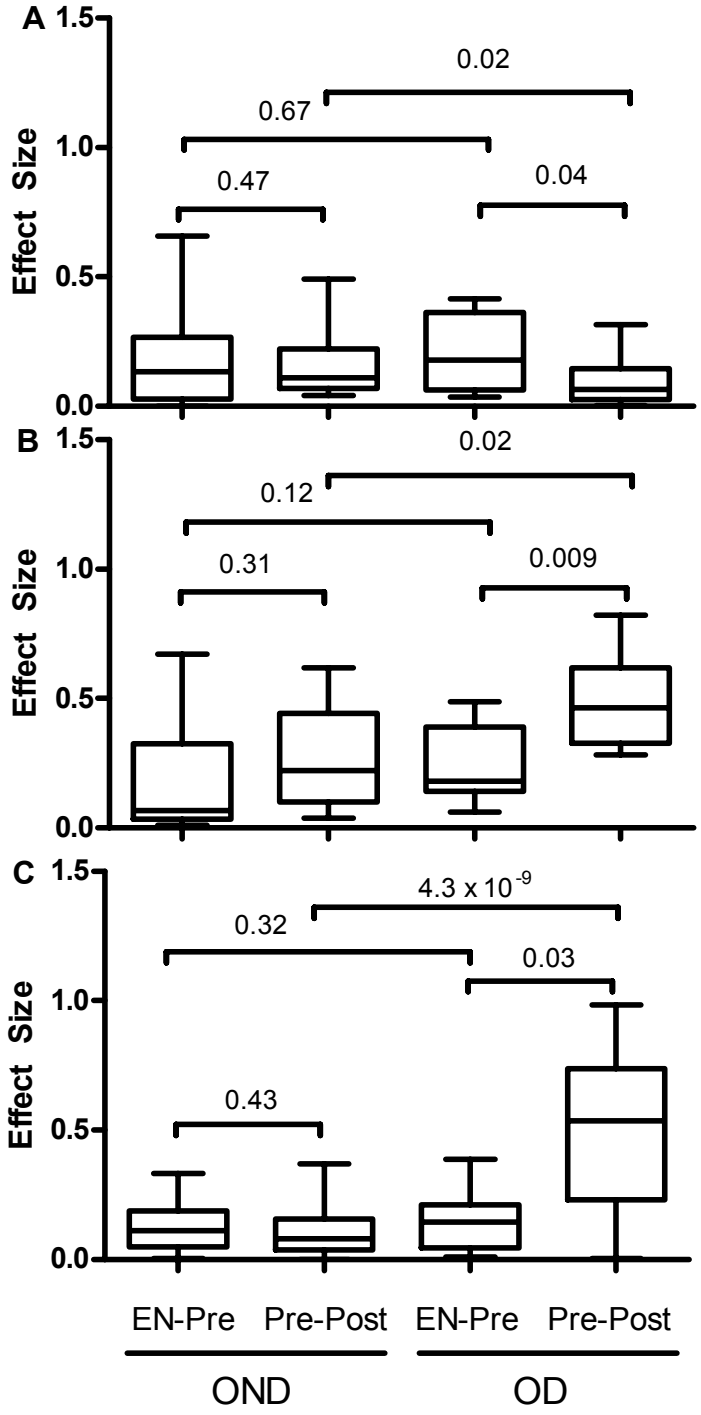
A
B
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Effect Size
1.5
1.0
0.5
0.0
0.02
0.67
0.47
0.04
0.12
0.31
0.009
4.3 x 10-9
0.32
0.03
0.43
EN-Pre
Pre-Post
EN-Pre
Pre-Post
OND
OD