

1 **Title**

2 Cranberries attenuate animal-based diet-induced changes in microbiota composition and
3 functionality: a randomized crossover controlled feeding trial

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26 **Keywords**

27 Clinical trial; cranberries; microbiota; polyphenols; bile acids; short-chain fatty acids

28

29 **Abstract**

30 Cranberries have multiple health effects but their impact on gut microbiota has not been
31 examined in randomized controlled feeding trials. We evaluated the relationship between the
32 microbiota and cranberries in the context of an animal-based diet. In a randomized, double-blind,
33 cross-over, controlled design trial, 11 healthy adults consumed for 5 days each a control diet
34 (animal-based diet plus 30 g/day placebo powder) and a cranberry diet (animal-based diet plus
35 30 g/day freeze-dried whole cranberry powder). The animal-based diet included meats, dairy
36 products, and simple sugars. Stool, urine, and blood samples were obtained before and after each
37 intervention phase. As compared to the pre-control diet, control diet modified 46 taxonomic
38 clades, including an increase in the abundance of *Firmicutes* and decrease in *Bacteroidetes*.
39 Moreover, it increased bacteria-derived deoxycholic acid and decreased acetate and butyrate in
40 stool. As compared to the post-intervention phase of control diet, the cranberry diet modified 9
41 taxonomic clades, including a decrease in the abundance of *Firmicutes* and increase in
42 *Bacteroidetes*. Further, the cranberry diet attenuated control diet-induced increase in secondary
43 bile acids and decrease in short-chain fatty acids (SCFA), and increased urinary anthocyanins
44 and bacterially derived phenolic acids. No changes were found in fecal trimethylamine and
45 plasma cytokines. In conclusion, an animal-based diet altered the microbiota composition to a
46 less favorable profile, increased carcinogenic bile acids, and decreased beneficial SCFA.
47 Cranberries attenuated the impact of the animal-based diet on microbiota composition, bile acids,
48 and SCFA, evidencing their capacity to modulate the gut microbiota.

49 **Keywords**

50 Clinical trial; cranberries; microbiota; polyphenols; bile acids; short-chain fatty acids

51

52 **Chemical compounds**

53 Trimethylamine (PubChem CID: 1146); trimethylamine *N*-oxide (PubChem CID: 1145);

54 lithocholic acid (PubChem CID: 9903); deoxycholic acid (PubChem CID: 222528); acetic acid

55 (PubChem CID: 176); propionic acid (PubChem CID: 1032); butyric acid (PubChem CID: 264).

56

57 **1. Introduction**

58 A higher consumption of plant relative to animal food based diets is associated with a lower
59 risk of all-cause mortality, particularly from cardiometabolic diseases [1]. In addition to being
60 rich in nutrients, plant foods are a source of bioactive phytochemicals and fiber, all of which
61 have beneficial effects on human health [2]. A growing body of evidence illustrates that the
62 nutritional value of foods is subject to the influence of the microbiota in the human gut [3].
63 Inversely, food shapes gut microbiota composition [4]. The complex interaction between foods
64 and the microbiota can modulate human health both positively and negatively. Gut bacteria have
65 the ability to metabolize inaccessible dietary components, synthesize essential vitamins, provide
66 protection from pathogens, and regulate the immune system [3]. However, dysbiotic
67 composition and decreased diversity and richness of the gut microbiota are also linked to several
68 chronic diseases, including atherosclerosis, hypertension, kidney disease, and type 2 diabetes [5-
69 7]. Thus, the gut microbiota has been considered an endocrine organ that has a dramatic impact
70 on the health of the host [6].

71 Secondary bile acids, short-chain fatty acids (SCFA), and trimethylamine (TMA) are well-
72 studied examples of microbiota-biosynthesized molecules [6, 8]. Bile acids are molecules that
73 facilitate digestion and lipid absorption in the small intestine but they also act as signaling
74 molecules. The liver synthesizes primary bile acids (cholic acid and chenodeoxycholic acid),
75 which are then dehydroxylated by intestinal bacteria to secondary bile acids (deoxycholic acid
76 and lithocholic acid) [9], which are considered possible carcinogens [8]. SCFA, a subset of
77 saturated fatty acids containing six or fewer carbon molecules, are the main metabolites
78 produced by colonic anaerobic bacteria following the fermentation of dietary fiber, and they have
79 been shown to exert beneficial effects on health [3, 10]. Acetate, propionate, and butyrate are the

80 primary SCFAs in the gut, constituting over 95% of the total production [11]. TMA is an organic
81 compound generated by gut bacteria from choline, phosphatidylcholine, and carnitine (all three
82 found at high concentrations in red meat, eggs, milk and fish) [12, 13]. Once absorbed and
83 transported to the liver, TMA is oxidized to trimethylamine *N*-oxide (TMAO), which is
84 considered a potential promoter of atherosclerosis and cardiometabolic diseases [6].

85 The wide variation in the gut microbiota between individuals is a result of modulations of
86 many dietary, environmental, physiological, and lifestyle factors. Clinical evidence has
87 illustrated that diet can alter the composition and diversity of human gut microbiota within 24
88 hours [14], and also modify their metabolic activity [15]. Cranberries (*Vaccinium macrocarpon*)
89 are fruits associated with multiple health benefits [16, 17], mainly attributed to (poly)phenolics,
90 including proanthocyanidins, anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids,
91 flavan-3-ols, flavonols, and terpenes. Among the 20 most commonly consumed fruits in the
92 American diet, cranberries have the highest total phenol content [18]. These (poly)phenolics
93 contribute to their noted antibacterial [19], antiviral [20], anti-tumorigenic [21], antiangiogenic
94 [22] and antioxidant activities [18]. Following ingestion of cranberries, a small proportion of
95 phenolic acids and flavonoids is bioavailable and have physiological consequences [23]. Those
96 that remain unabsorbed in the upper gastrointestinal tract can have a reciprocal relationship with
97 the microbes in the large intestine, a result of anti-microbial and bacteriostatic activities of
98 (poly)phenolics [24] and metabolic activity of gut bacteria towards (poly)phenolics [25, 26].
99 Emerging evidence including studies in animal models [27] and a recent prospective clinical trial
100 [28] showed that cranberries had an impact on gut microbiota. Nevertheless, randomized
101 controlled clinical trials with a well-controlled diet that diminishes the confounding effect of
102 individual background diet are needed to confirm these observations [29].

103 In the present work, we examined the impact of cranberry constituents on the bacteria profile
104 and bacterially derived products, including secondary bile acids, SCFAs, and TMA, in a small
105 scale, randomized, crossover, blinded, and controlled feeding trial. In the framework of the
106 study, we also aimed to confirm the absorption of cranberry anthocyanins, flavonoids, and
107 phenolic acids.

108

109 **2. Materials and methods**

110 **2.1. Study subjects and design**

111 We conducted a randomized, placebo controlled, double-blind, crossover study in 11 healthy
112 subjects (7 males, 4 females) with a regular bowel movement (≥ 3 times/week) and a mean age of
113 39.2 ± 12.3 years (ranging from 25 to 54 years). A detailed description of the inclusion and
114 exclusion criteria is available in the **Supplementary Material 1**. Baseline characteristics of
115 participants are presented in **Table 1**. The trial included two 5-day intervention phases with a 2-
116 week washout period between phases. After enrollment, eligible subjects were instructed to
117 consume their habitual diet during the one-week run-in period. Subsequently, subjects were
118 randomly assigned to receive either cranberry diet (basal diet plus 30 g/day of freeze-dried whole
119 cranberry powder) or control diet (basal diet plus 30 g/day of matched placebo powder). The
120 details on the interventional diets are described in section 2.2. During the 2-week washout
121 period, the subjects were instructed to consume their habitual diet, similar to the run-in period.
122 During the whole study, subjects were required to visit the study site for 12 times over 8 weeks
123 (Study timeline is depicted in **Fig. S1**).

124 A total of 4 stool, urine, and blood samples (one each before and after dietary intervention phase)
125 were collected from each subject for laboratory analyses. Subjects were provided with disposable

126 commode specimen containers (Claffin Medical Equipment, Warwick, RI) for collecting stool
127 samples. The whole evacuated stool was placed in a sealed bag, then in a cooler with ice packs,
128 and transported to the study site within 18 hours after the evacuation. Subjects were instructed to
129 not stop consuming the study meals until the first bowel movement of the post 5-day feeding
130 intervention. Morning spot urine samples were provided by volunteers during the study visits,
131 Urine samples were collected in 500-mL polypropylene containers and were stored at 5°C prior
132 to being aliquoted into cryovials for storage at -80°C. Fasted venous blood samples were
133 collected from a hand or arm vein. Blood was collected into 10 mL tubes containing EDTA and
134 centrifuged (1690 g, 15 min, 4 °C). The plasma samples were immediately separated and frozen
135 at -80 °C until analyses. The trial was approved by the Institutional Review Board of Tufts
136 University Health Sciences Campus and Tufts Medical Center. All participants signed a written
137 informed consent agreement before any study conducts were performed. This study was
138 registered with the public registry ClinicalTrials.gov (NCT02677649).

139 **2.1. Anthropometric measures**

140 Body weight, height, BMI, and waist and hip circumferences were measured according to
141 standardized procedures [30] and as detailed in **Supplementary Material**. The same apparatus
142 and equipment were used in all subjects.

143 **2.2. Control diet and cranberry diet**

144 Both interventional diets were prepared in the kitchen of the Metabolic Research Unit at Tufts
145 University and packed frozen study meals were provided to subjects to consume at home or
146 work. The basal diet was comprised of meats, dairy products, simple sugars, and stevia, and the
147 diet was formulated based on the David *et al.* study [15], in which an animal-based diet
148 significantly altered microbiota profile. Control diet and cranberry diet contained 30 g of placebo

149 powder and freeze-dried whole cranberry powder, respectively, whose nutrition composition is
150 presented in **Table S1**. An example of a one-day menu and its corresponding nutrient
151 composition are presented in **Tables S2 and S3**, respectively. The 30-g dosage was selected in
152 order to provide sufficient amounts of flavonoid and proanthocyanidins that were comparable to
153 the average flavonoid and proanthocyanidins intake at 157 mg and 95 mg/day, respectively, in
154 the United States [31, 32]. The caloric content of each diet was adjusted to each subjects' caloric
155 need for body weight maintenance using the Harris-Benedict equation and adjusting by activity
156 factor.

157 The freeze-dried whole cranberry powder was produced from a blend of cranberry varieties
158 similar to the market reality at the time of production (56% Stevens, and 11% each of Ben Lear,
159 Grygleski, Pilgrim, and HyRed). The berries were individually frozen after harvest, freeze-dried,
160 and ground into powder form. Silicon dioxide (3% total volume of powder) was added as an
161 anti-caking agent. The processing and packaging facilities are compliant with U.S. Food and
162 Drug Administration regulations. The placebo powder was produced from a blend of water,
163 maltodextrin (CPC Maltrin M-180), citric acid, artificial cranberry flavor (Lorann oils), fructose,
164 red color (Lorann oils), and grape shade (Esco Foods) that was then freeze-dried. The placebo
165 was manufactured by the United States Department of Agriculture (USDA), Agricultural
166 Research Service, Western Regional Research Center, Healthy Processed Foods pilot plant in
167 Albany, CA. Tea and other plant-based beverages were not allowed during the intervention
168 phases.

169 **2.3. Gut microbiota analysis**

170 Gut microbiota in the fecal samples was determined by 16S rRNA pyrosequencing in the
171 Phoenix laboratory of the Tufts Medical Center. Fecal DNA was extracted by enzymatic

172 digestion and bead-beating steps, followed by the use of a QIAamp Stool DNA Mini Kit
173 (Qiagen). 16S rRNA gene amplicons were generated from the extracted DNA using PCR with a
174 barcoded primer set targeted to the V4 variable region [33]. Amplicons were pooled in equimolar
175 amounts and then purified for 250 bp paired end sequencing using an Illumina MiSeq platform
176 (Illumina Inc.). A custom pipeline was used for sequence data analysis, using the Quantitative
177 Insights Into Microbial Ecology (QIIME) software v1.8.0 [34]. After quality filtering, paired-end
178 sequences were concatenated and demultiplexed. Closed reference Operational taxonomic units
179 (OTU) at 99% similarity were assigned using Greengenes v13_5 reference database [35] and
180 USEARCH v6.1 [36]. Alpha diversity indices Chao 1, Shannon, and Simpson's were calculated
181 using QIIME. Beta diversity was evaluated using unweighted and weighted UniFrac metrics
182 through QIIME. Differences in OTU abundance were analyzed by linear discriminant analysis
183 (LDA) effect size (LEfSe) method according to Segata *et al.* [37]. LEfSe analysis shows those
184 OTUs that were significantly differentially abundant between comparisons, ranked by effect size.
185 The bacterial taxa with significant differences were used to build the LDA model and to estimate
186 its effect as a discriminant feature. The threshold used to consider a discriminative feature for the
187 logarithmic LDA score was set to >2 (further details are provided in subheading 2.6. *Statistical*
188 *analysis*).

189 **2.4. Analysis of (poly)phenolics, bile acids, SCFA, and TMA**

190 **2.4.1. Urinary (poly)phenolics and anthocyanins**

191 Urinary phenolic acid and flavonoids were determined by a routine HPLC-electrochemical
192 detection method established in our laboratory as previously described [38]. Data were
193 normalized by creatinine concentrations and are expressed as $\mu\text{g}/\text{mg}$ creatinine.

194 Anthocyanins in urine were quantified by a LC-MS/MS as previously described [39]. Data were
195 normalized by creatinine concentrations and are expressed as pg/mg creatinine.

196 ***2.4.2. Fecal Bile acids***

197 Individual bile acids, including lithocholic, cholic, deoxycholic, and chenodeoxycholic acids, in
198 feces were determined by LC-QTOF/MS. Stool samples were freeze-dried and stored at -80°C
199 before the analysis. The sample preparation method requires a two-day procedure and included
200 overnight extraction from freeze-dried samples using a mixture of chloroform and methanol,
201 followed by a multi-stage purification process. In brief, a 10.0 mg aliquot of sample was
202 weighted and then spiked with 50 µL of internal standard solution (containing D₄-cholic acid and
203 D₄-lithocholic acid in methanol). The resulting mixture was incubated in 6 mL of
204 chloroform:methanol (2:1) solution overnight in a refrigerator. After centrifugation for 10 min at
205 4,000 g, the supernatant was transferred, dried under nitrogen gas, reconstituted with 1 mL of
206 mobile phase (10 mM ammonium acetate and 0.1% ammonium hydroxide in methanol at pH 9),
207 and then diluted 100 times (two sequential 1:10 dilutions) for LC-QTOF/MS analysis. For
208 further analytical details, see **Supplementary Material**. Data were normalized by dry weight
209 and are expressed as mg/g.

210 ***2.4.3. Fecal SCFA***

211 Fecal SCFA were measured by a QTRAP 5500 LC-MS/MS method as previously described. The
212 method employs a derivatization with 3-nitrophenylhydrazine and measures the SCFA in
213 stool samples [40]. Data are expressed as µmol/g.

214 ***2.4.4. TMA and TMAO***

215 TMA and TMAO in three different matrixes (plasma, urine and stool) were determined by a
216 QTRAP 5500 LC-MS/MS method as previously described [41].

217 **2.4.5. Water content and pH**

218 Water content in feces is the weight loss of wet stool samples after they were freeze-dried. It was
219 calculated using the following formula:

220
221 Water content = $\frac{\text{mass of the wet sample} - \text{mass of the dried sample}}{\text{mass of the wet sample}} \times 100$

222 pH value in feces was determined using a pH meter.

223 **2.4.6. Plasma cytokines**

224 Inflammatory cytokines IFN- γ , IL-1 β , IL-6, and TNF- α in plasma were determined following
225 manufacturer's instructions using a Meso Scale Discovery Multiplex kit (Rockville, MD). Data
226 are expressed as pg/mL.

227 **2.5. Sample size and power analysis calculation**

228 The sample size for this human trial (n=11) was estimated based on the effect of freeze-dried
229 whole cranberry powder on the animal-based diet induced increase in bile tolerant bacteria [15]
230 and on the increase in *Enterococcus*, *Bifidobacterium*, *Eggerthella lenta*, and *Blautia coccoides*–
231 *Eubacterium rectale* groups [42]. There are no data in the literature illustrating the effect of
232 freeze-dried whole cranberry powder on bile tolerant bacteria for power calculation, but our
233 sample size is comparable to the number of subjects in the studies reported by David *et al.* [15]
234 and Queipo-Ortuño *et al.* [42], respectively.

235 **2.6. Statistical analysis**

236 Statistical analyses of data generated during the study were performed using the current version
237 of the SAS statistical software package (SAS Institute Inc., Cary, NC). A Mixed ANOVA model,
238 including treatment, sequence, and period as the independent variables, was used to determine
239 statistically significant differences, followed by Tukey's Honest Significant Different (HSD)
240 multiple comparison tests. This model was employed for the statistical analysis of

241 (poly)phenolics, bile acids, SCFA, TMA, pH, water content, and inflammatory cytokines. The
242 effect of the control diet was evaluated comparing in *post-hoc* analysis differences between
243 values at baseline versus values after control diet. The cranberry effect was evaluated comparing
244 in *post-hoc* analysis differences between the values after control diet versus the values after
245 cranberry diet. A $p\text{-value} \leq 0.05$ was considered statistically significant. Pearson's and
246 Spearman's correlation coefficients (r) and p -values were used to determine correlations between
247 variables. Normality of continuous variables related to microbiota abundance and biochemical
248 parameters was assessed and, if required, data were log transformed. P -values were adjusted for
249 multiple comparisons to control the false discovery rate, generating p -adjusted values (also
250 known as q -values). Correlation analyses were carried out in R Microbiome package and graphs
251 were plotted with GraphPad Prism. Adonis (analysis of variance) and Anosim (analysis of
252 similarity) tests were performed using Vegan package in R on the post-interventional data.
253 Principal Coordinate Analysis (PCoA) was analyzed to illustrate beta-diversity using Phyloseq
254 package in R. LEfSe consists of an algorithm for high-dimensional biomarker discovery and
255 explanation that identifies genomic features (genes, pathways, or taxa) characterizing the
256 differences between two or more biological conditions. This algorithm uses firstly the non-
257 parametric factorial Kruskal-Wallis sum-rank test to detect features with significant differential
258 abundance with respect to the class of interest, then the (unpaired) Wilcoxon rank-sum test to
259 assess biological consistency and finally a linear discriminant analysis estimates the effect size of
260 each differentially abundant feature [37].

261 **3. Results**

262 **3.1. Participants baseline characteristics**

263 **Table 1** presents the baseline characteristics of the 11 healthy volunteers that completed the
264 study and the corresponding values grouped by sex. Of these subjects, 7 were male and 4 were
265 female, their average age was 39.2 ± 12.9 years and their average BMI was 22.2 ± 2.0 kg/m².
266 Their baseline waist circumference was 81.5 ± 8.3 cm and their hip circumference was 93.7 ± 4.3
267 cm. Diastolic blood pressure was 81.4 ± 7.9 mm Hg, systolic blood pressure was 116.5 ± 12.6
268 mm Hg and the heart rate was 70 ± 13 beats per minute.

269

270

271 **3.2. Gut microbiota analyses**

272 ***3.2.1. Diet effects on microbiota diversity***

273 Neither the control diet or the cranberry diet altered microbiota diversity. Within sample
274 diversity (alpha diversity) remained unaltered, as no differences in bacterial richness and
275 evenness were found using three different diversity metrics: Chao 1 (total number of species),
276 Shannon (heterogeneity), and Simpson's index (similarity) (**Fig. S2**). Between
277 environment/community diversity (beta diversity) was not altered, as no differences in weighted
278 and unweighted variants of UniFrac were found. PCoA plot (**Fig. S3**) also showed cranberry did
279 not affect beta diversity of the fecal microbiota as compared to control. The results of Adonis (R^2
280 = 0.0245, $P = 0.962$) and Anosim ($R = -0.1002$, $P = 0.998$) tests on the post-interventional
281 microbiome further confirmed that diversity of the fecal microbiome was not altered by the
282 addition of cranberry to the control diet.

283 ***3.2.2. Diet effect on relative phylum abundance***

284 The control diet altered the relative abundance of fecal bacteria with a decrease in the gram-
285 negative *Bacteroidetes* ($p = 0.018$) and an increase in gram-positive *Firmicutes* ($p = 0.023$), to

286 the extent that *Bacteroidetes* was the most abundant phylum before the diet and *Firmicutes*
287 became the most abundant after the diet. No differences were found in the relative abundance of
288 the phyla *Actinobacteria*, *Proteobacteria* nor *Verrucomicrobia* between groups (**Fig. 1**).

289 The addition of cranberries to the basal diet reversed the effect of the control diet on the relative
290 abundance of phyla, as shown by an increase in *Bacteroidetes* ($p = 0.032$) and a decrease in
291 *Firmicutes* ($p = 0.038$).

292 ***3.2.3. Diet effect on the overall relative abundances***

293 The control diet altered the relative abundance of gut microbiota. LEfSe analysis comparing the
294 relative abundances of microbiota at baseline vs. after 5 days of control diet identified 46 altered
295 bacterial clades. Specifically, the control diet induced an increase in 35 bacterial clades,
296 including 1 phyla (*Firmicutes*), 3 classes, 4 orders, 7 families, 15 genera and 5 species (**Fig. 2**).

297 The highest diet-induced increases in relative abundance were in the phyla *Firmicutes*, the class
298 *Clostridia* and the order *Clostridiales* (LDA >4.8). Furthermore, the control diet induced a
299 decrease in 11 bacterial clades, including 1 phyla (*Bacteroidetes*), 2 classes, 2 orders, 3 genera,
300 and 3 species. The highest diet-induced decrease in the relative abundance were in the phyla
301 *Bacteroidetes*, the class *Bacteroidia* and the order *Bacteroidales* (LDA >4.8) (**Fig. 2**).

302 A significant effect of cranberry diet on the relative abundance of fecal bacteria was observed, as
303 LEfSe analysis comparing relative abundances after control diet versus after cranberry diet
304 identified 9 differentially abundant taxonomic clades with LDA score larger than 2. Specifically,
305 after cranberry diet, there was an increase in the relative abundance of the phylum *Bacteroidetes*,
306 the class *Bacteroidia*, and the order *Bacteroidales*, as well as an increase in the genera
307 *Lachnospira* and *Anaerostipes*. Further, the phyla *Firmicutes*, the class *Clostridia*, the order

308 *Clostridiales* and the genus *Oribacterium* had lower relative abundances after the cranberry diet,
309 compared to the values after the control diet (**Fig. 3**).

310 **3.3. Urinary anthocyanins**

311 The urinary level of four major cranberry anthocyanins (cyanidin-3-galactoside, cyanidin-3-
312 arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside) and two minor cranberry
313 anthocyanins (cyanidin-3-glucoside and peonidin-3-glucoside) are depicted in **Fig. 4A** and **S4**,
314 respectively. Despite large inter-individual variability, all major cranberry anthocyanins were
315 significantly higher in morning spot urine at the end of cranberry phase (CRA post) compared to
316 those before the cranberry intervention (CRA pre) and to those before and after control, which all
317 had values generally close to zero (**Fig. 4A**). In the case of minor cranberry anthocyanins, no
318 significant differences were found between groups (**Fig. S4**).

319

320 **3.4. Urinary phenolic acids and flavonoids**

321 The urinary levels of 15 phenolic acids and flavonoids were measured before and after the two
322 different diets. A cranberry-induced treatment effect was observed as the concentration of 3,4-
323 dihydroxyphenylacetic acid (DOPAC) and its *ortho*-methylated metabolite 4-hydroxy-3-
324 methoxyphenylacetic acid (Homovanillic acid; HVA) were 160 and 72% higher, respectively, at
325 the end of the cranberry phase than those at the end of the control phase ($p < 0.01$) (**Fig. 4B**). No
326 other differences between treatments were found for the other phenolic acids and flavonoids
327 measured: protocatechuic, genistic, 3-hydroxybenzoic, 4-hydroxyphenylacetic, vanillic, 4-
328 hydroxybenzoic, caffeic, *p*-coumaric, ferulic, and sinapic acids, and catechin, epicatechin,
329 quercetin, and myricetin (**Fig. S4**).

330 **3.5. Fecal bile acids**

331 Among the bile acids measured, none of the primary (lithocholic and cholic), and only the
332 secondary bile acids (lithocholic and deoxycholic) were detected. Both secondary bile acids
333 followed a similar pattern, but the effects were more remarkable for deoxycholic acid, whose
334 concentrations after 5 days of control diet increased by 268%, compared to the pre-control diet
335 values (**Fig. 5A**). Interestingly, when this same basal diet was supplemented with whole
336 cranberry powder, the levels of these secondary bile acids did not increase and were statistically
337 lower than those after the control diet (**Fig. 5A**).

338 **3.6. Fecal SCFA**

339 The levels of SCFA quantified in the stools are depicted in **Fig. 5B**. No differences were found
340 in the levels of propionic acid between groups. The levels of acetic ($p < 0.005$) and butyric acids
341 ($p < 0.01$) decreased by 40 and 48%, respectively, after the consumption of the control diet for 5
342 days, compared to pre-control diet values. The addition of cranberry powder to the basal diet
343 attenuated the magnitude of the decreases to the extent that no differences were found before and
344 after the diet.

345 **3.7. TMA and TMAO**

346 The levels of TMA and TMAO in plasma, urine and stool are summarized in **Fig. S6**. No
347 significant changes between the 4 groups (before and after each intervention) were detected in
348 the levels of TMA and TMAO. No TMAO was detected in feces. This observation is in
349 agreement with the literature [43].

350 **3.8. Water content and pH**

351 Fecal water content and pH values were measured as these parameters can be modified by diet
352 [44]. Moreover, the measurement of fecal water content allows the normalization of the

353 concentration of biomarkers measured in stool samples when significant differences in water
354 content are found.

355 Fecal pH values increased significantly after the control diet, but the addition of cranberry
356 powder did not change the pH (**Fig. 6A**). Fecal water content presented a high inter-individual
357 variability and no diet-induced differences were noted.

358 **3.9. Inflammatory biomarkers**

359 Circulating levels of IFN- γ , IL-1 β , IL-6, and TNF- α after and before each diet are summarized in
360 **Fig. S5**. No differences on circulating cytokines were found as a consequence of the animal-
361 based diet or by the addition of cranberry.

362 **3.10. Correlation analyses**

363 Correlation analyses between different variables of taxonomic data from 16S rRNA sequencing
364 and biochemical parameters revealed some correlations that remained significant following the
365 false discovery rate correction. The most relevant findings are the positive correlation between
366 the levels of deoxycholic acid and the abundance of *Fusobacterium* (Spearman $r = 0.74$; p
367 <0.0005 ; adjusted $p <0.05$) (**Fig. 6B**), the negative correlation between deoxycholic acid and the
368 abundance of *Anaerostipes* (Spearman $r = -0.50$; $p <0.001$; adjusted $p <0.05$) (**Fig. 6C**), the
369 negative correlation between fecal pH values and the levels of butyric acid in stool (Pearson $r = -$
370 0.75 ; $p <0.0001$; adjusted $p <0.001$) (**Fig. 6D**), and the negative correlation between fecal pH
371 values and the levels of acetic acid in stool (Pearson $r = -0.63$; $p <0.0001$; adjusted $p <0.001$)
372 (**Fig. 6E**).

373 **4. Discussion**

374 In this small scale randomized controlled feeding study, we found that an animal-based diet
375 (comprising meats, dairy products, and simple sugars) altered in a short-period (5 days) the

376 microbiota relative abundance as well as the level of microbiota-derived compounds. Moreover,
377 the addition of cranberries to this diet restored part of the alterations in microbiota composition
378 and functionality towards a healthier profile, and increased urinary levels of anthocyanins and
379 phenolic acids.

380 Although it has been proposed that the beneficial effects of fruits in general [45] and polyphenols
381 in particular [46] could be mediated through their interaction with gut microbiota, there are only
382 a limited number of clinical studies evaluating the relationship between polyphenols and
383 microbiota composition and bacterial functionalities, such as palm dates [47], cocoa flavanols
384 [48], blueberries [49] and wine [42, 50].

385 In our study, the control diet decreased the relative abundance of the gram-negative
386 *Bacteroidetes* and increased the gram-positive *Firmicutes*, with a corresponding increase in the
387 *Firmicutes/Bacteroidetes* ratio. This ratio has consistently been found to associate positively with
388 weight gain and inversely with weight loss [51], and it has been proposed as a biomarker of
389 obesity [52]. In our study, the cranberry diet reversed the control diet effects on the
390 abovementioned changes, suggesting constituents in cranberries, yet to be fully identified could
391 counteract the animal-based diet induced changes in the fecal microbiome and potential
392 detrimental sequelae. An increase in *Bacteroidetes* and decrease in *Firmicutes*, similar to the
393 observed after the cranberry diet, has been observed with weight loss on two types of low-calorie
394 diets (fat- and carbohydrate- restricted diets) [53], after red wine intake over a 30-day period in
395 patients with metabolic syndrome [50], and in a recent prospective study with sweetened dried
396 cranberries [28].

397 At the genus and species level, the control diet significantly increased the relative abundance of
398 several microorganisms, some of which linked to detrimental effects on human health, like

399 *Solobacterium moorei* (associated with bacteraemia and colorectal cancer) [54], *Ruminococcus*
400 and *Ruminococcus gnavus* (inflammatory bowel disease [55]), *Clostridium clostridioforme*
401 (infection and antibiotic resistance [56]), *Dorea* and *Dorea formicigenerans* (obesity [57]), and
402 the genus *Bilophila* (bile acid metabolism [15] and associated with colitis in mice [58]), *Gemella*
403 (opportunistic pathogen related to infections [59]) and *Actinomyces* (infection [60]). On the other
404 hand, the control diet decreased the relative abundance of some bacteria linked to beneficial
405 health effects like the genus *Lachnospira* (associated with vegetable intake [61]) and
406 *Anaerostipes* (butyrate production [62]) as well as the species *Ruminococcus bromii* (resistant
407 starch degradation [63]).

408 The cranberry diet reversed, in part, the alterations induced by control diet on microbiota
409 abundances. Indeed, cranberry constituents not only shifted the abovementioned abundances in
410 *Firmicutes* and *Bacteroidetes* but also increased the abundance of *Lachnospira* and *Anaerostipes*
411 (which contribute to SCFA production [62]) and decreased that of *Clostridia* and *Oribacterium*.
412 It is worth noting that the 9 bacterial clades that were modified by cranberry diet altered the
413 relative bacteria abundances in the opposite direction of the control diet to the extent that the
414 effect of the latter was abolished. However, we did not see any changes in the beneficial genera
415 *Bifidobacterium*, whose abundance has been reported to be increased by a wild blueberry drink
416 [49], cocoa flavanols [48], and red wine polyphenols [42, 50]. Further, the increase in
417 *Lactobacillus* was noted by cocoa flavanols [48] and red wine polyphenols [50] but not in our
418 study. We speculate that the extreme basal diet administered in our trial might limit the favorable
419 effects of cranberry constituents on the growth of probiotic bacteria, although the treatment and
420 washout durations could also be contributing factors as recent studies in mice have shown that
421 diet switching can result in lasting effects on the gut microbiota [64-66].

422 In the previously mentioned prospective study evaluating the effect of sweetened dried cranberry
423 on fecal microbiome in healthy people [28], the authors did not observe treatment-induced
424 differences in the β -diversity of the fecal microbiome, but noted a cranberry-induced decrease in
425 the *Firmicutes/Bacteroidetes* ratio. These results are in line with our observations despite the
426 differences in administration form (freeze-dried cranberry powders vs. sweetened dried
427 cranberries), dosage (30 vs. 42 g), duration (5 days vs. 2 weeks), and design (randomized
428 crossover controlled vs. prospective). Interestingly, an increase in the *Akkermansia* relative
429 abundance after the sweetened dry cranberry treatment was noted in Nell *et al.* study [28], but
430 not in our study. On the contrary, we noted the increased relative abundance of the genera
431 *Anaerostipes* and *Lachnospira* after the cranberry diet. In order to (1) confirm the intake of
432 cranberries by the volunteers, (2) assess the bioavailability of cranberry (poly)phenols, and (3)
433 study potential correlations between cranberry constituents and microbiota-produced biomarkers,
434 we measured urinary levels of anthocyanins, flavonoids and phenolic acids. The levels of urinary
435 anthocyanins found after the cranberry diet are consistent with the anthocyanin profile in
436 cranberries. The 4 major cranberry pigments in the descending order of their contents are
437 peonidin-3-galactoside, cyanidin-3-galactoside, peonidin-3-arabinoside, and cyanidin-3-
438 arabinoside, whereas the 2 minor anthocyanins are peonidin-3-glucoside and cyanidin-3-
439 glucoside [67, 68]. The urinary levels of four major pigments were statistically higher after the
440 cranberry diet, confirming our previous observation in volunteers after the intake of cranberry
441 juice [23, 69]. However, the levels of minor anthocyanins did not reach statistical significance,
442 mainly attributable to a wide inter-individual variation in polyphenol pharmacokinetics.
443 Globally, these data provides solid evidence that cranberry anthocyanins are bioavailable, and
444 the major qualitative advantage compared to previous reports, is the controlled feeding design of

445 the present study. The measurement of 15 phenolic acids and flavonoids in urine before and after
446 both dietary interventions revealed a cranberry-mediated effect. We noted that the levels of 3,4-
447 dihydroxyphenylacetic acid and its analogue (4-hydroxy-3-methoxyphenylacetic acid) were
448 higher at the end of the cranberry diet. It is worth noting that, according to *in vitro* fermentation
449 studies with human fecal bacteria, these two phenolic acids are microbial metabolites derived
450 from cranberry procyanidins [25, 70].

451 In order to provide insights in the connection between the diet-induced microbiota alterations
452 and cardiometabolic and chronic diseases, we measured three families of compounds that are
453 produced by the gut microbiota and that are linked to specific pathologies: (1) secondary bile
454 acids (implicated in carcinogenesis [71]), (2) SCFA (known for their effects on suppressing
455 inflammation, obesity, carcinogenesis, amongst others [10, 72]), and (3) TMA, whose further
456 hepatic oxidation generates TMAO (which associated with atherosclerosis and cardiovascular
457 disease) [73, 74].

458 The exclusive detection of the secondary bile acids (lithocholic acid and deoxycholic acid) are in
459 agreement with the fact that primary fatty acids are deconjugated and dehydroxylated by bacteria
460 in the colon [75]. The control diet-induced increase in the secondary bile acids supports potential
461 harms of excess consumption of red meats and processed meats because of their positive
462 association with increased risk of colon cancer [76] and other gastrointestinal cancers [8, 75].
463 Moreover, the unchanged secondary bile acid levels after the cranberry diet suggest a protective
464 effect of cranberry constituents on the gastrointestinal health by modulating the levels of
465 secondary bile acids. Interestingly, we noted that there was a strong negative association between
466 the levels of secondary bile acids and the relative abundance of *Anaerostipes*. It is worth
467 mentioning that the previous correlations only provide descriptive information regarding the

468 functional capacity of the gut microbiota and do not establish causality, evidencing the need of
469 additional experiments using germ-free mice [77] or fecal microbiota transplants [78].

470 A significant decrease in acetic and butyric acid after the control diet was observed, and
471 cranberry constituents mitigated this change to the extent that no differences were noted before
472 and after the dietary intervention phase. Given that SCFA have an array of putative health
473 benefits [11], our data suggests cranberry constituents could exert their beneficial effects by
474 helping maintain SCFA production in the gut of people consuming diets low in fiber and high in
475 animal meats and simple sugars.

476 The levels of TMA and TMAO did not differ between treatments. The lack of significance could
477 be due to the high inter-individual variability observed and the small sample size.

478 Fecal pH values increased significantly after 5 days following the control diet, consistent with
479 the literature in which increased meat consumption elevated fecal pH [79]. This is of relevance
480 since fecal pH values that extend beyond the normal range are associated with the clinical course
481 and prognosis of critically ill patients [80]. A strong negative correlation was observed between
482 the fecal pH values and the concentrations of SCFA, being largest for butyric acid. This
483 observation (together with the acidic character of these molecules and their high fecal
484 concentrations) suggests the existence of a direct (or indirect) relationship between the levels of
485 SCFA and the fecal pH values.

486 Our study has strengths and limitations. One strength is the use of a fully characterized freeze-
487 dried whole cranberry powder and a matched placebo as standard reference materials. Their use
488 enables comparability and replicability, and overcomes previous drawbacks to ensure
489 consistency (e.g. confounding differences in the cranberry products and doses) [29]. Another
490 strength of our study is the crossover design, in which each subject serves as his or her own

491 control. Thus, such a design provides two main advantages: (1) it minimizes interferences on the
492 study outcomes from confounding variables, such as diet, lifestyle, genetics, and others, on study
493 outcomes because those may arise when subjects are only tested in one study treatment and (2) it
494 allows detecting a smaller effect size with a reduced sample size. One limitation of our study is
495 the high cranberry dosage that we used (30 g), which was selected to meet daily average
496 flavonoid and proanthocyanidins intakes in the United States. Additional limitations of our study
497 include the small sample size (11 healthy individuals), inability to test sex specific differences,
498 and the extreme diet which, although it has been described in pastoralist and high-latitude
499 cultures [15], is not commonly consumed. Additional measurements relevant to cardiometabolic
500 outcomes such as plasma fasting glucose, lipids, or circulating lipopolysaccharide-binding
501 protein could have been measured to provide a better understanding of the impact of the
502 intervention. Another limitation is the lack of a detailed mechanistic understanding (at the
503 molecular level) of the relationship between microbiota, microbiota-produced metabolites
504 (secondary bile acids, SCFA, and trimethylamine), and the final impact on human health. Future
505 studies assessing whether metabolic capacity of the gut microbiota is modified by the study diets
506 are needed to establish causality. The results from this study provide a proof of concept that the
507 cranberry phytochemicals could attenuate the changes in microbiota and specific biomarkers
508 induced by an animal food-based diet. Nevertheless, we anticipate that such positive finding can
509 be extrapolated to the impact of foods rich in phytochemicals, particularly polyphenols, on shifts
510 of the gut microbiota and related biochemical and physiological to more healthful profiles.
511 Further, the design and results of this pilot study provide a basis for future studies with a larger
512 sample size to further substantiate the effect of phytochemical rich plant foods or dietary patterns
513 on the gut microbiota and related health outcomes. In a similar way, future studies with a larger

514 sample size will be necessary to establish to which extent the results observed in this study could
515 be translated into clinical applications. Future studies with a larger sample size will be necessary
516 to establish to which extent the results observed in this study could be translated into clinical
517 applications.

518 **5. Conclusions**

519 In this randomized, cross-over, blinded, controlled feeding trial we observed that the
520 consumption of an animal-based diet for a short period of time (5 consecutive days) altered the
521 microbiota composition to a less favorable profile with the consequent alterations of microbiota-
522 derived compounds (increasing the levels of carcinogenesis-related deoxycholic acid, and
523 decreasing the levels of beneficial SCFA). Cranberry constituents modified the impact of the
524 animal-based diet on microbiota composition and prevented the animal-based diet-induced
525 increase in secondary bile acids and decrease in SCFA, evidencing their capacity to modulate the
526 gut microbiota.

527

528 **Conflict of interest**

529 The authors declare that there is no conflict of interest.

530 **Contributors**

531 CYOC conceived and designed the trial. NRM and JL performed the experiments and data
532 collection. JRM and CYOC analyzed the data. JRM drafted the first version of the manuscript.
533 NM, RT and CYOC contributed to the discussion and reviewed the manuscript. All authors read
534 and approved the manuscript.

535

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541

542 **References**

543 [1] Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, et al. Fruit and vegetable consumption
544 and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-
545 response meta-analysis of prospective cohort studies. *British Medical Journal*. 2014;349:g4490.

546 [2] Nyambe-Silavwe H, Williamson G. Polyphenol- and fibre-rich dried fruits with green tea
547 attenuate starch-derived postprandial blood glucose and insulin: a randomised, controlled, single-
548 blind, cross-over intervention. *British Journal of Nutrition*. 2016;116:443-50.

549 [3] Koppel N, Balskus Emily P. Exploring and Understanding the Biochemical Diversity of the
550 Human Microbiota. *Cell Chemical Biology*.23:18-30.

551 [4] Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut
552 microbiome, and immune system: envisioning the future. *Nature*. 2011;474:327-36.

553 [5] Röytiö H, Mokkala K, Vahlberg T, Laitinen K. Dietary intake of fat and fibre according to
554 reference values relates to higher gut microbiota richness in overweight pregnant women. *Br J*
555 *Nutr*. 2017;118:343-52.

556 [6] Tang WHW, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease.
557 *Circulation Research*. 2017;120:1183-96.

558 [7] Sonnenburg JL, Bäckhed F. Diet–microbiota interactions as moderators of human
559 metabolism. *Nature*. 2016;535:56.

560 [8] Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in
561 human gastrointestinal cancers. *Mutation Research/Reviews in Mutation Research*. 2005;589:47-
562 65.

563 [9] Yang C-H, Chen C-Y, Chou Y-Y, Chiu H-C, Tsai W-L, Shiesh S-C. Bile acid profiles in
564 neonatal intrahepatic cholestasis caused by citrin deficiency. *Clinica Chimica Acta*. 2017;475:28-
565 35.

566 [10] Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. Chapter Three - The
567 Role of Short-Chain Fatty Acids in Health and Disease. In: Alt FW, editor. *Advances in*
568 *Immunology*: Academic Press; 2014. p. 91-119.

569 [11] Van de Wouw M, Schellekens H, Dinan TG, Cryan JF. Microbiota-Gut-Brain Axis:
570 Modulator of Host Metabolism and Appetite. *Journal of Nutrition*. 2017;147:727-45.

571 [12] Taesuwan S, Cho CE, Malysheva OV, Bender E, King JH, Yan J, et al. The metabolic fate
572 of isotopically labeled trimethylamine-N-oxide (TMAO) in humans. *Journal of Nutritional*
573 *Biochemistry*. 2017;45:77-82.

574 [13] Battson ML, Lee DM, Weir TL, Gentile CL. The gut microbiota as a novel regulator of
575 cardiovascular function and disease. *The Journal of Nutritional Biochemistry*. 2018;56:1-15.

576 [14] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al. Linking Long-
577 Term Dietary Patterns with Gut Microbial Enterotypes. *Science (New York, Ny)*. 2011;334:105-
578 8.

579 [15] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet
580 rapidly and reproducibly alters the human gut microbiome. *Nature*. 2013;505:559-63.

581 [16] Blumberg JB, Camesano TA, Cassidy A, Kris-Etherton P, Howell A, Manach C, et al.
582 Cranberries and Their Bioactive Constituents in Human Health. *Advances in Nutrition*.
583 2013;4:618-32.

584 [17] Feliciano RP, Mills CE, Istaş G, Heiss C, Rodriguez-Mateos A. Absorption, Metabolism
585 and Excretion of Cranberry (Poly)phenols in Humans: A Dose Response Study and Assessment
586 of Inter-Individual Variability. *Nutrients*. 2017;9:268.

587 [18] Vinson JA, Su X, Zubik L, Bose P. Phenol Antioxidant Quantity and Quality in Foods:
588 Fruits. *Journal of Agricultural and Food Chemistry*. 2001;49:5315-21.

589 [19] Cavanagh HMA, Hipwell M, Wilkinson JM. Antibacterial Activity of Berry Fruits Used for
590 Culinary Purposes. *Journal of Medicinal Food*. 2003;6:57-61.

591 [20] Weiss EI, Hourı-Haddad Y, Greenbaum E, Hochman N, Ofek I, Zakay-Rones Z. Cranberry
592 juice constituents affect influenza virus adhesion and infectivity. *Antiviral Research*. 2005;66:9-
593 12.

594 [21] Seeram NP, Adams LS, Hardy ML, Heber D. Total Cranberry Extract versus Its
595 Phytochemical Constituents: Antiproliferative and Synergistic Effects against Human Tumor
596 Cell Lines. *Journal of Agricultural and Food Chemistry*. 2004;52:2512-7.

597 [22] Roy S, Khanna S, Alessio HM, Vider J, Bagchi D, Bagchi M, et al. Anti-angiogenic
598 property of edible berries. *Free Radicical Research*. 2002;36:1023-31.

599 [23] McKay DL, Chen CYO, Zampariello CA, Blumberg JB. Flavonoids and phenolic acids
600 from cranberry juice are bioavailable and bioactive in healthy older adults. *Food Chemistry*.
601 2015;168:233-40.

602 [24] Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. Benefits of
603 polyphenols on gut microbiota and implications in human health. *Journal of Nutritional*
604 *Biochemistry*. 2013;24:1415-22.

605 [25] Appeldoorn MM, Vincken J-P, Aura A-M, Hollman PCH, Gruppen H. Procyanidin Dimers
606 Are Metabolized by Human Microbiota with 2-(3,4-Dihydroxyphenyl)acetic Acid and 5-(3,4-
607 Dihydroxyphenyl)- γ -valerolactone as the Major Metabolites. *Journal of Agricultural and Food*
608 *Chemistry*. 2009;57:1084-92.

609 [26] Saura-Calixto F, Pérez-Jiménez J, Touriño S, Serrano J, Fuguet E, Torres JL, et al.
610 Proanthocyanidin metabolites associated with dietary fibre from in vitro colonic fermentation
611 and proanthocyanidin metabolites in human plasma. *Molecular Nutrition & Food Research*.
612 2010;54:939-46.

613 [27] Anhe FF, Roy D, Pilon G, Dudonné S, Matamoros S, Varin TV, et al. A polyphenol-rich
614 cranberry extract protects from diet-induced obesity, insulin resistance and intestinal
615 inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of
616 mice. *Gut*. 2015;64:872.

617 [28] Bekiaries N, Krueger CG, Meudt JJ, Shanmuganayagam D, Reed JD. Effect of Sweetened
618 Dried Cranberry Consumption on Urinary Proteome and Fecal Microbiome in Healthy Human
619 Subjects. *OMICS: A Journal of Integrative Biology*. 2017;22:145-53.

620 [29] Blumberg JB, Basu A, Krueger CG, Lila MA, Neto CC, Novotny JA, et al. Impact of
621 Cranberries on Gut Microbiota and Cardiometabolic Health: Proceedings of the Cranberry
622 Health Research Conference 2015. *Advances in Nutrition*. 2016;7:759S-70S.

623 [30] National Center for Health Statistics - The Third National Health and Nutrition Examination
624 Survey (NHANES III, 1988–1994)

625 <http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/NCHS/MANUALS/ANTHRO.PDF>
626 (Accessed May, 2018).

627 [31] Song WO, Chun OK. Tea is the major source of flavan-3-ol and flavonol in the U.S. diet.
628 *Journal of Nutrition*. 2008;138:1543S-7S.

629 [32] Wang Y, Chung S-J, Song WO, Chun OK. Estimation of Daily Proanthocyanidin Intake and
630 Major Food Sources in the U.S. Diet1–3. *Journal of Nutrition*. 2011;141:447-52.

631 [33] Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-
632 high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms.
633 *Multidisciplinary Journal of Microbial Ecology* 2012;6:1621-4.

634 [34] Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al.
635 QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*.
636 2010;7:335-6.

637 [35] DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a
638 Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Applied*
639 *and Environmental Microbiology*. 2006;72:5069-72.

640 [36] Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*.
641 2010;26:2460-1.

642 [37] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic
643 biomarker discovery and explanation. *Genome Biology*. 2011;12:R60-R.

644 [38] Chen C-Y, Milbury PE, Lapsley K, Blumberg JB. Flavonoids from Almond Skins Are
645 Bioavailable and Act Synergistically with Vitamins C and E to Enhance Hamster and Human
646 LDL Resistance to Oxidation. *Journal of Nutrition*. 2005;135:1366-73.

647 [39] Milbury PE, Vita JA, Blumberg JB. Anthocyanins are Bioavailable in Humans following an
648 Acute Dose of Cranberry Juice. *Journal of Nutrition*. 2010;140:1099-104.

649 [40] Han J, Lin K, Sequeira C, Borchers CH. An isotope-labeled chemical derivatization method
650 for the quantitation of short-chain fatty acids in human feces by liquid chromatography–tandem
651 mass spectrometry. *Analytica Chimica Acta*. 2015;854:86-94.

652 [41] Awwad HM, Geisel J, Obeid R. Determination of trimethylamine, trimethylamine N-oxide,
653 and taurine in human plasma and urine by UHPLC–MS/MS technique. *Journal of*
654 *Chromatography B*. 2016;1038:12-8.

655 [42] Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo
656 M, Estruch R, et al. Influence of red wine polyphenols and ethanol on the gut microbiota ecology
657 and biochemical biomarkers. *American Journal of Clinical Nutrition*. 2012;95:1323-34.

658 [43] Taesuwan S, Cho CE, Malysheva OV, Bender E, King JH, Yan J, et al. The metabolic fate
659 of isotopically labeled trimethylamine-N-oxide (TMAO) in humans. *The Journal of Nutritional*
660 *Biochemistry*. 2017;45:77-82.

661 [44] Rose C, Parker A, Jefferson B, Cartmell E. The Characterization of Feces and Urine: A
662 Review of the Literature to Inform Advanced Treatment Technology. *Critical Reviews in*
663 *Environmental Science and Technology*. 2015;45:1827-79.

664 [45] Rastmanesh R. High polyphenol, low probiotic diet for weight loss because of intestinal
665 microbiota interaction. *Chemico-Biological Interactions*. 2011;189:1-8.

666 [46] Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. Benefits of
667 polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional*
668 *Biochemistry*. 2013;24:1415-22.

669 [47] Eid N, Osmanova H, Natchez C, Walton G, Costabile A, Gibson G, et al. Impact of palm
670 date consumption on microbiota growth and large intestinal health: a randomised, controlled,
671 cross-over, human intervention study. *British Journal of Nutrition*. 2015;114:1226-36.

672 [48] Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Urbe C, Spencer JPE.
673 Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized,
674 controlled, double-blind, crossover intervention study. *The American Journal of Clinical*
675 *Nutrition*. 2011;93:62-72.

676 [49] Vendrame S, Guglielmetti S, Riso P, Arioli S, Klimis-Zacas D, Porrini M. Six-Week
677 Consumption of a Wild Blueberry Powder Drink Increases Bifidobacteria in the Human Gut.
678 *Journal of Agricultural and Food Chemistry*. 2011;59:12815-20.

679 [50] Moreno-Indias I, Sanchez-Alcoholado L, Perez-Martinez P, Andres-Lacueva C, Cardona F,
680 Tinahones F, et al. Red wine polyphenols modulate fecal microbiota and reduce markers of the
681 metabolic syndrome in obese patients. *Food & Function*. 2016;7:1775-87.

682 [51] Mathur R, Barlow GM. Obesity and the microbiome. *Expert Review of Gastroenterology &*
683 *Hepatology*. 2015;9:1087-99.

684 [52] De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact
685 of diet in shaping gut microbiota revealed by a comparative study in children from Europe and
686 rural Africa. *Proceedings of the National Academy of Sciences*. 2010;107:14691-6.

687 [53] Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity.
688 *Nature*. 2006;444:1022.

689 [54] Yu J, Feng Q, Wong SH, Zhang D, Liang QY, Qin Y, et al. Metagenomic analysis of faecal
690 microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut*.
691 2017;66:70-8.

692 [55] Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, et al. A novel Ruminococcus
693 gnavus clade enriched in inflammatory bowel disease patients. *Genome Med.* 2017;9:103.

694 [56] Finegold SM, Song Y, Liu C, Hecht DW, Summanen P, Könönen E, et al. Clostridium
695 clostridioforme: a mixture of three clinically important species. *European Journal of Clinical
696 Microbiology and Infectious Diseases.* 2005;24:319-24.

697 [57] Ottosson F, Brunkwall L, Ericson U, Nilsson PM, Almgren P, Fernandez C, et al.
698 Connection between BMI related plasma metabolite profile and gut microbiota. *The Journal of
699 Clinical Endocrinology & Metabolism.* 2018.

700 [58] Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, et al. Dietary-
701 fat-induced taurocholic acid promotes pathobiont expansion and colitis in *Il10*^{-/-} mice. *Nature.*
702 2012;487:104.

703 [59] Jayananda S, Gollol-Raju NS, Fadul N. Gemella Species Bacteremia and Stroke in an
704 Elderly Patient with Respiratory Tract Infection. *Case Reports in Medicine.* 2017;2017:1098527.

705 [60] Valour F, Sénéchal A, Dupieux C, Karsenty J, Lustig S, Breton P, et al. Actinomycosis:
706 etiology, clinical features, diagnosis, treatment, and management. *Infection and Drug Resistance.*
707 2014;7:183-97.

708 [61] Smith-Brown P, Morrison M, Krause L, Davies PSW. Dairy and plant based food intakes
709 are associated with altered faecal microbiota in 2 to 3 year old Australian children. *Scientific
710 Reports.* 2016;6:32385.

711 [62] Kumari M, Kozyrskyj AL. Gut microbial metabolism defines host metabolism: an emerging
712 perspective in obesity and allergic inflammation. *Obesity Reviews.* 2017;18:18-31.

713 [63] Ze X, Duncan SH, Louis P, Flint HJ. Ruminococcus bromii is a keystone species for the
714 degradation of resistant starch in the human colon. *The ISME Journal.* 2012;6:1535-43.

715 [64] Carmody Rachel N, Gerber Georg K, Luevano Jesus M, Jr., Gatti Daniel M, Somes L,
716 Svenson Karen L, et al. Diet Dominates Host Genotype in Shaping the Murine Gut Microbiota.
717 Cell Host & Microbe. 2015;17:72-84.

718 [65] Howe A, Ringus DL, Williams RJ, Choo Z-N, Greenwald SM, Owens SM, et al. Divergent
719 responses of viral and bacterial communities in the gut microbiome to dietary disturbances in
720 mice. The Isme Journal. 2015;10:1217.

721 [66] Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL.
722 Diet-induced extinctions in the gut microbiota compound over generations. Nature.
723 2016;529:212.

724 [67] Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF. Identification of
725 Procyanidins and Anthocyanins in Blueberries and Cranberries (*Vaccinium* Spp.) Using High-
726 Performance Liquid Chromatography/Mass Spectrometry. Journal of Agricultural and Food
727 Chemistry. 2001;49:1270-6.

728 [68] Zhou Y, Singh BR. Effect of Light on Anthocyanin Levels in Submerged, Harvested
729 Cranberry Fruit. Journal of Biomedicine and Biotechnology. 2004;2004.

730 [69] Milbury PE, Vita JA, Blumberg JB. Anthocyanins are Bioavailable in Humans following an
731 Acute Dose of Cranberry Juice¹⁻³. Journal of Nutrition. 2010;140:1099-104.

732 [70] Ou K, Sarnoski P, Schneider KR, Song K, Khoo C, Gu L. Microbial catabolism of
733 procyanidins by human gut microbiota. Molecular Nutrition & Food Research. 2014;58:2196-
734 205.

735 [71] Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer.
736 Nature Reviews Microbiology. 2014;12:661.

737 [72] Sivaprakasam S, Prasad PD, Singh N. Benefits of short-chain fatty acids and their receptors
738 in inflammation and carcinogenesis. *Pharmacology & Therapeutics*. 2016;164:144-51.

739 [73] Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal Microbial
740 Metabolism of Phosphatidylcholine and Cardiovascular Risk. *New England Journal of Medicine*.
741 2013;368:1575-84.

742 [74] Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, et al. Gut flora metabolism
743 of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472:57.

744 [75] Ajouz H, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of
745 colon cancer. *World Journal of Surgical Oncology*. 2014;12:164.

746 [76] Boada LD, Henríquez-Hernández LA, Luzardo OP. The impact of red and processed meat
747 consumption on cancer and other health outcomes: Epidemiological evidences. *Food and*
748 *Chemical Toxicology*. 2016;92:236-44.

749 [77] Martinez-Guryn K, Hubert N, Frazier K, Urllass S, Musch MW, Ojeda P, et al. Small
750 Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary
751 Lipids. *Cell Host & Microbe*. 2018;23:458-69.

752 [78] Zhou D, Pan Q, Shen F, Cao H-x, Ding W-j, Chen Y-w, et al. Total fecal microbiota
753 transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of
754 gut microbiota. *Scientific Reports*. 2017;7:1529.

755 [79] Silvester KR, Bingham SA, Pollock JR, Cummings JH, O'Neill IK. Effect of meat and
756 resistant starch on fecal excretion of apparent N-nitroso compounds and ammonia from the
757 human large bowel. *Nutrition and cancer*. 1997;29:13-23.

758 [80] Osuka A, Shimizu K, Ogura H, Tasaki O, Hamasaki T, Asahara T, et al. Prognostic impact
759 of fecal pH in critically ill patients. *Critical Care*. 2012;16:R119-R.

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763 **Figure captions**

764 **Fig. 1.** Taxonomic profile of 5 major bacterial phyla in study participants before (pre) and after
765 (post) consuming control diet (CON) or cranberry diet (CRA) for 5 days.

766 **Fig. 2.** Linear discriminant analysis score plot from linear discriminant analysis effect size
767 (LefSe) analysis of the gut microbiota composition before (pre) and after (post) the animal-based
768 diet. Positive LDA scores indicate bacterial clades with higher abundance at baseline (green
769 bars) whereas negative LDA scores indicate bacterial clades with higher abundance after the
770 control diet (red bars).

771 **Fig. 3.** Cladogram from linear discriminant effect size (LEfSe) analysis of stool samples after
772 study participants consumed control diet (CON) or the cranberry diet (CRA) for 5 days. Positive
773 LDA scores indicate bacterial clades with higher abundance after control diet (green) whereas
774 negative LDA scores indicate bacterial clades with higher abundance after the cranberry diet
775 (red).

776 **Fig. 4.** Urinary levels of major anthocyanins (**A**), and phenolic acids (**B**) before (pre) and after
777 (post) consuming control diet (CON, closed circles) or cranberry diet (CRA, open circles) for 5
778 days. *p <0.05; **p <0.01; ***p <0.005.

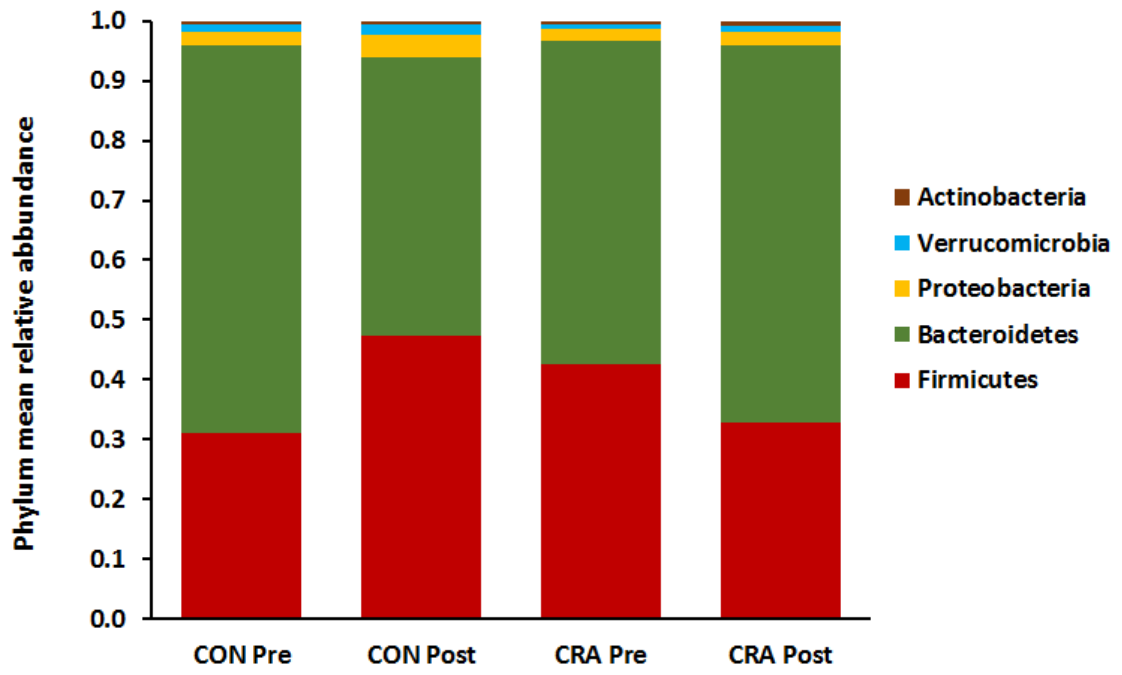
779 **Fig. 5.** Concentrations of secondary bile acids lithocholic acid and deoxycholic acid (**A**) and
780 short-chain fatty acids (**B**) in feces before (pre) and after (post) consuming either control diet
781 (CON) or cranberry diet (CRA) for 5 d. *p <0.05; **p <0.01; ***p <0.005.

782 **Fig. 6.** (**A**) Fecal pH values before (pre) and after (post) study participants consumed control diet
783 (CON) or the cranberry diet (CRA) for 5 days. Correlations between deoxycholic acid and
784 *Fusobacterium* (**B**), deoxycholic acid and *Anaerostipes* (**C**), Fecal pH and fecal acetic acid (**D**),
785 and Fecal pH and fecal butyric acid levels (**E**). P and r values are based on either Spearman or

786 Pearson correlation tests, as indicated in the Figure. Adjusted p-values (also known as q-values)
787 indicate the p-values after optimization using the false discovery rate approach. **P <0.01; ***P
788 <0.005.

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Fig. 1

Relative abundance differences before and after control diet

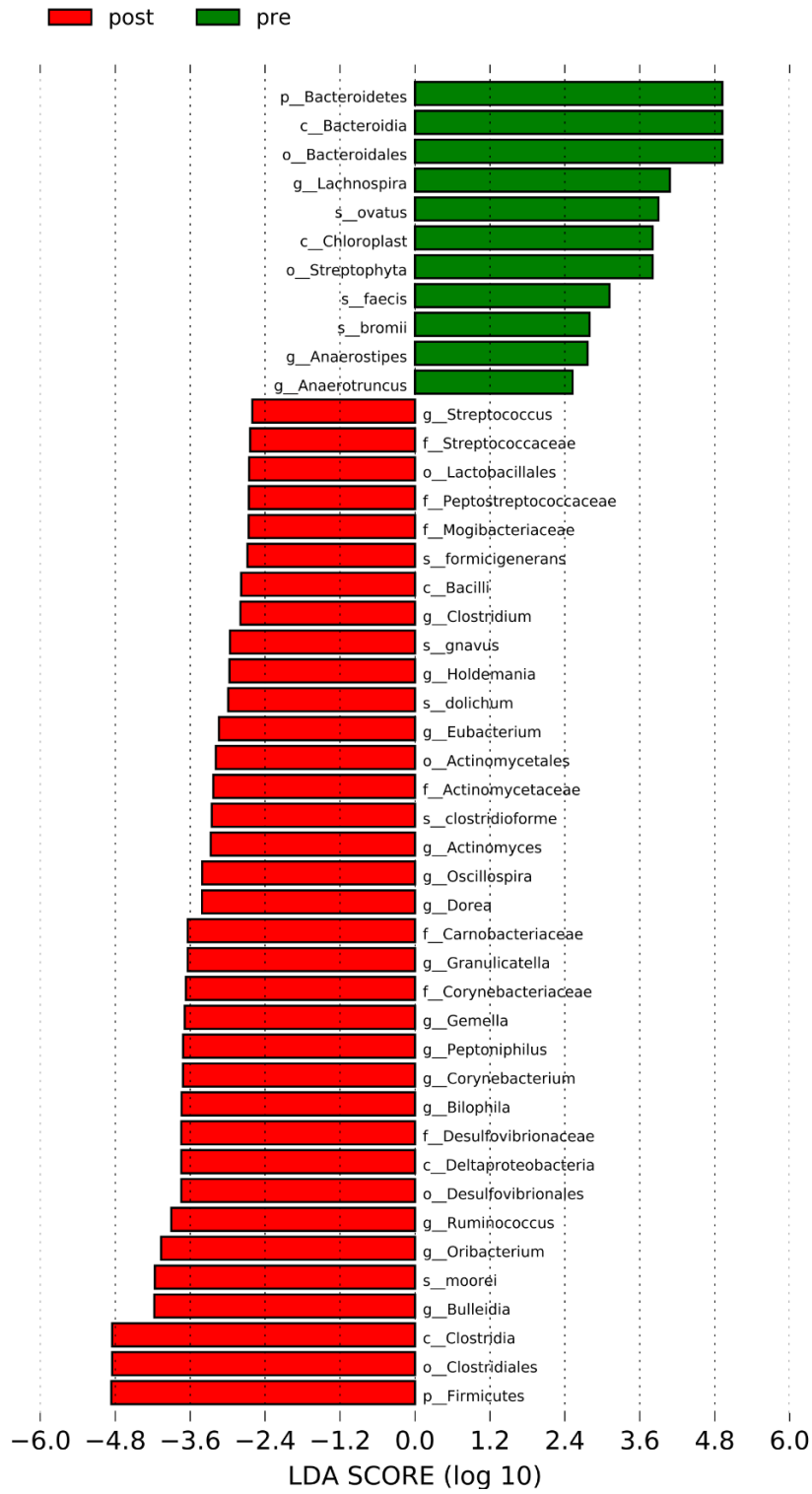


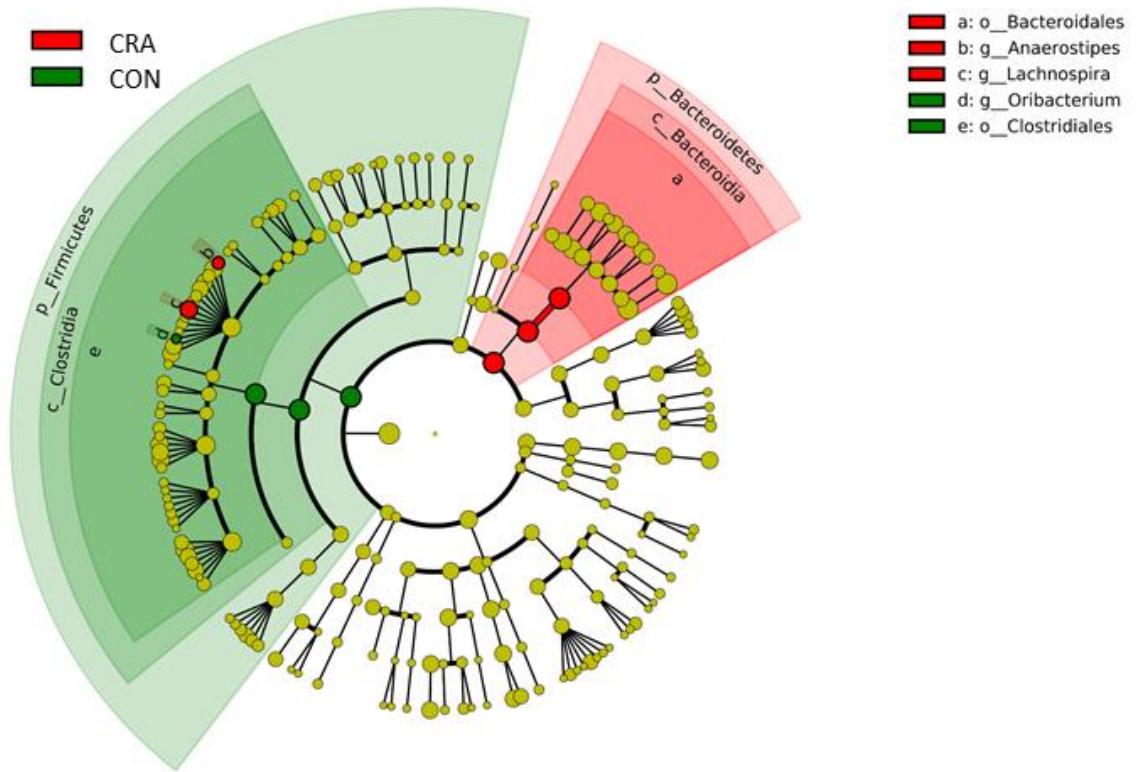
Fig. 2

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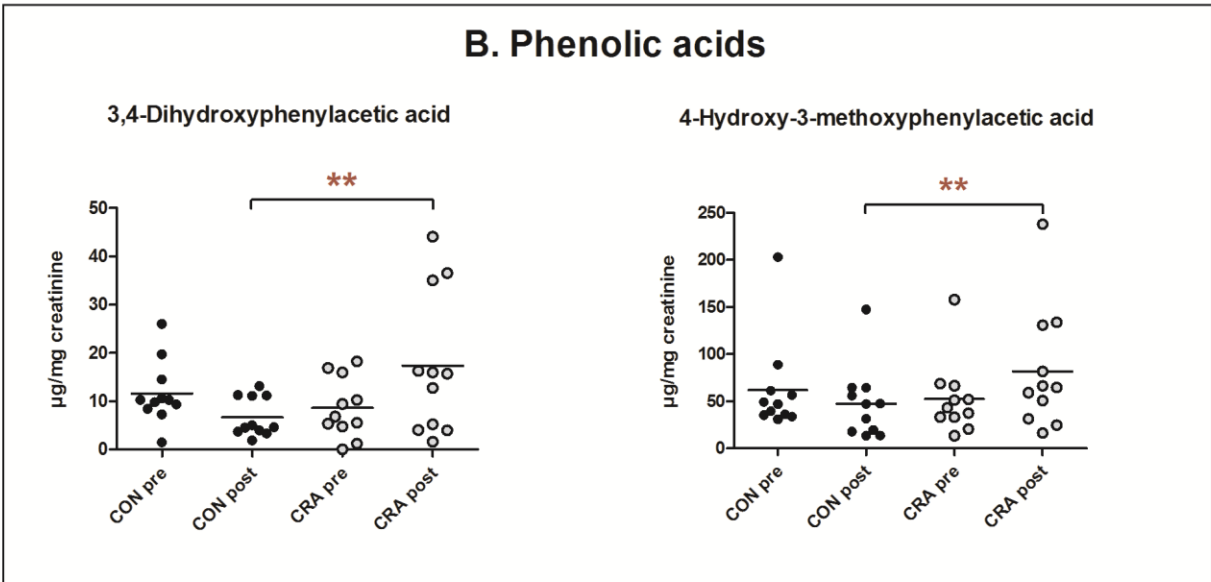
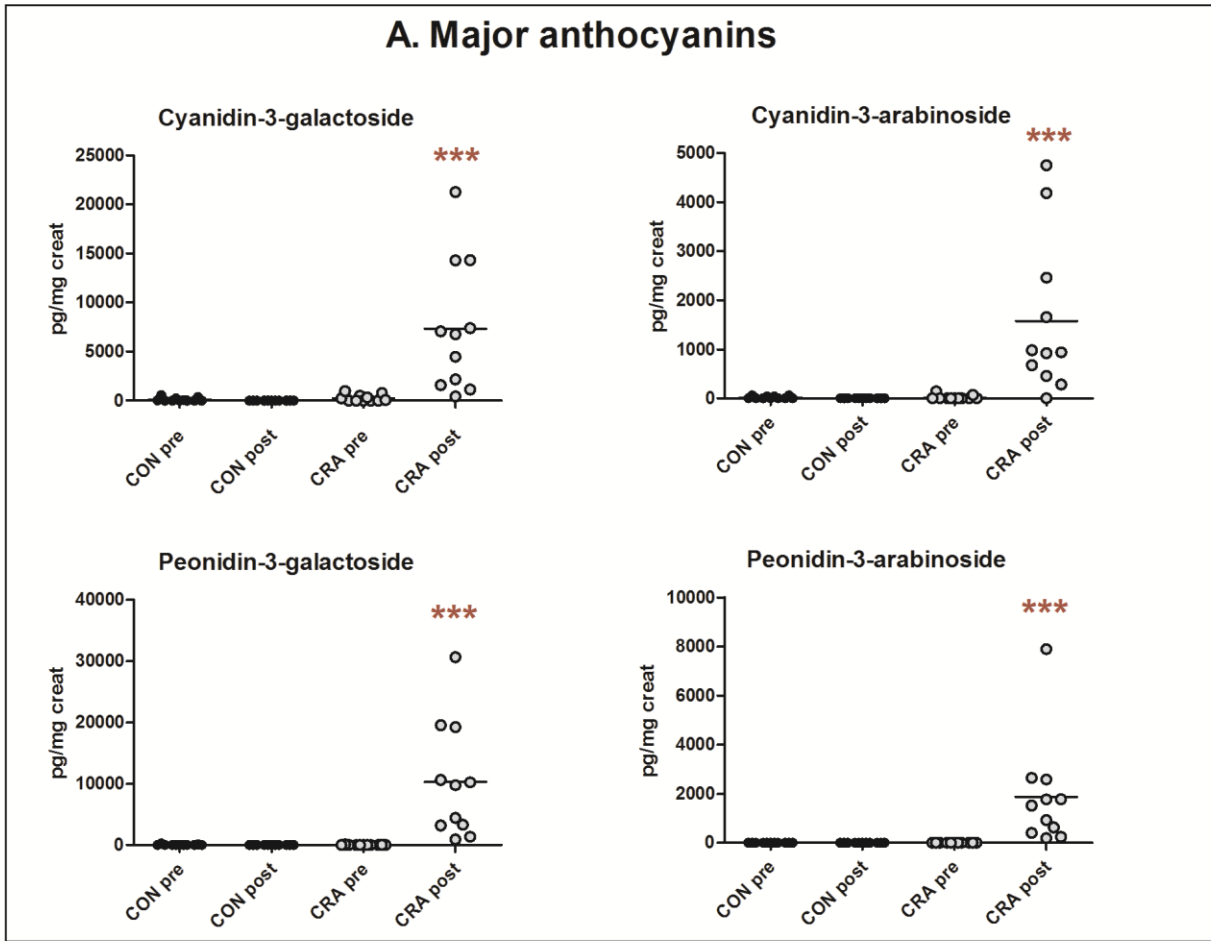
Cladogram of relative abundance differences between cranberry diet and control diet



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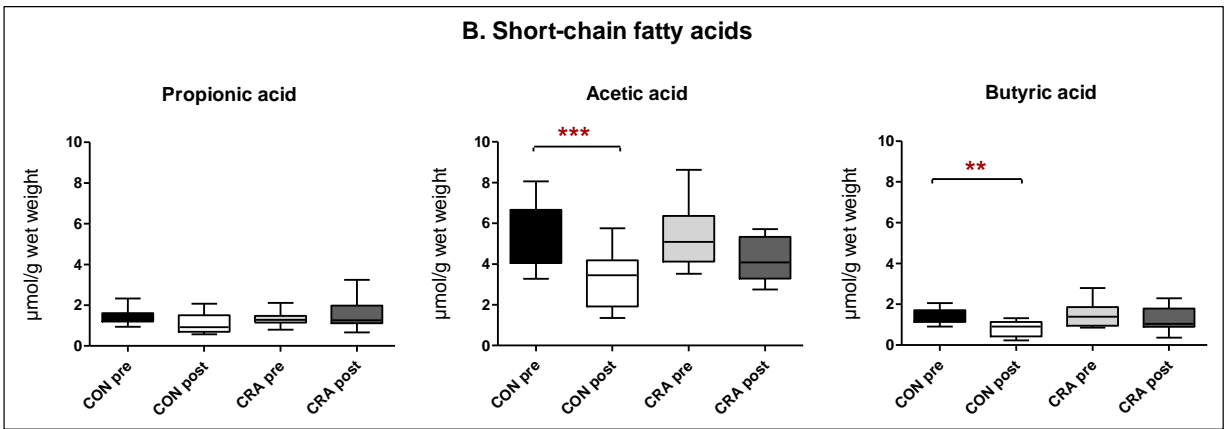
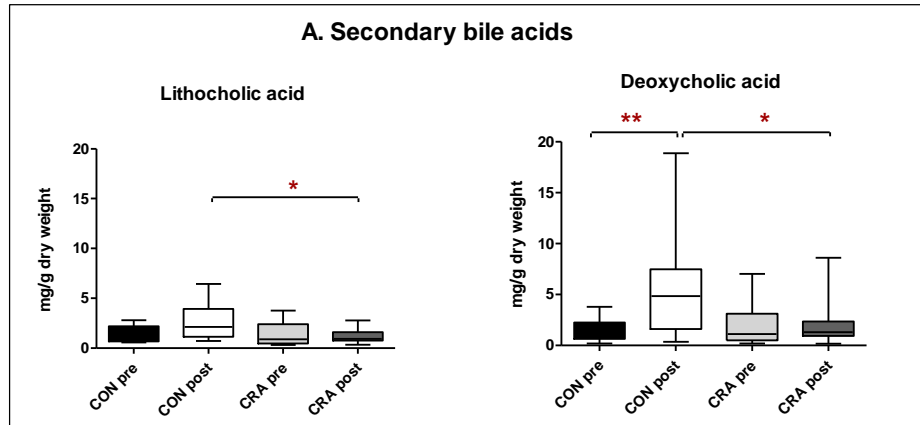
Fig. 3



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Fig. 4



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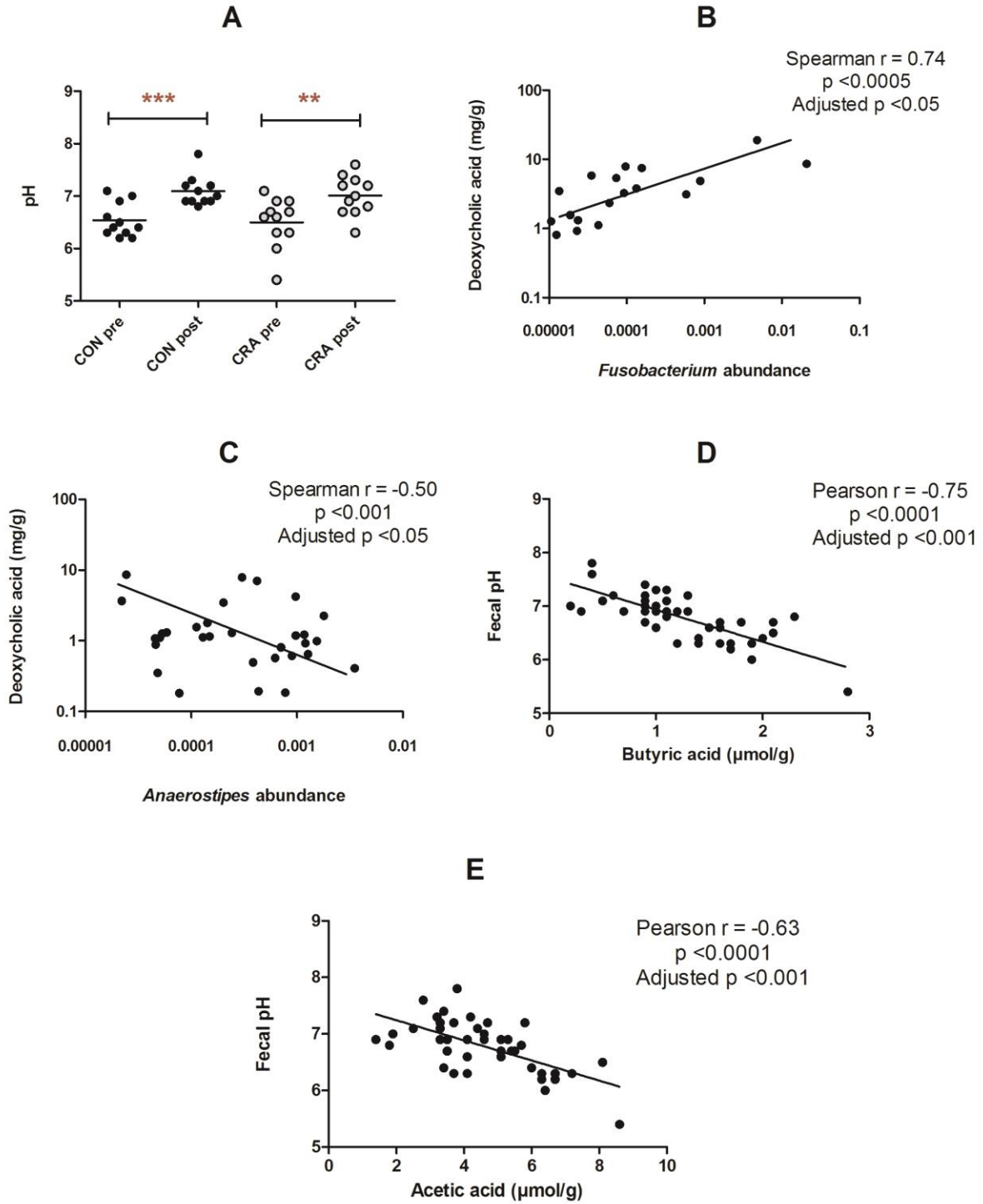
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Fig. 5



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Fig. 6

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815 **Table 1.** Baseline characteristics of participants

Variable	Mean ± SD		
	All (n=11)	Men (n=7)	Women (n=4)
Age (years)	39.2 ± 12.3	41.4 ± 12.4	35.3 ± 11.1
Height (cm)	171.6 ± 8.5	176.1 ± 4.9	163.8 ± 7.8
Weight (kg)	65.7 ± 8.9	70.7 ± 6.0	57.1 ± 5.8
BMI (kg/m ²)	22.2 ± 2.0	22.7 ± 1.9	21.3 ± 1.7
Waist circumference (cm)	81.5 ± 8.3	85.4 ± 8.1	74.6 ± 1.4
Hip circumference (cm)	93.7 ± 4.3	94.2 ± 4.8	92.7 ± 2.9
Diastolic blood pressure (mm Hg)	81.4 ± 7.9	83.6 ± 6.8	77.5 ± 8.0
Systolic blood pressure (mm Hg)	116.5 ± 12.6	122.4 ± 9.1	106.3 ± 11.0
Heart rate (beats per minute)	70 ± 13	70 ± 14	69 ± 11

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Supplementary Material

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842 **Text S1.** Subject inclusion and exclusion criteria

843 *Subject-inclusion criteria –*

- 844 1. Age between 20-55 years
- 845 2. BMI between 18.5-29.9 kg/m²
- 846 3. Bowel movement ≥ 3 times/wk
- 847 4. Consume ≥ 3 servings of fruits and vegetables daily on average
- 848 5. Willing to consume animal based diets for ≥ 10 days
- 849 6. Willing to consume freeze-dried whole cranberry powder
- 850 7. Willing to not take prebiotics and probiotics during the trial
- 851 8. Do not have allergic reaction to cranberries

852

853 *Subject-exclusion criteria –*

- 854 1. Colonoscopy 2 months prior to their enrollment or scheduled during the study
- 855 2. No antibiotic medications or drugs known to influence fecal microbiota were taken or
856 used 3 mo before the study
- 857 3. History of a bilateral mastectomy
- 858 4. History of autoimmune disorders (rheumatoid arthritis, lupus, multiple sclerosis,
859 vitiligo, psoriasis)
- 860 5. Consume < 3 servings of fruits and vegetables daily one average
- 861 6. Gastrointestinal diseases, conditions, or medications influencing gastrointestinal
862 absorption including active peptic ulcer disease or inflammatory bowel disease which
863 will be found based on the self-report during the screening visit
- 864 7. Regular use of any acid-lowering medications (≥ 3 times/week)

- 865 8. Use of ≥ 14 /wk serving of alcohol (168 oz beer, 56 oz wine, 14 oz hard liquor)
- 866 9. Intend to be pregnant, pregnancy, and breastfeeding
- 867 10. Infrequent (< 3 /wk) or excessive (> 3 /d) number of regular bowel movements
- 868 11. Active treatment for cancer (except non-melanoma skin cancer) and cardiovascular
869 disease of any type ≤ 1 y
- 870 12. Having diabetes and/or receiving medications for diabetic condition, which will be
871 found based on the self-report during the screening visit
- 872 13. Thyroid disease unstable or medication adjustments in past 6 months, which will be
873 found based on the self-report during the screening visit
- 874 14. Values of standard blood biochemistries are unacceptable for the study based on study
875 physician's discretion
- 876 15. Vegetarians and vegans, unwillingness or inability to consume animal-based foods
877 including chicken, turkey, beef, eggs, cheese, other milk products, etc. Allergy to eggs
878 or milk/dairy products.
- 879 16. Use anticoagulants, such as heparin, warfarin (Coumadin) in past 6 months
- 880 17. On or planning to be on a weight loss regimen
- 881 18. Regular use of any dietary supplements containing vitamins, minerals, herbal or other
882 plant-based preparations, fish oil supplements (including cod liver oil) or homeopathic
883 remedies; however, subjects who are willing to refrain from the use of these
884 supplements at enrollment and throughout the entire study may be considered eligible
- 885 19. Use of monoamine oxidase inhibitors (MAOIs)
- 886 20. Glomerular filtration rate (GFR) < 60 mL/min/1.73 m²
- 887 21. Total cholesterol (TC) > 250 mg/dL

888 22. Total triglycerides (TG) >300 mg/dL

889

890 **Text S2.** Waist and hip circumference measurements protocol

891 **Abdominal (Waist) Circumference Measurement**

- 892 • The volunteer is in a standing position.
- 893 • The volunteer is asked to hold up his gown.
- 894 • The examiner stands behind the volunteer and palpates the hip area for the
895 right iliac crest.
- 896 • The examiner marks a horizontal line at the high point of the iliac crest and
897 then crosses the line to indicate the mid axillary line of the body.
- 898 • The pants and underclothing of the volunteer must be lowered slightly for the
899 examiner to palpate directly on the hip area for the iliac crest.
- 900 • The examiner then stands on the volunteer's right side and places the
901 measuring tape around the trunk in a horizontal plane at this level marked on
902 the right side of the trunk.
- 903 • The recorder walks around the volunteer to make sure that the tape is parallel
904 to the floor and that the tape is snug, but does not compress the skin.
- 905 • The measurement is made at minimal respiration to the nearest 0.1 cm.

906

907 **Buttocks (Hip) Circumference Measurement**

- 908 • The volunteer stands erect with feet together and weight evenly distributed on
909 both feet.
- 910 • The volunteer is holding up the examination gown.

- 911
- The recorder stands in back of the volunteer and gathers the side seams of the
- 912
- exam pants together above the hips and places the thumb in the fabric to make
- 913
- a fold.
- 914
- The recorder holds the folded sides of the pants snugly while the examiner
- 915
- squats on the right side of the volunteer and places the measuring tape around
- 916
- the buttocks.
- 917
- The tape is placed at the maximum extension of the buttocks.
- 918
- The recorder then adjusts the sides of the tape and checks the front and sides
- 919
- so that the plane of the tape is horizontal.
- 920
- The zero end of the tape is held under the measurement value.
- 921
- The tape is held snug but not tight.
- 922
- The examiner takes the measurement from the right side and calls it to the
- 923
- recorder
- 924

925 **Table S1.** Nutrition composition of freeze-dried whole cranberry powder

	1 g	30 g
Moisture (g)	0.2353	7.059
Dry matter (g)	0.7647	22.941
Protein (g)	0.032	0.96
Fiber (g)	0.4235	12.705
Calcium (g)	0.0006	0.018
Phosphorus (g)	0.0011	0.033
Magnesium (g)	0.0006	0.018
Potassium (g)	0.0076	0.228
Sulfur (g)	0.0004	0.012
Fat (g)	0.0243	0.729
Ash (g)	0.0168	0.504
Lignin (g)	0.0555	1.665
Starch (g)	0.2217	6.651
Proanthocyanidins (mg)	23.54	706.2
Hydroxycinnmate (mg)	1.52	45.6
Flavonols (mg)	4.63	138.9
Anthocyanins (mg)	2.79	83.7

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927

928 **Table S2.** An example of a one-day study menu including items to which either freeze-dried
 929 cranberry or placebo powder were incorporated.
 930

Meal	Food	FWCP or placebo powder (gram)
Breakfast	• Ham steak with cranberry syrup	4
Lunch	• Cheeseburger	6
	• Ginger ale spritzer	4
Dinner	• Cranberry Chicken Satay	4
	• Cranberry Lemonade	6
Snack	• Cranberry pudding	6
	• Ice cream	
Total		30

931 FWCP: Freeze-dried whole cranberry powder

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Table S3. Nutrition composition of the example menu*

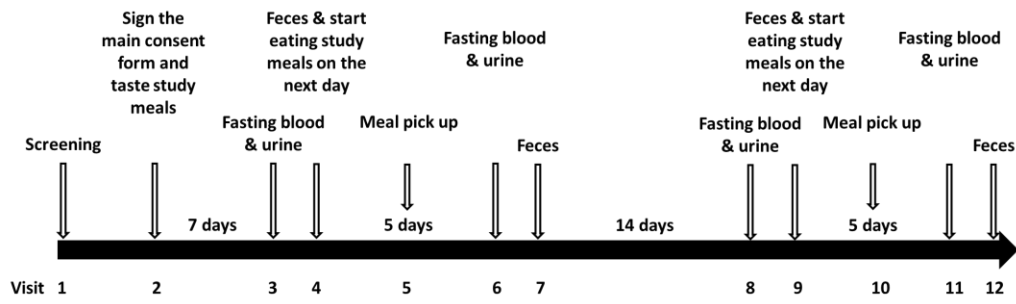
Nutrient	Values
Energy	2091 Kcal
Fats	94.5 g
Carbohydrates	217.1 g
Proteins	107.6 g
Saturated fats	50.2 g
Monounsaturated fats	28.5 g
Polyunsaturated fats	4.6 g
Cholesterol	495 mg
Fiber	9.8 g

934 *The actual amount of foods and nutrition compositions consumed by subjects were adjusted to

935 meet their energy and nutrient needs.

936

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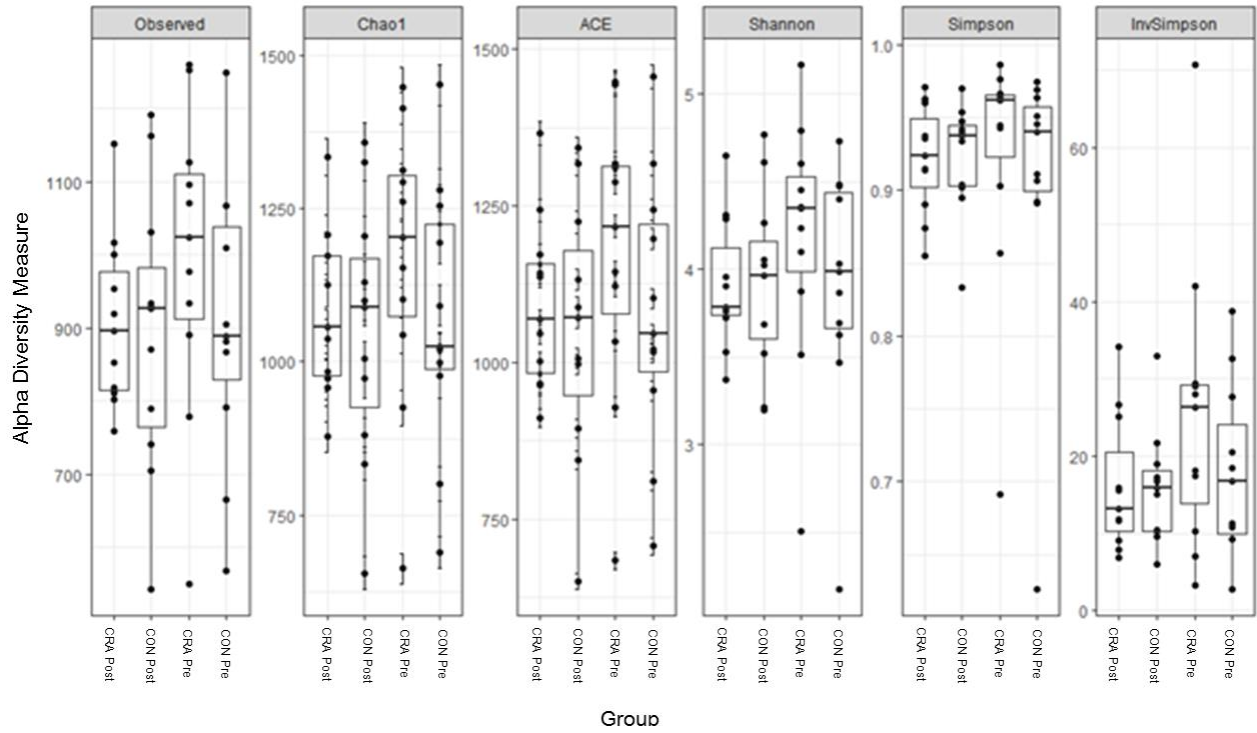
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Fig. S1. Study timeline, including 12 study visits over 8 weeks.

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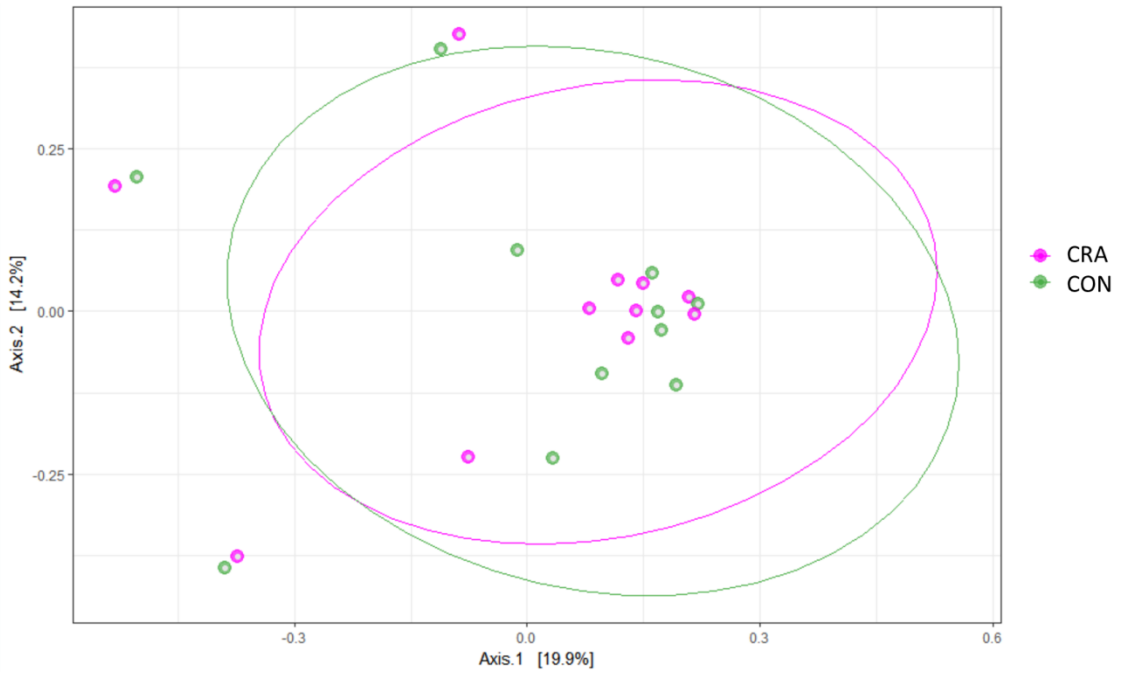


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943 **Fig S2.** Alpha-diversity indexes of stool collected from study participants before (pre) and after

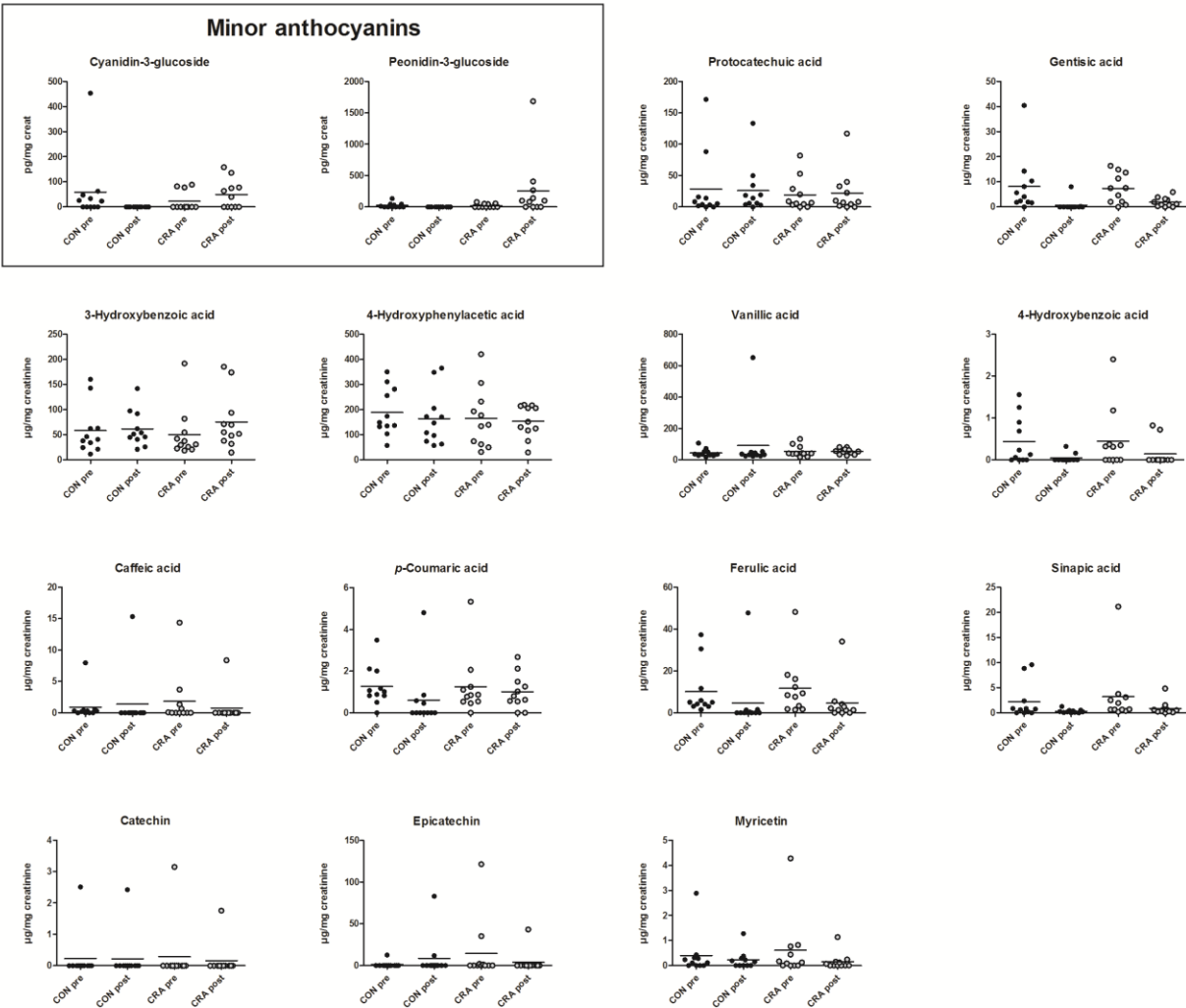
944 (post) consuming control diet (CON) or cranberry diet (CRA) for 5 days.

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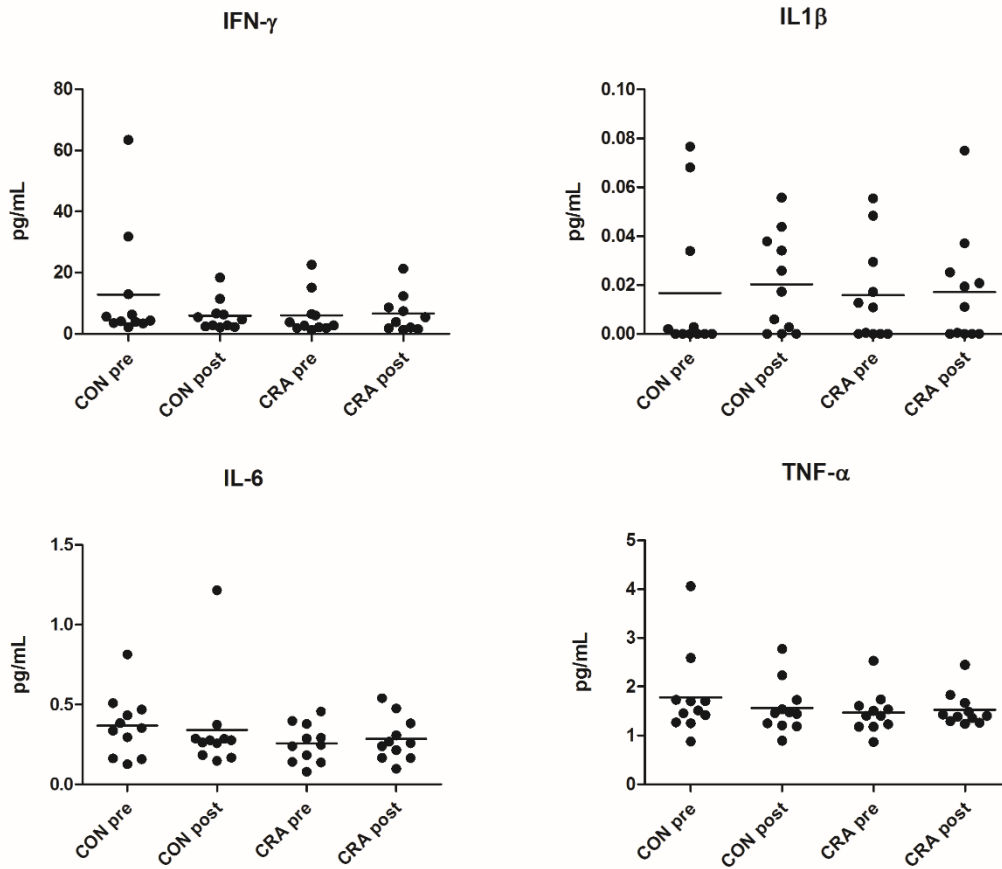
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950 **Fig. S4.** Urinary levels of minor anthocyanins, phenolic acids, and flavonoids in study
951 participants before (pre) and after (post) consuming control diet (CON) or cranberry diet (CRA)
952 for 5 days.

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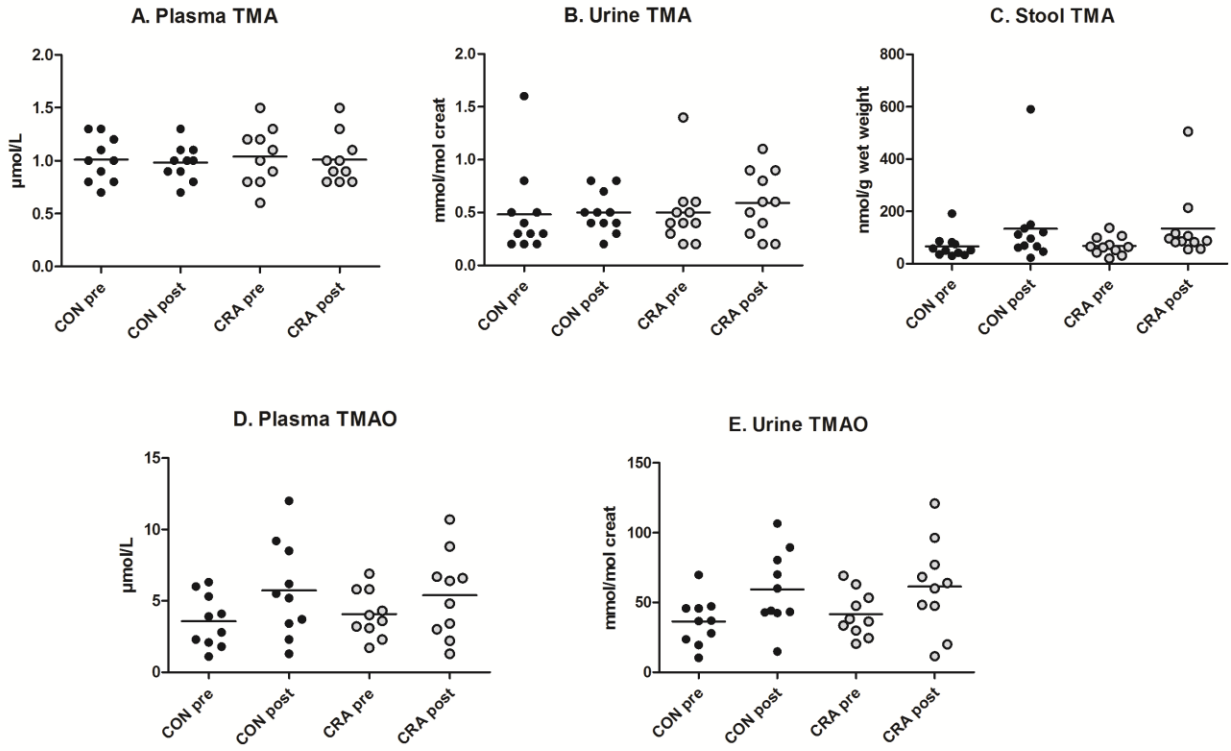
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955 **Fig. S5.** Levels of plasma inflammatory biomarkers before (pre) and after (post) consumption of
 956 the control diet (CON) and cranberry diet (CRA) for 5 days

957

958

959 **Fig. S6.** Trimethylamine (TMA) concentrations in plasma (A), urine (B) and stool (C);
960 trimethylamine-*N*-oxide (TMAO) concentrations in plasma (D) and urine (E).



961