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1 **Obesity influences composition of salivary and fecal microbiota and impacts the**
2 **interactions between bacterial taxa**

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38 **ABSTRACT**

39 Obesity is an increasing global health concern and is associated with a broad range of
40 morbidities. The gut microbiota are increasingly recognized as important contributors to obesity
41 and cardiometabolic health. This study aimed to characterize oral and gut microbial
42 communities, and evaluate host:microbiota interactions between clinical obesity classifications.
43 We performed 16S rDNA sequencing on fecal and salivary samples, global metabolomics
44 profiling on plasma and stool samples, and dietary profiling in 135 healthy individuals. We
45 grouped individuals by obesity status, based on body mass index (BMI), including lean (BMI 18-
46 24.9), overweight (BMI 25-29.9), or obese (BMI ≥30). We analyzed differences in microbiome
47 composition, community inter-relationships, and predicted microbial function by obesity status.
48 We found that salivary bacterial communities of lean and obese individuals were
49 compositionally and phylogenetically distinct. An increase in obesity status was positively
50 associated with strong correlations between bacterial taxa, particularly with bacterial groups
51 implicated in metabolic disorders including *Fretibacterium,* and *Tannerella.* Consumption of
52 sweeteners, especially xylitol, significantly influenced compositional and phylogenetic
53 diversities of salivary and fecal bacterial communities. In addition, obesity groups exhibited
54 differences in predicted bacterial metabolic activity, which was correlated with host's metabolite
55 concentrations. Overall, obesity was associated with distinct changes in bacterial community
56 dynamics, particularly in saliva. Consideration of microbiome community structure, and
57 inclusion of salivary samples may improve our ability to understand pathways linking microbiota
58 to obesity and cardiometabolic disease.

59 **IMPORTANCE**

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Obesity is a worldwide epidemic that is associated with a wide range of health issues. Microbiota were shown to influence metabolism and obesity development. Our study aimed to evaluate the interactions between obesity, salivary and fecal microbiota, and metabolite concentrations in healthy individuals. The oral bacterial community was more impacted by the obesity status of the host than fecal microbiota. Consistently for oral and fecal microbiota, the number of strong interactions between bacteria increased with the increase in the obesity status. Several predicted microbial metabolic pathways that were shown to be associated with metabolic health were uniquely enriched between obesity groups. In addition, these metabolic pathways were correlated with plasma and stool metabolites. Our results suggest that oral microbiota might better reflect the obesity status of the host than fecal microbiota, and that correlations between microbial taxa are altered during obesity.

INTRODUCTION

Obesity is a growing worldwide epidemic and is linked to a range of health issues including hypertension, type 2 diabetes, asthma, coronary heart disease, Alzheimer's disease and cancer [1-5]. Known risk factors include imbalances between calorie intake and expenditure, genetics, stress, and disruptions in the endocrine system [1, 6]; however much remains unknown. Better characterization of mechanisms predisposing to obesity could enable novel prevention and treatment strategies.

The composition of an individual's microbiota is increasingly being recognized as a contributor to obesity risk [7-9]. Microbiota can influence the host's metabolic phenotype both by directly affecting energy and nutrient availability [10-14], and through modulation of signaling pathways

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82 [15-22]. Previous studies suggested that the fecal symbiotic bacterial community of obese
83 individuals is less diverse than that of lean individuals [8, 23]. In addition, the abundance of
84 several bacterial taxa including *Lactobacillus*, *Pervotella, Alistipes, Akkermansia*, and others
85 vary with obesity status [7, 9]. Salivary microbiota of lean and obese individuals also differ in
86 diversity and composition [9, 24-27]. Abundance of several salivary bacterial taxa including
87 *Campylobacter*, *Aggregatibacter,* and *Veillonella* was reported to be positively associated with
88 obesity [28-30]. Higher abundances of Bacteroidetes, Spirochaetes, and Firmicutes were
89 observed in lean individuals [9, 31, 32]. However, data are contradictory, even for rather
90 abundant bacteria taxa. For example, the abundance of intestinal *Lactobacillus* was reported to
91 be both positively and negatively associated with obesity [7, 33-35]. These discrepancies may be
92 due in part to complex interactions between microbial community members, where metabolic
93 activity of individual bacterial taxa can vary based on the activity of other microbes in the
94 community [36-39]. Consideration of interactions between members of microbiota might be
95 essential to improve identification of bacterial mechanisms underlying obesity.

96 We hypothesized that the presence of obesity, in the absence of known disease, would
97 associate with differences in microbiome composition and function. We further hypothesized
98 that community structure and bacterial inter-relationships would differ by obesity status. We
99 evaluated the differences in compositional and phylogenetic diversity of salivary and fecal
100 microbiota between obesity groups in a well-characterized sample of healthy individuals. We
101 examined interractions between bacterial taxa based on obesity status of the host, and showed
102 that predicted bacterial metabolic activity varies between obesity groups and is correlated with
103 intestinal and circulating metabolite concentrations.

104 **MATERIALS AND METHODS**

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105 **Study population.**

106 We analyzed data from the ABO Study (n=135) as described previously [40-42]. Briefly, healthy
107 non-pregnant and non-lactating women and men were recruited to a cross-sectional study.
108 Participants completed dietary profiling (validated 3-day food records, and DHQ II food
109 frequency questionnaires [FFQ]), and provided stool, saliva, and blood samples. Height and
110 weight were measured at the study visit. Individuals were classified based on body mass index
111 (BMI, weight (kg)/height (m)-squared), including lean (BMI 18-24.9; fecal samples n=76, saliva
112 samples n=49), overweight (BMI 25-29.9; fecal samples n=34, saliva samples n=19), or obese
113 (BMI ≥30, fecal samples n=25, saliva samples n=16), to explore differences in composition and
114 function of microbiota by obesity. All participants provided written informed consent. The study
115 was approved by the Institutional Review Boards of the University of Pennsylvania and
116 Vanderbilt University.

117 **Sample Profiling.**

118 As we have previously described, 16S rDNA sequencing of the bacterial V4 fragment was
119 performed on Illumina MiSeq platform using 135 fecal and 85 saliva samples to identify
120 bacterial community composition [42]. Global metabolomics profiling of fecal and plasma
121 samples, from a subset of individuals (n=75) was performed at Metabolon (Metabolon Inc.,
122 Morrisville, NC, United States).

123 **Pre-analysis processing**.

124 Sequences alignment and normalization. Pre-analysis processing of 16SrRNA reads was
125 performed with R v4.0.2 [43]. Demultiplexed sequences were filtered, forward and reverse reads
126 were merged, and resulted sequences were assigned to amplicon sequence variants (ASVs), with

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127 the default settings of DADA2 pipeline v1.18.0 [44]. Chimeric sequences were also removed
128 with the dada2 package v1.18.0 [44]. Sequence variants were assigned taxonomy with dada2
129 and SILVA v138.1 database [44, 45]. ASVs counts were normalized with cumulative sum
130 scaling method implemented in the metagenomeSeq v1.32.0 package [46]. In the salivary
131 samples, we identified 1,932 ASVs that belonged to 12 phyla, 19 classes, 44 orders, 70 families,
132 134 genera, and 229 bacterial species. In our fecal samples, we identified 5,000 ASVs that
133 belonged to 16 phyla, 26 classes, 55 orders, 86 families, 270 genera, and 338 bacterial species.

134 Alpha diversity. Normalized ASVs counts were used to calculate species richness,
135 Shannon, and Gini–Simpson alpha diversity indices with the vegan v2.5.7 package [47]. Beta
136 diversity. Bray-Curtis distances were calculated with vegan v2.5.7 [47]. Unrooted neighbor-
137 joining tree was computed with the ape package v5.5 [48]. The tree was optimized based on
138 generalized time-reversible model implemented in the phangorn v2.5.5 package [49, 50]. Lastly,
139 weighted and unweighted Unifrac distances between each sample were calculated with the
140 phyloseq v1.30.0 package [51].

141 Functional potential of the bacterial communities was predicted with PICRUSt2
142 according with the default pipeline [52]. Predictions were made for Enzyme Commission
143 numbers (EC), Kyoto Encyclopedia of Genes and Genomes orthologs (KO), and MetaCyc
144 pathways [52-55]. In accordance with PICRUSt2 authors' recommendations, the resulting data
145 were transformed with centered-log ratio transformation implemented in the ALDEx2 v1.24.0
146 package [56].

147 **Statistical Analysis.**

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148 Statistical analysis and data visualization was done with R v3.6.1 [43]. Beta diversity
149 distances between obesity groups were compared with pairwise permutational multivariate
150 analysis of variance, based on the vegan package v2.5.7 [47]. The difference in alpha diversity
151 measurements was evaluated with Wilcoxon signed-rank test, implemented in the rstatix v0.7.0
152 package [57]. In order to evaluate if the obesity groups can be classified based on abundance of
153 bacterial taxa and inferred functional abundances (based on EC, KO, and MetaCyc
154 classification), we used linear discriminant analysis, implemented in in the MASS package v7.3-
155 51.4 [58]. In addition, we repeated linear discriminant analysis using only the 15 most abundant
156 bacterial taxa, in order to evaluate if the dominant bacterial taxa were sufficient for
157 discrimination of the communities, with the obesity status. The results were visualized by
158 plotting the first and second linear discriminants, with the ggplot2 v3.2.1 and the ggpubr v0.4.0
159 packages [59, 60]. The difference in abundances of bacterial taxa and predicted ECs, KOs, and
160 MetaCyc pathways, between obesity groups was evaluated with a pairwise t-test function,
161 implemented in R v3.6.1 [43]. The correlations between abundances of bacterial taxa were
162 calculated with Spearman's rank correlation test, included in the Hmisc v4.5.0 package [61].
163 Resulted correlation matrices were used to construct network plots, using the corrr v0.4.3
164 package [62]. In addition, the absolute values of correlation coefficients were compared between
165 obesity groups with a pairwise Wilcoxon signed-rank test, implemented in the rstatix v.7.0
166 package [57]. The influence of 133 recently consumed (from 3-day food records) and 185
167 habitually consumed (from FFQ) nutrients on beta diversity distances was evaluated with
168 permutational multivariate analysis of variance using a quadratic model [47]. The quadratic
169 model was used as most living organisms, including bacteria have an optimal range of
170 environmental conditions rather than a linear relationship [63-65].

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171 For enrichment analysis, we calculated the mean abundance of each KEGG ortholog for
172 obesity groups and used them as input for MicrobiomeAnalyst (2021-07-01) shotgun data
173 profiling tool, with the default settings [66]. False discovery rate (FDR) *P*-values were adjusted
174 using the Benjamini–Hochberg correction, implemented in rstatix v0.7.0 package [57]. We note
175 that usage of any particular FDR threshold is ambiguous and often varies between microbiome
176 studies; weaker correlations that fail to hold up to *p* adjustment methods often have biological
177 relevance. Premature rejection of associations falling below conservative p-value thresholds may
178 lead to loss of biologically meaningful data. [67-72]. For this reason, statistical results below
179 0.05 *p*-value threshold were considered to be significant. However, taking into account the
180 difference in opinions and for the readers' convenience, we report both unadjusted and FDR-
181 adjusted p-values in supplementary data.

182

183 **RESULTS**

184 **Lean, overweight and obese individuals can be separated into distinct groups based**
185 **on their oral and intestinal microbiota.**

186 Evaluating beta diversity distances, we observed that salivary microbiota communities of
187 obese and lean individuals were significantly different as measured with Bray-Curtis and
188 Weighted Unifrac distances (**Supplement Table 1**). Based on linear discriminant analysis (non-
189 overlapping confidence ellipses), obesity classes were separated by the abundances of bacterial
190 ASVs (**Fig. 1A**). Obesity groups were also clearly characterized based on abundance of
191 microbial species, genera, families, and orders but weaker based on classes and phyla
192 (**Supplement Figure 1**).

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193 In fecal samples, we did not observe a significant difference in beta diversity distances
194 between any of the obesity groups (**Supplement Table 1**). However, based on a linear
195 discriminant analysis, obesity groups could be classified based on abundance of bacterial ASVs
196 (**Fig. 1B**). Obesity groups were also clearly characterized based on abundance of bacterial
197 species, genera, families, and orders but weaker at class and phylum ranks (**Supplemental**
198 **Figure 2**). We did not observe any significant differences in alpha diversity indices between
199 obesity groups in saliva or feces (**Supplement Table 2**).

200 **Obesity status influences the abundance of individual bacterial taxa**

201 In saliva, we observed that abundances of Campylobacterota, Firmicutes, and
202 Spirochaetota were significantly different between obesity groups at the phylum rank. Obesity
203 groups were significantly different in the abundances of 5 bacterial classes, 10 orders, 17
204 families, 33 genera, 52 species, and 409 individual ASVs (**Supplement Table 3A**). Across all
205 taxonomic ranks, obese and lean individuals had the highest number of taxa that were
206 significantly different in their abundances (**Supplement Table 3A**). We evaluated which of the
207 15 most abundant bacteria taxa were the most influential for defining each of the obesity groups
208 with a linear discriminant analysis. At the genera taxonomic rank, *Campylobacter*, *Veillonella,*
209 *Aggregatibacter*, and *Prevotella* defined the obese group (**Fig. 2**). Although lean and overweight
210 groups were not distinct from each other, *Actinomyces* and *Haemophilus* were characteristic for
211 overweight group (**Fig. 2**). Overall, we note that the 15 most abundant bacteria taxa contribute
212 only modestly to discrimination of obesity groups (**Supplemental Fig. 3**).

213 In feces, at the phylum rank, only abundance of Fusobacteriota was significantly different
214 between overweight and lean groups. Obesity groups were significantly different in the
215 abundances of 2 bacterial classes, 8 orders, 10 families, 35 genera, 45 species, and 690 individual

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216 ASVs (**Supplement Table 3B**). The highest number of significant differences between groups
217 varied with taxonomic rank but was always between lean and one of the overweight/obese
218 groups. Linear discriminant analysis indicated that at the genus taxonomic rank *Agathobacter*
219 and *Parabacteroides* were influential in discriminating obese from lean groups (**Fig. 2**).
220 Although lean and overweight groups were not clearly separated, lean group was primarily
221 characterized by *Blautia* and *Ruminococcus* (**Fig. 2**). Similar to what we observed in salivary
222 samples, the most abundant fecal bacteria taxa were not the most influential variables for
223 discriminating samples based on obesity status (**Supplemental Figure 4**).

224

225 **The number of strong correlations between bacterial taxa vary by obesity status.**

226 We hypothesized that microbial community inter-relationships, as evidenced by
227 correlations between taxa, would differ by obesity status. We assessed the number of strong
228 correlations (>= |0.7|) between abundances of microbial taxa in saliva and stool samples by
229 obesity group and found evidence for increasing inter-dependence in the setting of obesity (**Fig**
230 **3**). Among microbiota genera in saliva, there were 67 strong correlations in the obese group, 32
231 in the overweight, and only 5 strong correlations in the lean group. The absolute means of
232 correlation coefficients were significantly different between all groups, and this observed pattern
233 remained across all taxonomic ranks (**Supplement Table 4**). We observed a similar pattern in
234 fecal samples, with 52 strong correlations between microbiota genera in the obese group, 20 in
235 the overweight group, and only 8 in the lean group. The absolute values of the correlation
236 coefficients, for abundances of the bacterial taxa were significantly different between all obesity
237 groups. Obese individuals had more strong correlations between bacterial taxa than lean

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238 individuals across all phylogenetic ranks except phylum, at which no group had strong inter-
239 bacterial correlations. (**Supplement Table 4**).

240 **Nutritional Factors Influencing Bacterial Communities**.

241 We examined the relationships between dietary variables and the overall bacterial
242 community, to identify influential nutrients from recent (3-day food records) and habitual (food
243 frequency questionnaire) consumption. We applied Bray-Curtis, weighted Unifrac, and
244 unweighted Unifrac distances, and assessed both linear and quadratic relationships. For recently-
245 consumed nutrient, xylitol and pectins had significant linear relationships across all 3 methods,
246 while inositol, glucose and omega-3 polyunsaturated fatty acids approached significance for
247 quadratic relationships across all 3 methods (**Supplement Table 5**). For habitually-consumed
248 nutrients, no nutrients displayed consistent linear relationships across all methods, while for
249 quadratic relationships, sorbitol and pinitol, as well as dairy cheese and yogurt were consistently
250 associated (**Supplement Table 6**). In the fecal bacterial community, recently-consumed pectins,
251 folate, and fiber had consistent significant linear relationships, while oxalic acid, formononetin,
252 biochanin A, and the ratio of polyunsaturated to saturated fat had consistent quadratic
253 relationships (**Supplement Table 5**). For habitually-consumed foods, there were consistent
254 linear relationships with cheese and vegetables, in addition to vegetable-derived nutrients (beta
255 carotene, oxalic acid, Vitamin K). Significant quadratic relationships were observed for grains
256 and processed meats, in addition to xylitol, caffeine, sodium and potassium (**Supplement Table
257 6**).

258 **Analysis of inferred metabolic pathways reveals enrichment in 2-oxocarboxylic acid
259 metabolism in lean individuals in oral and intestinal microbiota**

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260 We hypothesized that functional activity of microbiota, as predicted using PICRUSt2,
261 would differ by obesity status. We assessed differences in inferred function between obesity
262 groups, and found that obesity served as a good classifier for enzyme counts (ECs), KEGG
263 orthologs (KOs), and MetaCyc pathways abundances in saliva (Fig. 4). There were 969
264 significant differences in ECs, 3,915 in KOs and, 177 significant differences in the abundance of
265 MetaCyc pathways across all groups (**Supplement Table 7**). In all cases, lean and obese
266 individuals had the highest number of differences. 2-oxocarboxylic acid metabolism, terpenoid-
267 quinone biosynthesis, and D-glutamine and D-glutamate metabolism KEGG pathways were
268 enriched in lean individuals but not in obese group (**Supplement Table 8**). The obese group was
269 uniquely enriched in fluorobenzoate, sulfur, and several amino acid metabolic pathways.

270 Similarly, obesity groups could be characterized based on abundance of MetaCyc
271 pathways, KOs, and ECs in fecal samples (Fig. 4). We observed 128 significant differences
272 between the obesity groups in ECs, 391 in KOs, and 19 in MetaCyc pathways (**Supplement**
273 **Table 7**), spread across lean, overweight and obese groups. The lean group was uniquely
274 enriched in 2-oxocarboxylic acid metabolism, D-glutamine and D-glutamate metabolism, and
275 pentose and glucuronate interconversions, when compared with obese group. The obese group
276 was enriched in C5-branched dibasic acid, lipoic acid, and one-carbon KEGG metabolic
277 pathways (**Supplement Table 8**).

278

279 **Abundance of inferred bacterial metabolic enzymes/pathways influences host's**
280 **metabolites' concentrations.**

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281 We were interested in whether predicted functional activity would associate with
282 measured metabolic activity, as assessed by metabolomic profiling of plasma and stool. We
283 observed high numbers of correlations with predicted saliva microbial activity across all 3
284 databases (EC: 78,635 with plasma, 82,722 with stool; KO: 249,473 plasma, 263,616 stool;
285 MetaCyc: 15,633 plasma, 17,915 stool). The highest number of correlations was observed with
286 valerate and isoeugenol sulfate in plasma samples and with inosine in stool samples
287 (**Supplement Table 9**). We similarly observed high numbers of correlations between predicted
288 stool microbial activity and metabolites (EC: 92,852 with plasma, 109,830 with stool; KO:
289 299,557 plasma, 332,789 stool; MetaCyc: 18,179 plasma, 17,728 stool). The highest number of
290 correlations was observed with 1-palmitoyl-GPE and CMPF in plasma samples and steviol in
291 stool samples (**Supplement Table 9**).

292 **DISCUSSION**

293 Obesity has been linked to alterations in microbiota, however the relative importance of
294 gut and oral microbiota is unclear. We aimed to identify microbial signatures of obesity using
295 both stool and salivary samples in healthy individuals classified as normal weight, overweight or
296 obese based on their BMI. We observed that obesity status was associated with differences in
297 bacterial community composition and shifts in inter-microbial relations that were especially
298 evident in the salivary bacterial community. Although salivary and fecal microbiota were largely
299 impacted by different nutrients, dietary sweeteners were associated with both composition and
300 phylogenetic diversity of both the oral and gut bacterial communities. In addition, samples from
301 obese and lean individuals were enriched in several unique metabolic pathways, inferred activity
302 of which was correlated with plasma and stool metabolite concentrations.

303 **Obesity influences microbial community composition, especially in saliva.**

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304 In agreement with published research, we observed that oral bacterial community
305 composition was distinct between lean and obese individuals [24-27]. In our work, we also
306 observed that the difference in salivary bacterial composition between obese and lean individuals
307 extends to phylogenetic diversity measurements. Consistent with previous research, we also
308 observed some differences in gut bacterial communities between obese and lean groups, however
309 in our work the differences were not supported by Bray-Curtis or weighted Unifrac distances
310 [73, 74]. Our results suggest that at the level of the whole community, salivary microbiota
311 composition better reflects the difference in obesity status than fecal microbiota.

312 With the analysis restricted to the dominant bacterial taxa, we observed a strong influence
313 of *Campylobacter*, *Aggregatibacter, Veillonella*, and *Prevotella* on characterizing the obese
314 group in salivary samples. Interestingly, all of these bacterial genera have been shown to be
315 correlated not only with obesity but also with oral diseases, especially periodontitis [28-30, 75-
316 77]. Considering the whole bacterial community (abundance >20 reads), we observed that some
317 of the bacteria taxa with lower abundance had a stronger effect on differentiation of the obese
318 group than dominant bacteria, including *Shuttleworthia* at the genus rank and Mycoplasmataceae
319 at the family rank that were also significantly more abundant in the obese group. Previous studies
320 identified a correlation between Mycoplasmataceae and obesity [78, 79]. Although to the best of
321 our knowledge, no previous works associated *Shuttleworthia* with obesity in humans, it was
322 associated with obesity and elevated weight in model organisms [80-82]. In addition, similar to
323 what we observed with the dominant bacteria taxa, *Shuttleworthia* and Mycoplasmataceae are
324 associated with periodontitis [83, 84].

325 In the fecal samples, the dominant bacterial genera that characterized the obese group
326 were *Agathobacter* and *Parabacteroides. Agathobacter* and *Parabacteroides* were shown to be

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327 associated with metabolic disorders in humans and a murine model [85-88]. Similar to what we
328 observed in the saliva samples, several less abundant bacterial taxa that were previously
329 associated with obesity, including *Mitsuokella* and *Neisseria,* at the genus rank and
330 Fusobacteriaceae and Gemellaceae, at the family rank, produced more impact on separation of
331 obese and lean categories than dominant bacterial taxa [73, 89-92]. Proportionally to all
332 identified taxa, more organisms were significantly different in abundance between lean and
333 obese groups in saliva samples, when compared with fecal samples, which might suggest that
334 sampling oral microbiota may be more informative in identifying microbial biomarkers of
335 obesity. Given the relative ease of collection of saliva as compared with stool, this could
336 facilitate increased accessibility for research into the microbial contributors to obesity and
337 cardiometabolic disease; however this remains to be confirmed in independent studies.

338 **Number of strong correlations between bacterial taxa increases with the obesity**
339 **status.**

340 In saliva samples, bacterial taxa exhibited the highest inter-microbial connectivity (strong
341 correlations >= 0.7) in obese individuals. In the obese group, the highest connectivity was
342 observed for *Fretibacterium* (eight connections), *F0058* (seven connections), *Mycoplasma*
343 (seven connections), and *Tannerella* (seven connections). Several of these genera, including
344 *Fretibacterium, F0058*, and *Tannerella* were shown to be correlated with metabolic disorders
345 [31, 93-96]. In addition, all of the most connected bacterial taxa were associated with
346 periodontitis [83, 94, 97, 98]. In the lean group, the most connected bacteria exhibited less strong
347 connections than in obese group and were *Atopobium* (three connections), *Megasphaera* (two
348 connections), and *Prevotella* 7 (two connections). Abundance of *Atopobium* was shown to be
349 reduced in obese individuals [99]. Previous research indicated that the abundance of

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350 *Megasphaera* might increase after anti-obesity treatments [100, 101]. *Prevotella* was shown to
351 be associated with plant rich diet and increase in abundance after antidiabetic treatment, however
352 the genus is very diverse [102-104].

353 In the fecal samples, the most connected bacterial genera identified in obese group were
354 *Christensenellaceae R7* group (eight connections) and *Ruminococcaceae UCG-005* (five
355 connections). *Christensenellaceae R7* and *Ruminococcaceae UCG-005* were shown to be
356 associated with plasma lipoproteins and triglycerides [105]. *Ruminococcaceae UCG-005* was
357 also shown to be positively correlated with body weight and weight gain in a swine model [106,
358 107]. In addition, several bacterial taxa previously implicated in metabolic disorders, including
359 *Actinomyces*, *Ruminiclostridium,* and *Lachnospiraceae* exhibited strong inter-bacterial
360 correlations in the obese but not in the lean group [74, 108-110]. The most connected genus in
361 lean individuals was *Ruminococcaceae NK4A214* (three connections). Previous research
362 identified a negative correlation between *Ruminococcaceae NK4A214* and high fat diet and
363 hypertension [111, 112]. However, *Christensenellaceae R-7* group and *Ruminococcaceae UCG-*
364 *005* were also among few genera (total three) that had more than one strong correlation in lean
365 individuals.

366 The impact of the higher degree of microbial interconnectivity observed in obese
367 individuals is unclear but may represent a shift from relative independence of bacterial taxa to a
368 state more reliant on mutualistic relationships. Obesity is often associated with several
369 physiological and environmental conditions that have the potential to act as stressors for the
370 microbial community, including micronutrient deficiency, increased levels of reactive oxygen
371 species, and increase in c-reactive protein concentrations and inflammatory response in the host
372 [113-116]. In accordance with the stress gradient hypothesis, several studies demonstrated that

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373 presence of environmental stressors often increases positive facilitation between microbial taxa
374 in the community [117-120]. In addition, it was demonstrated that nutritional stress could
375 increase the number of connections, in a co-occurrence network of the microbiota members
376 [121]. In agreement with these observations, we found that in the obese individuals, almost all
377 of the strong inter-microbial correlations were positive.

378 **Sweeteners and other nutrients influence compositional and phylogenetic diversity**
379 **of salivary and fecal bacterial communities.**

380 We observed that recently and habitually consumed nutrients influenced bacterial
381 communities. For salivary samples, recently consumed nutrients influenced bacterial community
382 more than habitually consumed nutrients, for both compositional and phylogenetic beta diversity
383 distances. Sugars and sugar alcohols, especially xylitol, mannitol, sorbitol, and pectin were
384 especially influential factors impacting the bacterial community, based on compositional and
385 phylogenetic diversity measurements. Interestingly, all of the listed compounds with the
386 exception of pectin are used as sweeteners [122, 123]. Although the effect of sweeteners on gut
387 microbiota was extensively shown in humans and animal models, the studies on oral bacteria
388 community are limited [124, 125]. To the best of our knowledge, this work is the first report on
389 the correlation between dietary sweeteners and phylogenetic diversity of the human's salivary
390 bacterial community.

391 Fecal microbiota community was consistently more influenced by habitual nutrient
392 consumption than recently consumed nutrients, which might suggest a more stable microbial
393 community. Similar to the saliva samples, consumption of xylitol and pectin influenced
394 compositional and phylogenetic diversity of fecal microbiota. Consumption of sweeteners,
395 including xylitol was reported to influence intestinal bacterial community composition [124].

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396 Pectin consumption was also shown to be correlated with compositional changes in the intestinal
397 microbiota [126, 127]. In our study, compositional and phylogenetic measurements of the fecal
398 microbiota were also consistently influenced by consumption of vegetables and plant-derived
399 compounds including fiber, oxalic acid, formononetin, and daidzein. Consumption of fiber,
400 formononetin, and daidzein was show to have microbiota-mediated beneficial effects on host's
401 metabolic health [128, 129] In addition, habitual consumption of cholesterol and fatty acids also
402 produced a significant effect on compositional and phylogenetic diversity distances of the fecal
403 microbiota in our study.

404 **Bacterial communities of obesity groups are associated with enrichment in predicted**
405 **metabolic pathways, which are correlated with host's metabolite concentrations.**

406 In both saliva and fecal samples, microbiota of the lean individuals were enriched in 2-
407 oxocarboxylic acid metabolism and D-glutamine and D-glutamate metabolism, based on
408 functional prediction. 2-Oxocarboxylic acid metabolism is involved in ornithine and lysine
409 biosynthesis, supplementation of which were shown to have a potential for improving metabolic
410 health [130-132]. D-Glutamine concentrations were shown to be decreased in obese individuals
411 and glutamine supplementation may alleviate obesity symptoms [133, 134]. Metabolic pathways
412 enriched in the microbiota of obese individuals included one-carbon metabolism, which was
413 previously shown to contribute to the development of obesity[135]. In addition, steatosis was
414 shown to be associated with one carbon metabolism's gene expression [136]. Enrichment in
415 other pathways such as lipoic acid metabolism and degradation of valine, leucine, and isoleucine
416 might be a response to increase in oxidative stress and branched-chain amino acids
417 concentrations, often associated with obesity [137-139].

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418 Multiple host's metabolites were significantly correlated with abundance of KOs
419 involved in enriched pathways. For example, the abundance of KOs, predicted in salivary
420 samples and involved in 2-oxocarboxylic acid metabolism influenced the concentration of 435
421 plasma and 326 stool metabolites. Alpha-ketobutyrate was shown to be a biomarker of insulin
422 resistance and glucose intolerance and in our study exhibited a negative correlation with more
423 than half of the 2-oxocarboxylic acid metabolism pathway's KOs, predicted from saliva samples
424 [140, 141]. In addition, KOs involved in 2-oxocarboxylic acid metabolism were correlated with
425 adenosine and steviol in stool samples, both of which were shown to be beneficial for patients
426 with metabolic disorders [142, 143].

427 Our study had considerable strengths, including availability of salivary and fecal
428 microbial profiling, in addition to metabolic phenotyping, in a robust sample size. There were
429 also some limitations inherent in all microbiome projects that are based on 16S rRNA
430 sequencing. Namely, the necessity of choosing a specific segment of the gene, sequence filtering
431 methods, reference database for taxonomic identification, and even normalization methods are
432 all know to cause a degree of bias between studies. In addition, results presented in this study are
433 largely based on relative abundances of the identified microbial taxa and therefore might not be
434 interpreted as causative. Therefore, future studies would be necessary to demonstrate the
435 directions of interactions between the host and its oral and intestinal microbiota.

436 CONCLUSIONS

437 In this study we identified differences in salivary and fecal symbiotic bacterial
438 communities based on obesity status, in a population of otherwise healthy individuals. Our
439 results suggest that inter-correlations between bacterial taxa are altered in the setting of obesity
440 and suggest distinct differences in community dynamics at increasing levels of obesity.

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441 Consideration of microbial community correlation structure might be more informative than
442 measurement of relative abundances of bacteria taxa or diversity measurements alone. In
443 addition, across multiple comparisons, salivary microbiota provided a more distinct pattern of
444 differentiation between obese and lean individuals, than fecal microbiota. Previous studies have
445 primarily focused on analysis of gut microbiota in obesity, however our data suggest that
446 sampling oral microbiota might be a better choice in search of the bacterial biomarkers
447 associated with obesity.

448

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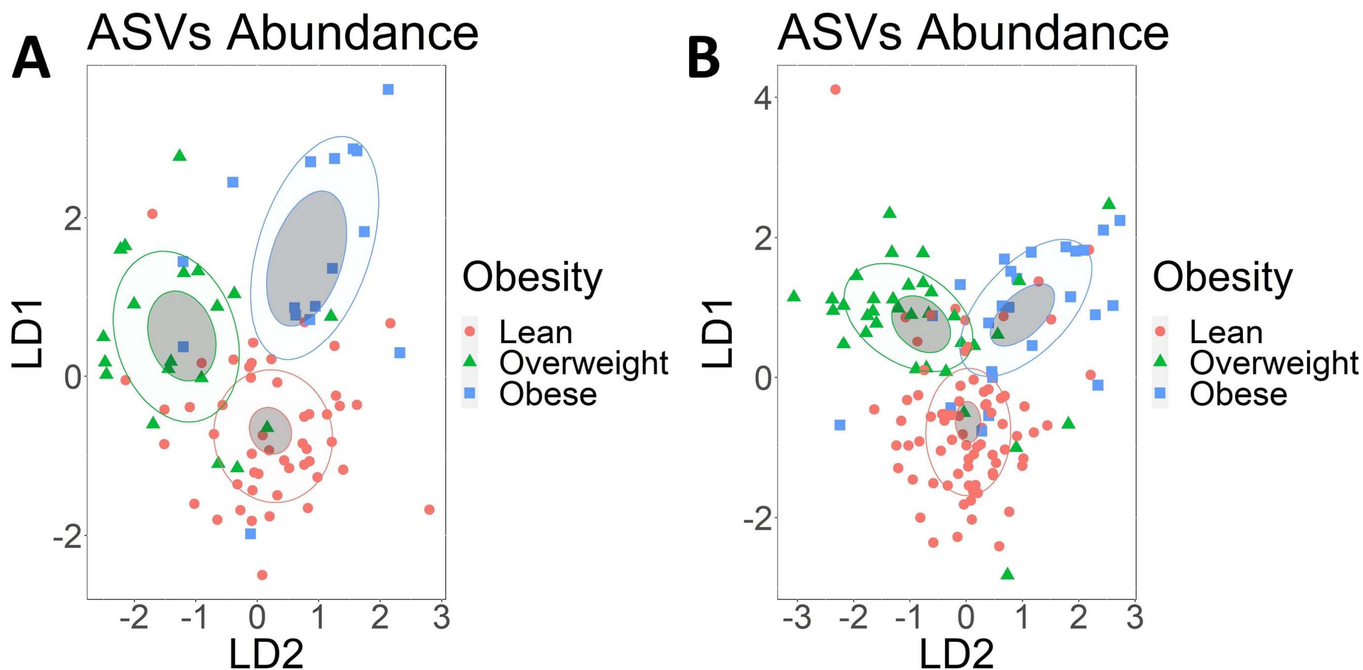
732 **TABLES AND FIGURES**



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735 **Figure 1: Obesity groups can be discriminated by the abundance of salivary or fecal**
736 **microbiota**. Linear discriminant analysis of A) ASVs identified in salivary samples B) ASVs
737 identified in fecal samples. ASVs with abundance of less than 20 sequences were filtered out.
738 Obesity groups are represented by color, lean group by red, overweight group by green, and
739 obese group by blue. Confidence ellipses are shaded. Normal data ellipses are unfilled and
740 leveled to include 50% of the samples.

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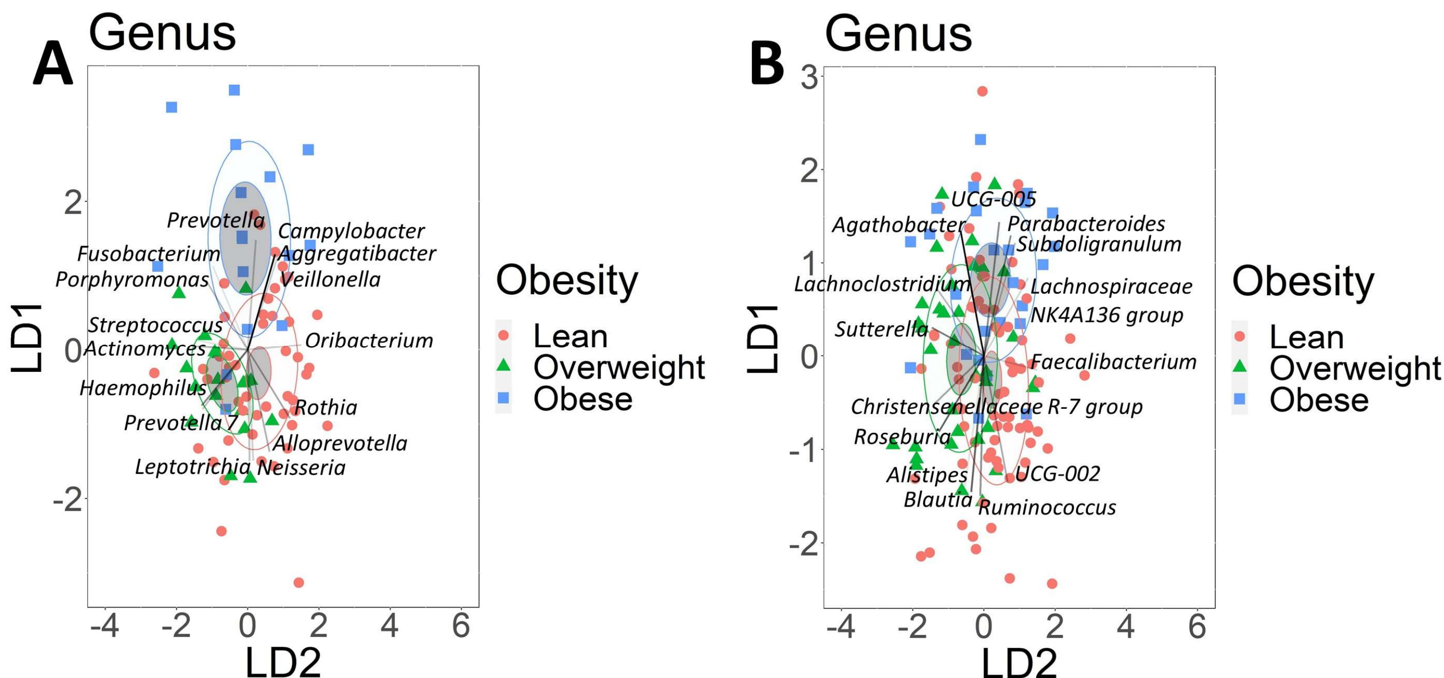

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748 **Figure 2: Obese and lean groups can be characterized by the abundance of**
749 **dominant bacteria genera**. Linear discriminant analysis of the 15 most abundant bacterial
750 genera identified in A) Salivary samples B) Fecal Samples. Obesity groups are represented by
751 color, lean group by red, overweight group by green, and obese group by blue. The higher
752 abundance of bacterial genera in the obesity groups is indicated by the direction of the vector
753 rays. The intensity of vector rays' color corresponds to the strength of the impact. Confidence
754 ellipses are shaded. Normal data ellipses are unfilled and leveled to include 50% of the samples.

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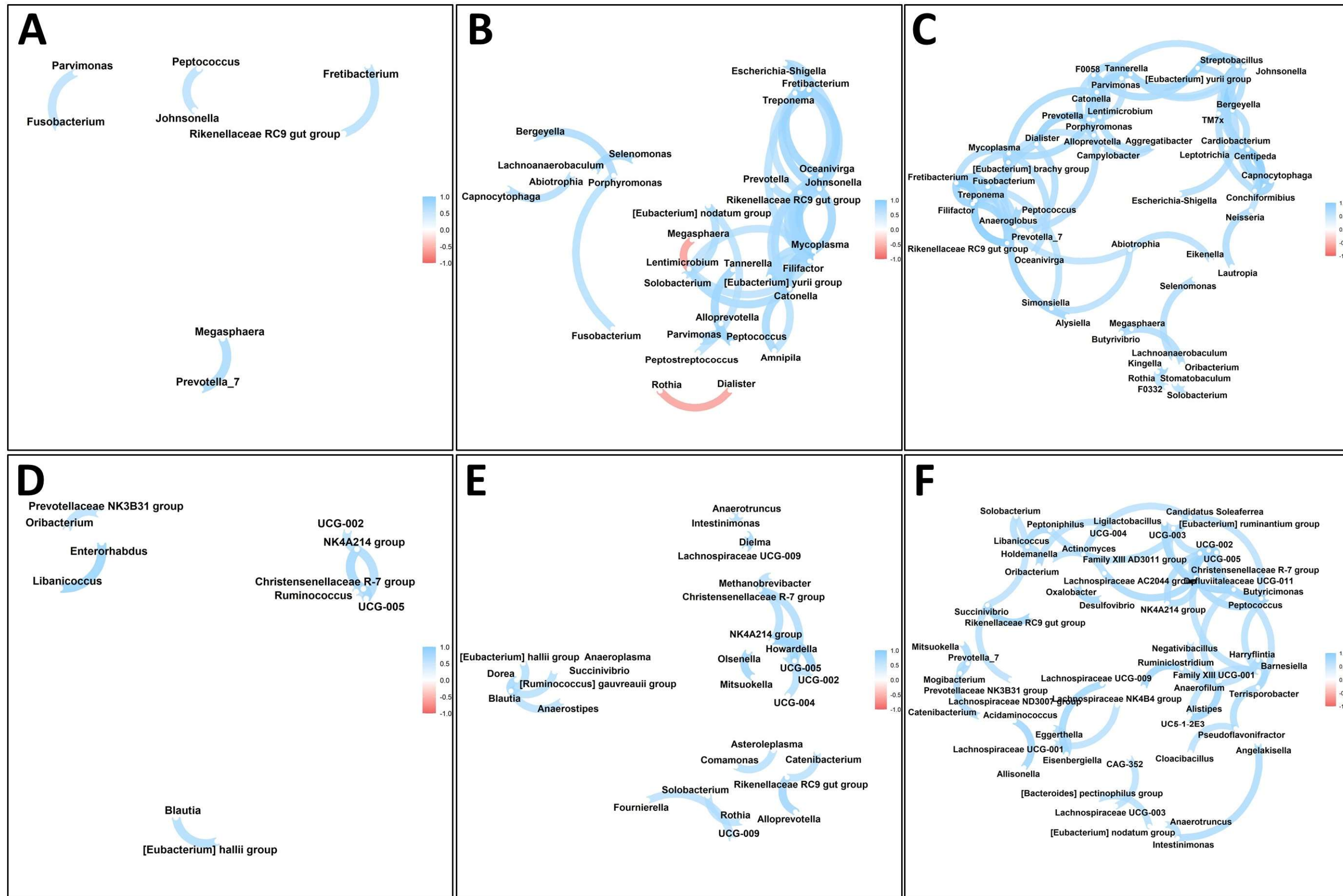

761 **Figure 3: Number of strong connections between bacterial genera increases with the**

762 **obesity status.** Spearman's rank correlation network between A) Salivary bacterial genera of

763 lean individuals; B) Salivary bacterial genera of overweight individuals; C) Salivary bacterial

764 genera of obese individuals; D) Fecal bacterial genera of lean individuals; E) Fecal bacterial

765 genera of overweight individuals; F) Fecal bacterial genera of obese individuals. For A-C

766 included genera had minimum abundance of 30 sequences and for D-F minimum abundance of

767 20 sequences.

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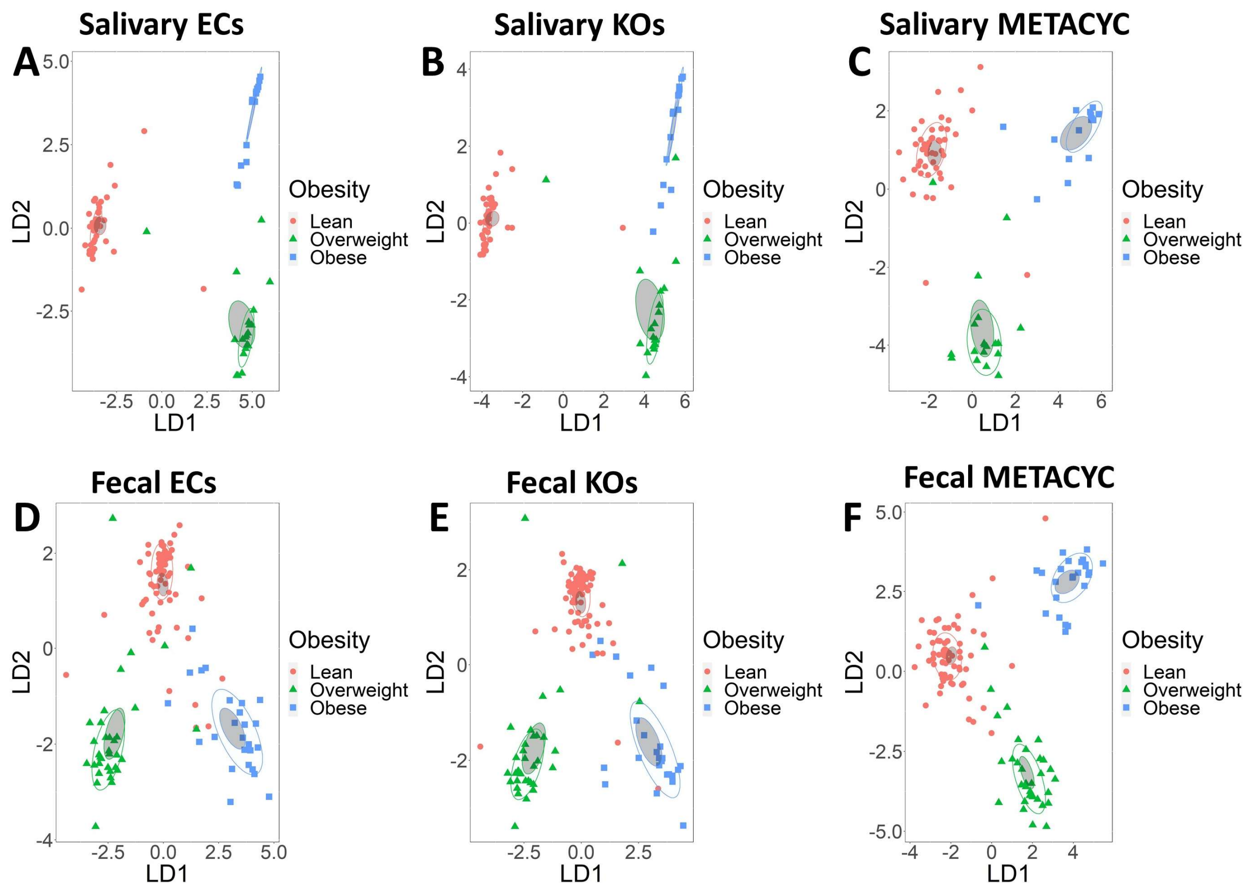

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770 **Figure 4: Obesity groups can be discriminated by metabolic potential predicted by**
771 **PICRUSt2**. Linear discriminant analysis of relative abundances of A) ECs inferred from saliva
772 samples B) KOs inferred from saliva samples, C) MetaCyc pathways inferred from saliva
773 samples, D) ECs inferred from fecal samples, E) KOs inferred from fecal samples, F) MetaCyc
774 pathways inferred from fecal samples. Obesity groups are represented by color, lean group by
775 red, overweight group by green, and obese group by blue. Confidence ellipses are shaded.
776 Normal data ellipses are unfilled and leveled to include 50% of the samples.

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780 **Supplementary Figures' Legends**

781 **Supplement figure 1: Obesity groups could be characterized based on abundance of**
782 **salivary bacterial taxa, especially at lower taxonomic ranks.** Linear discriminant analysis of
783 A) bacterial species, B) bacterial families, C) bacterial families, D) bacterial orders, E) bacterial
784 classes F) bacterial phyla. Taxa with abundance of less than 20 sequences were filtered out.
785 Obesity groups are represented by color, lean group by red, overweight group by green, and
786 obese group by blue. Confidence ellipses are shaded. Normal data ellipses are unfilled and
787 leveled to include 50% of the samples.

788 **Supplement figure 2: Obesity groups could be characterized based on abundance of fecal**
789 **bacterial taxa, especially at lower taxonomic ranks.** Linear discriminant analysis of A)
790 bacterial species, B) bacterial genera, C) bacterial families, D) bacterial orders, E) bacterial
791 classes F) bacterial phyla. Taxa with abundance of less than 20 sequences were filtered out.
792 Obesity groups are represented by color, lean group by red, overweight group by green, and
793 obese group by blue. Confidence ellipses are shaded. Normal data ellipses are unfilled and
794 leveled to include 50% of the samples.

795 **Supplement figure 3: Lean and obese groups can be characterized by the abundance of**
796 **dominant salivary bacterial taxa, across most taxonomic ranks.** The results also indicate that
797 the most abundant bacteria taxa are not the most influential for characterization of obesity
798 groups. Linear discriminant analysis of 15 most abundant salivary bacterial A) Species, B)
799 Families, C) Orders, D) Classes. Obesity groups are represented by color, lean group by red,
800 overweight group by green, and obese group by blue. The intensity of vector rays' color
801 corresponds to the strength of the impact. Confidence ellipses are shaded. Normal data ellipses
802 are unfilled and leveled to include 50% of the samples.

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803 **Supplement figure 4: Lean and obese groups can be characterized by the abundance of**
804 **dominant fecal bacterial taxa across most taxonomic ranks**. The results also indicate that
805 most abundant bacteria taxa are not the most influential for characterization of obesity groups.
806 Linear discriminant analysis of 15 most abundant fecal bacteria A) Species, B) Families, C)
807 Orders, D) Classes. Obesity groups are represented by color, lean group by red, overweight
808 group by green, and obese group by blue. The intensity of vector rays' color corresponds to the
809 strength of the impact. Confidence ellipses are shaded. Normal data ellipses are unfilled and
810 leveled to include 50% of the samples.

811 **Supplementary Tables' Legends**

812 **Supplement Table 1: Salivary but not fecal samples exhibited a significant difference in**
813 **compositional and phylogenetic distances between lean and obese groups**. Pairwise
814 comparisons of Bray-Curtis, Weighted and Unweighted Unifrac distances between obesity
815 groups in salivary and fecal samples.

816 **Supplement Table 2: Obesity groups did not exhibit a significant variation in alpha**
817 **diversity indices of salivary and fecal microbial communities**. Pairwise comparisons of
818 Shannon and Gini-Simpson indices', as well as species richness values between obesity groups
819 in salivary and fecal samples.

820 **Supplement Table 3: More salivary bacterial taxa exhibited a significant difference in their**
821 **abundances between obesity group than fecal bacterial taxa**. Pairwise comparisons of
822 microbiota taxa abundances between obesity groups in A) salivary samples and B) fecal samples.

823 **Supplement Table 4: The number of strong correlations between bacterial taxa in salivary**
824 **and fecal samples increased with the increase of the obesity status of the host**. Pairwise

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825 comparisons of absolute values for inter-bacterial correlation coefficients between obesity
826 groups.

827 **Supplement Table 5: More of the recently consumed nutrients produce a significant effect**
828 **on compositional and phylogenetic diversity distances of salivary samples than on fecal**
829 **samples.** The impact of changes in recently consumed nutrients on Bray-Curtis and Weighted
830 and Unwheighted Unifrac distances of salivary and fecal samples. Sq stands for quadratic effect
831 of the nutrient on beta diversity distances.

832 **Supplement Table 6: More of the habitually consumed nutrients produce a significant**
833 **effect on compositional and phylogenetic diversity distances of fecal samples than on**
834 **salivary samples**. The impact of changes in habitually consumed nutrients on Bray-Curtis and
835 Weighted and Unwheighted Unifrac distances of salivary and fecal samples. Sq stands for
836 quadratic effect of the nutrient on beta diversity distances.

837 **Supplement Table 7: Predicted metabolic potential of bacterial community varies between**
838 **obesity groups**. Pairwise comparisons of predicted bacterial enzymes, Kegg orthologs, and
839 MetaCyc abundances between obesity groups in A) salivary samples and B) fecal samples. ECs,
840 KOs, and MetaCyc pathways that were not significantly different (p >0.05) between obesity
841 groups were excluded from the table.

842 **Supplement table 8: Some of the predicted bacterial metabolic pathways are uniquely**
843 **enriched in obesity groups**. Pairwise comparisons of uniquely enriched bacterial metabolic
844 pathways identified from salivary and fecal samples between obesity groups.

845 **Supplement table 9: Predicted bacterial metabolic potential influences plasma and stool**
846 **metabolites concentrations**. The effect of A) ECs identified from salivary samples on plasma

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847 metabolites concentrations, B) ECs identified from salivary samples on stool metabolites
848 concentrations, C) ECs identified from fecal samples on plasma metabolites concentrations, D)
849 ECs identified from fecal samples on stool metabolites concentrations, E) KOs identified from
850 salivary samples on plasma metabolites concentrations, F) KOs identified from salivary samples
851 on stool metabolites concentrations, G) KOs identified from fecal samples on plasma metabolites
852 concentrations, H) KOs identified from fecal samples on stool metabolites concentrations, I)
853 MetaCyc pathways identified from salivary samples on plasma metabolites concentrations, J)
854 MetaCyc pathways identified from salivary samples on stool metabolites concentrations, K)
855 MetaCyc pathways identified from fecal samples on plasma metabolites concentrations, L)
856 MetaCyc pathways identified from fecal samples on stool metabolites concentrations.

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