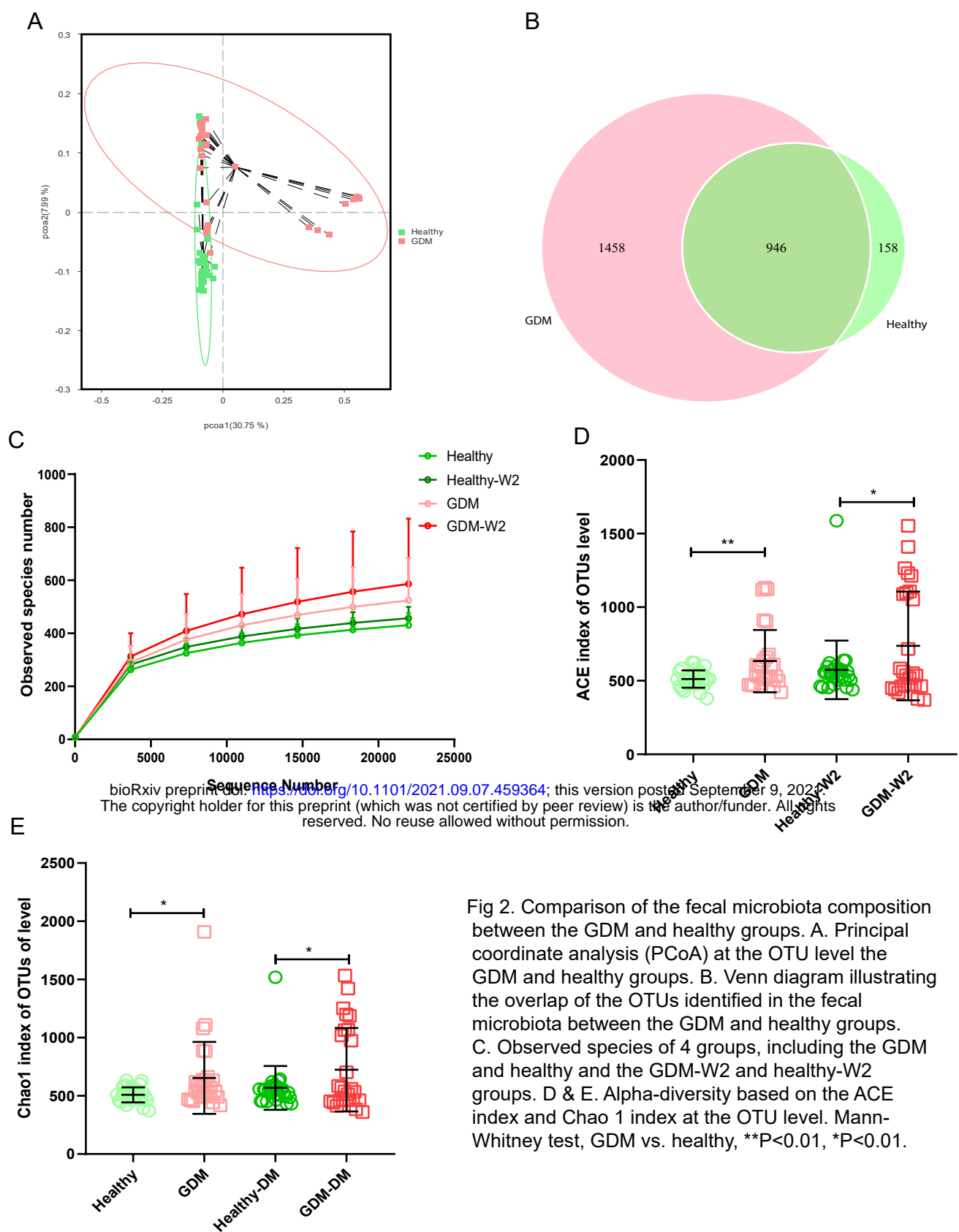


Figure 1. Flow chart illustrating the recruitment of GDM and healthy subjects.



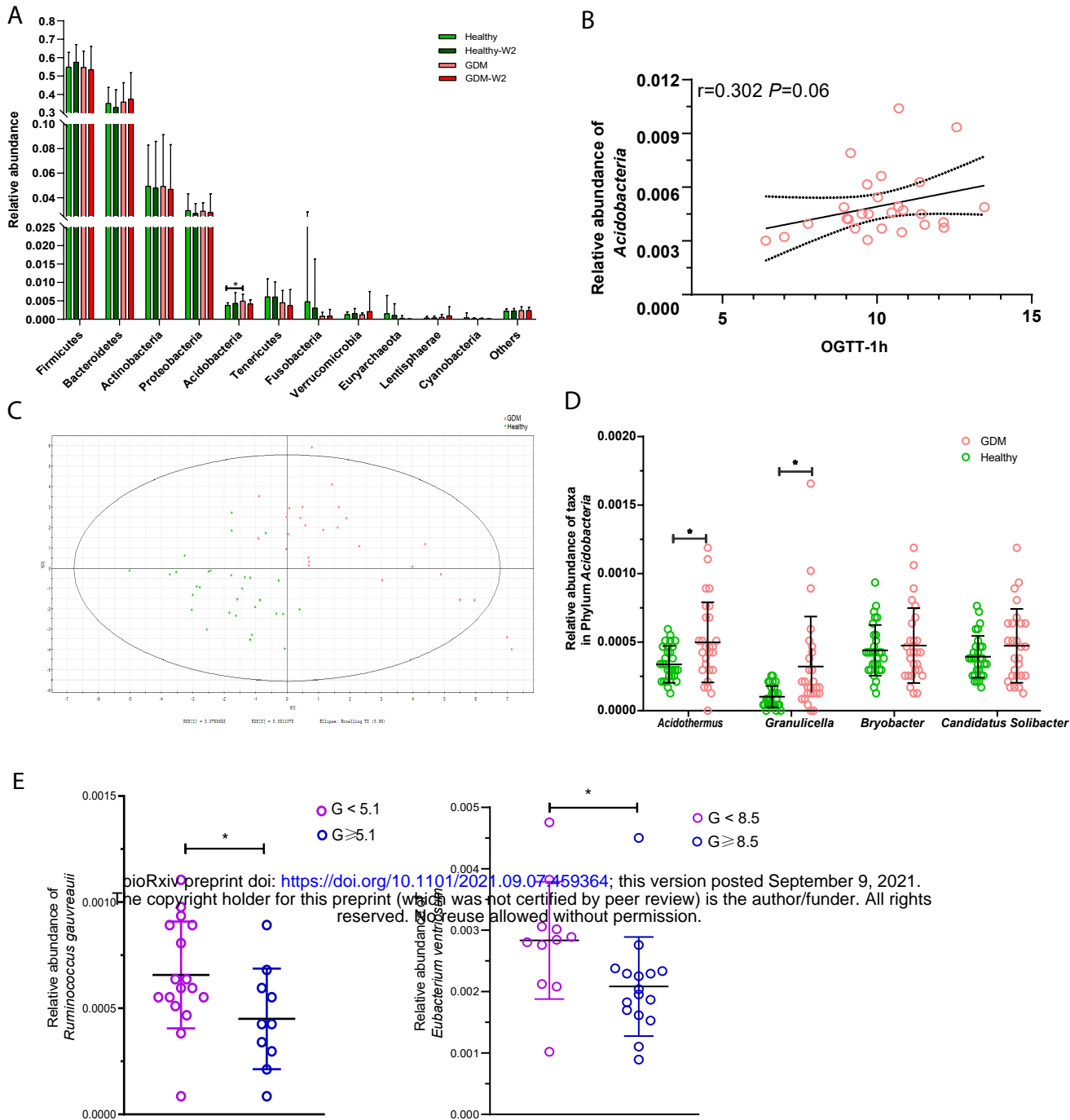
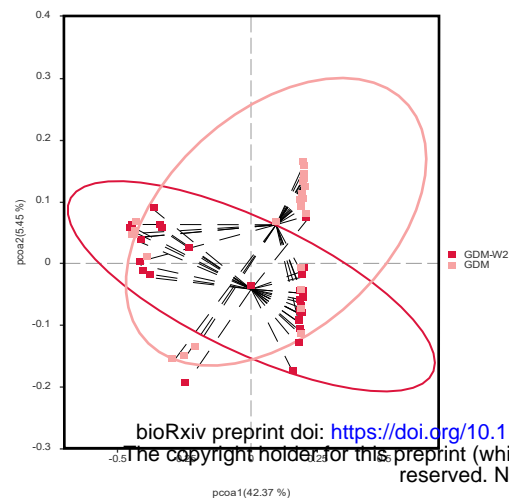
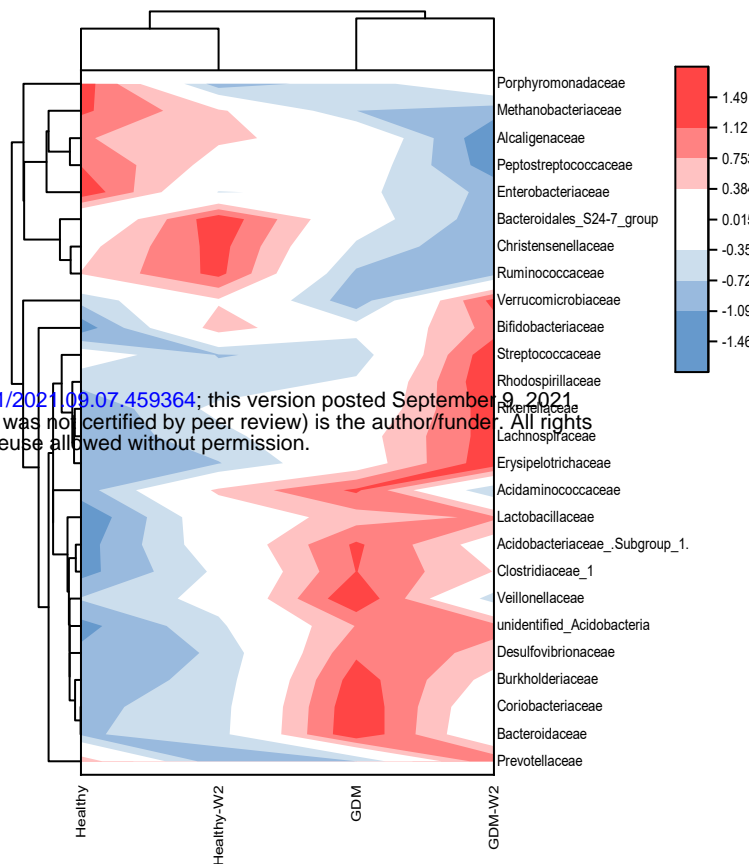


Fig 3. Abundances of taxa in GDM and healthy participants. A. Comparison of the relative abundances at the phylum level among the four GDM and non-GDM groups. The Mann–Whitney test was used to evaluate the two groups. * $P < 0.05$. B. PLS-DA score plots based on the relative abundances of microbiota between the GDM and healthy groups. C. Correlation between the relative abundance of the phylum *Acidobacteria* and the 1-h OGTT measurement. Spearman analysis, $R=0.302$, $P=0.06$. D. Comparison of the relative abundances of *Acidothermus*, *Granulicella*, *Bryobacter*, and *Candidatus Solibacter* in the phylum *Acidobacteria* in the GDM and healthy groups. Mann-Whitney test, GDM vs. control, ** $P < 0.01$, * $P < 0.01$. E. The relative abundances of *Ruminococcus gauvreaui* and *Eubacterium ventriosum* were highly correlated with the OGTT values at 0 h and 2 h. Mann-Whitney test, GDM vs. healthy, ** $P < 0.01$, * $P < 0.01$.

A



B



C

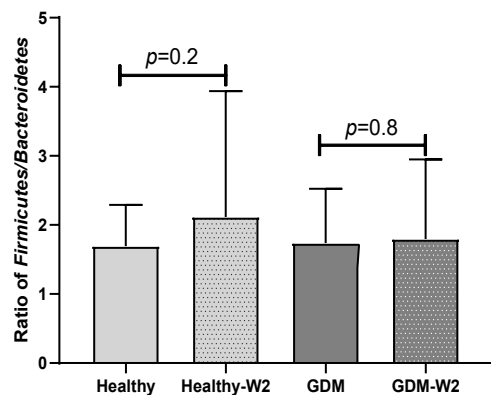


Fig 4. The microbial pattern after diet management. A. Principal coordinate analysis (PCoA) at the OTU level between the GDM-W2 and healthy-W2 groups. B. Heatmap analysis of the differentially expressed taxa at the family level. C. Ratio of *Firmicutes/Bacteroidetes* among the GDM and non-GDM groups with or without diet intervention.

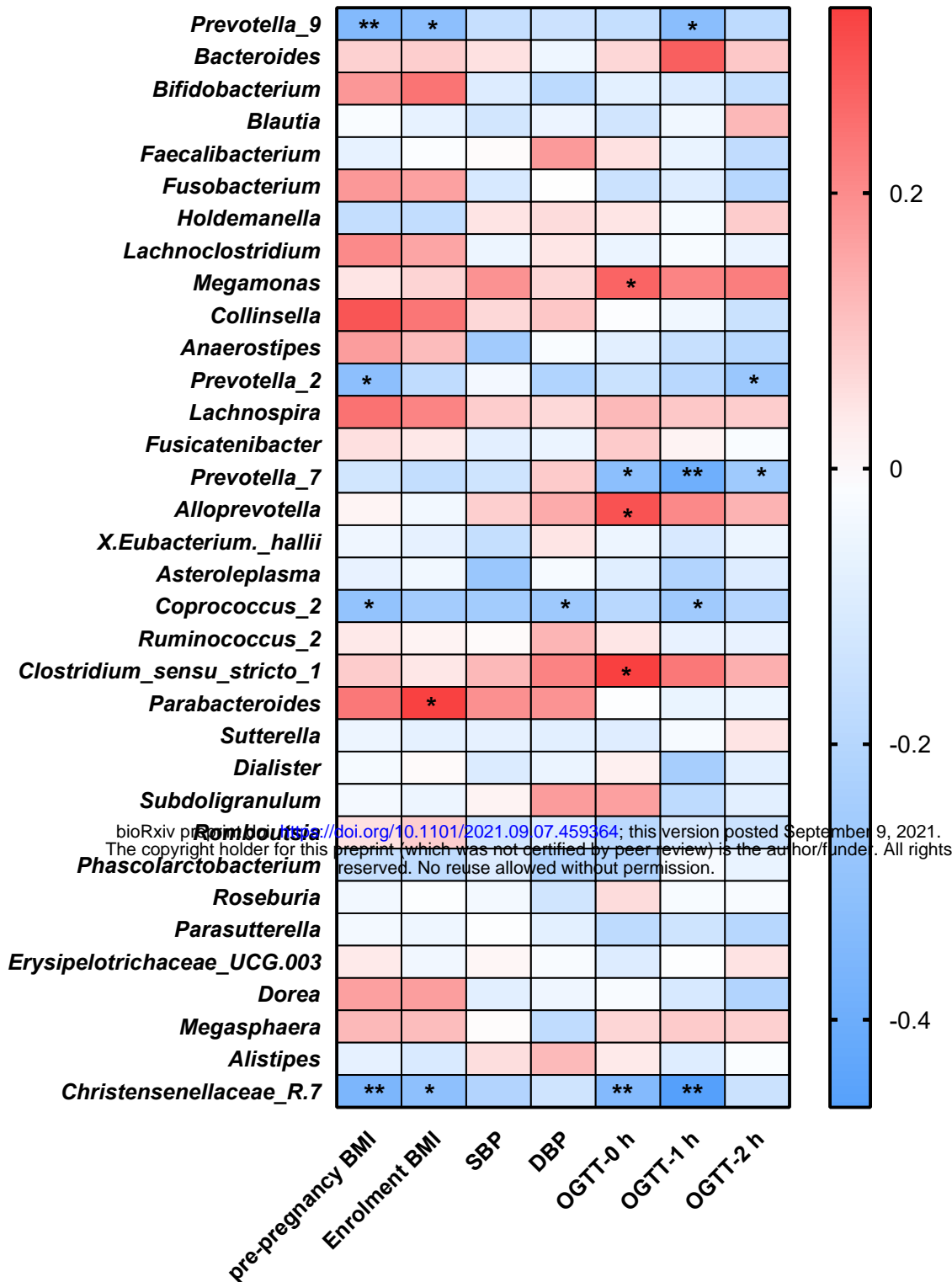


Fig 5. Heatmap analysis of the correlation between the gut microbiota composition and clinical scores.

1 **The gut microbial signature of gestational diabetes mellitus and the association with diet**
2 **intervention**

3

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22 **Abstract**

23 Gestational diabetes mellitus (GDM) is a high-risk pregnancy complication that is associated
24 with metabolic disorder phenotypes, such as abnormal blood glucose and obesity. The link
25 between microbiota and diet management contributes to metabolic homeostasis in GDM.
26 Therefore, it is crucial to understand the structure of the gut microbiota in GDM and to explore
27 the effect of dietary management on the microbiota structure. In this study, we analyzed the
28 composition of the gut microbiota between 27 GDM and 30 healthy subjects at two time points
29 using Illumina HiSeq 2500 platform. The taxonomy analyses suggested that the overall bacteria
30 clustered by diabetes status, rather than diet intervention. Of particular interest, the phylum
31 *Acidobacteria* in GDM was significantly increased, and positively correlated with blood glucose
32 levels. Moreover, Partial least-squares discriminant analysis (PLS-DA) revealed that certain
33 genera in the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Lentisphaerae* characterized
34 the GDM gut microbiota. Correlation analysis indicated that blood glucose levels and BMI index
35 were correlated with the relative abundance of SCFAS-producing genera. Through the
36 comparison between the GDM and healthy samples with or without diet intervention, we
37 discovered that the role of short-term diet management in GDM processes is associated with the
38 change in the *Firmicutes/Bacteroidetes* ratio and some specific taxa, rather than an alternative
39 gut microbial pattern. Our study have important implications for understanding the beneficial
40 effects of diet intervention on the specific gut microbiota and thus possibly their metabolism in
41 pregnant women with GDM.

42

43 **Importance**

44 Understanding the composition and dynamics of the gut microbiota in GDM women under
45 diet intervention is important because there may be opportunities for preventive strategies. We
46 examined the relationships between GDM gut microbiota at two times before and after the diet
47 intervention during second trimester of pregnancy and clinical characteristics in cohort of GDM
48 women. We found that short-term diet management in GDM processes is associated with
49 changes in the *Firmicutes/Bacteroidetes* ratio and some specific taxa rather than an alternative
50 gut microbial pattern. Our study highlights the importance of considering diet intervention as the
51 rescue of microbial dysfunction of GDM disease and can serve as a strategy for early prevention
52 in future study.
53

54 **Introduction**

55 The intestinal microbiota is a robust ecosystem inhabited by nearly 100 trillion bacteria (1). In
56 recent years, extensive attention has been given to the gut microbiota during pregnancy. Over the
57 course of a healthy pregnancy, the body undergoes substantial hormonal, immunological, and
58 metabolic changes (2, 3). In predisposed women, these physiological changes may lead to the
59 development of gestational diabetes mellitus (GDM). GDM is defined as abnormal glucose
60 regulation with onset or first recognition during pregnancy and is one of the most common
61 complications during pregnancy, with an incidence of 2–6% of all pregnancies (4, 5). The
62 clinical incidence of GDM in China is currently presenting a dramatic increasing trend (6). In the
63 context of nonpregnant obesity, recent work suggests a role for gut microbiota in driving
64 metabolic diseases, including diabetes, weight gain, and reduced insulin sensitivity (4, 5, 7, 8).
65 Researchers understand that the intestinal flora has an important function in the development of
66 GDM with the notions relating the intestinal flora to metabolic disease (3, 9, 10). GDM is a
67 transient state, and GDM patients are commonly treated by diet management to keep blood
68 glucose within the normal range and reduce the risk of GDM complications (11). However, very
69 few data from observational studies are available about whether diet interventions performed on
70 GDM patients affect the community structure of the gut microbiota. Diet, particularly long-term
71 eating habits, is known to be one of the drivers of microbiota variation (12, 13). Recent clinical
72 studies have shown the importance of routine dietary recommendations for GDM patients,
73 showing a better microbial pattern at the end of the study (14). However, the comparison
74 between healthy pregnant women without dietary recommendations and individuals with GDM
75 under routine dietary management remains uncertain.

76 In this study, we characterized the different patterns of the gut microbiota between GDM

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77 and healthy pregnancies in the second trimester of pregnancy. Then, comparison of microbial
78 structure between healthy pregnant women without dietary recommendations and individuals
79 with GDM under routine dietary management were assessed, to evaluate the role of short-term
80 diet management on GDM gut microbiota. The aim of the present study was to provide an update
81 on the existing knowledge of the specific structure of the gut microbiota in Chinese GDM
82 women and to elucidate the influence of diet management on the GDM gut microbiota.

83

84 **Material and methods**

85 *Patient recruitment*

86 This study was approved by the Conjoint Health Research Ethics Board of Peking University
87 People's Hospital, and informed consent forms were signed by all of the subjects prior to
88 participation in this study. All experiments were performed in accordance with the approved
89 guidelines and regulations.

90 Diagnosis of GDM is based on the results of the fasting 75 g OGTT at 24–28 weeks
91 gestation. One or more elevated level(s) is sufficient for a diagnosis of GDM. The threshold
92 values of OGTT (5.1 at 0 hour, 10.0 at 1 hour and 8.5 at 2 hours during OGTT) are based on the
93 diagnostic criteria recommended by the International Association of the Diabetes and Pregnancy

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95 Thirty healthy subjects were selected based on matched age and pregnancy period, no
96 complicating diseases and no antibiotic use during the 3-month period prior to sample collection.
97 All subjects who met the following criteria were excluded: complicating diseases (such as known
98 diabetes mellitus, hypertension, cardiovascular, pulmonary, autoimmune, joint, liver or kidney
99 diseases; thyroid dysfunction; or any other disease), prebiotics/probiotics use, and antibiotic use

100 during pregnancy.

101 The prepregnancy weight was self-reported; weight and height were measured at the time
102 of enrollment. BMI was calculated as weight divided by the square of height. Arterial blood
103 pressure (BP) was measured from the left arm with the participant in a sitting position after at
104 least 10 min of rest with a mercury sphygmomanometer with the appropriate cuff size. The
105 measurements for BP were taken by trained medical personnel at enrollment.

106

107 *Diet management for the GDM women*

108 The initial treatment of GDM involves diet modification, glucose monitoring, and moderate
109 exercise (15, 16). All the GDM participants in the study received 2 weeks of dietary management
110 and nutritional recommendations at enrollment, which showed the guidelines for the subjects.
111 Participants were considered as adhering to the given dietary recommendations in the presence
112 of all the following criteria: carbohydrates 35–45% of total energy, rapidly absorbed sugars <10%
113 of total energy, proteins 18–20% of total energy, fats 35% of total energy, fiber intake of at least
114 20–25 g/day, and no alcohol consumption. The nutritionist was in continuous contact with the
115 enrolled GDM subjects, through weekly telephone contact, to remain updated regarding the
116 nutritional condition of the subjects as the study progressed. Patients were instructed to

117 self-monitor their blood glucose by finger-prick capillary blood glucose tests at least 4 times per
118 day.

119 To reduce the effect of diet on the composition of the gut microbiota, general 2-week
120 dietary restrictions were imposed on the healthy participants, including no peppery food and no
121 yogurt intake and appropriate fat intake (the intake of calories from fat was no more than 35% of
122 the total calories).

123

124 ***Stool sample collection and DNA extraction***

125 After providing written informed consent, all subjects were contacted for detailed instructions on
126 how to collect and transport the stool sample. Stool samples of 57 subjects were collected at the
127 time of enrollment for the first time. The second stool samples for GDM subjects were collected
128 at the end of the study after the 2-week dietary intervention. For healthy pregnant women, the
129 second stool samples were collected at the end of 2 weeks without dietary management
130 intervention. Stool samples were self-collected by all the participants using the specimen
131 collection kit as instructed. The fecal samples were collected at home, transferred to the hospital
132 and immediately stored at -80°C until DNA extraction. DNA was extracted from stool samples
133 using the QIAamp DNA Stool Mini kit protocol (Qiagen, Germany). During the stool collection,
134 one GDM sample at enrollment from one patient (G28) were limited, and the second sample was
135 collected the other day, which changed the serial number to G28-2 at enrollment and G28-3 at
136 the end of study.

137

138 ***Illumina library generation***

139 The V4 region of the 16S rRNA gene was amplified using 515F

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G-CTGTGCCAGCAGCCGCGGTAAT-3' and 806R (5'-GGACTACGATGCGGTGCTCCT-3').

141 The V4-specific primer regions were associated with the adaptor and the sequences, which were
142 complementary to the Illumina forward and reverse sequencing primers. Each PCR product of
143 the appropriate size was purified and quantified using a Qubit fluorometer and then added to a
144 master pool of DNA for 250-bp nucleotide paired-end read assembly using the HiSeq 2500
145 genome analyzer (Illumina HiSeq 2500, USA).

146

147 ***Bioinformatics***

148 The RDP Classifier was used to assign all of the 16S rRNA gene sequences to a taxonomic
149 hierarchy. The assembled reads were analyzed. The relative abundances of the various phyla,
150 families and genera in each sample were computed and compared between the GDM patients and
151 the healthy subjects. The trimmed reads were clustered into operational taxonomic units (OTUs)
152 at 97 % identity. The comparison of the bacterial diversity of these samples was performed using
153 the Chao1 richness index, ACE index and observed species. The reads displaying greater than
154 0.1% abundance in both groups were further analyzed via partial least-squares discriminant
155 analysis (PLS-DA) to visualize the differences between two groups using the standard Simca-p1
156 software (version 12.0; <http://www.umetrics.com/>). The Principal Co-ordinates Analysis (PcoA)
157 analyzed were performed based on Unweighted Unifrac distance metric.

158

159 ***Statistical analysis***

160 The microbial comparisons between the GDM and healthy groups were performed using the
161 Mann-Whitney test. Associations between clinical indices and gut microbiota were evaluated by
162 the Spearman rank correlation coefficient method. The difference in alpha-diversity between

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The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission. groups during GDM and non-GDM was assessed using Student's t test. Statistical analysis of the

164 clinical data was performed using SPSS (Statistical Package for Social Sciences) 22.0 software
165 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significantly different.

166

167 ***Availability of data***

168 The raw sequences are available from the Genome Sequence Archive (GSA), the

169 accession is: CRA004782.

170

171 **Results**

172

173 *Characteristics of the patients*

174 A flow chart illustrating the recruitment strategy of GDM and healthy subjects is shown in Fig 1.

175 Clinical data from 27 GDM patients and 30 healthy controls are shown in Table 1. All 27 GDM

176 patients and 30 healthy pregnant women were from the Peking University People's Hospital. The

177 mean age of the subjects was 32.7 ± 3.3 years for the GDM group and 31.4 ± 2.9 years for the

178 healthy group. There were no differences in age or nulliparity rate between the two groups. The

179 prepregnancy BMI value of the GDM group was 24.2 ± 4.4 , which was significantly higher than

180 the value of 21.4 ± 2.8 of the healthy group ($P=0.0059$), and the same trend was observed for the

181 BMI at enrollment (27.1 ± 4.3 vs. 25.0 ± 2.9 , GDM vs. healthy, $P=0.038$). The GDM group had a

182 markedly higher systolic BP (SBP) value than that of the control group (mean 125.3 ± 11.8 vs.

183 115.8 ± 14.2 , GDM vs. healthy, $P=0.008$), and an increased diastolic BP (DBP) value was found

184 in GDM women compared to that of healthy women (mean 78.8 ± 9.5 vs. 73.6 ± 8.8 , GDM vs.

185 healthy, $P=0.038$). In the OGTT test, the GDM group had higher values at 0 h, 1 h and 2 h than

186 the values of the healthy group (all $P < 0.001$).

187

188 *Differences in fecal microbial communities between the healthy and GDM groups*

189 To demonstrate the GDM microbiota signature, we explored the microbial composition of

190 pregnant women with GDM. First, we performed PCoA using OTU relative abundance, and we

191 observed discrete clustering of intestinal microbiota in the GDM and healthy groups at

192 enrollment (Fig 2A). Additionally, shared or unique OTUs in the GDM and control groups were
193 assessed to detect whether GDM has an effect on the gut microbiota. We found that the GDM
194 group had more unique OTUs than the control group, with approximately 60.6% (1458/2404)
195 unique OTUs compared with 14.3% (158/1104) in healthy women, signifying that GDM patients
196 largely harbor unique inhabitant niches (Fig 2B).

197 The observed species of GDM samples were higher than non-GDM samples (Fig 2C).
198 The ACE and Chao1 indices for alpha-diversity were both significantly increased in the GDM
199 group (Fig 2D&2E), suggesting increased commensal diversity in GDM patients. Similar trends
200 of alpha-diversity were also observed between the Healthy-W2 and GDM-W2 (diet management)
201 groups, suggesting that the microbial pattern of women with GDM is distinct from that of
202 healthy subjects at enrollment and at the end of the study.

203

204 *Microbiota structure of GDM patients based on taxonomic comparison*

205 To further demonstrate these variations corresponding to the structure of the gut microbiota in
206 GDM, we compared the bacterial abundance between groups at the phylum level (Fig 3A). No
207 significant differences were observed between the healthy subjects and the GDM subjects at
208 enrollment for most of the phyla, with the exception of *Acidobacteria*, which was found to be

209 0.51% in the GDM group compared with 0.37% in the healthy group ($P=0.001$).
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210 The microbial compositions at the phylum level for each sample at enrollment and at the end of
211 the study are shown in Fig S1. Interestingly, *Acidobacteria* was associated with increased levels
212 of blood glucose in the 0-h OGTT (Fig 3B).

213 Next, we compared taxa at the genus level. The PLS-DA method was performed (Fig 3C).
214 Forty-nine key genera with variable importance in projection (VIP) scores >1 were identified

215 that differentiated the GDM and healthy groups (Table 2). We then clustered the samples
216 according to the relative abundance of the 49 genera. Twenty-seven genera were enriched in the
217 GDM microbiota samples, with 4 genera (*Acidothermus*, *Granulicella*, *Bryobacter*, and
218 *Candidatus_Solibacter*) belonging to the phylum *Acidobacteria*. Among them, *Acidothermus*
219 and *Granulicella* were significantly enriched in the GDM group (Fig 3D). Seven genera
220 belonging to *Proteobacteria*, including *Citrobacter*, *Burkholderia*, *Acidibacter*, and *Bilophila*,
221 were significantly highly expressed in the GDM intestinal microbiota ($P<0.05$). The genera
222 *Eubacterium*, *Holdemania*, and *Tyzzarella*, in the phylum *Firmicutes*, were rarely detected in
223 women with healthy pregnancy microbiota compared with women with GDM. The remaining 22
224 genera of the 49 key phlotypes were overexpressed in healthy pregnant microbiota, some of
225 which even disappeared in GDM patients. One genus, *Ruminococcaceae_UCG-010*, belonging
226 to *Firmicutes*, was highly enriched in the healthy group. Additionally, *Akkermansia* ($P=0.067$)
227 and *Coprococcus_2* ($P=0.027$) were increased in healthy subjects. *Akkermansia* was recently
228 proven to be a crucial player in maintaining the integrity of the gastrointestinal tract. In
229 nonpregnant adults with metabolic syndrome and type 2 diabetes, *Akkermansia* is reported to be
230 depleted as well (17-19). Our findings suggest that the gut microbiota of women with GDM has
231 similarities with the microbiota reported in patients with type 2 diabetes and associated

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intermediary metabolic traits. At the OTU level, a reduced abundance of *Akkermansia* has
233 previously been reported in the third trimester of healthy pregnant women (20).

234 To further examine the relationship between these VIP genera in GDM, we evaluated
235 their abundance based on the results of the OGTT. The threshold values (5.1 at 0 h, 10.0 at 1 h
236 and 8.5 at 2 h during the OGTT) are based on the diagnostic criteria recommended by the
237 International Association of the Diabetes and Pregnancy Study Groups in 2011. As shown in Fig

238 3E, two short chain fatty acids producing and anti-inflammatory bacteria were highly correlated
239 with the OGTT value at 0 h and 2 h. The relative abundance of *Ruminococcus gauvreauii* was
240 observed depleted in GDM women with abnormal OGTT value at 0 h ($P=0.046$), and the
241 relative abundance of *Eubacterium ventriosum* was decreased in GDM women with the
242 abnormal OGTT value at 2 h ($P=0.009$, Mann-Whitney test).

243

244 ***Microbiota signature after dietary intervention***

245 We found that GDM patients developed a microbial pattern with higher alpha-diversity after diet
246 management (Fig 2D & E). Compared with the GDM samples, the GDM-W2 samples showed
247 some distinct taxa with VIP scores >1 , according to the PLS-DA analysis (Fig S2).

248 At the family level, GDM-W2 samples showed decreased pathogenic taxa
249 (*Acidaminococcaceae*, *Enterobacteriaceae*, and *Bacteroidaceae*) and increased
250 *Bifidobacteriaceae* and butyric acid-producing bacteria (*Prevotellaceae* and *Lachnospiraceae*)
251 compared with the GDM microbial samples at enrollment, suggesting a better pattern driven by
252 the 2 weeks of diet management. One more interesting observation is that because the bacterial
253 lineages were constant within pregnancy over time, communities from the same GDM person
254 were generally more similar to one another than to those from other people from the healthy

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256 It is presumed that the influence of maternal gestational diet on the phylogenetic structure
257 of the intestinal microbiota during pregnancy remains underexplored in well-controlled models.
258 To investigate whether the microbiota can be driven by dietary management for GDM in
259 pregnancy, the two dominant groups of beneficial bacteria, *Bacteroidetes* and *Firmicutes*, were
260 analyzed. At the phylum level, a slightly increase in the *Firmicutes/Bacteroidetes* (F/B) ratio in

261 late pregnancy was exhibited in the GDM group compared with the non-GDM group (Fig 4C).
262 Previous studies indicated that a higher *Firmicutes/Bacteroidetes* ratio was associated with
263 obesity (21) and an aggravation of low-grade inflammation (22). Here, we showed that after 2
264 weeks of diet therapy, the relative abundance of *Bacteroidetes* in GDM samples increased, and
265 the abundance of *Firmicutes* decreased slightly (Fig 2A). More importantly, the ratio of
266 *Firmicutes/Bacteroidetes* did not increase in GDM-W2 fecal samples compared with GDM
267 samples at enrollment ($P=0.8$) (Fig 4C). However, without diet management, an obviously
268 increased proportion of *Firmicutes/Bacteroidetes* ($P=0.2$) developed in healthy pregnancies
269 (healthy-W2 samples).

270 Four genera (*Acidothermus*, *Granulicella*, *Bryobacter*, and *Candidatus_Solibacter*)
271 belonging to the phylum *Acidobacteria* were increased in the GDM group, compared with
272 healthy group. Furthermore, we evaluated the levels of the 4 genera in GDM with dietary
273 management (Fig S3). A total of 66.7% (18/27) of GDM subjects showed decreased levels of the
274 genus *Acidothermus* after 2 weeks of diet management. In contrast, 59.3% (16/27) of GDM
275 samples showed decreased levels of the genera *Granulicella*, *Bryobacter*, and *Candidatus*
276 *Solibacter* after 2 weeks of diet management.

277

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279 We examined the correlations between the OGTT values (0 h, 1 h and 2 h), BMI indices
280 (prepregnancy and at enrollment), blood pressure values (SBP and DBP) and the genera of the
281 fecal microbiota (Fig 5).

282 The distribution of correlation coefficients by heatmap analysis showed that the
283 *Coprococcus_2*, *Christensenellaceae_R.7*, and *Prevotella* groups (*Prevotella_2*, *Prevotella_7*

284 and *Prevotella_9*) were negatively correlated with the OGTT value, BP values and BMI index
285 ($P<0.05$); among them, *Coprococcus_2* was significantly increased in the healthy group
286 compared with the GDM group.

287 *Parabacteroides* showed positive correlations with BMI at enrollment ($P<0.05$).
288 Additionally, *Alloprevotella*, *Megamonas* and *Clostridium_sensu_stricto-1* showed positive
289 correlations with GDM-correlated clinical measures and OGTT values at 0 h ($P<0.05$). Previous
290 studies observed that the genus *Megamonas* was increased in GDM patients in late pregnancy.
291 Elevated genera of *Megamonas* have also been reported to be associated with higher blood
292 glucose at an individual level (9, 23-25).

293

294 **Discussion**

295 Studies support a causal role for the gut microbiota in the development of type 2 diabetes, insulin
296 resistance and obesity (26). In this study, we compared the composition of the human intestinal
297 microbiota between GDM patients and healthy subjects using a culture-independent Illumina
298 HiSeq 2500 platform. The aim of the present study was to identify gut microbiota dysbiosis in
299 GDM subjects and the associated microbial changes in GDM-W2 samples after diet intervention
300 for 2 weeks and compare them with the basal GDM microbial composition. We observed a

301 marked shift in the microbiota composition at the phylum and genus levels in GDM samples
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302 compared with healthy samples and identified the microbial pattern of GDM-W2 samples after a
303 2-week dietary intervention.

304 Gut dysbiosis in women with GDM was mainly characterized by changes in microbiota
305 diversity. It was previously reported that an increase was found in the alpha-diversity in the third
306 trimester of GDM women when compared to the level of the control group (24). Regarding

307 alpha-diversity, we used the ACE and Chao1 indices and found significant separation in the
308 alpha-diversity between GDM and non-GDM individuals at their enrollment and at the end of the
309 study, indicating dysbiosis of the gut microbiota in GDM women compared with healthy
310 pregnant women. To further identify gut microbial dynamics, the different bacterial taxa were
311 compared within the GDM and non-GDM groups. At the phylum level, the abundance of
312 *Acidobacteria* was significantly greater in the gut microbiota of GDM samples and was
313 associated with increased levels of blood glucose in the 0-h OGTT (Fig 3B). In particular, we
314 observed significant elevation of *Acidothermus* and *Granulicella* belonging to the phylum
315 *Acidobacteria* in the GDM group. The phylum *Acidobacteria* was reported in the gut microbiome
316 of obese individuals (27) and was shown to contain a host of genes involved in diverse metabolic
317 pathways, as evidenced by their pan-genomic profiles in the soil microbiota (28). Further
318 exploration of these genetic attributes and more in-depth insights into GDM mechanics and
319 dynamics would lead to a better understanding of the functions and biological significance of this
320 elevated phylum in the GDM gut environment.

321 Several bacterial groups at the genus level were detected to be different in the GDM and
322 healthy groups, such as *Megamonas* assigned to the phylum *Firmicutes*. The relationships
323 between gastrointestinal *Megamonas* and metabolic disorders such as obesity and type 2 diabetes

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The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission. have recently been discovered (29). Differential abundance testing showed that *Megamonas*,

325 *Bacteroides*, and *Eubacterium* were statistically associated with food addition (30). A recent
326 study also suggested that the abundance of *Megamonas*, which is closely related to childhood
327 obesity, increased in the gut microbiota of obese children (29). Of particular interest, we revealed
328 the association between gut *Megamonas* and GDM. Our results showed that *Megamonas* was
329 positively correlated with higher blood glucose in the OGTT test at 0 h in the GDM samples at

330 enrollment (Fig 5). Members of *Megamonas* are known to produce acetic and propionic acid,
331 which is beneficial for the balance of glucose uptake (31). Systemic disorders of glucose
332 metabolism might be modulated by the related gut microbiota. Further study to explore the
333 composition of *Megamonas* and the production of metabolites involved in glucose homeostasis
334 *in vitro* and *in vivo* is very important.

335 Short-chain fatty acids (SCFAs), especially acetate, propionate and butyrate, are the end
336 products of the intestinal microbial fermentation of dietary fibers and resistant starch. It is well
337 documented that plasma and colonic SCFAs are associated with metabolic syndromes, i.e.,
338 obesity and type 2 diabetes (32). SCFAs, namely, acetate, butyrate, and propionate, have been
339 reported to affect metabolic activities at the molecular level. Acetate affects the metabolic
340 pathway through the G protein-coupled receptor (GPCR) and free fatty acid receptor 2
341 (FFAR2/GPR43). The FFAR2 signaling pathway regulates insulin-stimulated lipid accumulation
342 in adipocytes and inflammation (33, 34). *Coprococcus_2*, an acetate-producing bacteria (25, 35),
343 was found to be negatively correlated with the OGTT value at 1 h, BP values and prepregnancy
344 BMI index ($P<0.05$) by Spearman analysis and was significantly higher in the healthy group than
345 in the GDM group. *Coprococcus* was also proven to be altered in the fecal microbiota of patients
346 with polycystic ovary syndrome, which is a metabolic disorder (36). Guo et al. (37) found that

347 *Coprococcus* deletion is implicated in many of the outcomes, including glucose homeostasis.
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348 The importance of an association between the deletion of the *Coprococcus* genus and high levels
349 of blood glucose at 1-h in the OGTT measure is therefore supported by the acetate-producing
350 effect. Furthermore, other SCFA-producing taxa, including *Prevotella_2*, *Prevotella_7*, and
351 *Prevotella_9*, were found to be negatively associated with OGTT measures and the BMI index
352 separately, indicating a beneficial effect on blood glucose in GDM subjects (38). We presumed

353 that acetate arising from *Coproccoccus_2* members and succinate from *Prevotalla* members are
354 important for energy metabolism and have a mainly protective role in relation to healthy
355 pregnancy. Thus, the observed absence of the *Coproccoccus_2* and *Prevotella* groups in the fecal
356 microbiota of GDM could be a possible microbial driving force for GDM. A better
357 understanding of the microbial ecology of colonic acetate- and succinate-producing bacteria,
358 especially the *Coproccoccus_2* and *Prevotella* groups, may help to explain the influence of diet
359 on the acetate and succinate supply and may contribute to the development of new approaches
360 for optimizing microbial activity for diet management for GDM subjects. *Eubacterium*
361 *ventriosum*, another SCFAs producer, had been found negative correlated with visceral fat area
362 (VFA) (39). Moraes et al. reported that the abundance of *E. ventriosum* were associated to better
363 cardiometabolic profile (40). Consistent with our study, the data demonstrated a significant
364 decrease of gut *Eubacterium ventriosum* from GDM subjects with abnormal OGTT values at 2 h
365 (Fig 3E). Combined with these findings, we presumed that the expression of the SCFAs
366 producers are critical for energy homeostasis during pregnancy. Further studies investigating the
367 targets and signaling pathways of SCFAs in the GDM microbial, and the modulation of
368 SCFAs-producing bacteria by diet intervention would benefit for GDM management.

369 Therefore, to further identify the role of diet intervention during GDM pregnancy, we

370 analyzed the ratio of *Firmicutes/Bacteroidetes* and a higher ratio was proposed as an eventual
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371 biomarker of obesity and other metabolic syndromes compared with normal-weight individuals
372 (41). Our data showed different increases in the *Firmicutes/Bacteroidetes* ratio between the
373 GDM and non-GDM groups. Healthy W2 samples without diet management showed a nearly
374 significant increase in the *Firmicutes/Bacteroidetes* ratio, indicating a change in energy
375 homeostasis during pregnancy. Similar to our findings on the *Firmicutes/Bacteroidetes* ratio in

376 healthy pregnant women, Zheng et al. (42) reported that there were elevations in the
377 *Firmicutes/Bacteroidetes* ratio in the second (T2) trimester compared with the first (T1) trimester.
378 Ley et al. (22) reported that the *Firmicutes/Bacteroidetes* ratio decreases with weight loss on a
379 low-calorie diet. In our observations, the *Firmicutes/Bacteroidetes* ratio did not change in
380 GDM-W2 samples under diet management compared to the ratio in GDM samples, suggesting
381 that the diet intervention could play a positive role during GDM pregnancy by affecting
382 *Firmicutes/Bacteroidetes* ratio. In particular, the gut microbial pattern was not altered in the
383 GDM group with or without 2 weeks of diet intervention (Fig 4A&B). In agreement with our
384 observation, a controlled-feeding study showed that enterotype identity remained stable during
385 the 10-day study, and alternative microbial states were associated with a long-term diet (43).
386 Thus, we presume that the role of short-term diet management in GDM processes is associated
387 with changes in the *Firmicutes/Bacteroidetes* ratio and some specific taxa rather than an
388 alternative gut microbial pattern.

389 It is well suggested that the diet contributes to the gut microbiota composition in GDM
390 (42). Microbiota-derived metabolites affect glucose homeostasis through intestinal
391 gluconeogenesis (38). A few studies have examined the gut microbiota of GDM and healthy
392 pregnant women before and after diet invention. Uniquely, in the present study, we could

393 compare gut microbiota in GDM fecal samples, allowing identification of taxa that exhibited
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394 differential abundance at the two time points. We discovered that a short-term diet had a
395 beneficial effect on GDM by modulating the *Firmicutes/Bacteroidetes* ratio and some taxa. This
396 first observation of the high expression of the phylum *Acidobacteria* in GDM offered an
397 important clue for further study on the subgroup of *Acidobacteria* and the mechanism of GDM.
398 Several limitations in our study should be considered. One was that we did not have fecal

399 samples after long-term dietary management. Additionally, our suggestion of the occurrence of
400 specific taxa with divergent metabolites calls for future metagenomic sequencing to reveal the
401 metabolic pathways of the key taxa. In conclusion, our results highlight the relevance of
402 characterizing gut microbial population differences and contribute to understanding the plausible
403 link between diet and specific gut bacterial species that are able to influence metabolic
404 homeostasis and GDM development. Modulating the gut microbiota via short-term diet
405 intervention, especially SCFA-producing bacteria, could be a promising strategy in the search for
406 alternatives for the treatment of metabolic disorders in GDM (44-46). Long-term observation
407 may be more valuable to study the dynamic alteration of the GDM gut microbiota.

408

409 **Declarations**

410

411 **Funding**

412 This work was supported by the National Natural Science Foundation of China (grant no.
413 32070116) and the Maternal and Infant Nutrition & Care Research Fund of the Institute of
414 Nutrition and Nursing of Biostime (grant no. 2015-Z-20).

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417 On behalf of all authors, the corresponding author states that there are no conflicts of interest.

418

419 **Acknowledgments**

420 We thank all the subjects who made this study possible.

421

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557

558 **Figure legends**

559 **Fig 1. Flow chart illustrating the recruitment of GDM and healthy subjects.**

560 **Fig 2. Comparison of the fecal microbiota composition between the GDM and healthy**
561 **groups. A.** Principal coordinate analysis (PCoA) at the OTU level between the GDM and
562 healthy groups. **B.** Venn diagram illustrating the overlap of the OTUs identified in the fecal
563 microbiota between the GDM and healthy groups. **C.** Observed species of 4 groups, including
564 the GDM and healthy and the GDM-W2 and healthy-W2 groups. **D & E.** Alpha-diversity based
565 on the ACE index and Chao 1 index at the OTU level. Mann-Whitney test, GDM vs. healthy,
566 **** $P < 0.01$, * $P < 0.01$.**

567 **Fig 3. Abundances of taxa in GDM and healthy participants. A.** Comparison of the relative
568 abundances at the phylum level among the four GDM and non-GDM groups. The Mann–
569 Whitney test was used to evaluate the two groups. *** $P < 0.05$.** **B.** PLS-DA score plots based on the
570 relative abundances of microbiota between the GDM and healthy groups. **C.** Correlation between
571 the relative abundance of the phylum *Acidobacteria* and the 1-h OGTT measurement. Spearman
572 analysis, $R = 0.302$, $P = 0.06$. **D.** Comparison of the relative abundances of *Acidothermus*,
573 *Granulicella*, *Bryobacter*, and *Candidatus_Solibacter* in the phylum *Acidobacteria* in the GDM
574 and healthy groups. Mann-Whitney test, GDM vs. control, **** $P < 0.01$, * $P < 0.01$.** **E.** The relative
575 abundances of *Ruminococcus gnavreanus* and *Eubacterium ventriosum* were highly correlated
576 with the OGTT values at 0 h and 2 h. Mann-Whitney test, GDM vs. healthy, **** $P < 0.01$, * $P < 0.01$.**

577 **Fig 4. The microbial pattern after diet management. A.** Principal coordinate analysis (PCoA)
578 at the OTU level between the GDM-W2 and healthy-W2 groups. **B.** Heatmap analysis of the
579 differentially expressed taxa at the family level. **C.** Ratio of *Firmicutes/Bacteroidetes* among the
580 GAM and non-GDM groups with or without diet intervention.

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581 **Fig 5. Heatmap analysis of the correlation between the gut microbiota composition and**
582 **clinical scores.**

583 **Fig S1.** Comparison of the relative abundance at the phylum level between the 27 GDM and 30
584 healthy individuals at the time of enrolment and study end.

585 **Fig S2.** PLS-DA analysis indicated 49 distinct taxa with VIP score>1 between GDM samples
586 and GDM-W2 samples. Mann-Whitney test, GDM vs. Healthy, ** $P<0.01$, * $P<0.01$.

587 **Fig S3.** The *Acidothermus*, *Granulicella*, *Bryobacter*, *Candidatus_Solibacter* belonging to the
588 phylum *Acidobacteria* were evaluated in GDM and GDM-W2 samples. The 66.7% (18/27)
589 GDM samples was showed decreased level of genus *Acidothermus* after two-week diet
590 management. While 59.3% (16/27) GDM samples was showed decreased level of genus
591 *Granulicella*, *Bryobacter*, *Candidatus_Solibacter* after two-week diet management.

592

593

594 **Tables**595 **TABLE 1** The clinical characteristics of all the GDM patients differ from those of the healthy

596 participants

	GDM (Mean ± SD)	Healthy (Mean ± SD)	<i>P</i> value
Number	27	30	
Age	32.7±3.3	31.4 ±2.9	0.11
Prepregnancy weight (kg)	63.5±12.2	57.3±8.9	0.031
BMI (kg/m ²)	24.2±4.4	21.4±2.8	0.0059
Enrollment weight (kg)	71.1±12.4	66.9±9.5	0.15
BMI (kg/m ²)	27.1±4.3	25.0±2.9	0.038
Nulliparous (number)	22/27	24/30	
Systolic BP (mmHg)	125.3±11.8	115.8±14.2	0.008
Diastolic BP (mmHg)	78.8±9.5	73.6±8.8	0.038
OGTT (mg/dL)			
0 min	5.2±1.4	4.3±0.3	0.001
60 min	10.1±1.6	7.3±1.4	<0.0001
120 min	8.8±1.3	6.4±1.2	<0.0001

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599 **TABLE 2** Forty-nine key genera with VIP >1 that were differentially expressed in the GDM and
600 healthy groups

Genus with VIP \geq 1	GDM mean	Healthy mean	GDM/Healt hy	P value	Phylum
<i>Citrobacter</i>	0.000316	2.41E-05	up	0.048	<i>Proteobacteria</i>
<i>Bradyrhizobium</i>	0.000422	8.64E-05	up	0.065	<i>Proteobacteria</i>
<i>Eubacterium</i>	0.00012	3.4E-05	up	0.001	<i>Firmicutes</i>
<i>Granulicella</i>	0.000323	0.000102	up	0.001	<i>Acidobacteria</i>
<i>Holdemania</i>	0.000187	7.5E-05	up	0.014	<i>Firmicutes</i>
<i>Succinivibrio</i>	8.81E-05	3.54E-05	up	0.212	<i>Proteobacteria</i>
<i>Oscillibacter</i>	0.000211	9.06E-05	up	0.44	<i>Firmicutes</i>
<i>Tyzzerella</i>	0.000856	0.000368	up	0.007	<i>Firmicutes</i>
<i>Holdemanella</i>	0.009481	0.004176	up	0.162	<i>Firmicutes</i>
<i>Paraprevotella</i>	0.000994	0.000578	up	0.126	<i>Bacteroidetes</i>
<i>Victivallis</i>	0.00056	0.000344	up	0.042	<i>Lentisphaerae</i>
<i>Desulfovibrio</i>	0.000458	0.00029	up	0.479	<i>Proteobacteria</i>
<i>Lachnospiraceae</i>	0.002291	0.001517	up	0.137	<i>Firmicutes</i>
<i>Burkholderia</i>	0.000824	0.000551	up	0.027	<i>Proteobacteria</i>
<i>Acidothermus</i>	0.000499	0.000338	up	0.034	<i>Acidobacteria</i>
<i>Acidibacter</i>	0.000677	0.000508	up	0.405	<i>Proteobacteria</i>
<i>Mucilaginibacter</i>	0.00037	0.00028	up	0.02	<i>Bacteroidetes</i>
<i>Candidatus_Solibacter</i>	0.000474	0.000394	up	0.404	<i>Acidobacteria</i>
<i>Ruminiclostridium_9</i>	0.001163	0.00098	up	0.141	<i>Firmicutes</i>
<i>Ruminococcus_gauvreauii</i>	0.000581	0.000491	up	0.214	<i>Firmicutes</i>
<i>undesignated_Ruminococcaceae</i>	0.001548	0.001359	up	0.949	<i>Firmicutes</i>
<i>Roseburia</i>	0.028429	0.025656	up	0.482	<i>Firmicutes</i>
<i>Bilophila</i>	0.002439	0.002216	up	0.179	<i>Proteobacteria</i>
<i>Alistipes</i>	0.011983	0.010959	up	0.354	<i>Bacteroidetes</i>
<i>Bryobacter</i>	0.000475	0.00044	up	0.968	<i>Acidobacteria</i>
<i>Odoribacter</i>	0.001495	0.001395	up	0.302	<i>Bacteroidetes</i>
<i>Dorea</i>	0.007676	0.007233	up	0.678	<i>Firmicutes</i>

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<i>Lachnospiraceae_NK4A136</i>	0.002712	0.002715	down	0.438	<i>Firmicutes</i>
<i>Eubacterium_ruminantium</i>	0.00633	0.006883	down	0.26	<i>Firmicutes</i>
<i>Bifidobacterium</i>	0.033865	0.038103	down	0.56	<i>Acidobacteria</i>
<i>Ruminococcaceae_UCG-013</i>	0.001251	0.00142	down	0.994	<i>Firmicutes</i>
<i>Tyzzerella_3</i>	0.002118	0.002482	down	0.073	<i>Firmicutes</i>
<i>Ruminococcaceae_UCG-005</i>	0.00281	0.003448	down	0.452	<i>Firmicutes</i>
<i>Ruminococcaceae_UCG-002</i>	0.005792	0.00715	down	0.056	<i>Firmicutes</i>
<i>Ruminococcaceae_NK4A214</i>	0.00144	0.001797	down	0.083	<i>Firmicutes</i>
<i>Eubacterium_ventriosum</i>	0.00239	0.003046	down	0.207	<i>Firmicutes</i>
<i>Enterococcus</i>	0.001193	0.001627	down	0.09	<i>Firmicutes</i>
<i>Megasphaera</i>	0.001971	0.00306	down	0.749	<i>Firmicutes</i>
<i>Lachnospiraceae_UCG-003</i>	0.000269	0.000419	down	0.11	<i>Firmicutes</i>
<i>Coprococcus_2</i>	0.005271	0.008583	down	0.027	<i>Firmicutes</i>
<i>Ruminiclostridium_5</i>	0.001904	0.003111	down	0.009	<i>Firmicutes</i>
<i>Ruminococcaceae_UCG-010</i>	0.000848	0.001403	down	0	<i>Firmicutes</i>
<i>Sarcina</i>	0.000126	0.000252	down	0.001	<i>Firmicutes</i>
<i>Butyrivibrio</i>	0.000455	0.000927	down	0.02	<i>Firmicutes</i>
<i>Intestinimonas</i>	3.93E-05	8.64E-05	down	0.07	<i>Firmicutes</i>
<i>Akkermansia</i>	0.000189	0.000435	down	0.067	<i>Verrucomicrobia</i>
<i>Weissella</i>	7.87E-05	0.000217	down	0.002	<i>Firmicutes</i>
<i>Prevotella_2</i>	0.001153	0.003598	down	0.108	<i>Bacteroidetes</i>

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