

[Regular article]

Relationship between gastrointestinal environment and physical conditions in elite athletes

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48 **Abstract**

49 Heavy exercise training may negatively affect the gastrointestinal status of athletes, often
50 leading to poor athletic performance and physical condition. The gut microbiome plays
51 an important role in the pathogenesis of gastrointestinal disorders. However, the
52 relationship between the gut environment and conditions in athletes remains to be
53 investigated. In this study, we aimed to determine the relationship between the gut
54 microbiome, stool pattern, and the physical condition of elite athletes. A total of 92 elite
55 athletes participated in this study, and their gut microbiomes, stool patterns, and physical
56 conditions were evaluated. The gut microbiome was analyzed using 16S rRNA
57 sequencing. The stool patterns and subjective physical conditions were evaluated using
58 questionnaires. The participants were divided into better and poorer physical condition
59 groups based on the median value of the physical condition score. The prevalence of
60 abnormal stool patterns was significantly higher in the poorer condition group than that
61 in the better condition group. The abundance of *Faecalibacterium* was significantly
62 higher and that of *Bifidobacterium* was significantly lower in the better condition group
63 than those in the poorer condition group. Principal component analysis revealed that the
64 stool pattern could be explained by the *Faecalibacterium* abundance and the physical
65 condition scores. Together, these findings suggest that the gut microbiome, especially the
66 *Faecalibacterium* and *Bifidobacterium* abundance, affects the stool patterns and physical
67 conditions of elite athletes, indicating that the gut microbiome may play a role in
68 controlling the gastrointestinal environment affected by physiological stress such as
69 intense exercise.

70

71 **Keywords:** gut flora, gastrointestinal symptom, defecation, conditioning, exercise

72

73 **タイトル:**

74 エリートアスリートにおける腸内環境とコンディションの関係

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86

87 **抄録:**

88 高強度運動トレーニングは腸内環境を悪化させ、身体コンディションや競技パフォー
89 マンスの低下と関連する。腸内細菌は胃腸障害の病因に寄与しているが、アスリート
90 の腸内環境とコンディションの関係性は明らかにされていなかった。そこで本研究で
91 は、日本人エリートアスリートにおける腸内細菌、排便状態、および身体コンディション
92 の関係性を検討することを目的とした。様々な競技のエリートアスリート 92 名を対象
93 に、腸内細菌、排便状態、および身体コンディションを評価した。腸内細菌を 16s
94 rRNA 技術により測定し、排便状態と身体コンディションは質問紙により測定した。さら
95 に、身体コンディションのスコアによりコンディション高値群とコンディション低値群に群
96 分けした。コンディション低値群は高値群に比べて、下痢や便秘などの排便症状の割
97 合が有意に高かった。また、コンディション高値群に比べて、コンディション低値群で

98 は *Faecalibacterium* 属の占有率は有意に低く、*Bifidibacterium* 属の占有率は有意に
99 高かった。さらに、排便症状の頻度の多さは *Facalibacterium* 属の占有率と関連するも
100 のであった。これらのことから、*Faecalibacterium* や *Bifidibacterium* などの腸内細菌は
101 アスリートの排便症状やコンディションと関連することが明らかになり、腸内環境を維持
102 することがコンディショニングに重要であることが示唆された。

103 **Introduction**

104 Athletes require intense exercise training to enhance their athletic performance. Many
105 athletes are under prolonged stress conditions due to training, competition, and/or their
106 lifestyle, which are major factors of decrease in physical condition, overtraining, and
107 underperformance in sports.¹ Deterioration of physical condition under high stress and
108 insufficient recovery is caused by a systemic dysfunction of the cardiovascular and
109 digestive systems through endocrine responses, autonomic nervous activity, and immune
110 function.² Athletes in poor physical condition often exhibit decreased heart rate and
111 maximal oxygen uptake during exercise, as well as gastrointestinal symptoms such as
112 nausea, vomiting, or diarrhea.³ Additionally, 30–50% of endurance athletes suffer from
113 gastrointestinal symptoms during training periods.⁴ Intense and prolonged exercise stress
114 are thought to induce mechanical force to abdominal organs and changes in sympathetic
115 and parasympathetic nerve activity leading decrease in splanchnic blood flow, which
116 contributes to damage gastrointestinal mucosal membrane.⁵ Further, this gastrointestinal
117 ischemia during exercise and reperfusion after exercise cause deteriorate mucosal
118 function including tight junction dysfunction, thereby increasing intestinal epithelial
119 permeability.⁶ Recently, it has been reported that four days of military training not only
120 increases intestinal permeability by 60 % but also alters the gut microbiome composition.⁷
121 Therefore, it is important to assess the possible relationships between physical condition
122 and the gastrointestinal environments in athletes.

123 The gut microbiome plays a crucial role in gastrointestinal homeostasis. Gut
124 bacteria break down dietary fibers, which cannot be digested by humans, and are involved
125 in the production of short-chain fatty acids (SCFA) and the synthesis of vitamins that are
126 beneficial to the host. Moreover, the gut microbiome plays a role in other biological
127 functions, such as immune functions, enzyme activity, and hormone secretion.⁸ The gut

128 microbiome forms a complex ecosystem, and its composition, profile, and diversity as an
129 aggregate reflects the health status of the host;² loss of the diversity and balance of the
130 gut microbiome may result in immune allergies and metabolic disorders, leading to
131 obesity and lifestyle-related diseases.⁹ Moreover, the compositional profile of the gut
132 microbiome is affected by several factors, such as aging, environmental factors, and
133 lifestyle factors.¹⁰ Exercise induces changes in the composition and function of the gut
134 microbiome and promotes increased energy metabolism and improved physical
135 function.¹¹ The gut microbiomes of elite rugby athletes are highly diverse and are
136 associated with enhanced pathways related to amino acid biosynthesis, carbohydrate
137 metabolism, and SCFA synthesis.¹² In addition, the extent of training in swimmer athletes
138 has been reported to exhibit a positive correlation with the gut microbiome, especially in
139 *Faecalibacterium* and *Coprococcus* genera, which are involved in SCFA synthesis.¹³
140 However, these studies did not evaluate stool conditions such as stool status (e.g.,
141 frequency, volume, form, color, feeling, etc.), stool pattern (e.g., constipation or diarrhea),
142 and physical condition (e.g., body condition and recovery status) of athletes, and the
143 association between these parameters and the gut microbiome remains unclear.

144 In this study, we aimed to investigate the relationship between the
145 gastrointestinal environment and the physical condition of athletes, and then detect the
146 gut microbiome affected by stool pattern and physical condition. In this study, we
147 conducted stool collection and a questionnaire survey on the stool status, stool pattern,
148 and physical condition of elite Japanese athletes in a cross-sectional design. We first
149 analyzed whether gut microbiome compositional profiles were different among groups
150 divided by stool pattern, and then analyzed whether gut microbiome composition and
151 stool pattern were different between groups divided by subjective physical condition.

152

153 **Materials and methods**

154 **Subjects**

155 We recruited elite Japanese athletes who belonged to a national team in their respective
156 sports disciplines and were competing at an international level. A total of 92 athletes (51
157 men and 41 women) aged 24 ± 5 years participated in the present study. All participants
158 were informed of the purpose and methods as well as the risks of the study, and each
159 provided informed consent for participation. This study was performed in accordance
160 with the principles of the Declaration of Helsinki and approved by the ethics committees
161 of the Japan Institute of Sports Sciences (049-01) and the National Institute of Biomedical
162 Innovation, Health, and Nutrition (KENEI 91). The participants belonged to the national
163 team of Olympic reinforcement-designed athletes in one of the following sports events
164 and disciplines and were classified into six categories according to their performance
165 characteristics based on a previous study:¹⁴ Power/sprint (track and field sprinters and
166 jumpers; n=13), endurance (race walkers, short-track ice speed skaters, bicycle track
167 cyclists, and alpine skiers; n=26), martial arts (fencers; n=38), artistic sports (rhythmic
168 gymnastics; n=1), ball games (soccer players; n=1), and others (sailing athletes, alpine
169 and halfpipe snowboarders; n=13). We collected fecal samples from all participants and
170 measured their stool status, stool pattern, and subjective physical condition using a cross-
171 sectional design.

172

173 **Measurements**

174 *Stool condition*

175 We evaluated stool volume, form, color, feeling, and frequency as stool status for one
176 month using the developed assessment tool.¹⁵ The stool volume was estimated by the
177 number of fecal units (response number 1 to 8, from 1, 0.5, to 8, >4 units) referring to the

178 stool model unit (2 cm diameter × 10 cm length, cylindrical in shape). Stool form was
179 measured using the modified Bristol Stool Scale. The seven types range from very hard
180 (type 1) to very loose (type 7). For stool color assessment, the closest of the six colors
181 indicated on the fecal assessment tool compared to the actual color was selected according
182 to the color standard Z8721: 1, 5Y8/12 (yellow); 2, 2.5Y7/12 (light yellowish-brown); 3,
183 10YR5/8 (yellowish-brown); 4, 7.5YR7/12 (brown); 5, 5Y4/4 (greenish-dark brown);
184 and 6, 2.5GY4/3 (dark brown). In addition, the questionnaire included questions on the
185 comfort of defecation (1, comfortable; 2, uncomfortable). The frequency of defecation
186 was evaluated as the number of times excretion occurred per week, using six responses
187 ranging from less than two to more than seven times per week. The stool pattern of
188 habitual gastrointestinal complaints was chosen within 1-4 patterns (1, normal; 2, tend to
189 constipation; 3, tend to diarrhea; and 4, repeated to constipation and diarrhea).

190

191 *Gut microbiome*

192 Fecal sampling and DNA extraction were performed as previously described.¹⁶
193 Briefly, fecal samples were collected using commercial vials containing guanidine
194 thiocyanate (GuSCN) solution (TechnoSuruga Laboratory Co., Ltd., Shizuoka, Japan)
195 and stored at room temperature. DNA was extracted from the fecal samples by the bead
196 beating method using a Cell Destroyer PS1000 (Bio Medical Sciences, Tokyo, Japan) and
197 a Gene Prep Star PI-80X device (Kurabo Industries Ltd., Osaka, Japan). The DNA
198 samples were stored at −30 °C until further use.

199 16S rRNA sequencing and analyses were performed as described in previous
200 studies.^{16,17} Briefly, the V3-V4 region of the 16S rRNA gene was amplified from fecal
201 DNA samples using KOD-plus-v2 (Toyobo, Osaka, Japan) and the following primers:
202 forward, 5'-

203 TCGTCGGCAGCGTCAGATGTGTATAAGCGACAGCCTACGGGNGGCWGCAG-
204 3' ; reverse, 5'-
205 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTA
206 ATCC-3'. Sequencing was performed by paired-end methods using the Illumina MiSeq
207 instrument and the MiSeq v3 Reagent Kit (Illumina, San Diego, CA, United States)
208 according to the manufacturer's instructions. Sequence reads from Illumina MiSeq were
209 analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) and Analysis
210 Automating Script (Auto-q) (<https://github.com/attayeb/auto-q>). The paired-end reads
211 obtained were selected, and chimeric sequences were removed using USEARCH v6.1.
212 Open-reference operational taxonomic unit (OTU) picking and taxonomy classification
213 were performed based on sequence similarity (>97%) using the UCLUST software with
214 the SILVA v128 reference sequence. The top 15 most abundant microbiomes at the genus
215 level are represented in this study.

216

217 *Physical condition*

218 We used two self-reported surveys to gauge their level of physical condition as
219 follows: (1) My condition was good physically, in addition to (2) I recovered well
220 physically, with values ranging from 1 (never) to 7 (always), modified from a previous
221 study.¹⁸ Scores were recorded on a 7-point Likert scale, and we evaluated a total of two
222 scores in this study.

223

224 *Dietary habit questionnaire*

225 Habitual diet during the preceding month was assessed using a brief self-
226 administered diet history questionnaire (BDHQ).¹⁹ The daily intake of total volume and
227 energy, protein, fat, carbohydrate, and fiber was calculated from the records.

228

229 **Statistical analysis**

230 All statistical analyses were performed using SPSS version 24 (IBM Inc.,
231 Chicago, IL, USA). The participants were divided into normal, constipation, or diarrhea-
232 compliant groups based on their habitual stool pattern, and better or poorer physical
233 condition groups based on their median physical condition score. Two subjects were
234 excluded from the analysis because they answered that both constipation and diarrhea
235 recurred. Continuous, ordinal, and categorical variables were analyzed using analysis of
236 variance (ANOVA), followed by an LSD post-hoc test, Kruskal-Wallis test, and χ^2 test
237 for the normal, constitution, and diarrhea groups, respectively. Unpaired t-tests and χ^2
238 tests were used to compare data between the better and poorer physical condition groups.
239 Statistical analyses of the gut microbiome were performed using R software (version 3.4.2,
240 <https://www.r-project.org/>). Alpha diversity indices, including Chao1 and Observed
241 index, were calculated using the *estimate_richness* function in the “phyloseq” package.
242 Principal coordinate analysis (PCoA) based on the Bray-Curtis distance metric was
243 performed using multivariate techniques of global view using the *dudi.pco* function in the
244 “vegan” and “ade4” packages. Principal component analysis (PCA) was conducted using
245 the *fviz_pca_biplot* function in the “FactoMineR” package to identify the relationship
246 between stool condition and gut microbiome in the better and poorer condition groups.
247 Figures, including boxplots, PCoA, and PCA plots, were created using the “ggplot”
248 package. The sample size was determined a priori, based on the expected differences in
249 the proportion of participants with abnormal stool patterns. Setting the verification power
250 to 95%, with an effect size of 0.5 (moderate) and a significance level of $\alpha = 0.05$, the
251 analysis determined a minimum total sample size of 62 participants to detect differences
252 between the better and poorer condition groups. Data are presented as mean \pm standard

253 deviation (SD) or counts with distribution percentage, as appropriate. Statistical
254 significance for all comparison was set *a priori* at $p < 0.05$ for all comparisons.

255

256 **Results**

257 Table 1 shows the participant characteristics and stool status of the three stool
258 pattern groups. The proportion of men and women, sports events, age, height, weight,
259 body mass index, and macronutrients did not significantly differ among the groups. The
260 frequency and form of stool defecation in the constipation group were significantly lower
261 than those in the normal and diarrhea groups ($p < 0.05$). The stool volume, color, and
262 odor did not differ between the groups. The percentage of athletes experiencing
263 comfortable defecation was lower in the diarrhea group than that in the normal and
264 constipation groups. Figure 1A shows the gut microbiome community structure at the
265 genus level obtained using PCoA. Figure 1B shows a comparison of the abundance of the
266 gut microbiome at the phylum level among stool pattern groups. The distribution of
267 *Bacteroidetes* was significantly lower and that of *Firmicutes* was significantly higher in
268 the constipation group than in the diarrhea group ($p < 0.05$). Figure 1C shows the
269 differences in alpha diversity among the groups. The Chao1 and observed indices in the
270 diarrhea group were significantly lower than those in the constipation groups ($p < 0.05$).

271 [Table 1 insert]

272 [Figure 1 insert]

273 Figure 2 shows the ratio of gastrointestinal complaints of stool patterns between
274 the better and poorer condition groups. The poorer condition group exhibited a
275 significantly higher frequency of abnormal stool patterns ($p < 0.05$). Table 2 shows the
276 characteristics of the participants in the better and poorer condition groups. The total
277 intake volume and intake of energy and fiber in the better condition group were higher

278 than those in the poorer condition group, whereas the proportion of sex and sports events,
279 height, weight, body mass index, and stool status did not differ significantly. Figure 3A
280 shows the comparison of the most abundant gut microbiome at the genus level in the
281 better and poorer condition groups. The distribution of *Faecalibacterium* was
282 significantly higher and that of *Bifidobacterium* was significantly lower in the better
283 condition group than those in the poorer condition group. Figure 3B shows the PCA of
284 stool status and patterns based on the fviz_pca_biplot function. The abundance of
285 *Faecalibacterium* was consistent with the physical condition score and adverse direction
286 for gastrointestinal complaints of stool patterns such as constipation and diarrhea.

287 [Figure 2 insert]

288 [Figure 3 insert]

289 [Table 2 insert]

290

291 **4. Discussion**

292 The present study investigated the relationship between the gut microbiome,
293 stool status and pattern, and the physical condition of elite Japanese athletes. We found
294 significant differences in the gut microbiome composition (*Bacteroidetes* and *Firmicutes*
295 phyla) and alpha diversity between constipation and diarrhea groups. We revealed that
296 athletes in the better physical condition group exhibited a lower frequency of habitual
297 gastrointestinal complaints of stool pattern, a higher abundance of *Faecalibacterium* and
298 a lower abundance of *Bifidobacterium* genus in the microbiome compared to athletes in
299 the poorer physical condition group. In addition, *Faecalibacterium* genus was associated
300 with stool pattern, physical condition score, and food intake in the PCA biplot analysis.
301 These results suggest that the physical condition of athletes is associated with their
302 gastrointestinal environment.

303 Athletes who perform intense and long-duration exercise suffer from a decrease
304 in functional digestive status and gastrointestinal issues such as irritable bowel syndrome
305 (IBS).⁴ Recently, several studies have revealed that the gut microbiome plays a key role
306 in the pathogenesis of gastrointestinal diseases.^{20,21} In a systemic review, the abundances
307 of *Faecalibacterium* and *Bifidobacterium* were lower and those of *Bacteroides* and
308 *Lactobacillaceae* and *Enterobacteriaceae* family were higher in patients with IBS than
309 those in healthy control.²¹ Vundeputte et al. have reported that species richness was
310 increased in the harder stool consistency, measured by the Bristol Stool Scale,²² and that
311 the stool moisture content was negatively correlated with microbial cell count.²³ In the
312 present study, we evaluated the stool patterns of the elite athlete and demonstrated that
313 the diarrhea group with relatively soft stool exhibited a lower alpha diversity, a higher
314 abundance of the *Bacteroidetes* phylum and a lower abundance of the *Firmicutes* phylum
315 (Figure 1) compared to the constipation group with relatively hard stool, which is
316 consistent with previous studies.^{22,23} These results suggest that gastrointestinal
317 complaints of stool patterns of elite Japanese athletes may be mediated, in part, by the gut
318 microbiome.

319 Athletes generally do not experience health issues when sufficient rest and
320 recovery are maintained, even under significant training stress. However, an imbalance
321 between stress and recovery may negatively affect the physical condition, athletic
322 performance, and skills development of athletes.²⁴ Intensive training can impair physical
323 conditions and is associated with gastrointestinal disorders, such as diarrhea, cramping,
324 bleeding, and flatulence, in over 50% of endurance athletes.²⁵ Furthermore, intense
325 exercise also delays gastric-emptying time, reduces vagal tone, elevates adrenergic
326 activity, and induces mechanical movement of intestinal contents, all of which contribute
327 to gastrointestinal symptoms.⁴ In terms of nutrition, athletes in the better physical

328 condition group, consumed a higher total volume of food, as well as more energy and
329 fiber, compared to those in the poorer physical condition group (Table 2). Low energy
330 availability has been linked to increased fatigue, injury, and illness, impaired adaptation
331 to training, and delayed recovery.²⁶ Consistent with this, King et al. demonstrated that
332 high carbohydrate consumption over 14 d reduced gastrointestinal complaints following
333 endurance exercise.²⁷ Additionally, dietary fiber plays a critical role in the health, as it is
334 metabolized into beneficial SCFAs, which support bowel movement regulation, protect
335 intestinal mucus barrier function, and enhance gut microbiome diversity.²⁸ In the present
336 study, athletes in the poorer physical condition group exhibited more abnormal stool
337 patterns than those in the better physical condition group (Figure 2). This distinction
338 highlights the relationship between physical condition and stool patterns based on self-
339 reported physical conditions, including recovery status. These findings suggest that
340 increasing food intake, particularly fiber-rich foods—a practice known as “gut
341 training”—could be beneficial in mitigating gastrointestinal disorders.

342 We observed differences in the abundance of *Bifidobacterium* and
343 *Faecalibacterium* in the gut microbiome between the better and poorer physical condition
344 groups (Figure 3A). *Bifidobacterium* is the predominant bacteria in Japanese individuals,
345 contributing to intestinal homeostasis via energy metabolism through the production of
346 SCFAs, which help maintain a luminal acidic environment that protects against harmful
347 bacteria and pathogenic substances colonization.^{2,29} Additionally, *Bifidobacterium* exerts
348 anti-inflammatory effects by promoting adiponectin and suppressing interleukin (IL)-6
349 which are involved in glucose and fatty acid oxidation metabolism.³⁰ Previous studies
350 have shown decreased *Bifidobacterium* level in patients with IBS suffering from
351 constipation or diarrhea.³¹ On the contrary, a Japanese cohort study did not observed
352 significant differences in *Bifidobacterium* levels among patients with IBS.³² In our study,

353 *Bifidobacterium* abundance was higher in the poorer physical condition group, which
354 exhibited many abnormal stool patterns, suggesting that the relationship between
355 gastrointestinal symptoms and *Bifidobacterium* abundance may be influenced by
356 confounding factors such as race or geographical location. However, we recently
357 demonstrated that *Bifidobacterium* levels are higher during exercise training season in
358 elite Japanese athletes.³³ These findings suggest that the interaction between the
359 gastrointestinal environment and intense exercise training could influence the levels of
360 *Bifidobacterium* in elite Japanese athletes.

361 *Faecalibacterium*, particularly the species *Faecalibacterium prausnitzii*, is one
362 of the most abundant microbiomes in the gut and plays a crucial role in maintaining gut
363 health.³⁴ This bacterium contributes to the prebiotic fermentation of non-digestible food
364 to produce butyrate, a primary energy source for colonocytes and involved in intestinal
365 metabolism. Additionally, butyrate exerts anti-inflammatory effects by inhibiting nuclear
366 factor kappa B (NF-kB) transcription and synthesizing pro-inflammatory cytokines like
367 IL-8.³⁴ *Faecalibacterium prausnitzii* has also been shown to inhibit NF-kB activation and
368 IL-8 secretion in a Crohn's disease model, even without butyrate production.³⁵ A recent
369 study using machine learning for multi-disease diagnosis demonstrated that
370 *Faecalibacterium prausnitzii* is associated with Crohn's disease in a large cohort
371 design.³⁶ Furthermore, *Faecalibacterium* abundance is reduced in patients with IBS
372 symptoms.³⁷ In the present study, we revealed an association between *Faecalibacterium*
373 and physical conditions as well as stool patterns in elite athletes. This suggests that
374 *Faecalibacterium* play a role in regulating gastrointestinal symptoms and influencing
375 physical conditions.

376 The biplot PCA analysis (Figure 3B) revealed that the clusters separated
377 depending on the physical condition; the better condition group tended to be in the upper

378 right and the poorer physical condition group tended to be on the left side. Moreover, the
379 constipation group gathered in the upper left, and the diarrhea group gathered in the lower
380 left, which is distributed among many athletes in poorer condition. This stool pattern
381 score showed an adverse direction on the physical condition score, food intake, and the
382 abundance of the genus *Faecalibacterium* abundance. *Faecalibacterium* affects
383 gastrointestinal functions including the maintenance of gut barrier integrity as described
384 above.³⁴ We revealed an association between *Faecalibacterium*, physical condition, and
385 stool patterns in elite athletes. Therefore, these results support the notion that the
386 relationship between the physical condition of athletes and gastrointestinal disorders is
387 mediated by the gut microbiome, especially the bacterium *Faecalibacterium*, which is
388 related to the amount of food intake, including fiber. However, it remains unclear how
389 the *Faecalibacterium* abundance is related to the physical conditions in this study.
390 *Faecalibacterium*, as a producer of SCFA like butyrate, plays a significant role in skeletal
391 muscle physiology and function via pathways that promote glycogen metabolism,
392 promotion of mitochondrial biogenesis, and the reduction of oxidative stress or
393 inflammation.³⁸ Moreover, SCFAs such as butyrate can influence serotonin synthesis,
394 which is associated with central higher-order behavior, mood disturbance, fatigue
395 sensation, and cardiovascular functions.² These effects suggest that *Faecalibacterium*
396 could potentially affect subjective physical condition through neurotransmitter and
397 hormonal responses.

398 The present study has several limitations. First, it was conducted using a
399 relatively small sample size. Previous studies have reported that gut microbiome is
400 affected by sex or sports characteristics.^{39.40} In this study, we recruited only international-
401 level athletes; therefore, the number of participants in each sport was limited. Although
402 the sample size was sufficient to analyze the differences in physical condition and the

403 proportion of sex and sports events did not differ between the groups, the findings of this
404 study may not be generalized to specific athletic group. Second, the present study relied
405 on a simple self-reported questionnaire based on a previous study¹⁸ to assess the physical
406 condition and gastrointestinal environment. While we aimed to compare the gut
407 microbiome between different subjective physical condition groups or subjective
408 gastrointestinal environments, future studies should incorporate objective parameters of
409 the physical condition and defecation status. Third, we could not control habitual diet. A
410 previous study showed that dietary intervention with high-fat/low-fiber or low-fat/high-
411 fiber for 10 days could affect gut microbiome composition in healthy individual.⁴¹ On
412 the contrary, other studies have shown that varying carbohydrate or protein intake 4 days
413 during intense training did not significantly affect the gut microbiome in military
414 personnel.⁷ These findings suggest that the effect of diet on the gut microbiome may vary
415 and interact with exercise training. Further studies are warranted to investigate the effect
416 of dietary “gut training” intervention. Lastly, the present study was used a cross-sectional
417 design, so a causal relationship, such as whether exercise-induced deterioration of
418 physical condition leads to decreased *Faecalibacterium* abundance or exercise-induced
419 decrease in *Faecalibacterium* abundance leads to deterioration of the physical condition,
420 could not be determined. Furthermore, we did not assess the functional or mechanistic
421 insight of gut microbiome, such as specific metabolomic pathways (e.g., organic enzymes,
422 carbohydrate degradation, or SCFA signaling). Further studies with longitudinal design
423 are needed to establish the precise mechanism and relationship between the gut
424 microbiome and the physical conditions of athletes.

425 In conclusion, we investigated the relationship between the gut microbiome,
426 stool status and pattern, and the physical condition of elite athletes. The alpha diversity
427 and compositional gut microbiome at the phylum level differed among the habitual stool

428 patterns. Subjective physical condition was associated with stool patterns. Furthermore,
429 the *Faecalibacterium* genus had a lower distribution in the poorer physical condition
430 group and was associated with stool patterns. This study suggests that the gut microbiome
431 profiles, especially those of *Faecalibacterium*, are one of the possible candidates
432 associated with progressing gastrointestinal disorders accompanied by deterioration of
433 physical condition due exercise training.

434

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439

440 **Contribution**

441 Experiment conception and design: NA and MM. Experiment implementation: NA, MN,
442 NE, HM, TN, HN, and MH. Data analysis: JP, KH, KM, and JK. Analyzing and advisory:
443 NA and MM. All authors approved the final version of the manuscript.

444

445 **Conflict of Interest**

446 The authors declare no conflict of interests.

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588

589 **Figure legends**

590 **Figure 1.** The gut microbiome profiles among the complaints of stool pattern groups. (A)
591 principal coordinate analysis of the gut microbiota profiles at the genus level, (B) alpha
592 diversity of the gut microbiome, and (C) differences in the relative abundance of phyla in
593 the microbiome of constipation, diarrhea, and normal groups. * $p < 0.05$ vs. normal group.
594 # $p < 0.05$ constipation vs. diarrhea groups.

595

596 **Figure 2.** The frequency of gastrointestinal complaint of stool patterns between the
597 poorer and better physical condition groups.

598

599 **Figure 3.** The gut microbiome profiles between the better and poorer physical condition
600 groups. (A) Comparison of the relative abundance of the gut microbiota at the genus level
601 between the poorer and better physical condition groups. * $p < 0.05$ better vs. poorer
602 groups. (B) Principal component analysis of gastrointestinal status profiles using
603 `fviz_pca_biplot` function.

604

605 **Supplemental Figure 1.** The sample of subjective questionnaire.

606

607 **Supplemental Figure 2.** The sample of stool sheet for volume, form, and color.

Table 1. The participants' characteristics, stool status, and macronutrients between normal, constipation, and diarrhea group.

	All		Normal		Constipation		Diarrhea	
Number, n	90		61		16		13	
Women, n (%)	39	(43)	24	(39)	11	(69)	4	(31)
Age, years	24 ± 4		24 ± 5		24 ± 4		25 ± 4	
Height, cm	170 ± 9		169 ± 8		168 ± 8		174 ± 11	
Weight, kg	64 ± 9		63 ± 9		62 ± 11		67 ± 8	
Body mass index, kg/m ²	22.1 ± 2.1		22.1 ± 2.0		21.9 ± 2.9		22.3 ± 2.1	
Sports event, n (%)								
Power/Sprint	13	(21)	8	(13)	1	(6)	4	(31)
Endurance	26	(43)	17	(28)	8	(50)	1	(8)
Martial arts	37	(61)	25	(41)	6	(38)	6	(46)
Artistic	1	(2)	0	(0)	1	(6)	0	(0)
Ball games	1	(2)	1	(2)	0	(0)	0	(0)
Others	12	(20)	10	(16)	0	(0)	2	(15)
Stool status								
Frequency, times/wk [#]	6.4 ± 1.2		6.5 ± 1		5.4 ± 1.5 ^{ab}		6.8 ± 0.8	
Volume, unit	3.8 ± 1.6		4.1 ± 1.5		3.5 ± 1.7		3.2 ± 1.6	
Color, unit	3.9 ± 1		3.9 ± 0.9		3.6 ± 1.3		3.8 ± 1	
Form, unit [#]	3.9 ± 0.9		3.9 ± 0.7		3.3 ± 0.8 ^{ab}		4.3 ± 1.3	
Comfortable, n (%) [†]								
Comfortable	75	(83)	55	(90)	12	(75)	8	(62) ^a
Uncomfortable	15	(17)	6	(10)	4	(25)	5	(48) ^a
Macronutrients								
Total intake volume, g	2744 ± 1212		2867 ± 1293		2407 ± 1032		2581 ± 970	
Total energy, kcal	2340 ± 930		2440 ± 965		2198 ± 977		2041 ± 625	
Protein, %	16 ± 3		16 ± 3		18 ± 5		16 ± 2	
Fat, %	26 ± 6		27 ± 6		26 ± 4		26 ± 4	
Carbohydrate, %	57 ± 8		58 ± 8		56 ± 7		58 ± 6	
Fiber, g	15 ± 7		16 ± 8		15 ± 7		12 ± 3	

[#] $p < 0.05$ by Kruskal-Wallis test and [†] $p < 0.05$ by X² test among the groups.

^a $p < 0.05$ vs. Normal group and ^b $p < 0.05$ vs. Diarrhea group

Table 2. The participants' characteristics, stool status, and macronutrients between better and poorer condition group.

	Better		Poorer	
Number, n	39		51	
Women, n(%)	14	(36)	25	(49)
Age, years	24	± 5	24	± 4
Height, cm	171	± 8	169	± 9
Weight, kg	65	± 9	63	± 10
Body mass index, kg/m ²	22.2	± 2.1	22.0	± 2.1
Sports event, n (%)				
Power/Sprint	5	(10)	8	(21)
Endurance	12	(24)	14	(36)
Martial arts	16	(31)	21	(54)
Artistic	0	(0)	1	(3)
Ball games	1	(2)	0	(0)
Others	5	(10)	7	(18)
Stool status				
Frequency, times/wk	6.4	± 1.2	6.3	± 1.2
Volume, unit	4.2	± 1.5	3.6	± 1.6
Color, unit	3.9	± 1.0	3.8	± 1.0
Form, unit	3.9	± 0.8	3.8	± 0.9
Comfortable, n (%)				
Comfortable	35	(90)	40	(78)
Uncomfortable	4	(10)	11	(22)
Macronutrients				
Total intake volume, g	3112	± 1363 ^a	2461	± 1008
Total energy, kcal	2636	± 1054 ^a	2113	± 758
Protein, %	16	± 3	16	± 3
Fat, %	26	± 6	26	± 5
Carbohydrate, %	57	± 8	57	± 7
Fiber, g	17	± 6 ^a	13	± 6

^a*p* < 0.05 vs. Poorer group.

Fig1

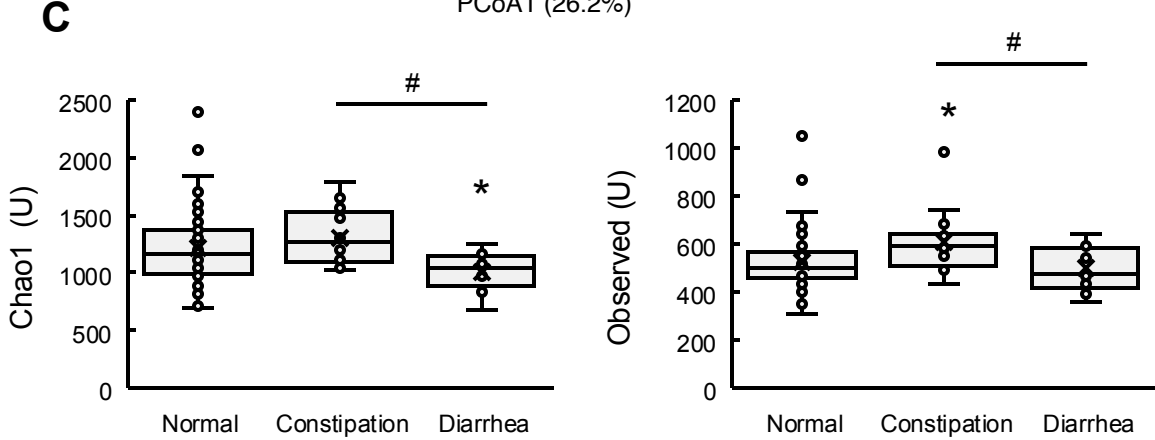
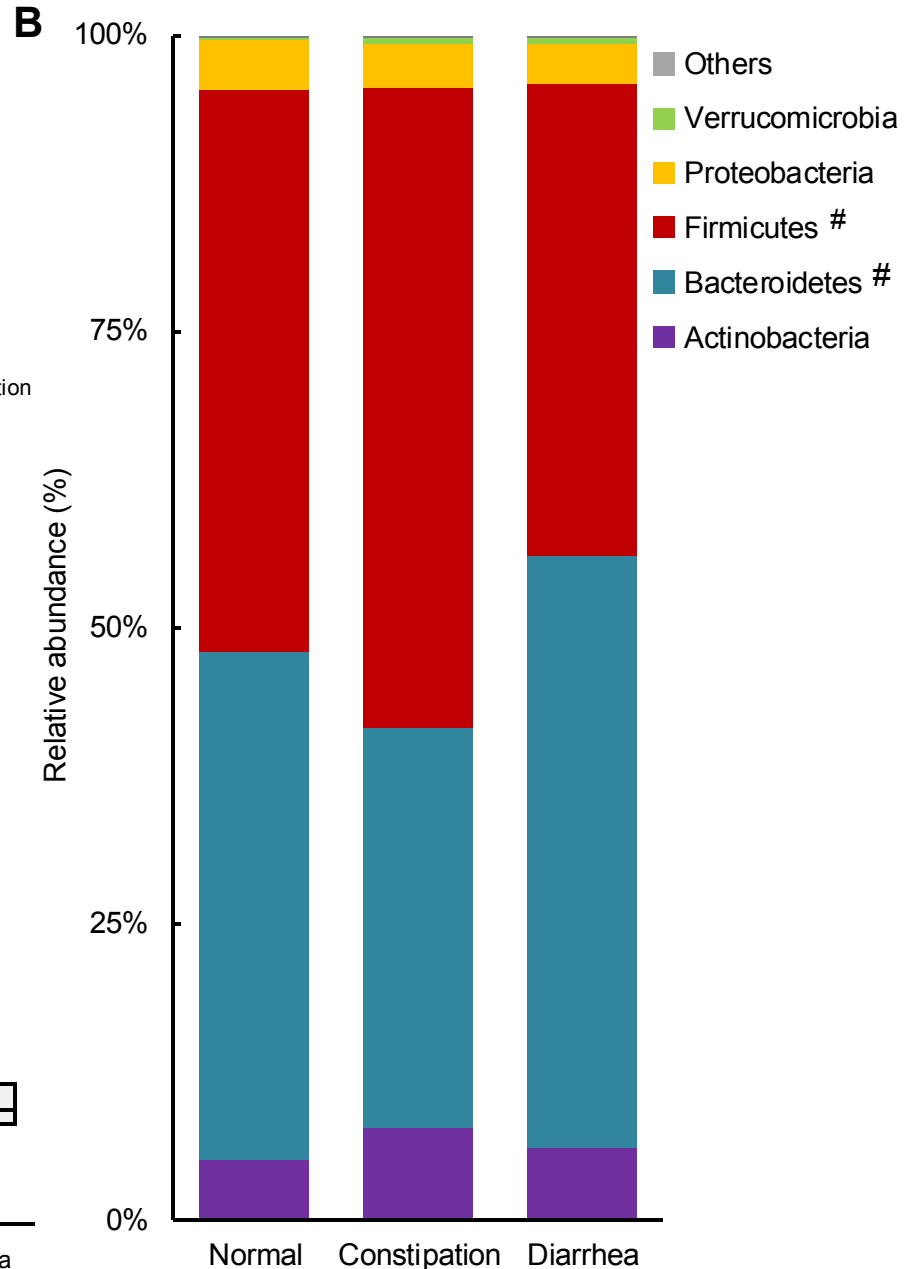
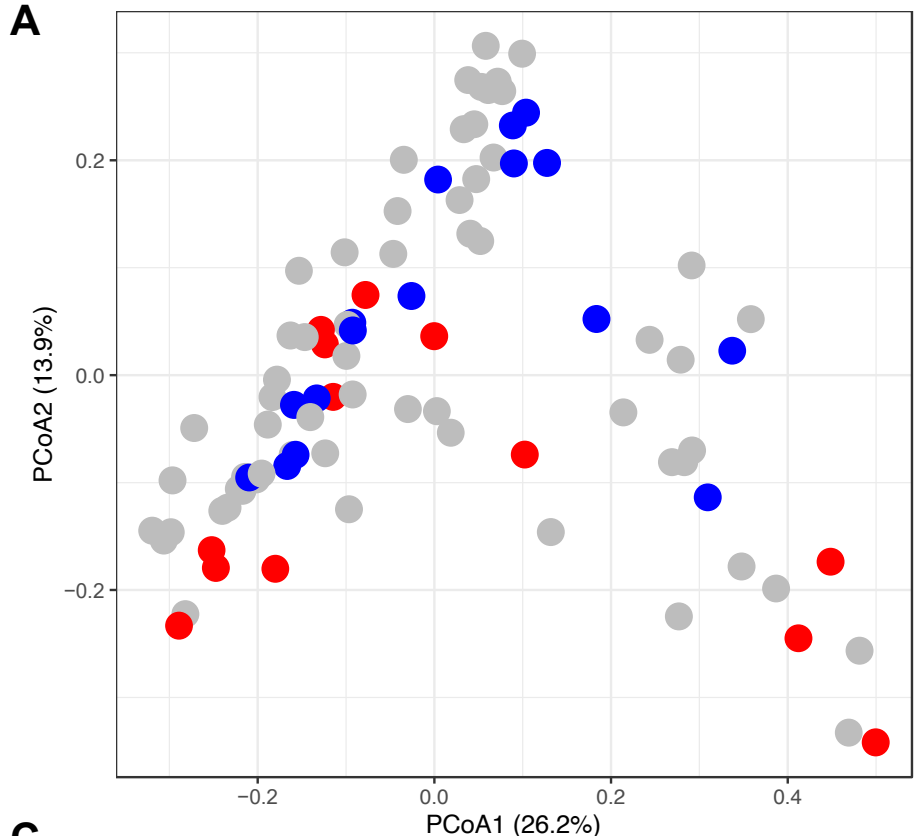
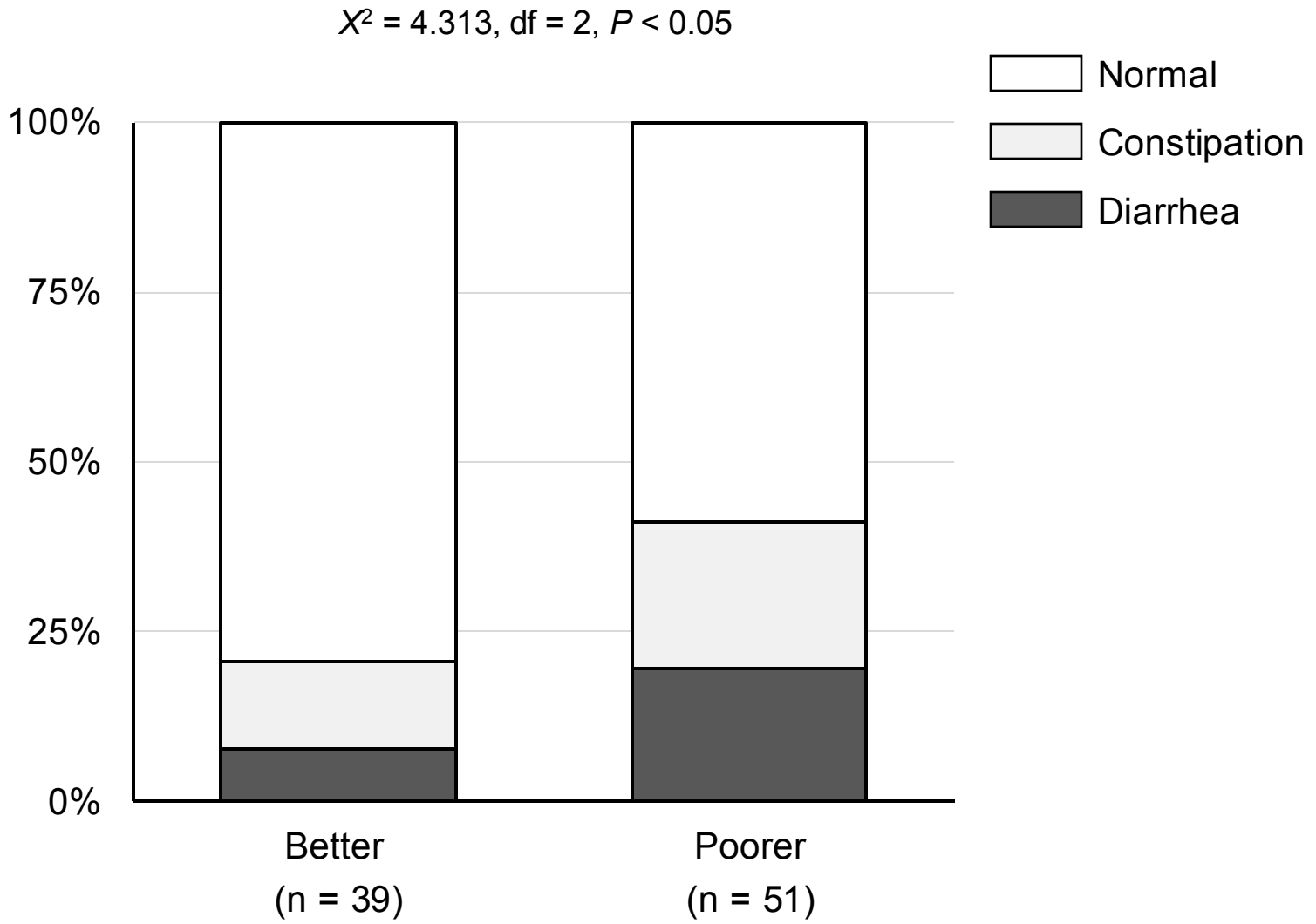
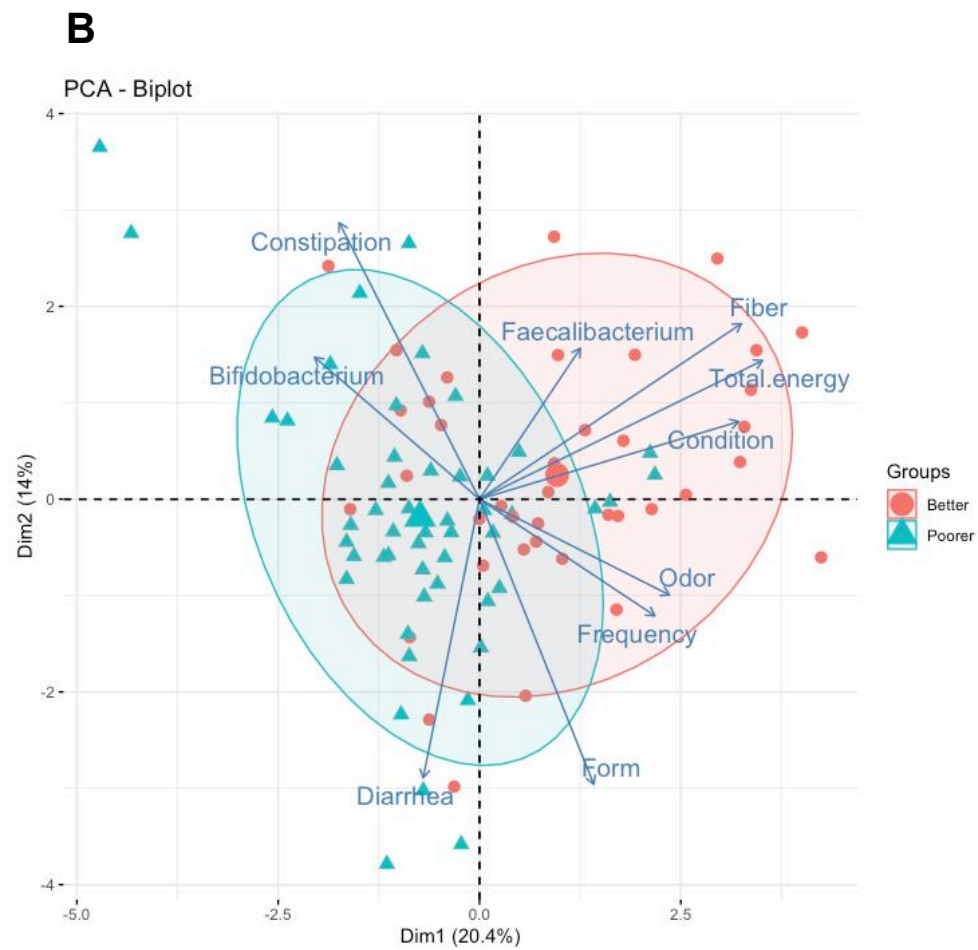
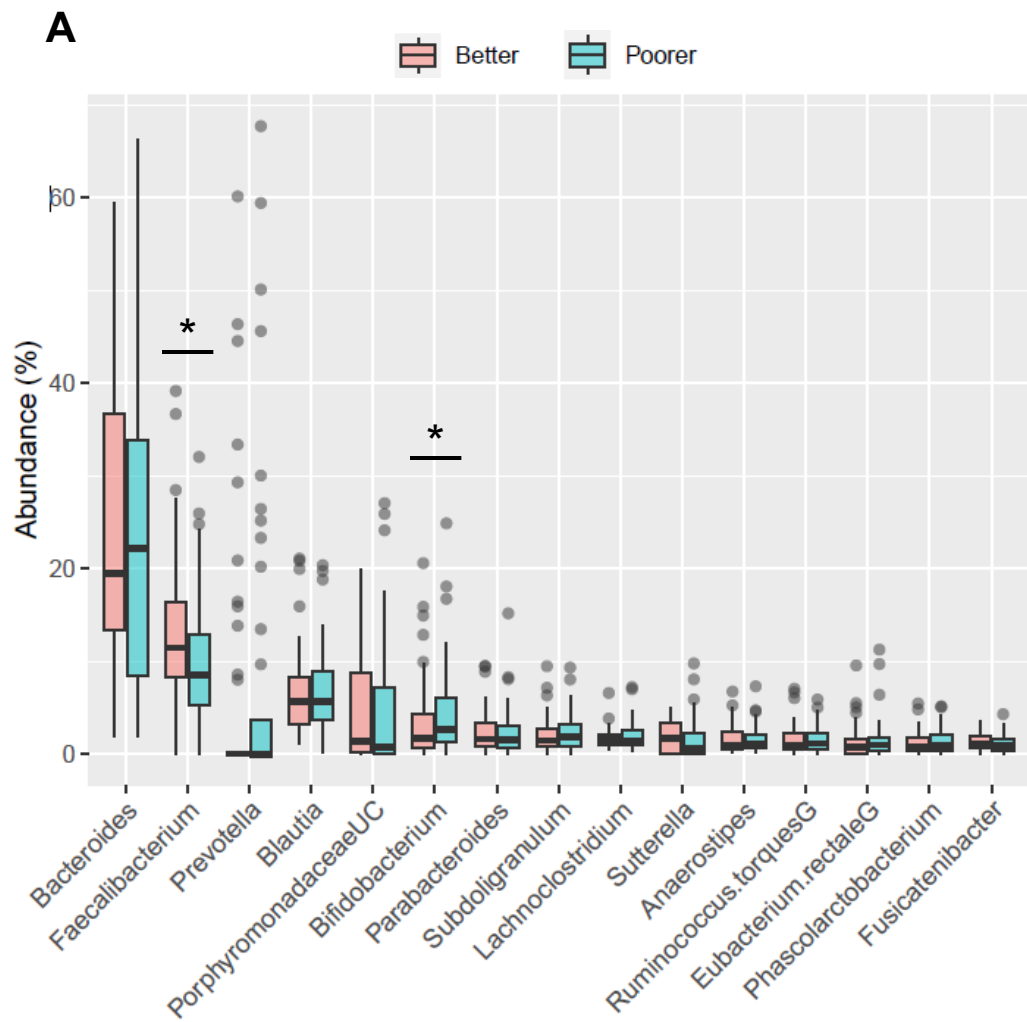


Fig2





ここ最近の習慣的な排便状況に関する質問 (Question for habitual stool status)

1. 1週間あたりの排便回数 (Frequency of defecation per week)

1. 週7回以上
(More than 7 times)
2. 6回
(6 times)
3. 5回
(5 times)
4. 4回
(4 times)
5. 3回
(3 times)
6. 週2回未満
(Less than 2 times)

2. 排便に関して、「排便量」「色」「形状」をシートから番号を回答ください。

(For stool status, please respond the corresponding number of "Volume" "Form" "Color" from stool sheet.)

- 1) 1回あたりの排便量
(Stool volume per one time)
- 2) 最も多い便の色
(Most common stool color)
- 3) 最も多い便の形状
(Most common stool form)

3. 排便後の爽快感 (Feeling comfort after defecation)

1. なし
(Uncomfortable)
2. あり
(Comfortable)

4. 排便パターン (Stoop pattern)

1. 快便
(Normal)
2. 便秘がち
(Tend to constipation)
3. 下痢がち
(Tend to diarrhea)
4. 便秘と下痢を繰り返す
(Repeated constipation and diarrhea)

ここ最近の身体コンディションに関する質問 (Question for physical condition)

1. 身体的なコンディションが良かった (My condition was good physically)

- 全くない
(Never)
- ほとんどない
(Seldom)
- 時々ある
(Sometimes)
- ある
(Often)
- よくある
(More often)
- 非常にある
(Very often)
- いつもある
(Always)

2. 身体的にリカバリーができていた (I recovered well physically)

- 全くない
(Never)
- ほとんどない
(Seldom)
- 時々ある
(Sometimes)
- ある
(Often)
- よくある
(More often)
- 非常にある
(Very often)
- いつもある
(Always)

1 **1回の排便量** あなたの排便量(うんち)は一回あたりどのくらいの量ですか？

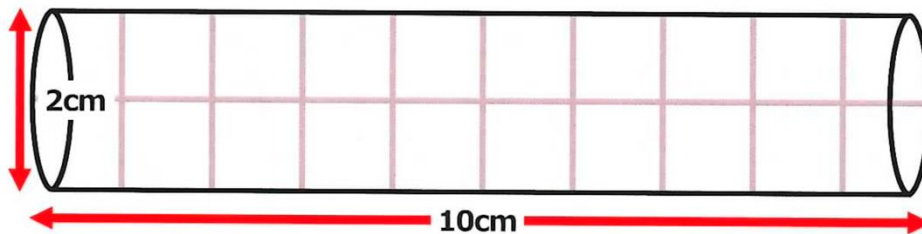
(Stool volume) (How much is your defecation volume each bowel movement?)

▶ **便モデル (2cm×10cm) の円柱に換算してみましょう**

(Please convert to a cylinder stool model unit (2cm × 10cm))

1	2	3	4	5	6	7	8
0.5 本	1 本	1.5 本	2 本	2.5 本	3 本	3.5 本	4本 以上

便モデル (Stool volume model)



2 **便の色** シートの色とうんちを比べてください。最も近い色の数字は何番ですか？

(Stool color) (Please compare your defecation to the color of the sheet.
What is the number of closest color?)



3 **便の形状** シートの形状とうんちを比べてください。最も近い形状の数字は何番ですか？

(Stool form) (Please compare your defecation to the form of the sheet.
What is the number of closest form?)

1	2	3	4	5	6	7
						
分離した硬い木の实のような便(排便困難を伴う)	硬便が集合したソーセージ状の便	表面にひび割れがあるソーセージ状の便	平滑で柔らかいソーセージ状あるいは蛇状の便	柔らかく割面が鋭い小塊状の便(排便が容易)	ふわふわした不定形の小片便、泥状便	固形物を含まない水様便

(Separate hard lumps, like nuts (Hard to pass))

(Sausage-shaped but lumpy)

(Like a sausage but with cracks on its surface)

(Like a sausage or snake, smooth and soft)

(Soft blots with clear-cut edges (passed easily))

(Fluffy pieces with ragged edges, a mushy stool)

(Watery, no solid pieces)