Gut Microbiota Composition Before and After Use of Proton Pump Inhibitors

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Abstract
Background Recently, problems associated with proton pump inhibitor (PPI) use have begun to surface. PPIs influence the gut microbiota; therefore, PPI use may increase the risk of enteric infections and cause bacterial translocation. In this study, we investigated fecal microbiota composition, fecal organic acid concentrations and pH, and gut bacteria in the blood of the same patients before and after PPI use.

Methods Twenty patients with reflux esophagitis based on endoscopic examination received 8 weeks of treatment with PPIs. To analyze fecal microbiota composition and gut bacteria in blood and organic acid concentrations, 16S and 23S rRNA-targeted quantitative RT-PCR and high-performance liquid chromatography were conducted.

Results Lactobacillus species were significantly increased at both 4 and 8 weeks after PPI treatment compared with bacterial counts before treatment ($P=0.011$ and $P=0.002$, respectively). Among Lactobacillus spp., counts of the L. gasseri subgroup, L. fermentum, the L. reuteri subgroup, and the L. ruminis subgroup were significantly increased at 4 and 8 weeks after treatment compared with counts before treatment. Streptococcus species were also significantly increased at 4 and 8 weeks after PPI treatment compared with counts before treatment ($P<0.01$ and $P<0.001$, respectively). There was no significant difference in the total organic acid concentrations before and after PPI treatment. Detection rates of bacteria in blood before and after PPI treatment were 22 and 28%, respectively, with no significant differences.

Conclusions Our quantitative RT-PCR results showed that gut dysbiosis was caused by PPI use, corroborating previous results obtained by metagenomic analysis.

Keywords Proton pump inhibitor · Bacterial translocation · Microbiota · Lactobacillus · Streptococcus

Introduction

The prevalence of gastroesophageal reflux disease (GERD) has increased worldwide, most likely due to changes in dietary patterns and increasing obesity [1]. The increasing prevalence of GERD has been associated with decreased prevalence of Helicobacter pylori infection, especially in Japan [2]. Proton pump inhibitors (PPIs) that suppress acid production and result in increased gastric pH are the most frequently used drugs for treatment of GERD [3]. The prophylactic use of aspirin to prevent coronary heart disease and cerebrovascular disease is commonly recommended [4], and PPIs are used to reduce upper gastrointestinal injuries associated with aspirin [5]. With the global growth of older populations, the prevalence of bone fractures [6] and osteoarthritis is increasing [7]. Nonsteroidal anti-inflammatory
drugs (NSAIDs) are often used as painkillers to alleviate pain associated with these diseases, and PPIs are also used to prevent NSAID-induced ulcers [8]. Moreover, in clinical trials, the tolerability of PPIs was similar to that of placebo, and PPIs have been concluded to be very safe drugs [9, 10]. Accordingly, the numbers of prescriptions for PPIs have increased significantly, and the duration of treatment has also increased.

Recently, problems associated with PPI use have begun to surface. Long-term PPI use may affect nutrient absorption including calcium malabsorption and the resulting increased risk of bone fracture [11]. PPI use may increase the risk of enteric infections, such as *Clostridium difficile* and *Campylobacter*, as well as community-acquired pneumonia [12–14]. PPI use may also increase the incidence of small intestinal bacterial overgrowth [15]. In addition, PPI use is potentially associated with development of spontaneous bacterial peritonitis in cirrhotic patients with ascites or cryptogenic liver abscess, which might be caused by bacterial translocation [16, 17]. The gut microbiota plays an important role in host resistance against colonization by exogenous enteric microbes and overgrowth of indigenous commensals [18]. Several observation and intervention studies found that PPIs altered the gut microbiota composition [19–23]. Accordingly, the increased risk of enteric infections in PPI users may be caused by the influence of PPIs on the gut microbiota. Moreover, intestinal bacterial overgrowth promotes bacterial translocation [24]; PPIs are therefore likely to be one of the risk factors for bacterial translocation.

Organic acids have various pathophysiological effects on mucosal blood flow in the gastrointestinal tract [25], intestinal epithelial proliferation [26], intestinal motility [27], and control of the intraluminal pH [28] and are major energy sources for intestinal epithelial cells [29]. Since organic acids are produced by colonic bacteria, any alteration in gut microbiota composition may be associated with a change in organic acid composition [30].

In this current study, we quantitatively investigated the fecal microbiota composition, fecal organic acid concentrations and pH, and the gut bacteria in the blood in the same patients before and after PPI use.

**Methods**

**Subjects**

This was an observational study. Study participants were recruited from patients who visited the outpatient clinic of the Department of Gastroenterology, Juntendo University Hospital, between October 2014 and September 2016. All patients were at least 20 years of age and had been shown to have ≥ grade A reflux esophagitis according to the Los Angeles classification [31] by endoscopic examination within 6 months prior to recruitment. Patients who had received PPIs within 1 month, who had taken antibiotics, a living bacterial preparation and/or yogurt within 1 month, who had a past history of gastrointestinal resection, and patients who had upper gastrointestinal ulcer(s) (except ulcer scars) or malignant lesion(s) were excluded from this study. The study protocol was reviewed and approved by the Juntendo University Ethics Committee (No. 13-096). Written informed consent was obtained from all patients. All participants received 8 weeks of treatment with PPIs [esomeprazole (20 mg), rabeprazole (10 mg), or lansoprazole (30 mg) once a day].

**Determination of the Bacterial Count by 16S and 23S rRNA-Targeted Quantitative Reverse Transcription-PCR (RT-qPCR)**

Fresh fecal samples were obtained from participants before treatment and 4 and 8 weeks after the start of treatment. Fecal samples were placed directly into two tubes (about 1.0 g/tube) by the participants; one tube contained 2 mL of RNaAlate® (Ambion, Austin, TX) for fecal bacterial analysis, and the other tube was used for fecal organic acid concentration and pH analysis. Samples were kept at −20 °C in a cooler box with refrigerants and sent or brought to Juntendo University by participants. Samples for bacterial analysis were stored in a refrigerator at 4 °C, and samples for organic acid concentration and pH analysis were kept in a freezer at −20 °C in Juntendo University.

Blood samples were obtained from participants before treatment and 8 weeks after the start of treatment. Blood (1 mL) was added to 2 mL of RNAProtect Bacterial Reagent (Qiagen, Hilden, Germany) immediately after collection and stored at −80 °C. Both fecal and blood samples were transported at −20 °C to Yakult Central Institute (Tokyo, Japan). To quantify the bacteria present in the samples, we extracted total RNA fractions from feces and blood using a modification of the acid guanidinium thiocyanate–phenol–chloroform extraction method [32–35] and examined the gut microbiota composition and plasma levels of gut bacteria using 16S and 23S rRNA-targeted RT-qPCR using the Yakult Intestinal Flora-SCAN analysis system (YIF-SCAN®, Yakult Honsha Co., Ltd., Tokyo, Japan). YIF-SCAN® analysis can quantify the abundance of a targeted bacterial population, including subdominant/dominant populations, with high resolution [32, 33]. Moreover, the YIF-SCAN® system has been shown to be highly effective for counting blood bacteria [32], and because RNA can be used as an indicator of bacterial cell viability, YIF-SCAN® analysis is capable of detecting viable bacteria [32, 36]. Three serial dilutions of each extracted RNA sample were used for rRNA-targeted RT-qPCR, and the threshold cycle values in the linear range
of the assay were applied to the standard curve to obtain the corresponding bacterial cell count for each fecal or blood sample. In the present study, predominant anaerobes present in the human intestine (Clostridium coccoides group, Clostridium leptum subgroup, Bacteroides fragilis group, Bifidobacterium, Atopobium cluster, and Prevotella) and intestinal subdominant populations (Clostridium difficile, Clostridium perfringens, Lactobacillus, Enterobacteriaceae, Enterococcus, Streptococcus, Staphylococcus, and Pseudomonas) were examined. The specificity of the RT-qPCR assay using group-, genus-, and species-specific primers was determined as described previously [32, 33]. Primers used in this study are listed in Table 1 [32–34, 37–39].

Measurement of Fecal Organic Acid Concentrations and pH

Fecal organic acid concentrations were determined as described previously [35] with slight modification. Briefly, frozen samples were homogenized in fourfold volumes of 0.15 mol/L perchloric acid and allowed to stand at 4 °C for 12 h. The suspension was then centrifuged at 20,400×g at 4 °C for 10 min. The resulting supernatant was passed through a filter with a pore size of 0.45 μm (Millipore Japan, Tokyo, Japan). The sample was analyzed for organic acids using a high-performance liquid chromatography system (432 Conductivity Detector; Waters Co., Milford, MA). The fecal pH was analyzed using an IQ 150 pH/Thermometer (IQ Scientific Instruments, Inc., Carlsbad, CA).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics Desktop version 22.0 software (IBM Japan Ltd., Tokyo, Japan). Half of the lower limit of detection was substituted as the fecal bacterial count for undetectable values [40, 41]. The Wilcoxon signed-rank test and Fisher’s exact probability test were used for data analysis. P < 0.05 was considered to be statistically significant.

Results

Baseline Characteristics and Prescribed PPIs

Twenty patients participated in this study. The characteristics of the patients in this study are summarized in Table 2. The male-to-female ratio was 13:7, the mean age was 60.2 years, and the mean body mass index was 23.9 kg/m². Only one patient was H. pylori-positive. The predominantly prescribed PPI was esomeprazole.

Fecal Bacteria, Organic Acid Concentrations, and pH

Fecal samples from all patients were examined. Fecal samples from three time points (before, 4 weeks after, and 8 weeks after the start of PPI treatment) from 19 patients were obtained. Because a fecal sample from one patient 4 weeks after the start of treatment could not be obtained, fecal samples from two time points (before and 8 weeks after the start of treatment) were obtained from this patient.

Total fecal bacteria counts before treatment, 4 weeks after the start of treatment, and 8 weeks after the start of treatment were 10.6 ± 0.6 log_{10} cells/g feces, 10.5 ± 0.5 log_{10} cells/g feces, and 10.5 ± 0.4 log_{10} cells/g feces, respectively (Table 3). Significant differences in total bacterial counts between pre-treatment and post-4 weeks of treatment and between pre-treatment and post-8 weeks of treatment were not observed. Similarly, significant differences in bacterial counts for each obligate anaerobe between pre-treatment and post-4 weeks or post-8 weeks of treatment were not observed. In contrast, the total counts of Lactobacillus, which are facultative anaerobes, were significantly different between pre-treatment and post-4 weeks of treatment and between pre-treatment and post-8 weeks of treatment (P = 0.011 and P = 0.002, respectively). Compared with counts before treatment, the bacterial counts increased significantly at 4 and 8 weeks after the start of treatment in the L. gasseri subgroup (P = 0.031 and P = 0.002, respectively), L. fermentum (P = 0.002 and P = 0.002, respectively), the L. reuteri subgroup (P = 0.001 and P = 0.001, respectively), and the L. ruminis subgroup (P = 0.022 and P = 0.011, respectively). Similarly, the bacterial counts of L. brevis after 4 weeks of treatment were significantly increased compared to counts before treatment (P = 0.025). Counts of facultative anaerobes in the genus Streptococcus were also significantly increased at both 4 and 8 weeks after the start of treatment compared with counts before treatment (P = 0.005 and P < 0.0001, respectively), and counts of facultatively anaerobic members of the family Enterobacteriaceae were significantly increased at 8 weeks after treatment compared with counts before treatment (P = 0.003). Counts and detection rates of facultative anaerobes in the genus Staphylococcus were also significantly increased after 8 weeks of treatment compared with those before treatment (P = 0.002).

Fecal total organic acid concentrations before treatment, 4 weeks after the start of treatment, and 8 weeks after the start of treatment were 102.8 ± 33.5, 122.9 ± 44.2, and 104.1 ± 44.1 μmol/g feces, respectively (Table 4). Significant differences both between pre-treatment and post-4 weeks of treatment and between pre-treatment and post-8 weeks of treatment were not observed. Formic acid and butyric acid
concentrations at 4 weeks after treatment were significantly increased compared with concentrations before treatment ($P = 0.022$ and $P = 0.033$, respectively). pH values were not significantly different among the three measurement points.

Table 1 16S and 23S rRNA gene-targeted specific primers used in this study

| Target bacteria                        | Primer  | Sequence (5′–3′)                             |
|----------------------------------------|---------|----------------------------------------------|
| *Clostridium coccoides* group          | g-Ccoc-F| AAAATGACGGTACCTGACTAA                       |
|                                        | g-Ccoc-R| CTTTGAGTTTCATTTGGCAAA                      |
| *Clostridium leptum* subgroup          | sg-Clept-F| GCACAAGGAGTGGAGT                   |
|                                        | sg-Clept-R3| CTTCTCGGTGGTCGCAATTT               |
| *Bacteroides fragilis* group          | g-Bfra-F2| AYAGCCTTTGGAAGRAAGAT                      |
|                                        | g-Bfra-R| CCAGTACCATCTGCAATTTTA                    |
| *Bifidobacterium*                      | g-Bifid-F| CTCTCTGGAAAAACGCGTGG                   |
|                                        | g-Bifid-R| GGTGTTCTTCGATATCTACA                     |
| *Atopobium* cluster                    | g-Atopo-F| CACRGTAAACGATGAGTCC                      |
|                                        | g-Atopo-R| GGTGCGTGGTGCGAC                       |
| *Clostridium difficile*                | Cd-Isu-F| GGGAGCTCTCCATACGGGTGG                   |
|                                        | Cd-Isu-R| TTGACTGCTCAATGCTGGGCC                   |
| *Clostridium perfringens*              | s-Clper-F| GGGGGTTCAACCTCC                           |
|                                        | CIPER-R| GCAAGGATGCTGATGT                      |
| *Lactobacillus gasseri* subgroup       | sg-Lgas-F| GATGCATAGCGGAAGACTGAT                     |
|                                        | sg-Lgas-R| TAAAGGGCGATTTACTCTATTCC                   |
| *Lactobacillus brevis*                 | s-Lbre-F| ATTTTGTGATGAAATGTCG                    |
|                                        | s-Lbre-R| ACCCTTGAACGTTACTCTCAAAGG                |
| *Lactobacillus casei* subgroup         | sg-Lcas-F| ACCCGATGACTCTCAGGTCGCC                   |
|                                        | sg-Lcas-R| CCGACACGTATCCTGCAA                      |
| *Lactobacillus fermentum*              | LFer-1| CCGTATCGTTCGTCGAGGC                     |
|                                        | LFer-2| ACYATCAAGCTCAGTCTCAGT                   |
| *Lactobacillus fructivorans*           | s-Lfru-F| TGGCCGCTAATGATGTCG                       |
|                                        | s-Lfru-R| GATACCGCTGCCAGCGTGG                     |
| *Lactobacillus plantarum* subgroup     | sg-Lpla-F| CTCCTGATGGATTGCGGTGTCGTGGCAT             |
|                                        | sg-Lpla-R| GTTCCGCACTCTCAATGTTGAA                  |
| *Lactobacillus reuteri* subgroup       | sg-Lreu-F| GAACGCGATGGCCCAA                         |
|                                        | sg-Lreu-R| TCCATTGTGCCGATAGC                       |
| *Lactobacillus ruminis* subgroup       | sg-Lrum-F| CACCGAATGCTTGCACTCACC                    |
|                                        | sg-Lrum-R| GCCGGCGCTCATCCAAA                       |
| *Lactobacillus sakei* subgroup         | sg-Lsak-F| CATAAAAACCTAMACCCGATGG                   |
|                                        | sg-Lsak-R| CATGTATCTGACTGTCGTCAA                   |
| *Enterobacteriaceae*                   | En-Isu-3F| TGGCGTAACCTTCGGAGAAAGCA                  |
|                                        | En-Isu-3R| TCAAGGACAGTGTTCAGTGC                   |
| *Enterococcus*                         | g-Encoc-F| ATCAGAGGGGGGATAACATT                    |
|                                        | g-Encoc-R| AACTCTCATCTTCTTCTCTCT                   |
| *Streptococcus*                        | g-Str-F| AGCTTGAAGCGATTTGATTC                    |
|                                        | g-Str-R| GGAATACCCCTTCGGGTCTC                    |
| *Staphylococcus*                       | g-Staph-F| TTTGGGTCAACAGCGTGTCACAATGGCAAA          |
|                                        | g-Staph-R| AACAACTTTATGAGGATTGTCGWTGA              |
| *Pseudomonas*                          | PSD7F| AAAACTCTGAGCTAGTACG                    |
|                                        | PSD7R| TAAAGTCTCAAGGATCCACCCACGCT             |

Group-, genus-, or species-specific primer sets were developed by using 16S rDNA sequences, except for Cd-Isu-F/R, En-Isu-3F/3′R, and g-Str-F/R, which targeted 23S rDNA.
Table 2  Study participant characteristics and prescribed PPIs

| Characteristics          | Patients n = 20 |
|--------------------------|-----------------|
| Sex (n) male/female      | 13:7            |
| Age (years), mean ± SD   | 60.2 ± 12.5     |
| Body mass index (kg/m²), mean ± SD | 23.9 ± 5.4 |
| Current smoker (n)       | 2               |
| Alcohol intake (n)       |                 |
| Nondrinker               | 10              |
| Occasional drinker       | 5               |
| Habitual drinker         | 5               |
| Helicobacter pylori (n)  |                 |
| Negative                 | 11              |
| Negative after eradication | 8          |
| Positive                 | 1               |
| Diseases (n)             |                 |
| Diabetes                 | 1               |
| Hypertension             | 4               |
| Dyslipidemia             | 4               |
| Prescribed PPIs (n)      |                 |
| Lansoprazole             | 2               |
| Rabeprazole              | 2               |
| Esomeprazole             | 16              |

PPIs proton pump inhibitors; SD standard deviation

Detection of Bacteria in Blood

Blood samples from 18 of the 20 participants were examined. A blood sample from one of the remaining participants could not be obtained, and one blood sample from a participant was not stored properly after collection. The minimum detectable number of bacteria was 1 bacterial cell per 1 mL of blood. Bacteria were detected in the blood of four of 18 subjects before treatment and in five of 18 subjects after 8 weeks of treatment (Table 5). There was no significant difference in the detection rate of bacteria in the blood before and after PPI treatment. No two blood samples from the same patient contained detectable numbers of bacteria at both time points pre-treatment and post-treatment. Two of the four subjects from whom bacteria were detected in the blood were habitual drinkers. However, bacteria were not detectable in the blood in any of the habitual drinkers after 8 weeks of treatment. Furthermore, bacteria were not detected in the blood of a diabetic patient either before or after treatment. The mean bacterial counts detected before treatment and after 8 weeks of treatment were 5.0 and 8.8 cells/mL, respectively. The Clostridium leptum subgroup, the Atopobium cluster, the genus Prevotella, and the genus Streptococcus were detected in patients before treatment, and the Atopobium cluster and genus Streptococcus were detected in patients after 8 weeks of PPI treatment.

Streptococcus spp. detected before treatment were S. salivarius and S. gordonii. The counts of S. salivarius were 8 cells/mL, and the counts of S. gordonii were 2 cells/mL. Streptococcus spp. detected after treatment were S. salivarius and S. oralis. S. salivarius was detected in two patients. The counts of S. salivarius were 1 cell/mL in each patient. S. oralis was also detected in two patients. The counts of S. oralis were 23 and 15 cells/mL (Fig. 1).

Discussion

The results of the present study showed that significant differences in the numbers of Lactobacillus, which is a subdominant population in the intestine, were observed between pre- and post-PPI treatment, while significant differences in the numbers of each predominant obligate anaerobe in the feces of PPI users between pre- and post-treatment were not observed. Recently, bacterial rRNA gene-based, metagenomic analyses have been conducted to analyze the composition of the human gut microbiome [22, 23, 42, 43]. However, it is difficult to quantify subdominant important intestinal genera using this approach. Therefore, we used the YIF-SCAN® system to perform bacterial analysis. The YIF-SCAN® system can quantify the abundance of the targeted bacterial population, including subdominant populations and dominant populations, with high sensitivity and has the ability to detect viable bacteria [32, 33, 36].

Previous large cohort studies of PPI users and intervention studies of PPI use for 4 weeks reported increases in bacteria from the genus Streptococcus [19, 20, 22, 23]. In the present study, we also observed an increase in the genus Streptococcus. Members of the genus Streptococcus are commensals of the human oral cavity, throat, and nasal cavity. Gastric acidity is known to inactivate ingested microorganisms [44]. Therefore, gastric acid may act as a barrier against bacterial influx down into the lower gastrointestinal tract from upper regions such as the oral cavity. Because PPIs reduce stomach acidity, the barrier function becomes weakened. This may explain the finding of increased Streptococcus counts detected in this study. Our results corroborate the findings of previous studies that employed metagenomic analyses. In addition, for the first time, the current pilot study verified the results of previous studies by using RT-qPCR. No significant differences in blood bacterial detection rates were observed before or after treatment; however, the mean counts of Streptococcus in blood before and after PPI treatment were 5 and 10 cells/mL, respectively. The Streptococcus spp. detected after PPI treatment were S. salivarius and S. oralis, which are commensals of the human oral cavity [45, 46]. This finding shows that bacteria present in the human oral cavity, throat, and nasal cavity increased in the intestine, implying that bacterial translocation may have occurred. Therefore,
PPI use may be associated with bacterial translocation. PPI use may also increase the risk of sepsis [47–49], as well as enteric infections. Four patients had bacteria in the blood before treatment. Although chronic alcohol consumption or diabetes is associated with bacterial translocation [50], neither alcohol nor diabetes was significantly associated with the presence of bacteria in the blood. The reason for bactemia before treatment is not currently clear. Further, because the method used for counting bacteria in this study is highly sensitive, a very small amount of bacteria in the blood due to unknown cause might be detected. The results of the present study show that PPI use may cause an increase in indigenous lactobacilli because patients who had received drugs and/or dietary items that affect the gut microbiota, such as antibiotics, living bacterial preparations, or yogurt, were excluded from the present study. Among lactobacilli, counts of the L. gasseri subgroup, L. fermentum, the L. reuteri subgroup, and the L. ruminis subgroup were significantly increased after PPI treatment. It is generally considered that these bacteria have probiotic influences on human health [51–54]. However, there are several case reports demonstrating that lactobacilli caused serious infections such as bacteremia and liver abscesses in susceptible immunocompromised patients [55, 56]. In addition to our results, increased numbers of Lactobacillus were also observed in patients with diseases such as diabetes mellitus type 2 [36] and Parkinson’s disease [57]. The biological effects of probiotics are strain specific [58], and whether these bacteria have probiotic features is determined by a coevolutionary relationship between the bacteria and their hosts [59]. Therefore, it is not clear at present whether increased numbers of such bacteria that are associated with PPI treatment provide harmful or beneficial

### Table 3
Comparisons of fecal bacterial counts before and after PPI treatment

|                     | Fecal bacterial count (detection rate, %) |               |               |               |
|---------------------|------------------------------------------|---------------|---------------|---------------|
|                     | Pre-treatment (n=20)                      | Post-4 weeks treatment (n=19) | Post-8 weeks treatment (n=20) |
|                     | Total bacteria 10.6 ± 0.6 (100)           | 10.5 ± 0.5 (100) | 10.5 ± 0.4 (100) |
| Obligate anaerobes  |                                          |               |               |               |
| Clostridium coccoidei group 10.2 ± 0.6 (100) | 9.8 ± 0.7 (100) | 9.9 ± 0.6 (100) |
| C. leptum subgroup    9.6 ± 0.9 (100)       | 9.6 ± 0.8 (100) | 9.5 ± 0.9 (100) |
| Bacteroides fragilis group 9.4 ± 0.7 (100) | 9.3 ± 1.0 (100) | 9.5 ± 0.6 (100) |
| Bifidobacterium      8.6 ± 1.9 (100)       | 8.9 ± 1.3 (100) | 8.9 ± 1.2 (100) |
| Atoptobium cluster   9.0 ± 1.0 (100)       | 9.1 ± 0.7 (100) | 9.1 ± 0.8 (100) |
| Prevotella           4.5 ± 2.6 (45)         | 4.9 ± 2.4 (58)  | 5.4 ± 2.4 (70)  |
| C. difficile         1.9 ± 1.6 (20)         | 2.2 ± 1.7 (32)  | 1.8 ± 1.4 (20)  |
| C. perfringens       2.5 ± 2.0 (35)         | 3.7 ± 2.5 (58)  | 3.2 ± 2.6 (45)  |
| Facultative anaerobes|                                          |               |               |               |
| Total Lactobacillus  5.7 ± 1.5 (95)        | 6.8 ± 1.3* (100) | 7.0 ± 1.0** (100) |
| L. gasseri subgroup  4.3 ± 2.0 (75)        | 5.3 ± 2.1* (89)  | 5.6 ± 1.5** (75) |
| L. brevis            1.7 ± 1.4 (15)         | 2.4 ± 1.7* (42)  | 2.4 ± 1.6 (55)  |
| L. casei subgroup    3.0 ± 1.8 (45)        | 2.9 ± 1.5 (53)  | 3.5 ± 1.5 (70)  |
| L. fermentum         3.3 ± 1.9 (35)         | 5.2 ± 2.2** (74*) | 4.8 ± 2.3** (65) |
| L. fructivorans      1.3 ± 0.5 (5)          | 1.3 ± 0.5 (5)   | 1.2 ± 0.4 (6)   |
| L. plantarum subgroup 4.1 ± 1.8 (80)      | 3.8 ± 1.3 (90)  | 4.2 ± 1.5 (90)  |
| L. reuteri subgroup  3.6 ± 1.7 (70)        | 5.0 ± 1.8** (89) | 4.8 ± 1.7** (85) |
| L. ruminis subgroup  3.1 ± 2.5 (40)       | 4.4 ± 2.9* (68)  | 4.4 ± 2.7* (70) |
| L. sakei subgroup    2.8 ± 1.4 (60)        | 3.5 ± 2.0 (74)  | 3.7 ± 2.0 (70)  |
| Enterobacteriaceae   5.9 ± 2.1 (85)        | 6.7 ± 1.0 (100) | 6.9 ± 1.6** (95) |
| Enterococcus         5.3 ± 1.7 (90)        | 6.0 ± 2.3 (84)  | 5.7 ± 2.5 (80)  |
| Streptococcus        8.7 ± 0.7 (100)       | 9.5 ± 0.8** (100) | 9.6 ± 0.7*** (100) |
| Staphylococcus       3.0 ± 1.5 (55)        | 3.7 ± 1.6 (74)  | 4.5 ± 1.0** (95**) |
| Aerobes              |                                          |               |               |               |
| Pseudomonas          2.2 ± 1.4 (25)         | 1.9 ± 1.0 (21)  | 2.2 ± 1.3 (30)  |

Values are the mean ± standard deviation (log10 cells/g feces)
*P < 0.05 versus Week 0, pre-PPI treatment
**P < 0.01 versus Week 0, pre-PPI treatment
***P < 0.001 versus Week 0, pre-PPI treatment
Table 4  Fecal organic acid concentrations and pH

|                  | Fecal organic acid concentrations (detection rate, %) |                  |
|------------------|------------------------------------------------------|------------------|
|                  | Pre-treatment ($n=20$)                                | Post-4 weeks treatment ($n=19$) | Post-8 weeks treatment ($n=20$) |
|                  | Values are the mean ± standard deviation (µmol/g feces) |                  |                  |
|                  |                                                      |                  |                  |
| Total organic acids | 102.8 ± 33.5 (100)                                   | 122.9 ± 44.2 (100) | 104.1 ± 44.1 (100) |
| Succinic acid     | 5.2 ± 9.0 (80)                                       | 5.1 ± 8.5 (68)   | 2.6 ± 5.2 (75)    |
| Lactic acid       | 1.3 ± 1.0 (15)                                       | 1.7 ± 1.3 (21)   | 2.7 ± 2.4 (40)    |
| Formic acid       | 0.6 ± 0.5 (75)                                       | 1.5 ± 1.2* (79)  | 1.3 ± 1.4 (90)    |
| Acetic acid       | 64.4 ± 20.7 (100)                                    | 74.6 ± 27.9 (100) | 62.4 ± 24.9 (100) |
| Propionic acid    | 19.2 ± 6.5 (100)                                     | 24.0 ± 10.6 (100) | 21.2 ± 9.8 (100)  |
| Butyric acid      | 12.5 ± 7.2 (90)                                      | 16.3 ± 10.3* (100) | 14.3 ± 10.0 (95) |
| Isovaleric acid   | 2.7 ± 2.0 (60)                                       | 2.4 ± 1.9 (68)   | 3.0 ± 1.8 (60)    |
| Valeric acid      | 2.7 ± 3.4 (55)                                       | 2.4 ± 1.8 (58)   | 1.8 ± 1.1 (55)    |
| pH                | 6.6 ± 0.4 (100)                                      | 6.4 ± 0.6 (100)  | 6.7 ± 0.7 (100)   |

Values are the mean ± standard deviation (µmol/g feces)

* $P < 0.05$ versus Week 0, pre-PPI treatment

** $P < 0.01$ versus Week 0, pre-PPI treatment

Table 5  Bacterial counts in blood samples

|                  | Pre-treatment ($n=18$) | Post-8 weeks treatment ($n=18$) |
|------------------|------------------------|---------------------------------|
|                  | Median (min–max) (cells/mL); $n$; detection rate | Median (min–max) (cells/mL); $n$; detection rate |
| Total bacteria   | 5.0 (1–15); 4; 22%   | 8.8 (1–23); 5; 28%             |
| Obligate anaerobes |                        |                                 |
| Clostridium cocoides group | ND          | ND                              |
| C. leptum subgroup | 4 (2–6); 2; 11%   | ND                              |
| Bacteroides fragilis group | ND          | ND                              |
| Bifidobacterium  | ND                     | ND                              |
| Atopobium cluster | 1; 1; 5.6%     | 4; 1; 5.6%                      |
| C. difficile      | ND                     | ND                              |
| C. perfringens    | ND                     | ND                              |
| Facultative anaerobes |                    |                                 |
| Total Lactobacillus | ND          | ND                              |
| L. gasseri subgroup | ND          | ND                              |
| L. brevis         | ND                     | ND                              |
| L. casei subgroup | ND                     | ND                              |
| L. fermentum      | ND                     | ND                              |
| L. fructivorans   | ND                     | ND                              |
| L. plantarum subgroup | ND         | ND                              |
| L. reuteri subgroup | ND          | ND                              |
| L. ruminis subgroup | ND          | ND                              |
| L. sakei subgroup | ND                     | ND                              |
| Enterobacteriaceae | ND          | ND                              |
| Enterococcus      | ND                     | ND                              |
| Streptococcus     | 5 (2–8); 2; 11%   | 10 (1–23); 4; 22%              |
| Staphylococcus    | ND                     | ND                              |
| Aerobes           |                        |                                 |
| Pseudomonas       | ND                     | ND                              |

ND not detected
influences on human health. However, if bacterial translocation was caused by PPI use, then PPI use would have a negative influence on human health.

With regard to total and individual organic acid concentrations and pH values, there were no significant differences between values at pre-treatment and post-8 weeks of PPI treatment. Although formic acid and butyric acid concentrations were significantly increased after 4 weeks of treatment compared with concentrations before treatment, significant differences disappeared after 8 weeks of treatment. PPI treatment therefore did not cause long-lasting changes in fecal organic acid concentrations. The reasons underlying the increases in formic acid and butyric acid concentrations after 4 weeks of treatment are currently not clear.

Regarding the limitations of this study, major drawbacks include the small sample size and short treatment period. Twenty patients and 8 weeks of treatment may be insufficient to evaluate the effects of PPI treatment. Moreover, because we used the proprietary YIF-SCAN® system, which may have biased results, studies using alternative approaches are needed.

In conclusion, our results by RT-qPCR demonstrate that gut dysbiosis was caused by PPI use, corroborating results obtained by previous metagenomic analyses. Further large-scale studies on longer-term PPI use, substantial effects of PPI use on human health caused by gut dysbiosis, and whether PPI use causes bacterial translocation to blood are needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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