Clinical Nutrition ESPEN 47 (2022) 70–77

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Randomized Controlled Trial

The effect of gastric acid suppression on probiotic colonization in a double blinded randomized clinical trial

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ARTICLE INFO

Article history:
Received 25 October 2021
Accepted 1 November 2021

Keywords:
PPI
Probiotics
Microbes
Metabolites

SUMMARY

Background & aims: Probiotics contain living microorganisms consumed for their putative benefits on the intestinal microbiota and general health and a concept is emerging to use probiotic as a therapeutic intervention to reduce proton pump inhibitors (PPIs) negative effects, but data is lacking. The use of PPIs can result in disordered gut microbiota, leading to a risk of enteric infections. PPIs are frequently prescribed in the general practice setting for gastroesophageal reflux disease (GERD), peptic ulcer disease, and related conditions. Despite the availability and widespread use of probiotics and acid-suppressing medications, the effect of PPIs-induced gastric acid suppression on the survival and colonization of probiotics bacterial species is currently unclear. We hypothesized that gastric acid suppression may improve intestinal colonization of probiotics bacterial species and probiotic intervention may have a potential role in mitigating untoward effects of PPI.

Methods: In a randomized, double-blind, placebo-controlled study, healthy subjects were given either proton pump inhibitor (PPI, n = 15) or placebo (n = 15) over 6 weeks. All subjects then consumed multi-strain probiotics from weeks 2–6. Thirty participants (10 males, 20 females, age range: 18–56 years) were enrolled in the study. Shotgun metagenomic sequencing and untargeted metabolomics analyses were performed on stool samples collected at week 0, 2, and 6.

Results: Short term PPI treatment increased the microbial abundance of *Streptococcaceae* ($p = 0.004$), *Leuconostacaceae* ($p = 0.001$), and *Pasteurellaceae* ($p = 0.020$) at family level and corresponding genus levels. The metabolomic analysis of the stools revealed a change in 10 metabolites where Gly Arg Val and phenylacetic acid were consistently increased compared to the baseline. Probiotic intervention inhibited PPI-induced microbial changes such as a decrease in Leuconostacaceae *family* ($p = 0.01$) and led to an increase in metabolite 1H-Indole-4-carbaldehyde. Notably, PPI enhanced the colonization of certain probiotic bacterial species like *Streptococcus thermophilus* ($p < 0.05$) along with other species present in the multi-strain probiotic.

Conclusion: Acid suppression enhanced certain probiotic associated bacterial colonization and probiotics in turn suppressed PPI-mediated intestinal microbial alterations. Thus, probiotics in combination with

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https://doi.org/10.1016/j.clnesp.2021.11.005

PPI might be a beneficial strategy that allows probiotic colonization and suppress PPI-induced microbial perturbations.
Clinical Trials.gov, number: NCT03327051.

1. Introduction

Acid-suppression medications, including proton pump inhibitors (PPIs), are some of the most prescribed medications in adult and pediatric medicine [1,2]. PPIs are frequently prescribed in the general practice setting for gastroesophageal reflux disease (GERD), peptic ulcer disease, and related conditions [3,4]. PPIs inhibit the hydrogen-potassium ATPase of parietal cells in the stomach, decreasing gastric acid production, thereby reducing acid-related gastric symptoms. The frequent use of PPIs and their effects on the gut microbiome has gained a lot of attention[5,6]. PPI users have shown a profound altered gastric and intestinal microbiome profile compared to non-PPI users [7,8]. The increasing incidences of enteric infections associated with PPI therapy and its effect on the gut microbiome have called into question repeated short-term and long-term use of acid suppression medications. Given PPI use is linked with disordered gut microbiota, modulating the microbiome with probiotic bacteria might be able to ameliorate the negative effects of PPIs.

Probiotics are composed of live microorganisms that confer health benefits when administered as supplements or added to dietary products. Research investigating the therapeutic potential of probiotics in maintaining human health has gained much attention in recent years [9,10]. Multiple studies suggest the prophylactic and therapeutic potential of probiotics, such as amelioration of irritable bowel syndrome (IBS) [11], inflammatory bowel disease (IBD) [12], prevention of pouchitis in adult patients [13], *Clostridium difficile* associated diarrhea [14], and neonatal late-onset sepsis and necrotizing enterocolitis [15]. The health benefits of probiotics have led to the supplementation of probiotics in foods such as breakfast cereals, dairy products, snacks, infant formulas, and also in cosmetic products [9].

However, the durability of these microorganisms is unclear, particularly when they pass through the stomach's highly acidic environment. Commercially available probiotics undergo over a 10^6-fold drop in colony-forming units within 5 min when incubated in gastric fluids lowering the chances of their therapeutic effect [16]. The combinatorial influence of PPI and probiotics on the gut microbiome is an area that has not been well explored.

We hypothesized that the effects of acid suppression on probiotic colonization would allow us to understand better the utility of probiotics used by individuals on acid suppression medications. We performed a randomized, double-blind, placebo-controlled study in cohorts where subjects were given PPI versus placebo and multi-strain probiotics over a 6-week study period. Metagenomic and metabolomic analyses of stool samples from enrolled subjects were performed to obtain a comprehensive understanding of probiotics' effect on PPI on microbiome and metabolome.

2. Material and methods

2.1. Study design

This study was a double-blind, parallel, placebo-controlled study conducted at Stanford University between March 2018 and December 2018.

2.2. Eligibility criteria for participants

This pilot study had a target of 30 participants. Healthy volunteers (age group 18 years and ≤75 years) without any pre-existing medical conditions, including gastrointestinal symptoms, who were able and willing to complete 3 study visits, answer study questionnaires, and provide stool samples, were recruited. To avoid any medication or probiotics related side effects, subjects with a history of dietary, soy, or gluten sensitivity were excluded. Subjects with a history of *Helicobacter pylori* infection, consuming herbs or probiotics, nursing or pregnant individuals, any chronic medical condition other than hypertension or hyperlipidemia were excluded. The subjects involved in the study were not on any antibiotic treatment or dietary restrictions. The randomization scheme in a 1:1 ratio was generated by a computer in blocks of four. Potential participants were screened and enrolled by research personnel, although the random allocation sequence and treatment assignment were performed by a third party and unknown to research personnel and participants throughout the study. An external pharmacist dispensed the PPI (Omeprazole: 20 mg/day) or placebo (422 mg of inactive ingredient lactose monohydrate NF into gelatin capsule) as unmarked pills with an identical appearance in identical containers. There was no difference in smell or taste. Initially, 39 participants were randomized, with 19 to the PPI and 20 to the placebo group. Four participants in the PPI group and five in the placebo group dropped before probiotic intervention. The remaining 30 participants (male:10, female:20, age group range~18–56 years) continued as per their randomization to receive either a PPI or placebo. Two weeks after randomization, all participants were administered 900 billion colony-forming units per day of multi-strain probiotic for 4 weeks (Supplementary Fig. 1). The baseline demographic data and unintended effects are shown in Table 1.

The commercially available multi-strain probiotic mix (VSL Pharmaceuticals, Inc. USA, batch no: 710012) is a mixture of 8 strains of bacteria: i) one strain of *Streptococcus thermophilus* BT01; ii) three strains of Bifidobacteria: *Bifidobacterium breve* BB02, *Bifidobacterium animalis* subsp. *lactis* BL03 and *B. animalis subsp. lactis* BI04; iii) four strains of Lactobacilli: *Lactobacillus acidophilus* BA05, *Lactobacillus plantarum* BP06, *Lactobacillus paracasei* BP07, and *Lactobacillus helveticus* BD08.

Survey data and stool samples were collected at weeks 0 (baseline) 2 and 6. The stool samples were snap-frozen on dry ice and stored at −80 °C until further use. The protocol was approved by the Institutional Review Board (IRB), Stanford University (IRB-41681). All patients gave their written informed consent to participate in the study. This trial is registered with http://www.ClinicalTrials.gov, number NCT03327051. All authors had access to the study data and reviewed and approved the final manuscript.

2.3. Shotgun metagenomics analysis and untargeted metabolomics

Shotgun metagenomic sequencing and analyses were performed to identify metagenomic differences between participants receiving PPI/placebo and multi-strain probiotic at the Children's

Table 1
Baseline demographics data, clinical characteristics and unintended effects on all participants (n = 39) who were randomized.

	PPI (n = 19)	Placebo (n = 20)
Gender	5 M, 14 F	6 M, 14 F
Race (Ethnicity)		
White/Caucasian	13	9
Asian	3	6
Black/African American	1	–
Native Hawaiian/Pacific Islander	–	1
Others/unidentified	2	4
AGE (SEM)	35 (3)	37 (3)
Antibiotic use	0	0
Dietary restriction	0	0
Bloating, n (%)		
Baseline	1 (5%)	0
2 week	1 (5%)	3 (15%)
6 week	0	2 (10%)
Flatulence, n (%)		
Baseline	0	0
2 week	1 (5%)	0
6 week	2 (11%)	0
Diarrhea, n (%)		
Baseline	0	0
2 week	1 (5%)	0
6 week	2 (11%)	0
Unintended effect		
Baseline	1 (5%) (nausea)	1 (5%) (tight throat)
2 week	1 (5%) (hungry)	1 (5%) (hungry)
6 week		1 (5%) (leg pain)

Hospital of Pennsylvania (CHOP) Microbiome Center (as described in supplementary information). Untargeted metabolomic analysis of the frozen stool samples (n = 90) was performed by the NIH-supported Michigan Regional Comprehensive Metabolomics Resource Core (MRC2). The methodology of untargeted metabolomics has been previously published [17] and further defined in the supplementary information.

2.4. Statistical analysis

Statistical analyses were performed with Prism 7 (GraphPad Software, Inc., La Jolla, CA). Differences with $p < 0.05$ were regarded as statistically significant. In RNA seq based differential abundance (DA) analysis, we used a t-test of log-transformed abundance values and applied a false discovery rate (FDR) correction to account for multiple comparisons. The methodology of Wilcoxon rank sum test and Linear discriminant analysis (LDA) effect size (LefSE) are mentioned in the supplementary information. For metabolite comparisons, one-way ANOVA with Tukey's posthoc correction was performed on the log-transformed values. A Student's t test was performed to compare two groups. Statistical analysis for microbial diversity inter-omics correlation analysis is described in the supplementary information. Spearman inter-omic correlation analysis of metabolomic and metagenomic data was performed in R according to Hardy et al., 2013 [18].

3. Results

3.1. Short-term PPI treatment induces microbial alterations: particularly in the Streptococcaceae family

The predominant phylum in the stool samples in subjects receiving PPI were Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria (Supplementary Fig. 2). Comparison of alpha and β-diversity between week 0 and 2 of PPI group revealed no significant differences (Supplementary Fig. 3). However, the PPI treatment increased the microbial abundance of *Leuconostacaceae* (adjusted $p = 0.001$), *Streptococcaceae* (adjusted $p = 0.004$), and *Pasteurellaceae* (adjusted $p = 0.020$) at family level and corresponding genus levels (Fig. 1A,B, based on edgeR, DA analysis). In addition to the RNA seq based DA analysis, we also validated our results with the commonly used Wilcoxon rank sum test and more stringent analyses like *LEfSe* (Linear discriminant analysis Effect Size).

The Wilcoxon rank sum test revealed 235 taxa that were differentially abundant between PPI 0 vs. PPI 2 weeks but did not meet statistical significance after FDR correction (Supplementary Table 1). *LEfSe* analysis revealed that 3 bacterial species were significantly altered with two weeks of PPI treatment ($p < 0.05$, LDA score: 2) (Fig. 1C, Supplementary Table 2). In all three different analyses, we found that the bacteria belonging to *Streptococacceae* family were significantly increased with two weeks of PPI treatment. The above microbial alterations were specific to 2 weeks of PPI treatment since there was no significant change in abundance of the *Streptococacceae* family between week 0–2 in the placebo group. Moreover, we also drew scatter plots to compare the common differentially abundant taxa between the Wilcoxon rank sum test and edgeR method. Mostly members of *Streptococaceae* family were significantly altered in both tests, although in Wilcoxon rank sum test, these features did not pass FDR correction (Supplementary Fig. 4).

3.2. PPI treatment leads to changes in microbial gene functions and metabolites

DA analysis shows that microbial genes annotated to different functional categories were altered with two weeks of PPI treatment (Supplementary Table 3). The metabolomic analysis of the stools revealed a change in 10 metabolites after two weeks of PPI treatment: Uracil, L-Tryptophan, L-Valine, Inositol hexanicotinate, L-Methionine, Iso-Olomoucine, Threoninyl-Leucine, and Gly Leu Leu were consistently decreased, while Gly Arg Val and phenylacetic acid were consistently increased compared to the baseline (Fig. 1D–F, Supplementary Table 4). Pathway enrichment analysis showed that the altered metabolites in the PPI group belonged to the Phenylalanine metabolism, Pantothenate, CoA biosynthesis, and Beta-Alanine metabolism pathways. However, this enrichment analysis did not pass FDR thresholds of <0.05 (Fig. 1F).

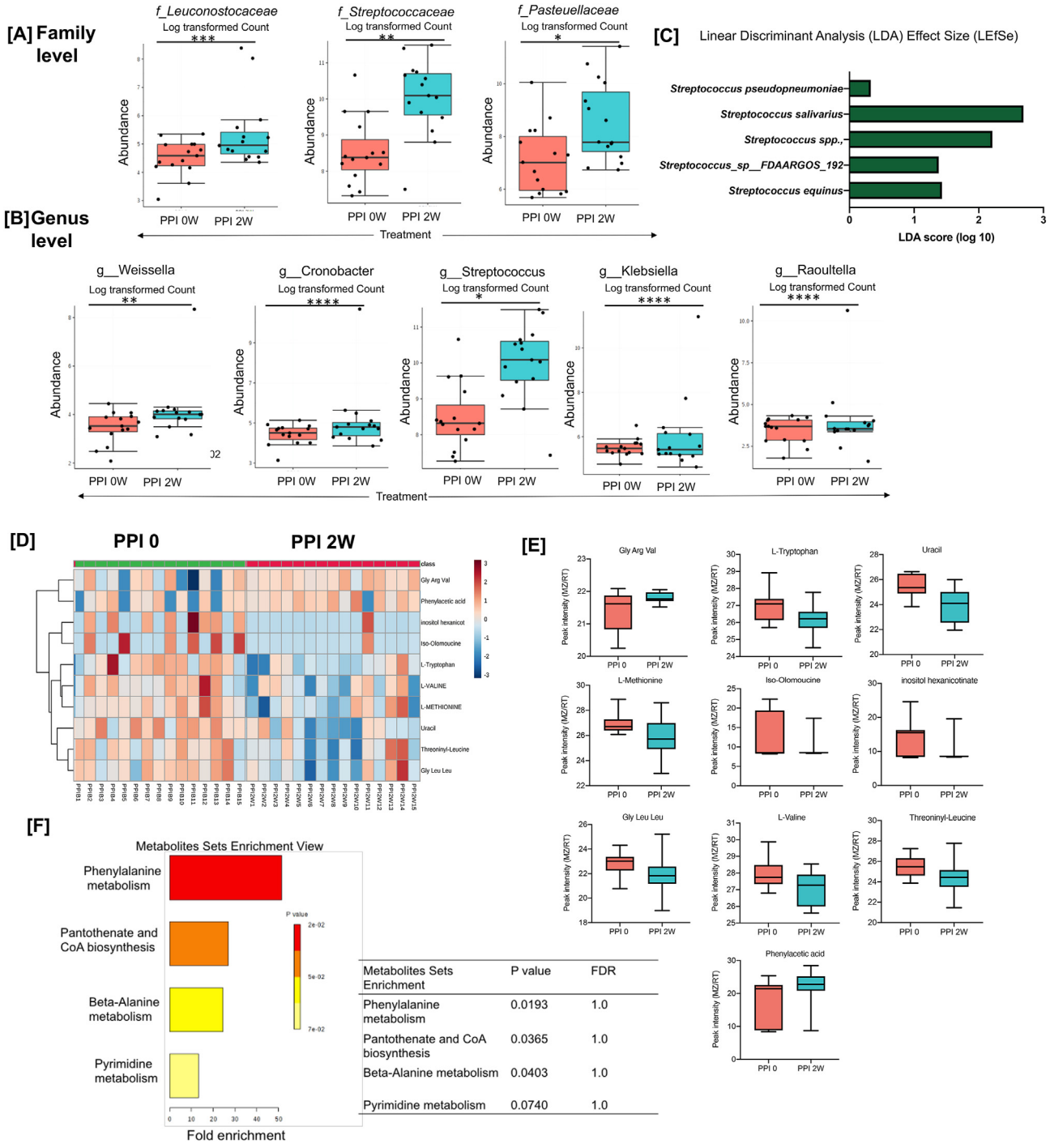
3.3. PPI facilitate certain probiotic species colonization

The differential abundance of probiotic species was compared between weeks 2 and 6 in PPI and placebo groups. In the PPI group (2 W vs. 6 W), four probiotic bacterial species *L. acidophilus* (adjusted $p = 0.0005$), *L. plantarum* (adjusted $p = 0.045$), *S. thermophilus* (adjusted $p = 0.025$), *B. animalis* adjusted $p = 0.0013$), were significantly increased with probiotic treatment (Fig. 2A). *L. paracasei*, showed an increasing trend but was not statistically significant (adjusted $p > 0.05$). The remaining probiotics species, *B. breve, L. helveticus* did not show any difference (Fig. 2A).

In the placebo group, the probiotic intervention increased the abundance of only three probiotic species (Supplementary Fig. 5A). The results indicate that PPI facilitates certain probiotic species like *S. thermophilus* colonization.

3.4. Probiotic treatment can suppress PPI induced microbial alterations and impacts microbial gene function and metabolites

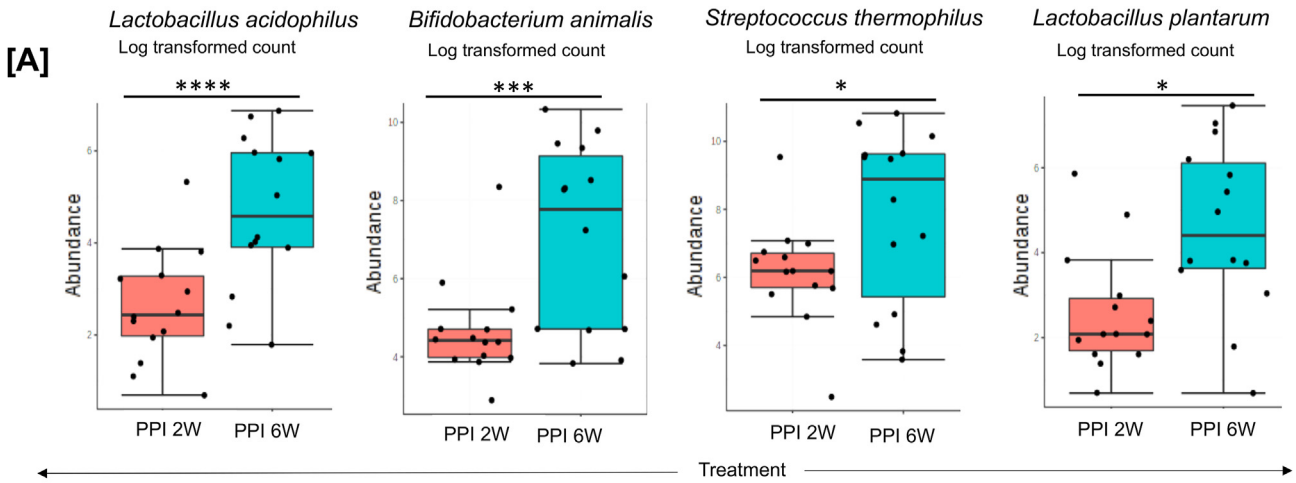
No significant change in alpha and β-diversity of microbial populations was found when PPI 2 W vs. 6 W was compared (Supplementary Fig. 6). DA analysis showed that there was a significant suppression in the abundance of the *Leuconostacaceae* family (adjusted $p = 0.007$), which was otherwise increased with the two weeks of PPI treatment (Fig. 3). The *Streptococcaceae* and



Metabolites Sets Enrichment	P value	FDR
Phenylalanine metabolism	0.0193	1.0
Pantothenate and CoA biosynthesis	0.0365	1.0
Beta-Alanine metabolism	0.0403	1.0
Pyrimidine metabolism	0.0740	1.0

Fig. 1. Short-term PPI treatment induces microbial alterations, A) DA analysis PPI 0 W vs. PPI 2 W at family B) genus, level C) Graphics of LEfSe for PPI group. Horizontal bars represent the effect size for each taxon. The length of the bar represents the log 10 transformed LDA score. The threshold on the logarithmic LDA score for discriminative features was set to 2.0. The taxon of bacteria with statistically significant change ($p < 0.05$) in the relative abundance is written alongside the horizontal lines. D) PPI treatment alters metabolites: Clustering result shown as a heatmap of stool metabolites, E) Box plot showing the comparison of metabolite levels, F) Metabolic pathway enrichment analysis. *represents $p < 0.05$; **represents $p < 0.01$, ***represents $p < 0.001$, ****represents $p < 0.0001$.

Pasteurellaceae families also showed a decreasing trend, although they did not reach statistical significance (Supplementary Fig. 7). Probiotic treatment had a profound effect on KEGG ortholog Hyaluronan synthase (K00752) in the PPI treated group (Supplementary Fig. 8). Probiotic treatment suppressed the abundance of some KEGG orthologs, such as dextransucrase K00689 [EC:2.4.1.5], which was increased with 2 weeks of PPI treatment. However, the p-value did not pass FDR correction (Supplementary Fig. 9). We found a single metabolite, 1H-Indole-4-carbaldehyde, that changed with the probiotic administration in those who continued PPI (Supplementary Fig. 8 D-E). This change was not observed in the probiotic treated group that did not take PPI (placebo group; data not shown). Thus, this effect appears to be due to combined probiotic and PPI therapy rather than probiotic alone.



[B]

Multi-strain probiotic bacterial species	Log2FC	p-value	FDR
Lactobacillus_paracasei	2.87	0.0004	0.09
Bifidobacterium_breve	0.47	0.3481	1.00
Lactobacillus_helveticus	0.20	0.6798	1.00

Fig. 2. PPI facilitate certain probiotic species colonization, A) DA analysis of probiotic bacterial species PPI 2 W vs. 6 W, B) Table showing differential abundance analysis of remaining probiotic species.

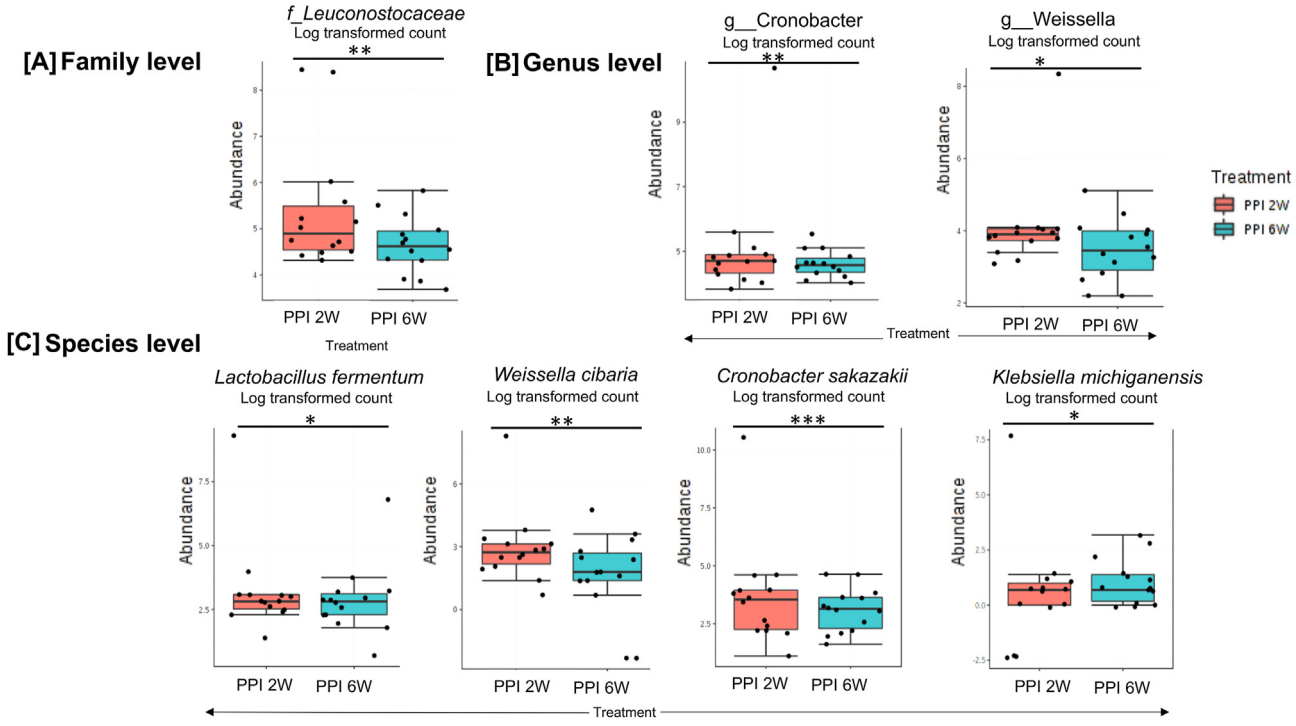


Fig. 3. Probiotic treatment suppress PPI induced microbial alterations, A) DA analysis between week 2 and 6 in PPI group at family level, B) at genus level, C) at species level.

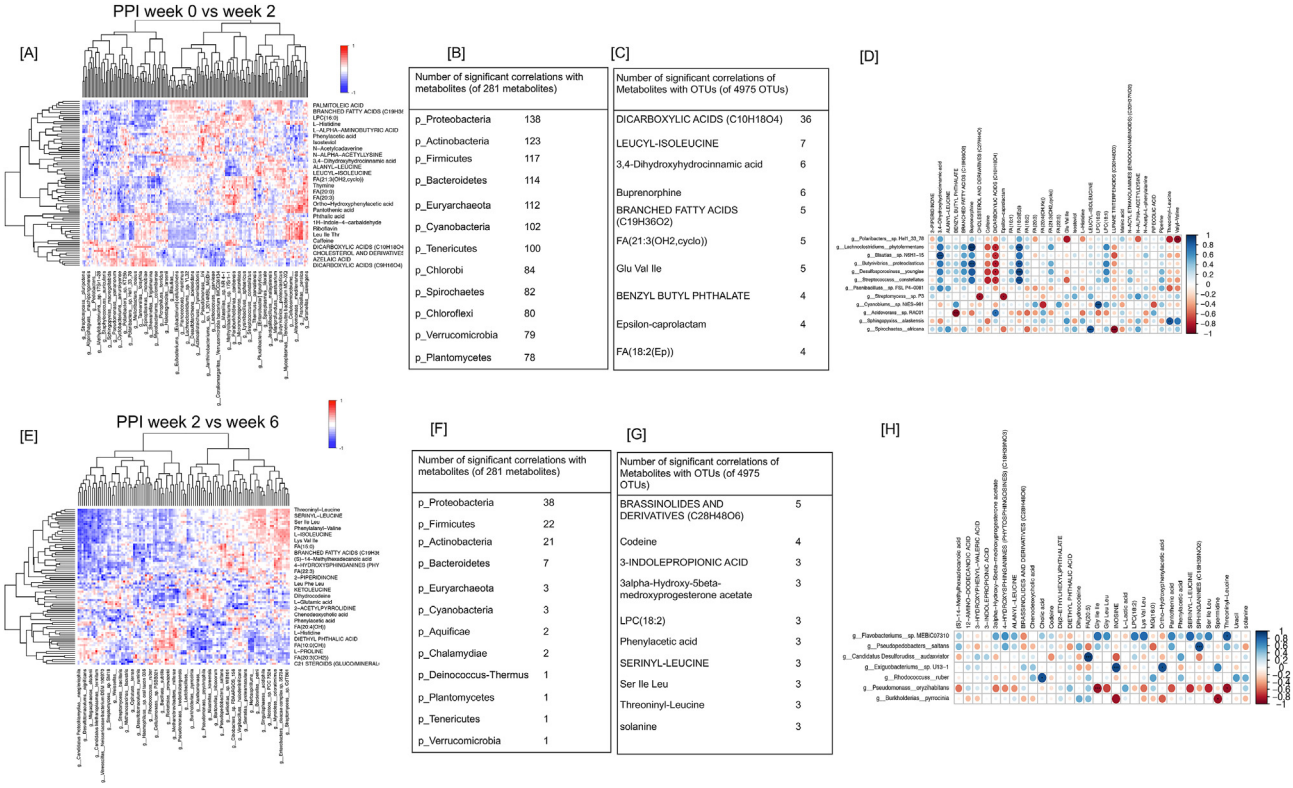
3.5. Correlations between metabolites and microbial genera

Next, we analyzed possible correlations between metabolites and microbial genera based on Spearman's correlation. In general, a large number of OTUs belonging to different phylum were correlated to metabolites with two weeks of PPI treatment, whereas number of OTU-metabolite correlations decreased when probiotics were introduced (Fig. 4A–C & Fig. 4 E-G). With 2 weeks of PPI treatment, threnoninyl leusine metabolite was positively correlated with *Sphingopyxiss alaskensis* of Proteobacteria phylum whereas for the PPI 2 vs 6-week comparison, threnoninyl leusine was positively correlated with flavobacterium and negatively correlated with *Psuedomonas* sp. (Fig. 4D). Negative correlation of dicarboxylic acid (DCAs) with *Streptococcus constellatus,* which had an increasing trend (although not significant after FDR correction) with two weeks of PPI was also noted (Fig. 4D and Supplementary Table 1). There was also a negative correlation between e-caprolactum, cholesterol derivates with *Streptomyces* genera with two weeks of PPI treatment (Fig. 4D). The probiotic intervention for four weeks after two weeks of PPI, resulted in negative correlation of Gly Ile Ile with *Psuedomonas oryzihabitans* (Fig. 4H). However, Shinganines and fatty acids (20.5) were positively correlated with *Pseudopedobacters saltans* and *Candidatus Desulforudiss audaxviator* respectively in the same PPI and probiotic group (Fig. 4H).

4. Discussion

The use of probiotics has increased immensely due to their prophylactic and therapeutic potential in treating various gastrointestinal disorders [11,12,14,15]. However, the viability of probiotic species in the gut is always a concern, particularly when they pass through the stomach's highly acidic environment. The health-promoting effects of probiotics generally depend upon their survival during passage through the gastrointestinal tract [16]. In this randomized and double-blind study, we tested the colonization of multi-strain probiotic microorganisms among individuals who consumed probiotics with and without acid suppression therapy. Prior studies reported that the use of probiotics with PPI reduced "dysbiosis" in patients on PPI therapy and children with GERD [19,20]. None of these reports had investigated the role of PPI in probiotic species colonization. Notably, our study examined the synergistic effect of PPI and probiotics, available both as prescription and over the counter. Our DA analysis in this pilot randomized, double-blind, placebo-controlled indicates that PPI facilitate certain probiotic species colonization and that probiotics can suppress PPI-mediated microbial perturbation. These encouraging results will need to be validated in future large cohort studies not only in "healthy" but also patients with diseases that would benefit from treatment with either or both of the agents used in this study.

Consistent with prior investigations in the literature, there is remarkable stability on consistent species richness and diversity, indicating limited or no effect of PPI on alpha and β-diversity [7,21]. However, similar to "dysbiosis" described with long-term PPI use, our findings confirm that significant changes in bacterial composition occur at different taxonomic levels even with short-term PPI treatment. Since there is wide availability of PPIs over the counter and their repeated short-term uses, our findings have significant implication. Our results are also consistent with prior reported investigations [22–25] where PPI therapy increased the abundance of oral cavity bacteria such as *Streptococcaceae*. Many *Streptococcaceae*



[B]

Number of significant correlations with metabolites (of 281 metabolites)	
p_Proteobacteria	138
p_Actinobacteria	123
p_Firmicutes	117
p_Bacteroidetes	114
p_Euryarchaeota	112
p_Cyanobacteria	102
p_Tenericutes	100
p_Chlorobi	84
p_Spirochaetes	82
p_Chloroflexi	80
p_Verrucomicrobia	79
p_Plantomycetes	78

[C]

Number of significant correlations of Metabolites with OTUs (of 4975 OTUs)	
DICARBOXYLIC ACIDS (C10H18O4)	36
LEUCYL-ISOLEUCINE	7
3,4-Dihydroxyhydrocinnamic acid	6
Buprenorphine	6
BRANCHED FATTY ACIDS (C19H36O2)	5
FA(21:3(OH2,cyclo))	5
Glu Val Ile	5
BENZYL BUTYL PHTHALATE	4
Epsilon-caprolactam	4
FA(18:2(Ep))	4

[F]

Number of significant correlations with metabolites (of 281 metabolites)	
p_Proteobacteria	38
p_Firmicutes	22
p_Actinobacteria	21
p_Bacteroidetes	7
p_Euryarchaeota	3
p_Cyanobacteria	3
p_Aquificae	2
p_Chalamydiae	2
p_Deinococcus-Thermus	1
p_Plantomycetes	1
p_Tenericutes	1
p_Verrucomicrobia	1

[G]

Number of significant correlations of Metabolites with OTUs (of 4975 OTUs)	
BRASSINOLIDES AND DERIVATIVES (C28H48O6)	5
Codeine	4
3-INDOLEPROPIONIC ACID	3
3alpha-Hydroxy-5beta-medroxyprogesterone acetate	3
LPC(18:2)	3
Phenylacetic acid	3
SERINYL-LEUCINE	3
Ser Ile Leu	3
Threoninyl-Leucine	3
solanine	3

Fig. 4. Spearman correlation analysis: PPI 0 vs 2 weeks and 2 vs 6 week. (A & E): Heatmap showing correlation of the metabolite abundance PPI (week 2–0) with the OTU abundance of PPI (week 2–0) and PPI (week 6–2) with the OTU abundance of PPI (week 6–2) respectively. Only those features were selected where only OTUs with at least 1 significant OTU correlated with a metabolite and only metabolites with at least 1 significant OTU correlated with an OUT. (B and F) Number of significant phylum level correlations with metabolites (of 281 metabolites), (C and G) Number of significant correlations of metabolites with OTUs (of 4975 OTUs), (D & H) Heatmap showing features only OTUs with at least 2 significant OTU correlation with a metabolite and only metabolites with at least 2 significant OTU correlation with an OTUs.

bacterial species like *Streptococcus* sp. *NPS 308, S parasanguinis, S australis, S oralis, S sanguinis, Streptococcus* sp. *Oral taxon 431, Streptococcus* sp. *FDAARGOS_192, Streptococcus* sp. *Oral taxon 064, Streptococcus* sp. *I–P16* were increased with two weeks of PPI treatment although didn't pass the FDR correction in the wilcoxon rank test. In general, gastric acidity inactivates ingested microorganisms and acts as a barrier against bacterial influx down into the lower gastrointestinal tract from upper regions such as the oral cavity. PPI use facilitates the survival and colonization of oral cavity bacteria into the lower gastrointestinal tract by abrogating the stomach acid barrier. In this study of healthy subjects, these PPI-induced microbial alterations described above may be mitigated by probiotic combination therapy. The extension of this study in non-healthy subjects is an area of interest in future investigations.

Negative correlation of DCAs with *S. constellatus* shows that PPI disrupts microbial balance in the gut. DCAs are known to be associated with intestinal microbial "dysbiosis" and the analysis of urine DCAs is a vital diagnostic tool of metabolic disorders [26]. Negative correlation of Lupane triterpenoids with *Spirochaetes* bacteria also shows the disordered gut microbiome with PPI treatment. Lupane triterpenoids and their derivatives have shown a wide range of potential health benefits with many bioactivities against cancer cell lines and hold encouraging antitumor effects [27]. Sphingolipids and their derivatives are bioactive compounds with anti-cancer, bacteriostatic and cholesterol-lowering properties [28]. The significant correlation of *P. saltans* (bacteria that has capability to produce sphingolipids) with sphingolipids indicates that probiotic intervention supports positive correlation between microbes and metabolites beneficial to health.

PPI treatment was associated with several altered stool metabolites. L-tryptophan has been shown to improve the mucosal barrier and dampen inflammatory cytokine production [29]. L-valine is one of the preferred amino acids of gut bacteria, which can influence the epithelial cells and modulate the mucosal immune system [30]. One of the notable changes observed with PPI treatment involved phenylalanine metabolism in both the metagenomics and metabolomics data sets. Stool phenylacetic acid has been correlated with a more proinflammatory status, as shown by the increase in serum level of C-reactive protein (CRP), IL17 and IL8 [31]. Our study was likely limited in size to show the effect of probiotic intervention on metabolite alterations since many of the changes observed did not meet statistical significance when corrected for multiple testing (FDR correction). Given that PPI use can trigger a shift in microbial composition and gut metabolites, exploring the effect of probiotics intervention on the above altered metabolites and disease outcome in a larger number of patients would be an important future exploration to consider.

Our study was adequately powered to test the primary hypothesis that PPIs enhance probiotic colonization as shown by the metagenomic analysis, however, our study had limitations due to its relative sample size for breath of the metabolomic analysis. Our study was limited in correlating dietary intake to microbiome composition, however a heatmap showing the dietary self-reported intake of all groups is presented in the Supplementary Fig. 10). Dietary intake is a major regulator of gut microbiota composition and long-term dietary intake can influence the microbial abundance and activity of the microorganisms residing in the human gut [32,33]. Although the use of PPI is encouraged in the lowest effective dose, citing the beneficial effects of PPI therapy [34], concerns over repeated dosing, higher doses and long term use of PPIs and their potential adverse effect on the gut microbiome is well described [35]. The therapeutic implications of combination therapy of probiotics and PPI in correcting microbial changes through the restoration of key bacterial species (or microbial gene functions and metabolites) hold a clinical promise. Our results show that PPI-induced microbial alterations might be substantially reduced by adding probiotic intervention. In addition, PPI facilitate certain probiotic species colonization of the gut and likely optimize probiotic efficacy. In this study, the PPI treatment was relatively short-term (2–6 weeks) but relevant to real-world situations where there is a wide usage of over-the-counter PPIs and not to mention healthy subjects that experience transient heart burn/reflux symptoms and don't necessarily present for medical assessment but use PPIs intermittently. This study lays foundation for future studies to assess the short- and long-term effects of PPI and probiotics intervention on the gut microbiome and metabolome in patients with and without gastrointestinal disorders.

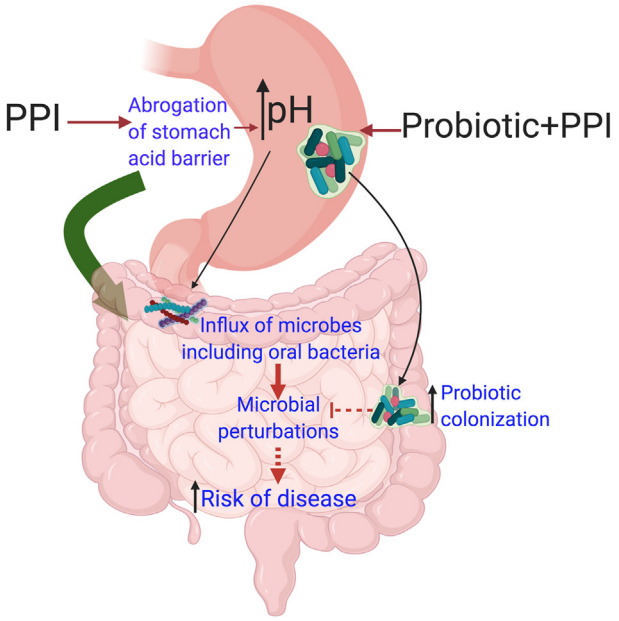


Fig. 5. Schematic diagram showing the potential combinatorial effect of PPI and probiotics on gut microbiota (created with BioRender.com).

5. Conclusion

Based upon our primary findings, short term PPI therapy may facilitate probiotic species colonization and, thus, likely to enhance probiotic efficacy. Importantly, probiotic supplementation suppresses PPI-induced microbial shifts and thus mitigate potential risks associated with PPI mediated perturbation of the microbial community (Fig. 5). Findings from this pilot study suggest that if there is a need for intermittent or short-term PPI therapy, combining it with probiotics might provide dual benefit by minimizing the potential negative effects of PPIs on the microbiota and enhancing the potential benefit of probiotics by improving their colonization.

Funding statement

This work was in part funded through a philanthropic support to AH and BNL.

Authors contribution

AH and BNL designed the study; SH assisted with study blinding; ES consented subjects to participate in this study, ES and YH collected and processed samples, GS and YH performed experiments, GS, YH, AP, and AH analyzed and interpreted the data; PS

helped in analyzing the metabolomic data; LB and NRG helped in differential abundance and correlation analysis, GS, YH, AH wrote the paper with assistance from all authors had the opportunity to discuss the results, review, and comment on the final manuscript.

Declaration of competing interest

None to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2021.11.005.

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