Gastro Hep Advances 2025;4:100533

ORIGINAL RESEARCH—CLINICAL

Thiamine-Reduced Fatigue in Quiescent Inflammatory Bowel Disease Is Linked to *Faecalibacterium prausnitzii* Abundance

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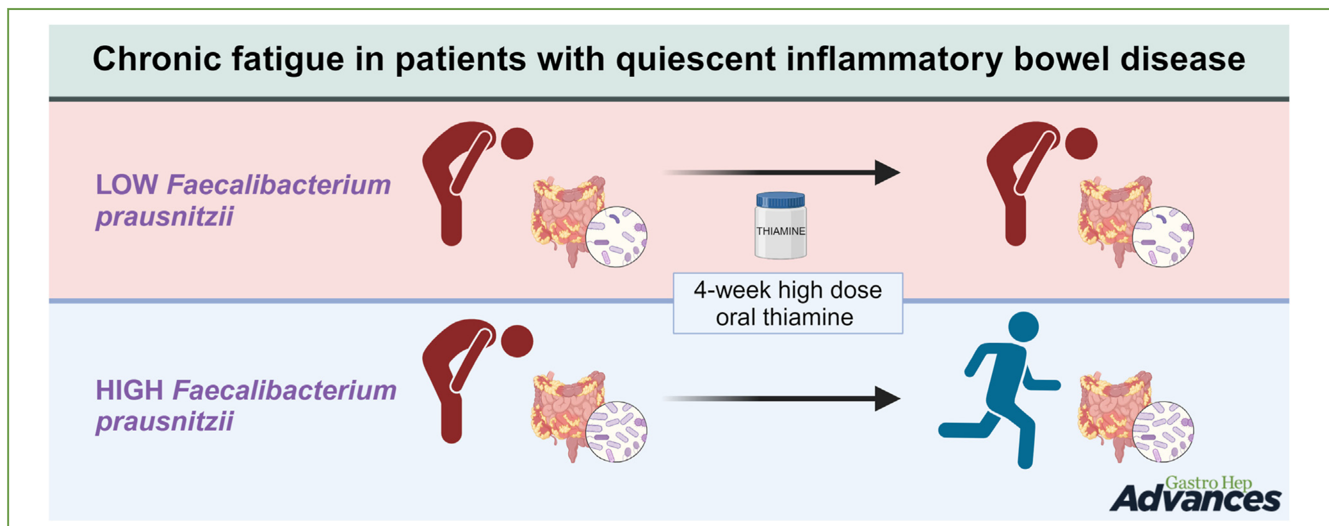

BACKGROUND AND AIMS: Chronic fatigue is common in patients with inflammatory bowel disease (IBD). The gut microbiota, specifically, microbial diversity and butyrate-producing bacteria have been linked to the fatigue pathogenesis. High-dose oral thiamine reduces fatigue, potentially through gut microbiota modification. In this study, we investigated how the gut microbiota influences the efficacy of high-dose thiamine in alleviating chronic fatigue in quiescent IBD (qIBD). **METHODS:** We analyzed the microbiota and short-chain fatty acids concentrations in fecal samples from patients with qIBD, with (n = 40) or without (n = 20) chronic fatigue. The 40 patients with qIBD and fatigue were included in a randomized, placebo-controlled, crossover trial to assess a 4-week high-oral-dose thiamine regimen. **RESULTS:** Butyrate and butyrate-producing bacteria were similar in patients with and without fatigue and did not change with high-dose thiamine treatment. Notably, *Faecalibacterium prausnitzii* was more abundant in thiamine responders compared with nonresponders both pretreatment ($P = .019$) and post-treatment ($P = .038$). The relative abundances of *Faecalibacterium prausnitzii* and *Roseburia hominis*, both pretreatment and post-treatment, inversely correlated with IBD fatigue score changes for patients with chronic fatigue (PRE; $R = -0.48$, $P = .004$, and $R = -0.40$, $P = .018$; POST; $R = -0.42$, $P = .012$, and $R = -0.40$, $P = .017$) respectively. **CONCLUSION:** *Faecalibacterium prausnitzii* and *Roseburia hominis* may serve as markers for response to high-dose thiamine in alleviating chronic fatigue in patients with qIBD. The mechanistic role of gut bacteria and butyrate in patients with chronic fatigue and qIBD should be further explored.

Keywords: Gut Microbiota; Chronic Fatigue; Crohn's Disease; Colitis; Ulcerative; Thiamine

Introduction

Chronic fatigue is the most common extraintestinal symptom in patients with inflammatory bowel disease (IBD). Patients with fatigue report symptoms such as

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Abbreviations used in this paper: **ANOVA, analysis of variance; ASV, amplicon-sequencing variant; BW, body weight; IBD, inflammatory bowel diseases; qIBD, quiescent inflammatory bowel disease; IBD-FQ1, Inflammatory Bowel Disease-Fatigue Questionnaire section 1; PCA, principal component analysis; PCoA, principal coordinate analysis; PCR, Polymerase chain reaction; PERMANOVA, permutational multivariate analysis of variance; qIBD, quiescent IBD; SCFAs, short-chain fatty acids; TCA, tricarboxylic acid; dim, dimension.**

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2772-5723
https://doi.org/10.1016/j.gastha.2024.08.012

overwhelming exhaustion, reduced energy levels, and a persistent feeling of physical and mental weariness that negatively affects their quality of life.[1] The etiology of chronic fatigue in IBD remains unexplained. Recent studies that investigated factors contributing to chronic fatigue in IBD focused on the gut-brain axis and the associated microbiota.[2,3]

Thiamine (vitamin B1) is essential for all organisms and acts as a cofactor for several enzymes involved in glycolysis and the tricarboxylic acid cycle.[4] Most microorganisms, plants, and fungi synthesize thiamine, while vertebrates cannot produce thiamine. In humans, diet is the main source of thiamine, most being absorbed in the small intestine. The consequence of thiamine deficiency, or its aberrant transport to the mitochondria, is an energy deficit that may lead to organ failure mainly in the central nervous system.[5]

Metagenomic analyses of human gut microbiota predict that thiamine synthesis pathways are present in many gut bacteria,[6] while bacteria unable to produce it possess a vast number of transporters to incorporate it. Thiamine is important for bacterial growth and competition,[7] since it is an essential cofactor of pyruvate:ferredoxin 2-oxidoreductase, part of the metabolic pathway for the production of butyrate and other short-chain fatty acids (SCFAs).[8,9] Bacteria belonging to *Faecalibacterium* spp. are the main butyrate producers in the intestine, and these do not possess the thiamine synthesis pathway. Therefore, these bacteria acquire their thiamine from other bacteria or from the host diet via thiamine transporters.[6]

Butyrate, one of the major SCFAs produced by the gut microbiota, exerts multiple health benefits, including anti-inflammatory properties.[10] Several studies found lower abundances of *Fecalibacterium prausnitzii, Roseburia hominis,* and butyrate in fecal samples from patients with chronic fatigue, either with quiescent IBD (qIBD),[11] or with encephalomyelitis/chronic fatigue syndrome[12,13] when compared to their controls. A decrease in butyrate-producing bacteria, and butyrate, is not restricted to patients with chronic fatigue, but also occurs in patients with active IBD compared with healthy controls.[14]

In an pilot study including 12 patients with IBD and normal blood thiamine levels, high-dose thiamine significantly improved chronic fatigue.[15] Recently, we conducted a randomized, double-blinded, placebo-controlled, crossover study including 40 patients with qIBD experiencing severe chronic fatigue. The study aimed to assess the impact of a 4-week high-dose thiamine supplementation and revealed a notable treatment effect, as determined by the IBD fatigue questionnaire section 1 score (IBD-FQ1).[16]

The present study was conducted to unravel potential pathways connecting gut microbiota to the observed positive impact of high oral thiamine intake on chronic fatigue in patients with qIBD. We analyzed the fecal microbiota and SCFAs concentration in samples collected during the clinical trial. The aim was to investigate the role of the gut microbiota in mediating the effects of high-dose thiamine in reducing chronic fatigue in qIBD and its associated effects on the gut microbiota.

Material and Methods

Study Design and Participants

Patients with qIBD and chronic fatigue were included in a randomized, double-blinded, placebo-controlled, crossover trial conducted between 2018 and 2020 at Aarhus University Hospital, Denmark. The participants had a diagnosis of Crohn's disease or ulcerative colitis for more than 3 months and had disease in remission, documented by fecal calprotectin < 200 mg/kg feces and clinical activity scores within normal range or for patients with a diverting stoma, in their habitual range. The clinical disease activity indexes applied were the Harvey–Bradshaw Activity Index[17] for Crohn's disease and the Simple Clinical Colitis Activity Index for ulcerative colitis.[18] Patients were monitored according to routine clinical standards and did not undergo project-related endoscopies or imaging. Fatigue severity was assessed using the fatigue questionnaire IBD-FQ1; and the study included patients with a IBD-FQ1 > 12 for more than 6 months.[19] As controls, 20 patients without chronic fatigue were included in the study to validate whether the fecal microbiota differed between patients with qIBD with or without chronic fatigue.[11]

Briefly, the trial comprised 3 4-week periods: Treatment period 1 (P1), washout period, and treatment period 2 (P2). Forty patients were randomized 1:1 into group 1 (G1) and group 2 (G2). Both groups received a high-dose oral thiamine (600–1800 mg/d) and placebo. Using a crossover design, patients in G1 received high-dose thiamine during P1 and placebo in P2 while G2 received placebo during P1 and high-dose thiamine during P2. Details of the study participants, trial, and outcomes were previously reported.[16] Thiamine dose was adjusted for gender and body weight (BW): 1) females: BW $<$ 60 kg: 600 mg, BW 60–70 kg: 900 mg, BW 71–80 kg: 1200 mg, and BW $>$ 80 kg: 1500 mg and 2) males: BW $<$ 60 kg: 900 mg, BW 60–70 kg: 1200 mg, BW 71–80 kg: 1500 mg, and BW $>$ 80 kg: 1800 mg.

Fecal samples were collected at baseline, week 4, 8, and 12. The fecal samples from patients with qIBD and no fatigue were included once for comparison (Figure 1). Fecal samples were collected by the patients at home and stored at -20 °C. At the following scheduled visit, samples were brought to the hospital and thereafter stored at -80 °C until analysis.

SCFAs Extraction and Analysis

SCFAs were extracted from 100 to 150 mg fecal material. Samples was weighed in a 1.5-ml Eppendorf tube and 4x wt/vol MilliQ® water was added to each sample. Fecal samples and extracts were kept on ice or at 4 °C throughout sample preparation. Samples were homogenized at 30 cycles/s for 1 min using a bead beater MM300 (Retsch, VWR, Haan, Germany). Samples were centrifuged at 15.000 g/4 °C/10 min, and then the supernatant was transferred into a labeled SpinX (Costar) centrifuge filter. Samples were filtered by centrifugation at 15.000 g/4 °C/5 min. The extracted fecal water was stored at -80 °C until analysis. Sample analysis was carried out by MS-Omics (Vedbæk, Denmark) as follows. Samples were

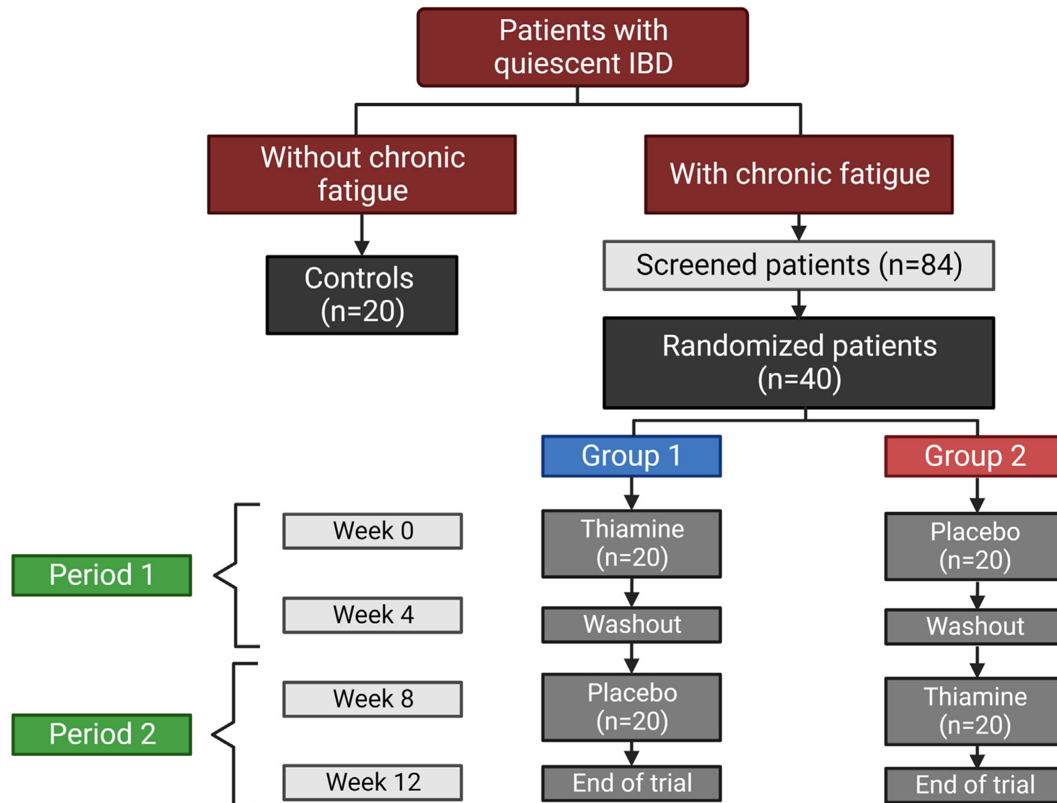

Figure 1. Study overview. Fecal samples were collected at week 0, 4, 8, and 12 from 40 patients with qIBD and chronic fatigue who received high-dose oral thiamine (600–1800 mg/d) or oral placebo for a 4-weeks period in a crossover trial. Fecal samples were collected at week 0 from 20 patients with qIBD and without chronic fatigue.

acidified using hydrochloride acid and deuterium-labeled internal standards were added. All samples were analyzed in a randomized order. Analysis was performed using a high-polarity column (Zebron™ ZB-FFAP, GC Cap. Column 30 m × 0.25 mm x 0.25 μm) installed in a gas chromatograph (7890B, Agilent) coupled with a time of flight mass spectrometry (Pegasus® BT, LECO). The system was controlled by ChromaTOF® (LECO). Raw data were converted to netCDF format using Chemstation (Agilent), before the data were imported and processed in Matlab R2021b (Mathworks, Inc.) using the PARADISe software.[20]

DNA Extraction

DNA was extracted from approximately 200 mg feces using the DNeasy® PowerLyzer® PowerSoil® DNA isolation kit (Qiagen, Germany) according to the manufacturer's instructions. Mechanical lysis of bacteria was conducted at 30 cycles/s for 10 min on bead beater MM300 (Retsch, VWR, Haan, Germany). DNA was eluted in 80 μL elution buffer. DNA concentrations were measured by the Qubit dsDNA BR assay kit (Life Technologies, Carlsbad, CA, United States). DNA samples were diluted to 5ng/μl and stored at −20 °C.

16S rRNA Gene Amplicon Sequencing

The V3-region of the 16S rRNA gene, hereafter referred to as "16S", was sequenced using the IonS5 Torrent platform (Life Technologies) as previously described.[21] In summary, 16S was amplified using a universal forward primer (PBU 5′-A- adapter-TCAG-barcode-CCTACGGGAGGCAGCAG-3′) with a unique 10–12 bp barcode for each sample (Ion Xpress barcode as suggested by Life Technologies) and a universal reverse primer (PBR 5′-trP1-adapter-ATTACCGCGGCTGCTGG-3′). For the polymerase chain reaction (PCR) reactions, Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, United States) was used following manufactured protocol, adding 5 ng/μL template in a total reaction volume of 20 μL. The reaction conditions consisted of an initial denaturation step (30s/98 °C), then 24 cycles of denaturation (15s/98 °C) and annealing (30s/72 °C), followed by extension (5 min/72 °C), and finally cooling to 4 °C. PCR products were purified by HighPrep™ PCR Clean-up System (Magbio, Gaithersburg, MD, United States) according to the manufacturer's protocol, and DNA concentrations were determined with Qubit dsDNA HS assay kit. Finally, a library with equimolar concentration of amplicons from each sample was sequenced using the Ion 520/530™ Kit 520-chip for Ion Torrent sequencing on the Ion OneTouch™ 2/Ion S5™ sequencing system.

Bioinformatics Analysis

16S sequencing data were processed via our in-house pipeline.[22] Briefly, raw FASTQ files were demultiplexed and trimmed using cutadapt (v. 4.1),[23] denoised using DADA2 (v. 1.22) and amplicon-sequencing variant (ASV) table constructed, which contains the counts of each sequence variant in each sample.[24] ASVs were classified against rdp_train_set_18.[25]

Further processing was done using Phyloseq (v.1.42.0)[26] running in R (v. 4.2).[27] ASVs were decontaminated using decontam,[28] with the minimum probability for frequency and prevalence based approach for each sequencing run and combined using the Fisher's method.

Statistical Analysis

We compared alpha diversity (Observed richness, Shannon diversity index) using Wilcoxon test or 2-way analysis of variance (ANOVA) as appropriate. Permutational multivariate analysis of variance (PERMANOVA) was used to compare beta diversity differences (weighed UniFrac distances) between groups.[29] Principal component analysis was used to analyze the fecal concentration of SCFAs and t-test to assess significant differences between clusters. The Wilcoxon test or Kruskal–Wallis was used to compare individual concentrations of SCFAs. Differential abundance analyses were performed at all taxonomic levels after removing rare taxa (present in less than 10 samples and/or with fewer than 30 reads). We used unpaired Wilcoxon test followed by P value correction using the Benjamini–Hochberg step-up method with a false discovery rate of 0.05.[30] Nonparametric Spearman correlations were calculated for specific variables. For all statistical analyses, significance was set at $P < .05$.[31] All analysis were performed in R (v.4.2)[27] (Supplementary document TARIF_complete_analysis.html).

Results

Participants

Forty patients with qIBD and chronic fatigue were included in this placebo-controlled crossover study. An additional 20 patients with qIBD without chronic fatigue were included at baseline. Patient characteristics are summarized in Table 1. Feces samples were obtained related to

Table 1. Baseline Characteristics of Patients With IBD With (n = 40) or Without (n = 20) Chronic Fatigue

Variable	Patients with chronic fatigue (n = 40)	Patients without chronic fatigue (n = 20)
Age, y, mean (SD)	37 (12)	33 (10)
Sex, male (%)	5 (12%)	3 (15%)
Disease		
Ulcerative colitis, n (%)	20 (50%)	10 (50%)
SCCAI score, median (IQR)	3 (2–6)	2 (1–2)
F-calprotectin, mg/kg, median (IQR)	22 (13–58)	42 (35–48)
Montreal classification of disease extent		
E1 (ulcerative proctitis)	2	2
E2 (left-sided UC/distal UC)	2	3
E3 (extensiv UC/pancolitis)	15	3
Not applicable	1	2
Crohn's disease, n (%)	20 (50%)	10 (50%)
HBAI score[a], median (IQR)	3 (2–6)	3 (2–3)
F-calprotectin, mg/kg, median (IQR)	41 (11–109)	40 (33–41)
Montreal classification		
Location		
L1 (only ileal disease)	2	2
L2 (only colonic disease)	4	1
L3 (ileocolonic disease)	14	7
L4 (upper gastrointestinal tract disease)	0	0
Behavior		
B1 (nonstricturing, nonpenetrating)	14	8
B2 (stricturing)	5	1
B3 (penetrating)	1	1
Comorbidity		
Stoma	3	1
Osteoporosis	11	5
Rheumatoid arthritis	5	0
Depression (mild)	3	0
Medication		
5-Aminosalicylates	14	2
Azathioprine/6-mercaptopurine	17	11
Tumor necrosis factor alpha inhibitors	21	11
Vedolizumab	6	4

HBAI, Harvey–Bradshaw Activity Index; IQR, interquartile range; SCCAI, Simple Clinical Colitis Activity Index; SD, standard deviation; UC, ulcerative colitis.
[a]HBAI, score data do not include 4 patients with Crohn's disease who had a diverting stoma where stool frequency could not be quantified. All were evaluated as in clinical remission, based on low fecal calprotectin levels (6, 14, 36, 54 mg/kg feces, respectively).

Table 2. Number of Fecal Samples Collected, Successfully Microbiota and Metabolite Analyzed From 40 Patients (Fatigue-G1 and Fatigue-G2) With qIBD and Chronic Fatigue (Randomized and Treated in a Crossover Trial With Peroral High-Dose Thiamine and Placebo) and From 20 Patients (Nonfatigue) With qIBD Without Chronic Fatigue

	Collected				Microbiota				Metabolite			
	Samples				Samples				Samples			
Timepoint (wk)	T-0	T-4	T-8	T-12	T-0	T-4	T-8	T-12	T-0	T-4	T-8	T-12
Fatigue-G1[a]	19	20	20	20	18	18	17	20	19	20	20	20
Fatigue-G2[b]	19	18	20	20	19	17	19	19	19	18	20	19
Nonfatigue	17	-	-	-	17	-	-	-	17	-	-	-

[a]G1: group 1 (20 patients): 4 wk thiamine—4, wk washout—4 wk placebo.
[b]G2: group 2 (20 patients): 4 wk placebo—4, wk washout—4 wk thiamine.

the 2 treatment periods (week 0–4 and week 8–12) and a washout period (week 4–8), as outlined in Table 2).[16]

Chronic Fatigue was Not Associated with Gut Microbiota Composition

Comparing microbiota in fecal samples from patients with qIBD with or without chronic fatigue, we observed no statistically significant differences in alpha diversity, neither for observed richness nor Shannon diversity index (Wilcoxon test, $P = .44$, $P = .15$, respectively, Figure 2A and B). For beta diversity, weighted UniFrac distances showed no differential clustering of the fecal microbiota from patients with chronic fatigue compared to patients without chronic fatigue (PERMANOVA, R = 0.020, P = .385, Figure 2C).

Chronic Fatigue Does Not Affect SCFAs Concentration

The total fecal SCFAs concentration did not differ significantly between patients with or without chronic fatigue (t-test, Dim 1, P = .521, Figure 3). Comparing each SCFA between the patients with or without chronic fatigue revealed no significant differences (Supplementary material section 4.1.4.2.3).

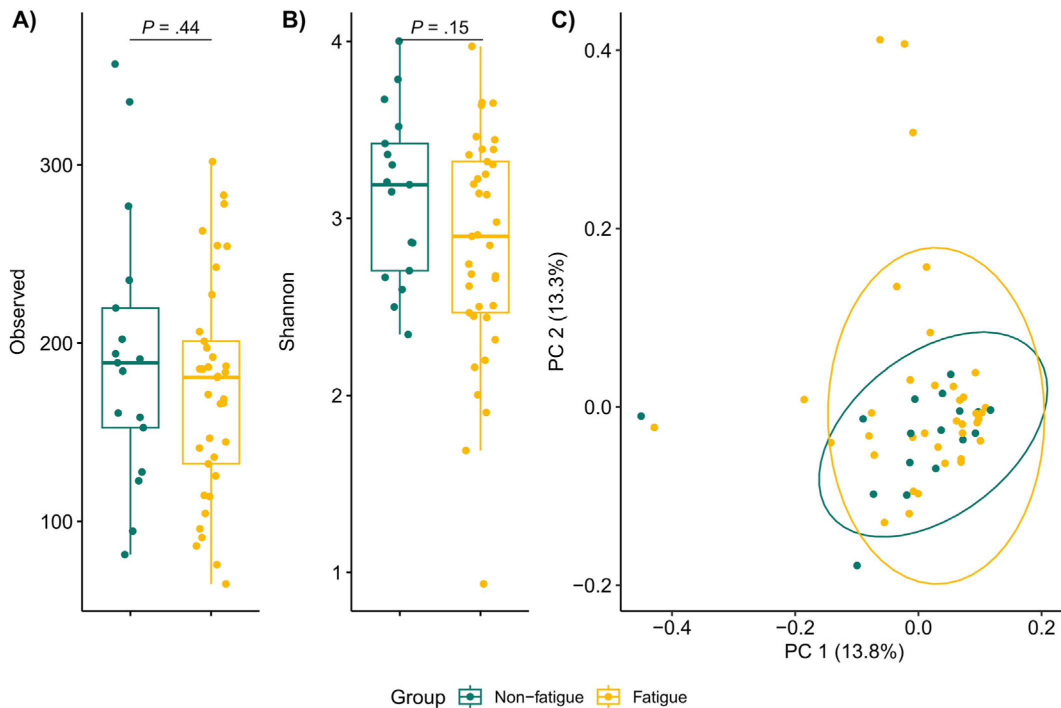

Figure 2. Box plots for alpha diversity indices A) Observed richness, and B) Shannon diversity index of patients with qIBD with chronic fatigue (Fatigue) and without chronic fatigue (Nonfatigue). No significant differences were observed between the groups (Wilcoxon test). C) PCoA plot of beta diversity between fecal samples using Weighted UniFrac distances. PCoA, principal coordinate analysis.

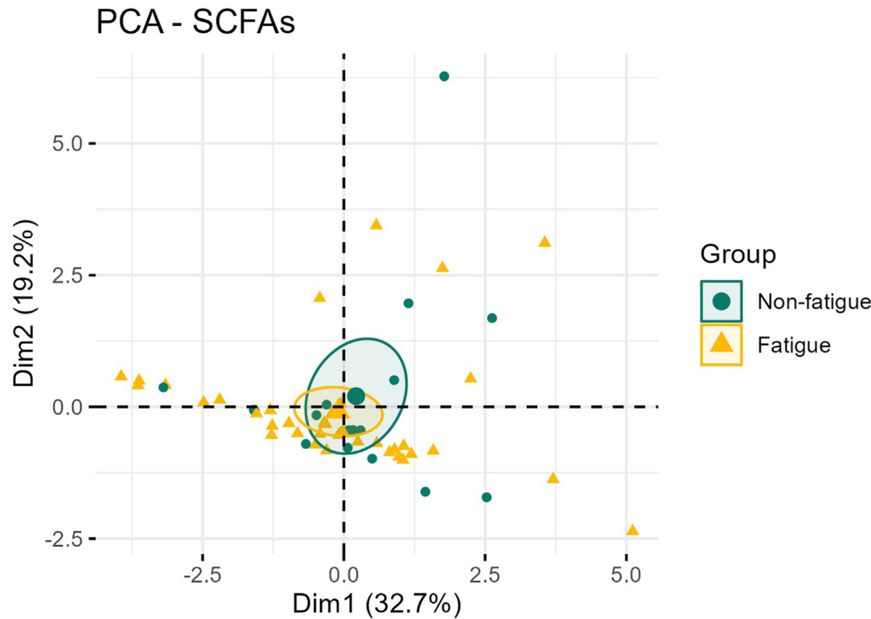

Figure 3. PCA plot of SCFAs indicates no significant differences between non-fatigued vs chronic fatigued patients with qIBD. PCA, principal component analysis.

Thiamine Treatment did Not Affect Fecal Microbiota, nor SCFAs, at Any Time Point

We compared fecal microbiota from patients after high-dose thiamine or placebo for all time points. Two-way ANOVA test showed no statistically significant differences in alpha diversity between the groups G1 vs G2 (Shannon $P = .752$, observed richness $P = .448$) or between time points (Shannon $P = .574$, observed richness $P = .849$). Significant differences between time points were seen including all groups for observed richness ($P = .043$)

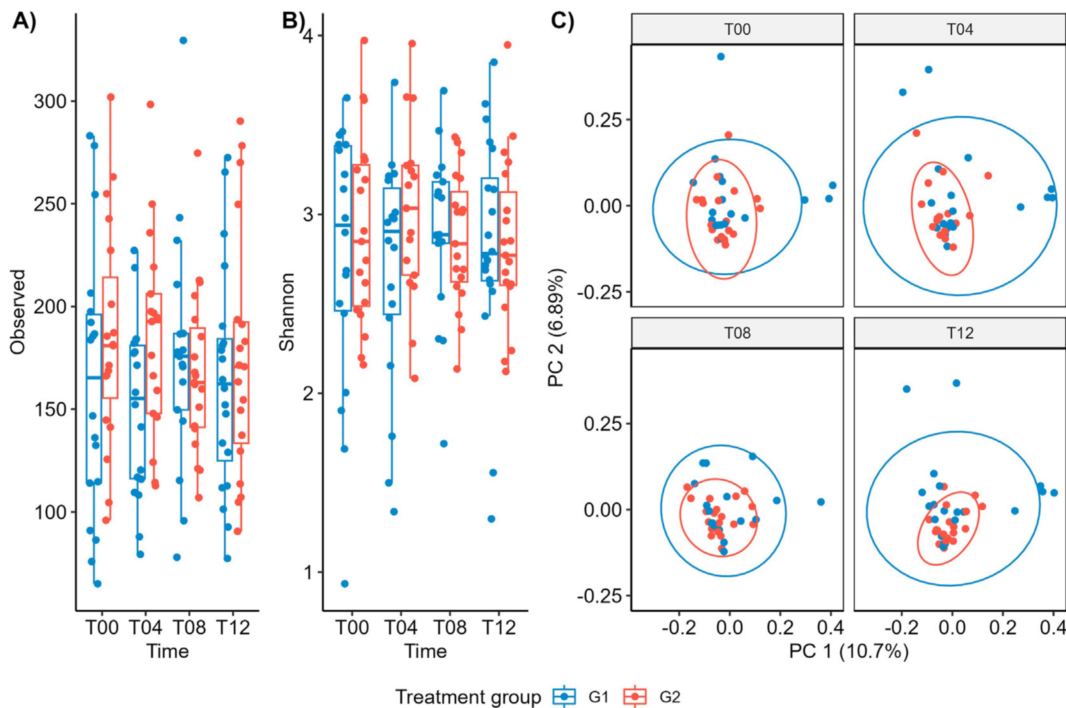

Figure 4. Box plots for alpha diversity indices A) Observed richness, B) Shannon diversity index of patients with qIBD and chronic fatigue who received high-dose oral thiamine (600–1800 mg/d) or oral placebo for 4 weeks in a crossover trial. Two-way ANOVA was used to compare both thiamine and placebo groups at the different time points, additional t-test was used to compare between groups at the same time point. *P* values were adjusted for false discovery rate. C) PCoA plot of beta diversity between fecal samples using Weighted UniFrac distances indicate no differential clustering between groups at any time point. PCoA, Principal coordinate analysis.

indicated overall effect of time. Further pairwise t-test comparisons, adjusted for multiple testing, did not identify significant differences between time points for each group (Figure 4A and B).

A PERMANOVA test identified significant beta diversity differences between treated groups (G1 and G2, $R^2 = 0.035$, $P = .001$), but this difference could be driven by the significantly greater dispersion of the group G1 at all time points ($P < .001$). No differences were observed when the tested variable was time; (P1 vs P2, $R^2 = 0.008$, $P = .998$), or treated groups over time; (G1 vs G2, $R^2 = 0.006$, $P = .999$, Figure 4C).

SCFAs concentrations in fecal samples showed no statistically significant differences at any time point between the treatment groups, nor between time points within each group (Supplementary material section 4.2.4.2.3).

Effect of Thiamine on Chronic Fatigue Correlates with the Relative Abundances of Faecalibacterium prausnitzii and Roseburia hominis

No significant differences were observed at any taxa level when comparing nonfatigue vs patients with chronic fatigue, or the different treated groups (G1 vs G2) at P1 and P2 (Supplementary material, section 5). To investigate if there was any correlation between the microbiota and improvement in IBD-FQ1 during the period they received thiamine, we classified individuals who decreased 3 or more points as responders (n = 17) and individuals who decreased less as nonresponders (n = 18).

There were no significant differences in SCFA or fecal microbiota beta diversity between responders and nonresponders pretreatment or post-treatment (Supplementary material Sections 4.3.4 and 4.3.5).

Analysis of the relative abundance of specific species associated with chronic fatigue (*Faecalibacterium prausnitzii*, *Ruminococcus* species and *Roseburia hominis*) revealed that *Faecalibacterium prausnitzii* was significantly more abundant in responders, both pretreatment ($P = .019$) and post-treatment ($P = .038$) (Figure 5). Consistently, the relative abundance of *Faecalibacterium prausnitzii* was negatively correlated to IBD-FQ1 both pretreatment (R = −0.48, $P = .004$ and post-treatment (R = −0.42, $P = .012$). This was also observed for *Roseburia hominis* pretreatment (R = −0.4, $P = .018$) and post-treatment (R = −0.4, $P = .017$). Interestingly, *Faecalibacterium prausnitzii* abundance was not significantly different for the patients that improved their fatigue symptoms when given placebo (Wilcoxon test, $P = .926$, $P = .714$).

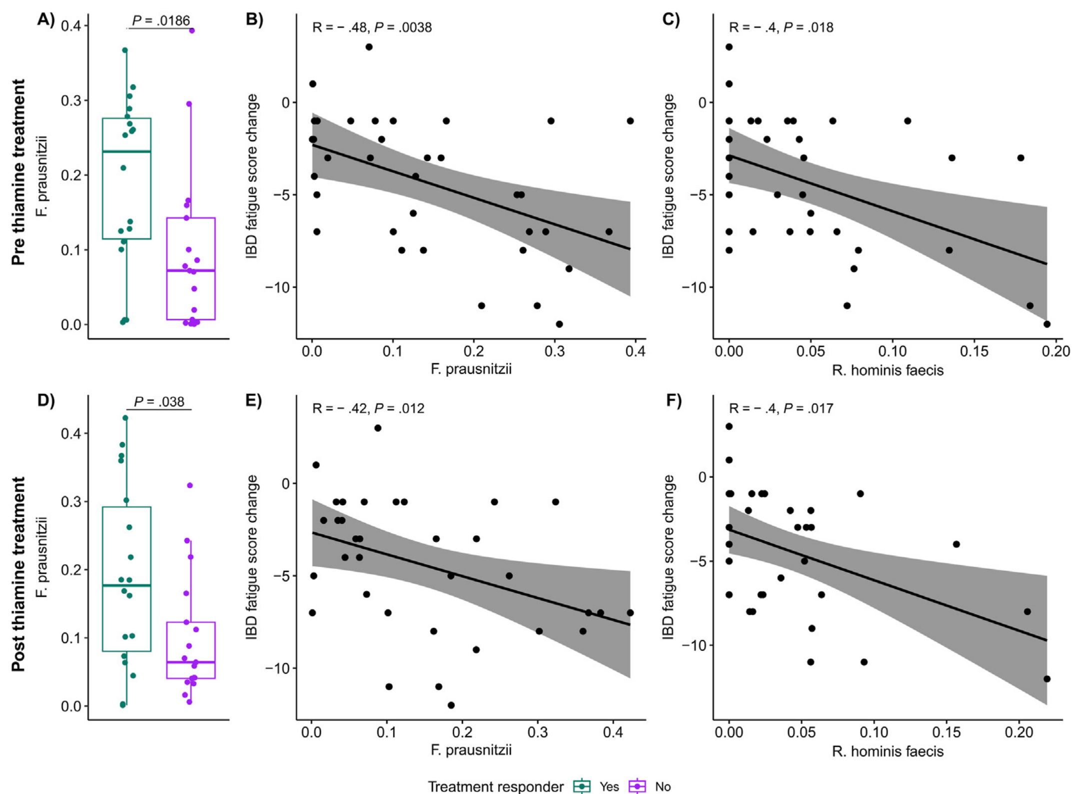

Figure 5. Box plots of relative abundance for *Fecalibacterium prausnitzii* in feces of patients with qIBD and chronic fatigue grouped as treatment responders (IBD-FQ1 score ≥3) and nonresponders, before (A) and after thiamine treatment (D, Wilcoxon test). B and C: scatter plots, with linear regression and Spearman correlation statistics, of the IBD-FQ1 change vs relative abundance of *Fecalibacterium prausnitzii* and *Roseburia hominis faecis* before thiamine treatment—with E and F after thiamine treatment.

Discussion

In this exploratory study of feces samples from a randomized clinical trial, we found comparable microbiota patterns in patients with qIBD with or without chronic fatigue. We identified a correlation between high abundance of *Faecalibacterium prausnitzii* or *Roseburia hominis* and the effectiveness of thiamine treatment on reducing fatigue in patients with qIBD and chronic fatigue. Thiamine treatment did not affect the relative abundance of *Faecalibacterium prausnitzii* or *Roseburia hominis.* Rather, *Faecalibacterium prausnitzii* relative abundance, before and after thiamine treatment, was significantly greater in those patients whose IBD-FQ1 decreased $\geq$3 points after the treatment. In addition, we observed that the relative abundance of *Faecalibacterium prausnitzii* and *Roseburia hominis* negatively correlated with the precise IBD-FQ1 change.

Faecalibacterium prausnitzii is one of the most abundant bacteria in healthy adults and has an average relative abundance of approximately 5%,[32] 6.5% for the *Faecalibacterium* genus, with 92% prevalence in adults, decreasing to 78% in the elderly population.[33] Generally, *Faecalibacterium prausnitzii* is consistently less abundant in patients with intestinal disorders compared to healthy controls,[34] being considered for some researchers, as biomarker of good intestinal health.[35,36] Its relative abundance has been negatively correlated with the severity of IBD,[14] and general fatigue in patients with myalgic encephalomyelitis/chronic fatigue.[12]

Defining the gut microbiota profile in IBD poses a challenge due to the substantial interindividual variation in bacterial composition. Despite this numerous studies have consistently shown an imbalance within the gut microbiota among patients with IBD.[37] Borren et al. described a “chronic fatigued microbiota profile” in patients with qIBD and chronic fatigue, characterized by lower diversity of the gut microbiota, and lower levels of the butyrate-producing bacteria *Fecalibacterium prausnitzii* and *Roseburia hominis* in fecal samples of patients with qIBD with chronic fatigue, compared to those patients without symptoms of chronic fatigue.[11] In the present study, we found no evidence for a “chronic fatigued microbiota profile”. We did not find differences in alpha or beta diversity between patients with or without chronic fatigue and found that no individual bacteria differed in abundance between the groups.

Borren et al. also found a depleted pathway of butyrate synthesis in patients with chronic fatigue. Since bacterial butyrate synthesis depends on thiamine as a cofactor for the enzyme pyruvate:ferredoxin 2-oxidoreductase,[9] we hypothesized that supplementation with this vitamin could enhance microbial butyrate production. Importantly, we did not see an increase of butyrate-producing bacteria. Thiamine supplementation could have increased the *Faecalibacterium prausnitzii* butyrate production, without increasing its abundance, but fecal metabolite analysis revealed no increase in butyrate concentration following thiamine treatment. One reservation to this finding is that since SCFAs are utilized by the colonocytes, the levels measured in feces may not give the true picture of the microbial production.

In mammals, thiamine is necessary for energy metabolism and to maintain nerve cell function. Supplementation has been studied for different fatigue-related conditions, such as exercise-induced fatigue.[38] Thiamine was also reported to improve symptoms of reduce chronic fatigue related to mitochondrial myopathy in 1 patient.[39] Thiamine turnover is generally incompletely understood in the gut environment, and there is still much to learn about its acquisition by microbes.[40] Overall, this study suggests that thiamine’s mechanism of action in chronic fatigue does not involve the microbiota. While a larger relative abundance of *Faecalibacterium prausnitzii* predicted a positive response to thiamine treatment, we remark that improved fatigue levels were not associated with changes in abundance of *Fecalibacterium prausnitzii.* In addition, no significant differences were found in alpha or beta diversity between responders and nonresponders. The mechanism for the beneficial effect of thiamine supplementation remains unknown, but potential pathways for the effect of thiamine supplementation have been hypothesized, including facilitation of aerobic cellular respiration and promotion of lactate clearance.[41]

In conclusion, we found no differences in gut microbiota between patients with qIBD with or without chronic fatigue. The structure and stability of the fecal microbiota were not affected by thiamine or associated with improvement of fatigue symptoms. Importantly, the correlation between change in IBD-FQ1 and relative abundance of *Faecalibacterium prausnitzii* or *Roseburia hominis* indicated that *Faecalibactium prausnitzii* or *Roseburia hominis* might serve as predictors of thiamine treatment effect for chronic fatigue likely because they are markers of a better general gut health in patients who respond to high-dose thiamine. Validation studies conducted in large cohorts are essential to apply these insights in clinical settings.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2024.08.012.

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Received March 1, 2024. Accepted August 16, 2024.

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Acknowledgments:
The authors wish to thank the patients who participated in the study, both those included in the randomized clinical trials and those providing control samples for the present study.

Authors' Contributions:
Sandra Bermúdez-Sánchez: generation, assembly, analysis, and interpretation of the data, writing – original draft. Palle Bager: Study conceptualization and design and generation, collection, assembly, and interpretation of data. Jens Frederik Dahlerup: Study conceptualization and design and generation, analysis, and interpretation of data. Simon Mark Dahl Baunwall: Study conceptualization and design and generation, analysis, and interpretation of data. Tine Rask Licht: Study conceptualization and design and interpretation of data. Martin Steen Mortensen: Study supervision and analysis and interpretation of data. Christian Lodberg Hvas: Conceptualization, design, and supervision of the study and as well as generation, collection, and interpretation of data.

Conflicts of Interest:
The authors disclose no conflicts.

Funding:
Innovation Fund Denmark (grant no. 8056-00006B). CL Hvas received funding from Novo Nordisk Foundation (grant no. NNF22OC0074080).

Ethical statement:
The protocol and consent forms were approved by the Central Denmark Research Ethics Committee (j.no. 64207) and the Danish Medical Agency (EudraCT j.no. 2018-002324-17). The trial was conducted in accordance with the Declaration of Helsinki and adhered to the principles for good clinical practice and was monitored by the good clinical practice unit at Aarhus and Aalborg University Hospitals. The trial was registered in the ClinicalTrials.gov, study identifier NCT03634735, before initiation of patient enrollment.

Data transparency statement:
All requests regarding clinical data or clinical analyses may be addressed to the corresponding author. Data access may be granted if scientifically justified and to the extent that is allowed according to person data protection regulation and patient consent. The raw sequencing files have been made available at NCBI (https://www.ncbi.nlm.nih.gov/sra/PRJNA1036806). Supplementary file 1 contains the complete bioinformatics analysis with all steps and all code necessary to reproduce this study together with the results so that it can be read without running the analysis.

Reporting guidelines:
None.