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Possible Role of Butyric Acid in Long-Term Symptom Relief in Irritable Bowel Syndrome Patients Following Fecal Microbiota Transplantation

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ABSTRACT

Background: We previously found that the fecal levels of short-chain fatty acids (SCFAs) changed in irritable bowel syndrome (IBS) patients at 1 month and 1 year after fecal microbiota transplantation (FMT). This study analyzed SCFAs at 2 and 3 years after FMT in the same IBS patients included in those previous studies.

Methods: This study randomized 113 IBS patients into placebo, 30-g, and 60-g groups, who received FMT with 30 g of their own feces and with 30 g and 60 g of the donor's feces, respectively. The patients completed four questionnaires to assess IBS symptoms, fatigue, and quality of life, and supplied fecal samples at the baseline and at 2 and 3 years after FMT. The fecal SCFA levels were measured using gas chromatography.

Results: The butyric acid level was significantly increased at 2 and 3 years after FMT in the 30-g and 60-g groups, and was significantly higher than that in the placebo group. The total SCFA and acetic acid levels decreased significantly in the 30-g and 60-g groups at 2 and 3 years after FMT, while the propionic acid level decreased in the 60-g group at both time points. The butyric acid level was inversely correlated with IBS symptoms and fatigue.

Conclusion and Inferences: The increased butyric acid levels in IBS patients at 2 and 3 years after FMT and their inverse correlation with both IBS symptoms and fatigue suggest that butyric acid contributes to the long-term improvement seen after FMT (www.clinicaltrials.gov: NCT03822299).

1 | Introduction

Short-chain fatty acids (SCFAs) are produced by the bacterial fermentation of undigested and unabsorbed carbohydrates and fiber in the human intestine [1, 2]. These SCFAs are absorbed by the colonic epithelial cells via both passive and active transport [2]. The SCFAs that remain unconsumed by colonic epithelial cells are transported through the basolateral membrane to the circulating blood [2]. Acetic, propionic, and butyric acids are the main intestinal SCFAs, and they exist in a ratio of 60:20:20 [2]. SCFAs are an energy source for colonic epithelial cells, and they regulate gut barrier functions, immune defenses, intestinal motility, and visceral senstivity [3–7]. SCFAs also affect the metabolism of glucose, lipid, and cholesterol in several tissues of the body [2].

There are some reports of the fecal SCFA levels in patients with irritable bowel syndrome (IBS) differing from those in healthy

Summary

- Fecal butyric acid level increased in IBS patients who received the donor's feces at 2 and 3 years after FMT, and it was higher than that in patients in the placebo group who received their own feces.
- The fecal butyric acid level was inversely correlated with the total score for IBS symptoms and with the scores for abdominal pain, abdominal distention, diarrhea, and constipation. These findings suggest that butyric acid plays a significant role in the long-term improvement in IBS symptoms following FMT.

subjects, with the fecal propionic acid level being markedly higher in IBS patients [3]. However, other studies have found no differences in the SCFA levels between IBS patients and healthy subjects [8, 9]. A recently published systematic review and meta-analysis found that the fecal propionic level was higher in IBS patients than in healthy controls [10].

Previous randomized double-blind placebo-controlled studies performed by our group found that fecal microbiota transplantation (FMT) reduced IBS symptoms and fatigue, and improved the quality of life in patients with IBS for up to 3 years after FMT [11–13]. These improvements were accompanied by marked changes in the fecal SCFA levels at 1 month and 1 year after FMT [13, 14]. The present study investigated whether the improvements in symptoms and quality of life of IBS patients at 2 and 3 years after FMT are associated with changes in fecal SCFAs in the same cohort of patients who were included in our previous studies [11–13].

2 | Materials and Methods

2.1 | Study Design

The design of this study has been described in detail for our previous clinical trial [11]. In brief, patients provided a fecal sample and completed four questionnaires to assess IBS symptoms, fatigue, and quality of life at the baseline, and then subsequently provided further fecal samples and completed new sets of questionnaires at 2 and 3 years after FMT. The rescue medications allowed in the study were polyethylene glycol and loperamide. The patients were randomized at 1:1:1 into the following three groups: placebo group (FMT with 30 g of their own feces), 30-g group (FMT with 30 g of the donor's feces), and 60-g group (FMT with 60 g of the donor's feces) [11].

2.2 | Patients

The study included 113 of the 164 IBS patients who participated in our previous clinical trial [11], and 37, 37, and 39 of them were allocated to the placebo, 30-g, and 60-g groups, respectively. The characteristics of these patients are given in Table 1. The patient enrollment method has been described in detail previously [11]. In brief, patients were recruited from those attending the outpatient clinic at Stord Hospital. The inclusion criteria were fulfilling the Rome IV criteria for a diagnosis of IBS, age > 18 years, and having moderate-to-severe IBS symptoms based on a total score on the IBS Severity Scoring System (IBS-SSS) of ≥ 175. The exclusion criteria were having a systemic disease or immune deficiency, being pregnant, planning pregnancy or lactating, having alcohol or drug abuse, suffering from a severe psychiatric disorder, taking

TABLE 1 | Characteristics of the included patients at the baseline.

	Overall	Placebo group	30-g group	60-g group	*p*
Number	113	37	37	39	
Age, years	40.6 ± 13.5	41.8 ± 14.5	39.7 ± 12.4	40.5 ± 13.6	0.9
Sex, female/male	94/19	33/4	29/8	32/7	0.5
IBS-D	43	12	15	16	
IBS-C	43	15	14	14	0.9
IBS-M	27	10	8	9	
IBS duration, years	15.5 ± 7.9	14.5 ± 8.0	17.2 ± 9.3	14.7 ± 5.7	0.3
PPI medication	39 (34.5)	14 (37.8)	12 (32.4)	13 (33.3)	0.9
Birth-control medication	62 (54.9)	17 (45.9)	23 (62.2)	22 (56.4)	0.4
Antimigraine medication	9 (8.0)	2 (5.4)	4 (10.8)	3 (7.7)	0.8
Medication against asthma/allergies	14 (12.4)	4 (10.8)	6 (16.2)	4 (10.3)	0.8
Medication with levothyroxine	3 (2.7)	1 (2.7)	0 (0)	2 (5.1)	0.4
Medication with heart/vascular drugs	6 (5.3)	3 (8.1)	2 (5.4)	1 (2.6)	0.6

Note: Data are mean ± SD, *n* (%), or *n* values. Patients in the placebo, 30-g, and 60-g groups received FMT with 30 g of their own feces and with 30 g and 60 g of the donor's feces, respectively.
Abbreviations: FMT, fecal microbiota transplantation; IBS-C, constipation-predominant IBS-C; IBS-D, diarrhea-predominant IBS; IBS-M, mixed-diarrhea-and-constipation IBS; PPI, proton-pump inhibitor.

antibiotics or probiotics, or taking IBS medications within 8 weeks prior to study inclusion. The patients were advised to return to a normal Norwegian diet as soon as possible, and to continue with their baseline medication.

2.3 | Donor

The single male donor used in this study has been described in detail previously [11]. In brief, he was screened according to the European guidelines for FMT donors [15]. He was a healthy non-smoking 36-year-old Caucasian male born via a vaginal delivery, breastfed, and not taking any medication regularly. He had a normal BMI, exercised regularly, and took sport-specific dietary supplements. Examinations of his feces before and regularly during the previous clinical trial revealed that he was normobiotic.

2.4 | Collection, Preparation, and Administration of Fecal Samples

Fecal samples were frozen immediately and kept at −20°C until they were delivered frozen to the laboratory, where they were kept at −80°C. The FMT protocol has been described in detail previously [11]. In brief, patients who were randomized to the placebo group received 30 g of their own feces, while those in the 30-g and 60-g groups received 30 g and 60 g of the donor's feces, respectively. The fecal samples were thawed for 2 days at 4°C, mixed with 40 mL of sterile saline, and filtered. Each fecal sample was administered by a gastroscope to the distal duodenum.

2.5 | Assessments of IBS Symptoms, Fatigue, and Quality of Life

IBS symptoms were assessed using the IBS-SSS questionnaire and the Birmingham IBS Symptom Questionnaire, while fatigue was measured using the Fatigue Assessment Scale (FAS) questionnaire [16–18]. The quality of life was measured using the IBS Quality of Life (IBS-QoL) questionnaire [19]. Patients who showed a decrease of ≥ 50 points in the IBS-SSS total score after FMT were considered responders.

2.6 | Determination of Fecal SCFA Levels

The fecal samples were weighed and 0.5 g of each sample was homogenized with 2.5 mL of a solution containing 3 mmol/L 2-ethylbutyric acid and 0.5 mmol/L H_2SO_4. Each sample was vacuum distilled, and the total SCFA level and the levels of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocapronic, and capronic acids were measured using gas chromatography (Agilent 7890 A, Agilent, CA, USA) [20, 21]. SCFAs were analyzed in the donor's fecal samples obtained at the baseline and at 3, 6, 12, 18, 24, and 36 months after FMT.

2.7 | Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics West, Bergen, Norway (approval no. 2017/1197/REK vest). Patients provided both oral and written consents to participate. The study was registered at www.clinicaltrials.gov (NCT03822299).

2.8 | Statistical Analyses

The sample size required in each arm of the study was 37 patients, as calculated by assuming that a placebo effect was 40% and an effect response was 80% ($\alpha = 0.05$, $\beta = 0.2$). Differences between the placebo, 30-g, and 60-g groups in sex, IBS subtypes, and medications were analyzed using Fisher's exact test. Differences in scores on the IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, and IBS-QoL and in SCFA levels were analyzed using the nonparametric Kruskal-Wallis multiple-comparisons test, Dunn's Post Hoc Tests with Bonferroni adjustment. *p*-values across all tests were adjusted using the Benjamini-Hochberg (BH) method to control the FDR. To quantify the effect size, Cohen's *d* test was used. Differences in the SCFA levels between females and males as well as between responders and nonresponders were assessed using the nonparametric Mann–Whitney test. The correlations between the changes in SCFA levels and in the scores on the IBS-SSS, Birmingham IBS Symptom Questionnaire, and FAS were analyzed using the nonparametric Spearman test, and *p*-values were adjusted for multiple correlations using Bonferroni correction. Since measurements are taken of 3 groups (placebo, 30-g, and 60-g groups) repeatedly at different intervals (baseline, 2 and 3 years) and considering the skewness of the data, we used a generalized linear mixed model (GLMM) and linear mixed-effects models (LMMs).

All of the authors had access to the study data and reviewed and approved the final version of the manuscript.

3 | Results

3.1 | Assessments of IBS Symptoms, Fatigue, and Quality of Life

The total scores and the subitem scores on the IBS-SSS and Birmingham IBS Symptom Questionnaire were reduced significantly at 2 and 3 years after FMT in both 30-g, and 60-g groups, but not in the placebo group (Tables S1 and S2). FMT had a large-moderate effect size on IBS-SSS total scores at 2 and 3 years in both the 30-g and 60-g groups (Cohen's $d = 1.1$, 0.6, 0.8, and 1.0, respectively). The corresponding values for Birmingham total scores showed also a large-moderate effect size (Cohen's $d = 0.7$, 0.7, 0.8, and 1.0, respectively).

The FAS total score was reduced while that on the IBS-QoL was significantly increased at 2 and 3 years after FMT (Tables S3 and S4). FMT had a moderate effect size on FAS total scores at 2 and 3 years after FMT in the 30-g group (Cohen's $d = 0.5$, and 0.5, respectively), and in the 60-g group (Cohen's $d = 0.6$, and 0.7, respectively). FMT had a moderate effect size on IBS-QoL total scores at 2 and 3 years in the 30-g group (Cohen's $d = -0.6$, and −0.7, respectively), and large effect size in the 60-g group (Cohen's $d = -^{1}$ and −0.9, respectively).

GLMMs and LMMs showed a significant decrease in IBS-SSS scores, Birmingham total scores, and FAS scores, and an increase

TABLE 2 | Fecal short-chain fatty acid (SCFA) levels in the placebo, 30-g, and 60-g groups at the baseline and at 2 and 3 years after FMT.

	Placebo group				30-g group				60-g group			
SCFAs	**Baseline**	**2 years after FMT**	**3 years after FMT**	***p*[a]**	**Baseline**	**2 years after FMT**	**3 years after FMT**	***p*[a]**	**Baseline**	**2 years after FMT**	**3 years after FMT**	***p*[a]**
Total SCFAs	72.7 ± 36.7	60.4 ± 23.4	61.8 ± 19.3	0.291	76.6 ± 39.6	58.1 ± 24.2	59.2 ± 24.2	**0.031**	80.8 ± 44.4	49.2 ± 22.1	53.8 ± 20.8	**0.0001**
Acetic acid	40.3 ± 15.1	36.3 ± 14.3	32.6 ± 11.6	0.060	44.3 ± 21.4	24.5 ± 13.8	26.0 ± 10.8	**0.0001**	43.6 ± 19.7	24.6 ± 13.3	26.0 ± 10.8	**0.00011**
Propionic acid	12.1 ± 7.6	12.4 ± 5.2	12.1 ± 5.3	0.697	13.4 ± 9.5	11.4 ± 6.7	12.0 ± 5.3	0.679	11.9 ± 6.5	8.1 ± 3.5	8.9 ± 4.9	**0.02**
Iso-butyric acid	1.5 ± 1.5	1.4 ± 0.6	1.4 ± 0.8	0.190	1.4 ± 0.9	1.3 ± 0.6	1.4 ± 0.8	0.973	1.5 ± 1.2	1.3 ± 0.5	1.3 ± 0.6	0.9197
Butyric acid	10.6 ± 8.0	10.8 ± 6.2	11.8 ± 5.0	0.281	10.6 ± 8.0	16.2 ± 9.2	17.4 ± 7.8	**0.0001**	12.5 ± 7.8	15.7 ± 9.3	18.5 ± 6.9	**0.00**[2]
Iso-valeric acid	1.5 ± 1.5	1.4 ± 0.6	1.4 ± 0.8	0.190	1.4 ± 0.9	1.3 ± 0.6	1.4 ± 0.8	0.973	1.5 ± 1.2	1.3 ± 0.5	1.3 ± 0.6	0.9197
Valeric acid	1.7 ± 1.5	1.4 ± 0.8	1.6 ± 0.8	0.719	1.9 ± 1.9	1.7 ± 1.1	1.6 ± 0.6	0.960	1.8 ± 1.4	1.4 ± 0.6	1.5 ± 0.8	0.1707
Iso-capronic acid	0.010 ± 0.037	0.016 ± 0.039	0.012 ± 0.044	0.584	0.0 ± 0.0	0.004 ± 0.02	0.0 ± 0.0	0.247	0.020 ± 0.082	0.417 ± 1.037	0.000 ± 0.000	0.2674
Capronic acid	0.467 ± 0.708	0.321 ± 0.559	0.559 ± 0.927	0.858	0.690 ± 1.265	0.415 ± 0.506	0.370 ± 0.565	0.351	0.558 ± 0.784	0.535 ± 0.763	0.478 ± 0.722	0.3434

Note: Data are mean ± SD values in units of mmol/kg wet weight. Boldface indicates a significant difference.
[a]Nonparametric Kruskal–Wallis multiple-comparisons test.

in IBS-QoL scores in 30- and 60-g groups at 2 and 3 years after FMT, but not in the placebo group (Data S1: longitudinal analysis).

3.2 | Fecal SCFA Levels

In the placebo group, there were no significant differences in the fecal total SCFA level or in the fecal levels of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocapronic, and capronic acids between the baseline and at 2 and 3 years after FMT (Table 2). In both the 30-g and 60-g groups, the total SCFA level and the acetic acid level decreased significantly at 2 and 3 years after FMT (Table 2). The propionic acid level decreased significantly in the 60-g group at 3 years after FMT, while the butyric acid level increased significantly at 2 and 3 years after FMT in both the 30-g and 60-g groups (Table 2).

The total SCFA level did not differ significantly between the placebo, 30-g, and 60-g groups at 2 and 3 years after FMT (Table 3). The acetic, and propionic acid levels were lower in the 30-g and 60-g groups than placebo group at both 2 and 3 years after FMT (Table 3). Butyric acid level was higher in the 30-g and 60-g groups than placebo group at both 2 and 3 years after FMT (Table 3). In The 30-g group, while FMT had a moderate effect size at 2 years (Cohen's $d = -0.6$), it had a large effect size at 3 years (Cohen's $d = -1.2$). In the 60-g group, FMT had a large effect size both at 2 and 3 years after FMT (Cohen's $d = -0.8$, and -1.4, respectively).

GLMMs, and LMMs showed a significant increase in fecal butyric acid levels in 30-g and 60-g groups at 2 and 3-year intervals after FMT, but not in the placebo group (Data S1: longitudinal analysis).

3.3 | Differences in SCFA Levels Between Responders and Nonresponders

The butyric acid level was significantly higher in the responders than in nonresponders at both 2 and 3 years after FMT (Table 4). There were no differences between responders and nonresponders in the total SCFA level or in the levels of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocapronic, and capronic acids between the baseline and at 2 and 3 years after FMT (Table 4).

3.4 | Differences in SCFA Levels Between IBS Subtypes

In the placebo group, the butyric acid level was significantly lower in patients with constipation-predominant IBS (IBS-C) and mixed-diarrhea-and-constipation IBS (IBS-M) than in patients with diarrhea-predominant IBS (IBS-D) (Table S5). The total SCFA level was higher in IBS-C patients in the 60-g group at 3 years after FMT (Table S7). There were no other differences in the SCFA levels in the placebo, 30-g, and 60-g groups at the baseline and at 2 and 3 years after FMT (Tables S6 and S7).

3.5 | Differences in SCFA Levels Between Females and Males

There were no significant differences between females and males in the total SCFA level or in the levels of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocapronic, and capronic acids at the baseline and at 2 and 3 years after

TABLE 3 | Fecal SCFA levels in the placebo, 30-g, and 60-g groups at 2 and 3 years after FMT.

	2 years after FMT				3 years after FMT				
SCFAs	Placebo group	30-g group	60-g group	p[a]	Placebo group	30-g group	60-g group	p	Donor
Total SCFAs	59.3 ± 21.9	58.1 ± 23.5	52.1 ± 23.1	0.137	62.1 ± 19.1	59.2 ± 24.2	53.8 ± 20.8	0.127	95.7 ± 21.8
Acetic acid	32.6 ± 11.6	25.0 ± 11.8*	26.0 ± 0.8*	**0.02**	31.4 ± 13.5	24.5 ± 13.8*	25.6 ± 15.4*	**0.02**	57.0 ± 10.0
Propionic acid	11.8 ± 4.8	11.4 ± 6.7	8.1 ± 3.5**	**0.003**	12.1 ± 5.3	12.0 ± 5.8	8.9 ± 4.9**	**0.01**	14.2 ± 3.9
Isobutyric acid	1.4 ± 0.6	1.3 ± 0.6	1.3 ± 0.5	0.868	1.4 ± 0.7	1.4 ± 0.8	1.3 ± 0.6	0.610	0.7 ± 0.1
Butyric acid	10.4 ± 6.2	16.2 ± 9.2*	15.7 ± 9.3*	**0.014**	11.5 ± 4.6	17.4 ± 7.8**	18.6 ± 6.7****	**0.0001**	24.9 ± 6.7
Isovaleric acid	2.1 ± 1.0	1.9 ± 0.9	1.9 ± 0.7	0.668	2.1 ± 1.1	2.2 ± 1.0	2.2 ± 0.9	0.950	0.8 ± 0.2
Valeric acid	1.4 ± 0.8	1.7 ± 1.1	1.4 ± 0.6	0.472	1.6 ± 0.8	1.6 ± 0.6	1.5 ± 0.8	0.508	1.4 ± 0.5
Isocapronic acid	0.01 ± 0.03	0.01 ± 0.02	0.3 ± 1.7	0.295	0.01 ± 0.04	0.0 ± 0.0	0.0 ± 0.0	0.126	
Capronic acid	0.3 ± 0.5	0.5 ± 0.7	0.5 ± 0.6	0.228	0.6 ± 0.9	0.4 ± 0.6	0.5 ± 0.7	0.732	0.0 ± 0.0

Note: Data are mean ± SD values in units of mmol/kg wet weight. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ relative to placebo using Dunn's multiple comparisons as a posttest. Boldface indicates a significant difference.
[a]Nonparametric Kruskal–Wallis multiple-comparisons test.

TABLE 4 | Fecal SCFA levels in the nonresponders and responders to FMT at 2 and 3 years after FMT.

	2 years after FMT			3 years after FMT		
SCFAs	**Non-responders**	**Responders**	***p***	**Non- responders**	**Responders**	***p***
Total SCFAs	54.5 ± 28.4	52.9 ± 21.5	0.828	58.4 ± 17.1	55.3 ± 22.4	0.326
Acetic acid	32.6 ± 14.7	28.1 ± 13.7	0.6	30.6 ± 9.6	29.5 ± 12.6	0.343
Propionic acid	9.0 ± 5.4	9.7 ± 5.3	0.516	11.3 ± 5.9	10.5 ± 5.3	0.582
Isobutyric acid	1.3 ± 0.7	1.3 ± 0.5	0.857	1.4 ± 0.6	1.4 ± 0.7	0.446
Butyric acid	7.2 ± 3.8	11.7 ± 5.4	**0.0001**	10.5 ± 3.9	15.1 ± 8.0	**0.0001**
Isovaleric acid	1.9 ± 1.0	2.1 ± 0.8	> 0.999	2.2 ± 0.9	2.1 ± 1.0	0.432
Valeric acid	1.5 ± 0.7	1.7 ± 0.8	0.666	1.7 ± 0.9	1.5 ± 0.6	0.381
Isocapronic acid	0.0 ± 0.0	0.0 ± 0.0	> 0.999	0.018 ± 0.048	0.010 ± 0.38	0.423
Capronic acid	0.567 ± 1.0	0.643 ± 0.9	0.667	0.685 ± 0.996	0.417 ± 0.518	0.285

Note: Data are mean ± SD values in units of mmol/kg wet weight. Boldface indicates a significant difference.

FMT (Tables S8–S10). However, the isovaleric acid level in the 30-g group was lower in males than males at the baseline (Table S8).

3.6 | Correlations

The fecal butyric acid level was inversely correlated with the IBS-SSS total score and the scores for its subitems of abdominal pain, abdominal distension, dissatisfaction with bowel habits, and interference with quality of life (Figure 1). Furthermore, the fecal butyric acid level was inversely correlated with the total Birmingham IBS Symptom Questionnaire score and the scores for its three subitems: abdominal pain, diarrhea, and constipation (Figure 2). The fecal butyric acid level was also correlated with the FAS total score and the scores for its subitems of physical fatigue and mental fatigue (Figure 3).

The fecal acetic acid level was not correlated with the IBS-SSS total score ($r = 0.09$, $p = 0.2$) or with the scores for its subitems of abdominal pain, ($r = 0.09$, $p = 0.2$), abdominal distension ($r = -0.1$, $p = 0.2$), dissatisfaction with bowel habits ($r = -0.01$, $p = 0.8$), or interference with quality of life ($r = 0.05$, $p = 0.5$). Similarly, the fecal propionic acid level was not correlated with the IBS-SSS total score ($r = 0.1$, $p = 0.8$) or with the scores for its subitems of abdominal pain ($r = 0.07$, $p = 0.3$), abdominal distension ($r = 0.01$, $p = 0.9$), dissatisfaction with bowel habits ($r = -0.08$, $p = 0.3$), or interference with quality of life ($r = -0.01$, $p = 0.9$).

4 | Discussion

The present findings that FMT induced changes in fecal SCFAs at both 2 and 3 years after FMT and that these changes varied with the dose of the donor's feces administered are in agreement with previous findings at 1 month and 1 year after FMT [13, 14]. The increase of butyric acid levels at 1 month after FMT is most probably due to the engraftment of the donor's bacteria, especially those producing butyric acid [13]. However, the increase in fecal butyric acid observed here at 2 and 3 years after FMT could be a reflection of patients' dietary changes, as FMT-induced improvement in symptoms might have enabled patients to expand and enrich their diet. While it was previously found that the fecal total SCFA level increased after 1 month and 1 year [13, 14], it decreased in the present study at 2 and 3 years after FMT. This discrepancy suggests that it is the proportion of SCFAs rather than their total level that is associated with the improvement in IBS symptoms seen after FMT [11–13]. In the present study, the butyric acid level was higher after FMT in both the 30-g and 60-g groups at 2 and 3 years after FMT. These increases in butyric acid after FMT had moderate to large effect sizes in both 30-g and 60-g groups at both 2 and 3 years post FMT, indicating its clinical relevance. Similarly, we previously observed that the butyric acid level was higher in the 30-g and 60-g groups at both 1 month and 1 year after FMT [13, 14]. Thus, the increase in the butyric acid level after FMT is independent of the dose of fecal sample administered and is constant over time. This increase can be explained by our previous finding that the levels of *Faecalibacterium prausnitzii* and *Eubacterium biforme* increased at 1 month, 1, 2, and 3 years after FMT [11–13]. These two species are the main butyrate producers in the human colon [22–26].

This study also found that the fecal butyric acid level was inversely correlated with the total IBS-SSS and Birmingham IBS Symptom Questionnaire scores. In a prospective multicenter clinical trial, administering 150 mg of microencapsulated sodium butyrate twice daily for 12 weeks to IBS patients improved their abdominal pain, diarrhea, and constipation [27]. The present observation that the fecal butyric acid level was inversely correlated with abdominal pain as measured by scores on both the IBS-SSS and Birmingham IBS Symptom Questionnaire is in agreement with the previous observation that administering butyrate as an enema decreased the visceral sensitivity in healthy subjects [28]. Moreover, treatment with sodium butyrate has been shown to improve abdominal pain in IBS patients [27], which seems to be due to its regulation of serotonin expression by intestinal enterochromaffin cells [3–7]. Serotonin activates the sensory branch of the enteric nervous system, which is located in the submucosal plexus and the myenteric plexus. These sensory branches

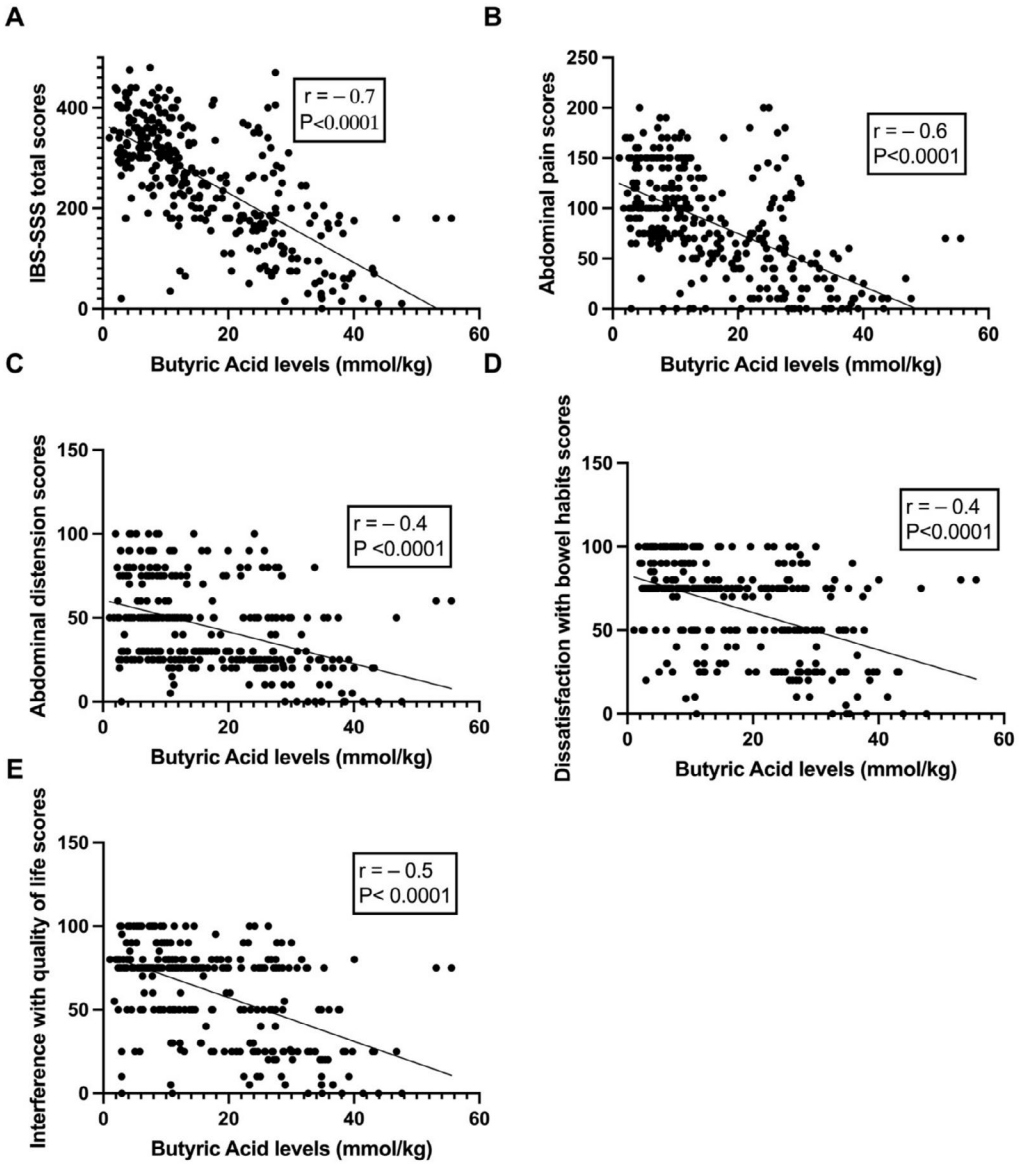

FIGURE 1 | Correlations of the butyric acid level with the IBS-SSS (Irritable Bowel Syndrome Severity Scoring System) total score (A) and the scores for its subitems of abdominal pain (B), abdominal distension (C), dissatisfaction with bowel habits (D), and interference with quality of life (E).

convey sensation from the gut to the CNS via the sympathetic and parasympathetic nervous systems [29]. Pain stimuli activate the cerebral cortex through the thalamus for the recognition of visceral pain [29]. In the present study, the fecal butyric acid level was also found to be inversely correlated with dissatisfaction with bowel habits according to IBS-SSS score and with both diarrhea and constipation scores on the Birmingham IBS Symptom Questionnaire. The present findings are supported by the previously reported observations that the butyrate level was inversely correlated with intestinal transit time in healthy subjects, and inversely correlated with colon transit time and positively correlated with stool frequency in IBS patients [8, 30]. Butyric acid seems to regulate intestinal motility by modulating the expression of serotonin in enterochromaffin cells, as well as the expression levels of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) in intestinal L-cells through activating FFAR2, FFAR3, GPR43, and GPR41 receptors [2–7, 31]. Serotonin, PYY, and GLP-1 delay gastric emptying [29, 32, 33]. Serotonin also accelerates the transit through the small intestine and colon, and PYY and GLP-1 are major mediators for the ileal brake [29, 32, 33]. The fecal butyric acid level has previously been reported to be inversely correlated with the FAS total score at 1 month after FMT [14]. Such a correlation was also found in the present study between the fecal butyric acid level and the FAS total score and the scores for its subitems of physical fatigue and mental fatigue. The mechanisms via which butyric acid affects fatigue in IBS patients remain to be determined.

It was previously found that the fecal levels of acetic and propionic acids did not change at 1 month after FMT in the 30-g and 60-g groups [14]. At 1 year after FMT, while the fecal acetic acid level decreased in both the 30-g and 60-g groups, the propionic acid level decreased only in the 60-g group [13]. The present study found that fecal acetic acid level decreased in both the 30-g and 60-g groups at both 2 and 3 years after FMT. On the other hand, the fecal propionic acid level decreased only in the 60-g group at both time points. The fecal levels of acetic and

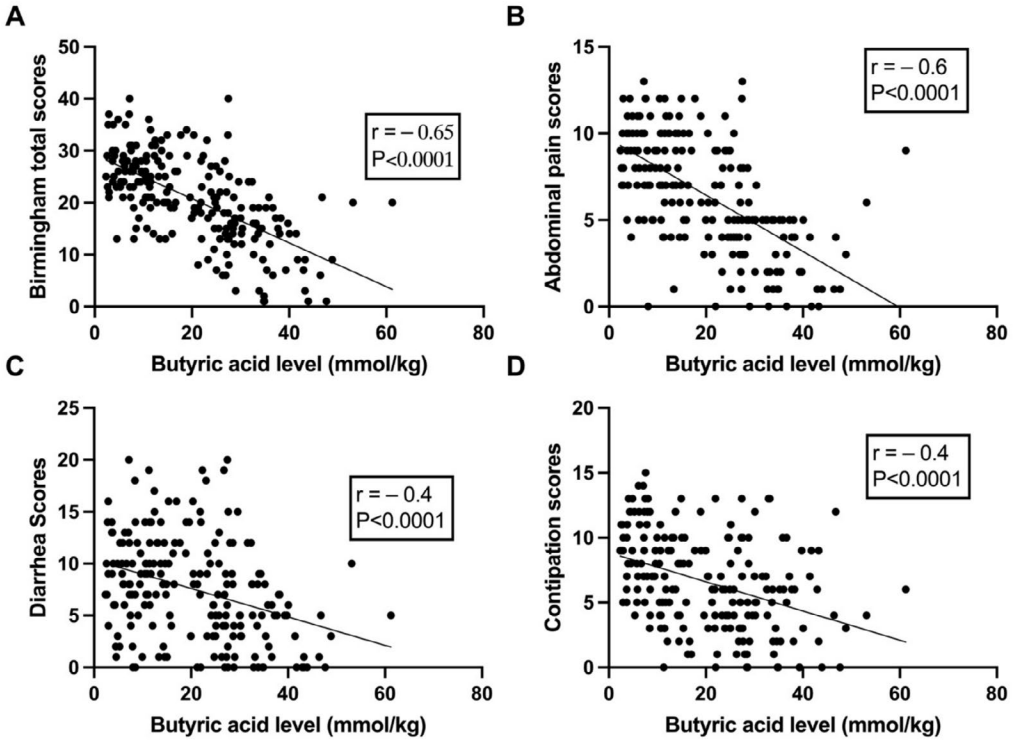

FIGURE 2 | Correlations of the butyric acid level with the total Birmingham IBS Symptom Questionnaire score (A) and the scores for its subitems of abdominal pain (B), diarrhea (C), and constipation (D).

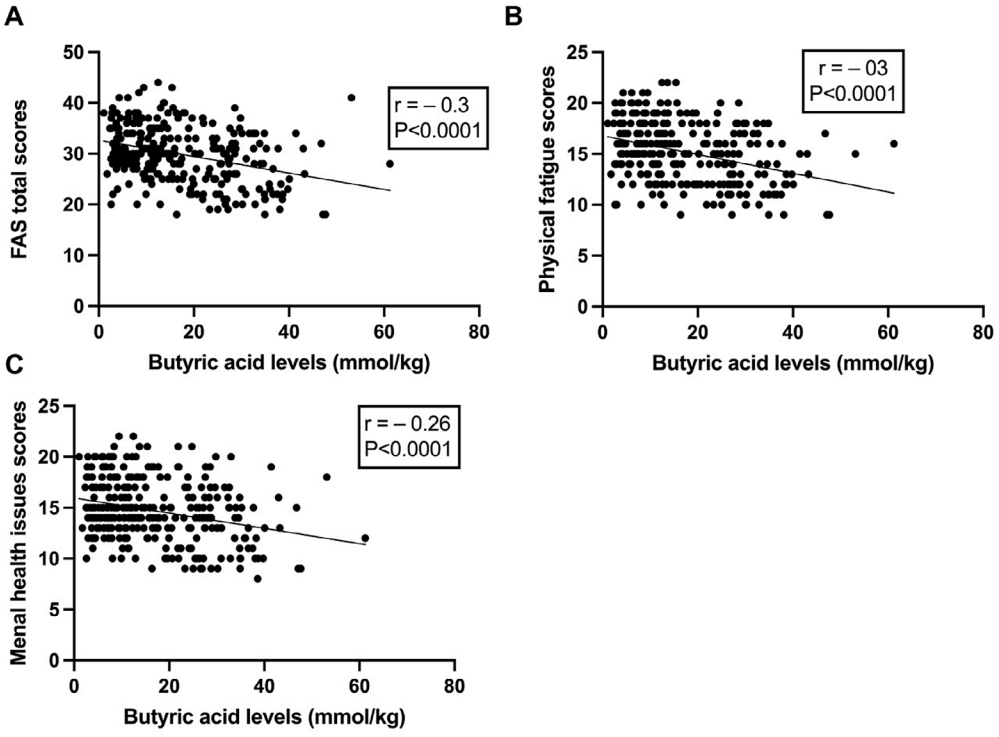

FIGURE 3 | Correlations of the butyric acid level with the FAS (Fatigue Assessment Scale) total score (A) and the scores for its subitems of physical fatigue (B) and mental fatigue (C).

propionic acids were not correlated with either the total IBS-SSS score or the scores for its four subitems. Moreover, there was no difference in the fecal levels of acetic and propionic acids between responders and nonresponders at 2 and 3 years after FMT. These observations suggest that the changes in acetic and propionic acids had no impact on the symptom relief observed after FMT. However, it should be noted that acetic acid was found to induce visceral hypersensitivity in rodents [34].

The fecal SCFA levels have been reported to vary between IBS subtypes [1, 6, 8, 10], with the total SCFA level being significantly lower in IBS-C patients than in IBS-D and IBS-M patients [6, 8]. Furthermore, the fecal levels of acetic, propionic, and butyric acids were lower in IBS-C patients than in healthy subjects, and that of butyric acid was higher in IBS-D patients [1, 10]. However, a very recent study found that the total SCFA level did not differ between IBS-D, IBS-C, and IBS-M patients [9]. In the present study, the butyric acid level at the baseline was higher in IBS-C and IBS-M patients than in IBS-D patients. Moreover, the total SCFA level was higher in IBS-C patients in the 60-g group at 3 years after FMT. Further studies are needed to determine the significance of the differences in the SCFA levels between IBS subtypes.

The main strengths of this study are that it investigated a relatively large cohort of IBS patients and that it included three of the four IBS subtypes, namely IBS-D, IBS-C, and IBS-M. The limitations of the study were that it did not include the unclassified IBS subtype (IBS-U), it was dominated by female IBS patients, and the patients were recruited from outpatient clinic, which may not represent the broader IBS population. Moreover, it excluded patients with systemic diseases, or recent probiotic/antibiotic.

In conclusion, FMT induced long-term changes in the levels of SCFAs. The levels of butyric acid increased at 2 and 3 years after FMT and were significantly higher than those of the placebo group. The levels of butyric acid were inversely correlated not only with total IBS symptom scores, but also with those of abdominal pain, diarrhea and constipation. Butyric acid seems to play a significant role in the long-term improvement in abdominal pain and bowel habits seen after FMT.

Author Contributions

M.E.S. designed the study, obtained the funding, administered the study, recruited the patients, performed FMT, collected, analyzed, and interpreted the data, and drafted the manuscript. J.V. contributed to the design of the study, analyzed the SCFAs, and critically revised the manuscript for important intellectual content. I.B, T.H., and J.G.H. contributed to the design of the study and to the analysis and interpretation of the data, and critically revised the manuscript for important intellectual content.

Conflicts of Interest

M.E.S., J.V., I.B., and J.G.H. have nothing to disclose. O.H.G. has received speaker honoraria from Bracco, GE Healthcare, Takeda AS, Ferring AS, Allergan, and Janssen-Cilag. He has served as a consultant for Bracco, GE Healthcare, Takeda, and Samsung.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Endnotes

[1] 1.1.

[2] 0.001.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1.**