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ORIGINAL ARTICLE

Clinical Trials Study

Responses to faecal microbiota transplantation in female and male patients with irritable bowel syndrome

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Abstract

BACKGROUND

Faecal microbiota transplantation (FMT) seems to be a promising treatment for irritable bowel syndrome (IBS) patients. In Western countries (United States and Europe), there is a female predominance in IBS. A sex difference in the response to FMT has been reported recently in IBS patients.

AIM

To investigate whether there was a sex difference in the response to FMT in the IBS patients who were included in our previous randomized controlled trial of the efficacy of FMT.

METHODS

The study included 164 IBS patients who participated in our previous randomized controlled trial. These patients had moderate-to-severe IBS symptoms belonging to the IBS-D (diarrhoea-predominant), IBS-C (constipation-predominant) and IBS-M (mixed) subtypes, and had not responded to the National Institute for Health and Care Excellence (NICE)-modified diet. They belonged in three groups: placebo (own faeces), and active treated group (30-g or 60-g superdonor faeces). The patients completed the IBS severity scoring system (IBS-SSS), Fatigue Assessment Scale (FAS) and the IBS quality of life scale (IBS-QoL) questionnaires

This study is registered at www.clinicaltrials.gov (NCT03822299) and www.cristin.no (ID657402).

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at the baseline and 2 wk, 1 mo and 3 mo after FMT. They also provided faecal samples at the baseline and 1 mo after FMT. The faecal bacteria profile and dysbiosis were determined using the 16S rRNA gene polymerase chain reaction DNA amplification covering V3-V9; probe labelling by single nucleotide extension and signal detection. The levels of short-chain fatty acids (SCFAs) were determined by gas chromatography and flame ionization.

RESULTS

There was no sex difference in the response to FMT either in the placebo group or active treated group. There was no difference between females and males in either the placebo group or actively treated groups in the total score on the IBS-SSS, FAS or IBS-QoL, in dysbiosis, or in the faecal bacteria or SCFA level. However, the response rate was significantly higher in females with diarrhoea-predominant (IBS-D) than that of males at 1 mo, and 3 mo after FMT. Moreover, IBS-SSS total score was significantly lower in female patients with IBS-D than that of male patients both 1 mo and 3 mo after FMT.

CONCLUSION

There was no sex difference in the response to FMT among IBS patients with moderate-to-severe symptoms who had previously not responded to NICE-modified diet. However, female patients with IBS-D respond better and have higher reduction of symptoms than males after FMT.

Key Words: Dysbiosis; Fatigue; Microbiome; Quality of life; Short-chain fatty acids; Superdonor

Core Tip: A sex difference in the response to faecal microbiota transplantation (FMT) was previous reported for a subgroup of refractory irritable bowel syndrome (IBS) patients with severe bloating who had not responded to at least three conventional therapies for IBS. This subgroup only contained patients with diarrhoea-predominant (IBS-D) or mixed (IBS-M) IBS. The present study found no sex difference in the response to FMT among IBS patients with moderate-to-severe symptoms of IBS-D, constipation-predominant (IBS-C) and IBS-M. However, female patients with IBS-D respond better and have higher reduction of symptoms than males after FMT.

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INTRODUCTION

The gut microbiota plays an important role in the pathophysiology of irritable bowel syndrome (IBS)[1,2]. The composition of the gut bacteria in IBS patients differs from that of healthy subjects[2-6]. IBS patients have lower abundances of the butyrate-producing bacteria, *Erysipelotrichaceae* and *Ruminococcaceae* compared with healthy controls[7,8]. Methane-producing bacteria, *Methanobacteriales* were found to be more abundant in IBS patients with constipation as a predominant symptom (IBS-C) and less abundant in IBS patients with diarrhoea as a predominant symptom (IBS-D) compared with healthy individuals[7,8]. Moreover, IBS patients have been found to have increased abundances of *Veillonella*, *Lactobacillus* and *Ruminococcus* bacteria and decreased abundances of *Bifidobacterium*, *Faecalibacterium* and *Erysipelotrichaceae* methanogens[7,8]. IBS patients also have a lower diversity of gut bacteria (dysbiosis) than healthy subjects[4-6,9].

Faecal microbiota transplantation (FMT) has previously been performed in IBS patients in seven randomized controlled trials (RCTs)[10-16]. Four of these RCTs

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showed that FMT had good effects on symptoms and the quality of life[10,12,15,16]. while the other three RCTs found no effects[11,13,14]. It soon became clear that carefully selecting the donor based on clinical and microbial criteria as well as the dose of the transplant are important for a successful outcome of FMT[17].

In Western countries (United States and Europe) there is a sex difference in IBS, with a female:male ratio of 2:1[18-20]. However, in Asia there is no such female predominance[21-24]. A recently published RCT on FMT in IBS found that females responded better to FMT than did males[16]. A recent RCT of IBS patients performed by our group found that FMT led to marked reductions in IBS symptoms and fatigue and an improvement in the quality of life[12]. These improvements were accompanied by marked changes in the faecal bacteria profile and the profile of short-chain fatty acids (SCFAs) of the patients[12,25].

The present study investigated whether there is a sex difference in the response to FMT in terms of symptoms, dysbiosis, and bacteria and SCFA profiles in the same cohort of patients that we had investigated in our previous study[12].

MATERIALS AND METHODS

Study design and randomization of patients

The design of this study has been described in detail previously[12]. In brief, patients completed three questionnaires to assess their symptoms and quality of life at the baseline and 2 wk, 1 mo and 3 mo after FMT. They also provided faecal samples at the baseline and 1 mo after FMT. Polyethylene glycol and loperamide were allowed as rescue medication during the study. The patients were randomized 1:1:1 to placebo (own faeces), 30-g (superdonor faeces) or 60-g (superdonor faeces) FMT[12]. The 30- and 60-g superdonor-faeces groups were pooled together and called the active treated group in order to increase the sample size and reduce the probability of type-II statistical errors.

Patients

This study included 164 patients who had participated in our previous study[12]. The characteristics of these patients are given in Table 1. The patients enrolled in this study have been described in detail previously[12]. In brief, patients attending the outpatient clinic at Stord Hospital who fulfilled the Rome IV criteria for a diagnosis of IBS were recruited. All of the recruited patients had previously not responded to consuming the National Institute for Health and Care Excellence (NICE)-modified diet for at least 3 mo[12]. They also received a course of IBS treatment that slightly improved their symptoms.

The inclusion criteria were being aged between 18 and 75 years and having moderate-to-severe IBS symptoms, as indicated by a score of 175 on the IBS severity scoring system (IBS-SSS). The exclusion criteria were being pregnant or planning pregnancy, lactating, the presence of systemic disease, having immune deficiency or being treated by immune-modulating medication, or having a psychiatric illness, excessive alcohol consumption or drug abuse. Patients who took probiotics, antibiotics or IBS medications within 8 wk prior to study inclusion were also excluded[12].

Donor

The single superdonor used in this study has been described in detail previously[12]. Briefly, he was screened according to the European guidelines for FMT donors[26]. He was a healthy 36-year-old male, non-smoker, not taking any medication regularly and had a normal body mass index. He had been born *via* a vaginal delivery, breastfed and had taken only a few courses of antibiotics during his life. He exercised regularly and took sport-specific dietary supplements, which made his diet richer than average in protein, fibre, minerals and vitamins. He was normobiotic, but his faecal bacteria profile deviated from the healthy subjects abundance in 14 of the 39 bacteria markers[12].

Collection, preparation and administration of faecal samples

Faecal 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 process of FMT has been described in detail previously[12]. In brief, the patients randomized to the placebo FMT group received 30 g of their own faeces (autologous), while those in the 30-g and 60-g FMT groups received 30 g and 60 g of the superdonor's faeces (allogenic),

Table 1 Characteristics of the patients in the placebo and active treated groups

	Placebo				Active treated			
	Total	Females	Males	*P* value	Total	Females	Males	*P* value
n	**55**	**47**	**8**		**109**	**85**	**24**	
Age, yr (median, range)	38.5 (18-75)	38.0 (18-73)	47.0 (20-75)	0.3	39.0 (18-73)	40.0 (18-73)	32.0 (21-65)	0.07
IBS-D	21	19	2	0.7	42	30	12	0.4
IBS-C	22	18	4		40	32	8	
IBS-M	12	10	2		27	23	4	
IBS duration, yr	15.5 ± 7.9	16.2 ± 8.0	15.0 ± 9.0	0.9	17.3 ± 8.9	16.8 ± 8.2	18.0 ± 9.2	0.9
Age at IBS onset, yr (median, range)	20.0 (15-35)	20.5 (16-35)	19.0 (15-30)	0.4	20.0 (15-36)	20.0 (16-35)	20 (15-33)	0.6
IBS-SSS total score	315.2 ± 77.1	320.1 ± 77.8	286.9 ± 69.3	0.5	312.9 ± 82.0	319.1 ± 77.3	297.7 ± 82.0	0.4
Moderate symptoms[1] (%)	23 (42)	17 (36)	6 (75)	0.06	45 (41)	30 (35)	13 (54)	0.1
Severe symptoms[2] (%)	32 (58)	30 (64)	2 (25)		64 (59)	55 (65)	11 (46)	

Data are *n*, *n* (%) or mean ± SD values.

[1]Irritable bowel syndrome severity scoring system total score between 175 and 300.

[2]Irritable bowel syndrome severity scoring system total score of ≥ 300. IBS: Irritable bowel syndrome; IBS-D: Irritable bowel syndrome with diarrhoea-predominant; IBS-C: Irritable bowel syndrome with constipation-predominant; IBS-M: Irritable bowel syndrome with mixed diarrhoea and constipation; IBS-SSS: Irritable bowel syndrome severity scoring system.

respectively. The transplant was administered to the distal duodenum *via* a gastroscope[12].

Symptom and quality-of-life assessments

Symptoms were assessed using the IBS-SSS and the Fatigue Assessment Scale (FAS)[27-31]. Quality of life was measured using the IBS quality of life scale (IBS-QoL)[32-34]. Response was defined as a decrease of ≥ 50 points in the IBS-SSS total score after FMT.

Microbiome analysis and dysbiosis index

The faecal bacteria profile and dysbiosis were determined by the GA-map Dysbiosis Test (Genetic Analysis, Oslo, Norway) using the 16S rRNA gene polymerase chain reaction DNA amplification covering V3-V9; probe labelling by single nucleotide extension and signal detection by BioCode 1000A 128-Plex Analyzer (Applied BioCode, Santa Fe Springs, CA, United States)[6]. The bacterial markers used detected bacteria within 5 phyla (Firmicutes, Proteobacteria, Bacteroidetes, Tenericutes and Verrucomicrobia) that cover 10 bacterial classes, 36 genera and 32 species[6]. This test assesses > 300 bacteria at different taxonomic levels[9]. The dysbiosis index (DI) was measured on a 5-point scale from 1 to 5, where DI values 1-2 indicates normobiosis, 3-5 indicates dysbiosis[6].

Determination of faecal SCFA levels

The method used to determine faecal SCFA levels has been described in detail previously[25]. Briefly, the faecal samples were homogenized with a solution containing 3 mmol/L 2-ethylbutyric acid and 0.5 mmol/L H_2SO_4. The homogenate was vacuum distilled, and the SCFA levels were determined by gas chromatography (Agilent 7890 A, Agilent, CA, United States) using a capillary column (serial no. USE400345H, Agilent J&W GC columns, Agilent) and flame ionization[35,36] levels of total SCFAs, acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric acid, isocapronic and n-capronic acids, were determined and were expressed in units of mmol/kg wet weight.

Statistical analysis

The sample size required in each arm of the previously published trial was calculated by assuming that a placebo effect was 40% and an effect response was 80%. The total

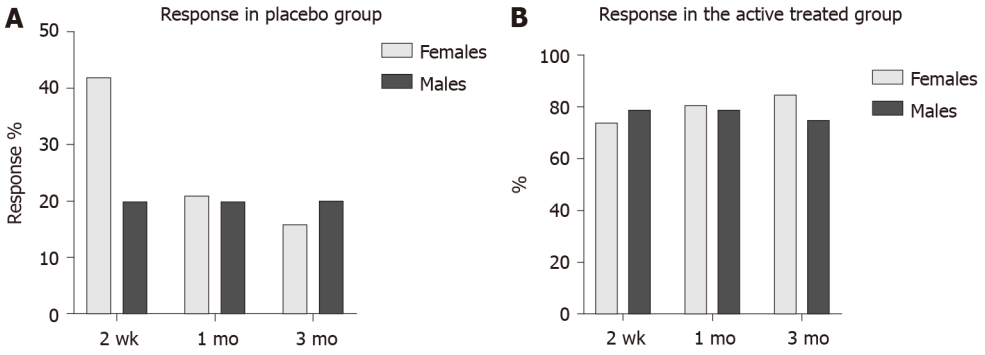

Figure 1 Response to faecal microbiota transplantation in females and males at different intervals after faecal microbiota transplantation in the two groups. A: Placebo group; B: Active treated group.

sample size was estimated to be 60 patients, with 20 in each arm ($\alpha = 0.05$, $1\text{-}\beta = 0.80$)[12]. In the present study a new calculation for the sample size was done based on the response rates obtained from our previous RCT[12]. Thus, assuming that the females' response is 90% and males' response is 60%, The total sample size was estimated to be 22 with 11 females and 11 males ($\alpha = 0.05$, $1\text{-}\beta = 0.80$). The 30- and 60-g superdonor-faeces groups were pooled together and called the active treated group in order to increase the sample size and reduce the probability of type-II statistical errors. Differences in response and dysbiosis between females and males in the placebo and the active treated group were analyzed using the χ^2 test. Differences between females and males in the total scores on the IBS-SSS, FAS and IBS-QoL, and in faecal bacteria and SCFA levels were analyzed using the Mann-Whitney test. These analyses were performed using GraphPad Prism (version 8, La Jolla, CA, United States).

Ethics

The Regional Committee for Medical and Health Research Ethics West, Bergen, Norway approved the study (approval No. 2017/1197/REK vest). All subjects provided both oral and written consents to participate. The study was registered at www.clinicaltrials.gov (NCT03822299) and www.cristin.no (ID657402).

RESULTS

Symptom and quality-of-life assessments

In the placebo group, the response did not differ between females and males at 2 wk, 1 mo and 3 mo after FMT ($P = 0.4$, 0.9 and 0.8, respectively). The responses in the active treated group did not differ between females and males after 2 wk, 1 mo and 3 mo ($P = 0.6$, 0.8 and 0.3, respectively) (Figure 1). The response rate was significantly higher in females with IBS-D than that of males at 1 mo, and 3 mo after FMT (Table 2 and Figure 2). There was no significant difference of response rates between female and male patients with either moderate or severe IBS symptoms (Table 3 and Figure 3).

The IBS-SSS total score did not differ significantly between female and male IBS patients in either the placebo or the active treated group (Table 4 and Figure 4). However, IBS-SSS total score was significantly lower in female patients with IBS-D than that of male patients both 1 mo and 3 mo after FMT (Table 5 and Figure 5). The IBS-SSS total score did not differ significantly between females and males in patients with moderate or severe IBS symptoms (Table 6 and Figure 3).

The FAS total score also did not differ significantly between female and male IBS patients in the active treated group (Table 7 and Figure 6), but it was lower in males than females in the placebo group at 3 mo after FMT. This could have been due to a type-I statistical error. There was no significant difference between female and male IBS patients belonging to different IBS-subtypes IBS symptoms (Table 8 and Figure 7). However, the FAS total score was lower in males IBS patients with IBS-D than that of females 2 wk after FMT.

The IBS-QoL total score did differ between females and males in both the placebo and active treated groups (Table 9 and Figure 8), being higher in males than in females at the baseline. IBS-QoL total scores did not differ significantly between female and male patients belonging to different IBS-subtypes (Table 10 and Figure 9).

Table 2 The response rates of females and males in different irritable bowel syndrome-subtypes at different intervals after faecal microbiota transplantation

Time after FMT	IBS-D			IBS-C			IBS-M		
	Females	Males	*P* value	Females	Males	*P* value	Females	Males	*P* value
2 wk (%)	73	58	0.3	65	50	0.7	72	55	0.3
1 mo (%)	90	42	**0.0003**	69	75	0.7	65	60	0.9
3 mo (%)	90	42	**0.0003**	70	75	0.3	63	80	0.6

IBS-D: Irritable bowel syndrome with diarrhoea-predominant symptom; IBS-C: Irritable bowel syndrome with constipation-predominant symptom; IBS-M: Irritable bowel syndrome with mixed diarrhoea and constipation; FMT: Faecal microbiota transplantation.

Table 3 The response rates in females and males with either moderate or severe irritable bowel syndrome symptoms

Time after FMT	Moderate symptoms[1]			Severe symptoms[2]		
	Females	Males	*P* value	Females	Males	*P* value
2 wk (%)	58	61	0.999	78	91	0.4
1 mo (%)	61	61	0.999	78	91	0.4
3 mo (%)	63	56	0.778	77	82	0.999

[1]Irritable bowel syndrome severity scoring system total score between 175 and 300.
[2]Irritable bowel syndrome severity scoring system total score of ≥ 300. FMT: Faecal microbiota transplantation.

Table 4 Irritable bowel syndrome severity scoring system total scores of females and males in the two study groups at different times after faecal microbiota transplantation

Time	Placebo		*P* value	Active treated		*P* value
	Females	Males		Females	Males	
0	320 ± 78	287 ± 69	0.2	319 ± 77	297 ± 82	0.3
2 wk	254 ± 106	256 ± 90	0.9	199 ± 102	205 ± 95	0.6
1 mo	277 ± 98	272 ± 89	0.8	196 ± 108	193 ± 94	0.9
3 mo	288 ± 90	266 ± 100	0.6	173 ± 116	183 ± 105	0.5

Data are mean ± SD values.

Microbiome analysis

The faecal bacteria levels in the placebo group did not differ between female and male IBS patients at the baseline and 1 mo after FMT (Table 11 and Figure 10). Similarly, there were no differences in the faecal bacteria levels between female and male IBS patients in the active treated group (Table 12 and Figure 11).

In the placebo group, 26 females (55%) and 4 males (50%) had dysbiosis ($P = 0.8$) at the baseline, while 25 females (53%) and 4 males (50%) had dysbiosis ($P = 0.9$) at 1 mo after FMT. In the active treated group, 52 females (61%) and 13 males (54%) had dysbiosis ($P = 0.3$) at the baseline, while 41 females (48%) and 9 males (38%) had dysbiosis ($P = 0.2$) at 1 mo after FMT.

Faecal SCFA levels

The faecal levels of total SCFAs and acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocapronic and capronic acids did not differ between female and males IBS patients in both the placebo and active treated groups at the baseline and 1 mo after FMT (Table 13 and Figure 12).

Table 5 The irritable bowel syndrome severity scoring system total scores in females and males belonging to different irritable bowel syndrome-sub-types

Time after FMT	IBS-D			IBS-C			IBS-M		
	Females	Males	*P* value	Females	Males	*P* value	Females	Males	*P* value
2 wk	190.5 ± 191.4	204.0 ± 92.2	0.6	228.1 ± 116.2	239.2 ± 113.8	0.5	202.8 ± 121.3	225.0 ± 65.8	0.5
1 mo	177.8 ± 94.9	226.9 ± 73.3	**0.02**	228.8 ± 118.1	215.8 ± 115.7	0.6	219.9 ± 136.6	197.0 ± 65.2	0.8
3 mo	157.8 ± 102.9	212.3 ± 96.9	**0.03**	212.8 ± 124.0	234.6 ± 131.8	0.5	219.2 ± 146.3	149.0 ± 36.0	0.5

Data are mean ± SD values. IBS-D: Irritable bowel syndrome with diarrhoea-predominant symptom; IBS-C: Irritable bowel syndrome with constipation-predominant symptom; IBS-M: Irritable bowel syndrome with mixed diarrhoea and constipation; FMT: Faecal microbiota transplantation.

Table 6 Irritable bowel syndrome severity scoring system total scores in females and males with moderate or severe irritable bowel syndrome symptoms

Time after FMT	Moderate symptoms[1]			Severe symptoms[2]		
	Females	Males	*P* value	Females	Males	*P* value
2 wk	166.5 ± 70.0	167.2 ± 55.8	0.8	225.1 ± 114.0	259.5 ± 102.3	0.3
1 mo	162.1 ± 73.1	179.4 ± 63.8	0.5	229.2 ± 120.2	214.1 ± 118.1	0.7
3 mo	155.3 ± 76.9	175.3 ± 100.2	0.5	214.6 ± 131.8	202.3 ± 120.6	0.8

Data are mean ± SD values.

[1]Irritable bowel syndrome severity scoring system total score between 175 and 300.

[2]Irritable bowel syndrome severity scoring system total score of ≥ 300. FMT: Faecal microbiota transplantation.

Table 7 Fatigue Assessment Scale total scores of females and males in the two study groups at different times after faecal microbiota transplantation

Time	Placebo			Active treated		
	Females	Males	*P* value	Females	Males	*P* value
0	31 ± 5	29 ± 4	0.3	32 ± 5	30 ± 5	0.09
2 wk	31 ± 6	29 ± 6	0.2	28 ± 6	28 ± 5	0.5
1 mo	31 ± 6	27 ± 7	0.1	27 ± 7	29 ± 5	0.5
3 mo	30 ± 4	26 ± 4	**0.01**	29 ± 6	27 ± 5	0.7

Data mean ± SD values.

DISCUSSION

The present study found that the response to FMT did not differ between females and males. Furthermore, the total scores on the IBS-SSS, FAS and IBS-QoL did not differ between females and males in the active treated groups before FMT and at different times after FMT. In the placebo group, the total score of IBS-QoL was higher in males than males and the FAS total score was lower in males than females 3 mo after FMT. This indicates that the effects of active treated FMT did not differ between males and females regarding IBS symptoms, fatigue and quality of life. Moreover, there was no difference between females and males regarding dysbiosis or the faecal bacteria.

SCFA profiles following FMT did not differ between females and males in both the placebo and the active treated groups. SCFAs regulate intestinal motility and the secretion and absorption of water and electrolytes[37,38]. Moreover, SCFAs increase also the secretion and up-regulate the gene expression of peptide YY (PYY)[39,40]. PYY is a mediator of the ileal brake and stimulates the absorption of water and electrolytes in the colon[37]. The faecal level of total SCFAs increased significantly in IBS patients after 1 mo and remained elevated at 1 year after FMT (unpublished

Table 8 Fatigue Assessment Scale total scores of females and males irritable bowel syndrome patients belonging to different irritable bowel syndrome-subtypes

Time after FMT	IBS-D			IBS-C			IBS-M		
	Females	Males	*P* value	Females	Males	*P* value	Females	Males	*P* value
2 wk	30.1 ± 3.6	27.0 ± 3.6	**0.04**	28.0 ± 6.3	29.3 ± 7.4	0.5	26.7 ± 5.4	26.3 ± 2.1	0.9
1 mo	27.1 ± 5.2	26.6 ± 4.6	0.6	27.1 ± 6.7	31.3 ± 6.1	0.1	28.3 ± 8.3	28.3 ± 4.0	0.9
3 mo	27.5 ± 5.7	27.8 ± 5.0	0.4	26.0 ± 6.2	29.2 ± 5.0	0.2	26.8 ± 7.6	24.8 ± 2.2	0.7

Data are mean ± SD values. IBS-D: Irritable bowel syndrome with diarrhoea-predominant symptom; IBS-C: Irritable bowel syndrome with constipation-predominant symptom; IBS-M: Irritable bowel syndrome with mixed diarrhoea and constipation; FMT: Faecal microbiota transplantation.

Table 9 Irritable bowel syndrome quality of life scale total scores of females and males in the two study groups at different times after faecal microbiota transplantation

Time	Placebo			Active treated		
	Females	Males	*P* value	Females	Males	*P* value
0	116 ± 20	130 ± 11	**0.03**	111 ± 23	114 ± 21	0.9
2 wk	123 ± 29	120 ± 23	0.7	122 ± 24	118 ± 27	0.6
1 mo	123 ± 26	121 ± 26	0.8	126 ± 24	119 ± 29	0.4
3 mo	112 ± 24	118 ± 26	0.2	132 ± 23	131 ± 25	0.9

Data are mean ± SD values.

Table 10 Irritable bowel syndrome quality of life scale total scores of females and males irritable bowel syndrome patients belonging to different irritable bowel syndrome-subtypes

Time after FMT	IBS-D			IBS-C			IBS-M		
	Females	Males	*P* value	Females	Males	*P* value	Females	Males	*P* value
2 wk	123.3 ± 98	123.7 ± 25.5	0.8	120.5 ± 23.1	102.4 ± 28.0	0.06	123.1 ± 24.6	131.5 ± 13.0	0.4
1 mo	131.3 ± 20.8	129.6 ± 28.2	0.9	121.9 ± 24.4	111.4 ± 29.1	01	125.4 ± 25.2	130.5 ± 11.3	0.8
3 mo	136.4 ± 16.6	134.5 ± 22.7	0.9	128.7 ± 24.4	129.1 ± 31.3	0.6	129.5 ± 28.6	124.8 ± 22.0	0.5

Data are mean ± SD values. IBS-D: Irritable bowel syndrome with diarrhoea-predominant symptom; IBS-C: Irritable bowel syndrome with constipation-predominant symptom; IBS-M: Irritable bowel syndrome with mixed diarrhoea and constipation; FMT: Faecal microbiota transplantation.

data)[25]. Similarly, the faecal level of butyric acid increased in IBS patients after 1 mo and remained elevated at 1 year after FMT (unpublished data)[25]. Butyrate is a major energy source for colonic epithelial cells, interacts with the immune response, modulates the oxidative stress, and decreases both intestinal-cell permeability and intestinal motility[41]. Butyrate modulates also colonic hypersensitivity[42-44]. At 1 year after FMT levels of isobutyric and isovaleric acids were increased in IBS patients, indicating a shift in microbial fermentation from a saccharolytic to a proteolytic pattern[45]. It is worthy of note that in IBS patients, who adhered to a low-FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet an increase in the levels of isobutyric and isovaleric acids have been found[46]. Moreover, the level of acetic acid which induces visceral hypersensitivity decreased significantly at 1 year after FMT[47].These changes in SCFAs after FMT appear to be one of the mechanisms underlying the effects seen in IBS patients after FMT. That is why the difference between females and males regarding the changes in SCFAs was assessed.

Holvoet *et al*[16] reported that females responded better to FMT than did males. That RCT differed from the present study in terms of the characteristics of the included patients, the size of the patient cohort and the dose of the faecal transplants[16]. The trial of Holvoet *et al*[16] included a subgroup of refractory IBS

Table 11 Faecal bacteria levels in the female and male irritable bowel syndrome patients in the placebo group at the baseline and 1 mo after faecal microbiota transplantation

Bacteria	Baseline		1 mo after FMT	
	Females	Males	Females	Males
Actinobacteria	-0.235 ± 0.763	-0.365 ± 0.768	-0.250 ± 0.954	-0.375 ± 0.838
Actinomycetales	0.118 ± 0.382	-0.212 ± 0.536	0.175 ± 0.594	0.100 ± 0.496
Bifidobacterium spp.	-0.020 ± 0.607	-0.154 ± 0.539	0.025 ± 0.660	-0.075 ± 0.572
Alistipes	-0.863 ± 0.895	-0.885 ± 0.900	-0.875 ± 0.853	-0.800 ± 0.709
Alistipes onderdonkii	-0.667 ± 0.792	-0.615 ± 0.718	-0.650 ± 0.834	-0.550 ± 0.783
Bacteroides fragilis	-0.255 ± 0.689	-0.212 ± 0.637	0.175 ± 0.501	0.050 ± 0.221
Bacteroides spp. and *Prevotella spp.*	-0.980 ± 1.157	-0.885 ± 1.182	-0.750 ± 1.032	-0.900 ± 0.687
Bacteroides stercoris	-0.137 ± 0.448	-0.154 ± 0.415	0.025 ± 0.158	0.100 ± 0.304
Bacteroides zoogleoformans	0.078 ± 0.272	0.038 ± 0.194	0.025 ± 0.158	0 ± 0
Parabacteroides johnsonii	0.039 ± 0.196	0.077 ± 0.269	0.050 ± 0.221	0.100 ± 0.304
Parabacteroides spp.	-0.451 ± 0.642	-0.327 ± 0.706	-0.425 ± 0.747	-0.225 ± 0.480
Firmicutes	-0.431 ± 0.575	-0.385 ± 0.566	-0.325 ± 0.526	-0.400 ± 0.591
Bacilli	0.235 ± 1.124	0.192 ± 0.991	0.271 ± 1.132	0.150 ± 1.001
Catenibacterium mitsuokai	0.000 ± 0.400	0.135 ± 0.525	0.050 ± 0.289	0.100 ± 0.441
Clostridia	-0.020 ± 0.244	-0.077 ± 0.269	-0.025 ± 0.276	0.0 ± 0.036
Clostridium spp.	0.039 ± 0.196	0.038 ± 0.194	0.0 ± 0.0	0.050 ± 0.316
Dialister invisus	0.118 ± 0.381	-0.173 ± 0.474	0.200 ± 0.405	0.225 ± 0.158
Dialister invisus and *Megasphaera micronuciformis*	0.059 ± 0.238	0.173 ± 0.474	0.125 ± 0.335	0.025 ± 0.158
Dorea spp.	0.569 ± 0.700	0.500 ± 0.700	0.625 ± 0.628	0.667 ± 0.806
Eubacterium biforme	0.412 ± 0.753	0.269 ± 0.598	0.275 ± 0.640	0.400 ± 0.633
Eubacterium hallii	0.804 ± 0.939	0.673 ± 0.879	0.655 ± 0.730	0.650 ± 0.597
Eubacterium rectale	0.078 ± 0.337	0.058 ± 0.235	0.050 ± 0.221	0.025 ± 0.158
Eubacterium siraeum	-1.412 ± 0.963	-1.288 ± 0.161	-1.475 ± 1.086	-1.200 ± 1.265
Faecalibacterium prausnitzii	-0.431 ± 0.671	-0.500 ± 0.804	-0.550 ± 0.745	-0.500 ± 0.599
Lachnospiraceae	0.196 ± 0.566	0.269 ± 0.630	0.325 ± 0.730	0.275 ± 0.640
Lactobacillus ruminis and *Pediococcus acidilactici*	0.059 ± 0.311	0.077 ± 0.334	0.0 ± 0.0	0.025 ± 0.158
Lactobacillus spp.	0.353 ± 0.594	0.269 ± 0.528	0.325 ± 0.616	0.475 ± 0.680
Phascolarctobacterium spp.	0.078 ± 0337	0.077 ± 0.337	0.125 ± 0.404	0.075 ± 0.350
Ruminococcus albus and *Ruminococcus bromii*	0.353 ± 0.658	0.404 ± 0.721	0.325 ± 0.616	0.450 ± 0.749
Ruminococcus gnavus	0.431 ± 0.878	0.577 ± 0.878	0.450 ± 0.815	0.325 ± 0.764
Streptococcus agalactiae & Eubacterium rectale	0.157 ± 0.367	0.250 ± 0.480	0.110 ± 0.304	0.125 ± 0.345
Streptococcus salivarius ssp. Thermophilus and *Streptococcus sanguinis*	0.412 ± 0.606	0.346 ± 0.556	0.675 ± 0.888	0.475 ± 0.751
Streptococcus salivarius ssp. thermophilus	0.628 ± 0.871	0.577 ± 0.915	0.500 ± 0.934	0.600 ± 0.928
Streptococcus spp.	0.471 ± 0.833	0.423 ± 0.696	0.400 ± 0.709	0.450 ± 0.815
Veillonella spp.	-0.177 ± 0.518	-0.173 ± 0.648	-0.175 ± 0.385	-0.150 ± 0.534
Proteobacteria	0.294 ± 0.576	0.289 ± 0.499	0.275 ± 0.599	0.325 ± 0.616
Shigella spp. and *Escherichia spp.*	-0.275 ± 0.940	-0.212 ± 0.893	-0.200 ± 0.853	-0.335 ± 0.920
Mycoplasma hominis	-0.451 ± 0.503	-0.404 ± 0.496	-0.450 ± 0.504	-0.450 ± 0.503

Akkermansia muciniphila	0.471 ± 0.644	0.365 ± 0.627	0.450 ± 0.714	0.650 ± 0.802

The bacterial levels are mean ± SD relative values to a normobiotic microbiota profile of 165 healthy subjects. FMT: Faecal microbiota transplantation.

patients with severe bloating who had not responded to at least three conventional therapies for IBS. This subgroup contained only patients with the IBS-D or mixed (IBS-M) subtypes. The patients included in the present study had moderate-to-severe IBS symptoms belonging to the IBS-D, IBS-C and IBS-M subtypes, and had not responded to the NICE-modified diet. The patient cohort investigated by Holvoet *et al*[16] included 62 patients: 19 in the placebo group and 43 in the active treated group. The present study investigated a cohort of 164 patients: 55 in the placebo group and 109 in the active treated group. It is worthy of note that in the trial of Holvoet *et al*[16] included 30 females and 13 males in the active treated group and 8 females and 11 males in the placebo group. The present study included 85 females and 24 males in the active treated group and included 47 females and 8 males in the placebo group. Thus, this makes the present study less constrained than the RCT of Holvoet *et al*[16] regarding power and sample sizes. Moreover, the dose of the faecal transplant from the donor was not reported for the RCT by Holvoet *et al*[16], while in the present study the active treated group received either 30 g or 60 g of a superdonor transplant. In our previously published RCT we showed that the response rates increased with increased dose[12]. These differences make it difficult to compare the outcomes of the present study with those of Holvoet *et al*[16]. However, in the present study, female patients with IBS-D had a significant higher response rate to FMT and lower IBS-SSS score after FMT than males. These observations could explain the discrepancy between the findings of Holvoet *et al*[16] and the present study as in Holvoet *et al*[16] study the cohort of patients included were only IBS-D and IBS-M IBS-subtypes.

CONCLUSION

In conclusion, there is no sex difference in the response to FMT in IBS patients with moderate-to-severe IBS symptoms belonging to the three of IBS subtypes of IBS-C and IBS-M in patients who did not responded to NICE-modified diet. Female patients with IBS-D had a significant higher response rate to FMT and lower IBS-SSS score after FMT than males.

Table 12 Gut bacteria levels in female and male irritable bowel syndrome patients in the active treated group at the baseline and 1 mo after faecal microbiota transplantation

Bacteria	Baseline		1 mo after FMT	
	Females	Males	Females	Males
Actinobacteria	-0.250 ± 0.954	-0.375 ± 0.838	-0.250 ± 0.719	-0.2350 ± 0.636
Actinomycetales	0.175 ± 0.594	0.100 ± 0.496	0.068 ± 0255	0.145 ± 0.412
Bifidobacterium spp.	0.025 ± 0.660	-0.075 ± 0.572	-0.045 ± 0.526	-0.063 ± 0.433
Alistipes	-0.875 ± 0.853	-0.800 ± 0.709	-0.886 ± 0.869	-0.783 ± 0.821
Alistipes onderdonkii	-0.650 ± 0.834	-0.550 ± 0.783	-0.523 ± 0.699	-0.354 ± 0.565
Bacteroides fragilis	0.175 ± 0.501	0.050 ± 0.221	0.159 ± 0.480	0.104 ± 0.371
Bacteroides spp. and *Prevotella spp.*	-0.750 ± 1.032	-0.800 ± 0.687	-1.091 ± 1.996	-0.708 ± 0.967
Bacteroides stercoris	0.025 ± 0.158	0.100 ± 0.304	0.023 ± 0.151	0.146 ± 0.357
Bacteroides zoogleoformans	0.025 ± 0.158	0 ± 0	0.091 ± 0.291	0.083 ± 0.347
Parabacteroides johnsonii	0.050 ± 0.221	0.100 ± 0.304	0.045 ± 0.302	0.021 ± 0.144
Parabacteroides spp.	-0.425 ± 0.747	-0.225 ± 0.480	-0.455 ± 0.504	-0.313 ± 0.468
Firmicutes	-0.325 ± 0.526	-0.400 ± 0.591	-0.546 ± 0.627	-0.454 ± 0.483
Bacilli	0.271 ± 1.132	0.150 ± 1.001	0.205 ± 1.047	0.042 ± 0.824
Catenibacterium mitsuokai	0.050 ± 0.289	0.100 ± 0.441	0.023 ± 0.151	0.104 ± 0.515
Clostridia	-0.025 ± 0.276	0.0 ± 0.036	0.068 ± 0.255	0.021 ± 252
Clostridium spp.	0.0 ± 0.0	0.050 ± 0.316	0.223 ± 0.151	0.063 ± 0.245
Dialister invisus	0.200 ± 0.405	0.225 ± 0.158	0.091 ± 0.362	0.146 ± 0.505
Dialister invisus and *Megasphaera micronuciformis*	0.125 ± 0.335	0.025 ± 0.158	0.068 ± 0.034	0.104 ± 0.308
Dorea spp.	0.625 ± 0.628	0.667 ± 0.806	0.727 ± 0.758	0.663 ± 0.796
Eubacterium biforme	0.275 ± 0.640	0.400 ± 0.633	0.477 ± 0.791	0.563 ± 0.769
Eubacterium hallii	0.655 ± 0.730	0.550 ± 0.597	0.886 ± 0.993	0.979 ± 1.021
Eubacterium rectale	0.050 ± 0.221	0.025 ± 0.158	0-068 ± 0.255	0.042 ± 0.202
Eubacterium siraeum	-1.475 ± 1.086	-1.200 ± 1.265	-1.295 ± 0.930	-1.208 ± 0.988
Faecalibacterium prausnitzii	-0.550 ± 0.745	-0.500 ± 0.599	-0.568 ± 0.759	-0.521 ± 0.825
Lachnospiraceae	0.325 ± 0.730	0.275 ± 0.640	0.205 ± 0.553	0.125 ± 0.489
Lactobacillus ruminis and *Pediococcus acidilactici*	0.0 ± 0.0	0.025 ± 0.158	0.021 ± 0.146	0.188 ± 0.571
Lactobacillus spp.	0.325 ± 0.616	0.475 ± 0.680	0.500 ± 0.731	0.583 ± 0.679
Phascolarctobacterium spp.	0.125 ± 0.404	0.075 ± 0.350	0.091 ± 0.362	0.083 ± 0.347
Ruminococcus albus and *Ruminococcus bromii*	0.325 ± 0.616	0.450 ± 0.749	0.205 ± 0.553	0.271 ± 0.574
Ruminococcus gnavus	0.450 ± 0.815	0.325 ± 0.764	0.364 ± 0.810	0.250 ± 0.636
Streptococcus agalactiae & Eubacterium rectale	0.110 ± 0.304	0.125 ± 0.345	0.267 ± 0.495	0.083 ± 0.279
Streptococcus salivarius ssp. thermophilus and *Streptococcus sanguinis*	0.675 ± 0.888	0.475 ± 0.751	0.455 ± 0.504	0.292 ± 0.459
Streptococcus salivarius ssp. thermophilus	0.500 ± 0.934	0.600 ± 0.928	0.523 ± 0.821	0.604 ± 0.844
Streptococcus spp.	0.400 ± 709	0.450 ± 0.815	0.444 ± 0.841	0.396 ± 0.610
Veillonella spp.	-0.175 ± 0.385	-0.150 ± 0.534	-0.273 ± 0.544	-0.208 ± 0.504
Proteobacteria	0.275 ± 0.599	0.325 ± 0.616	0.717 ± 0.750	0.583 ± 0.498
Shigella spp. and *Escherichia spp.*	-0.200 ± 0.853	-0.335 ± 0.920	-0.151 ± 1.077	-0.188 ± 0.790
Mycoplasma hominis	-0.450 ± 0.504	-0.450 ± 0.503	-0.500 ± 0.506	-0.479 ± 0.505

Akkermansia muciniphila	0.450 ± 0.714	0.650 ± 0.802	0.741 ± 0.713	0.813 ± 0.915

The bacterial levels are relative values to a normobiotic microbiota profile of 165 healthy subjects (mean ± SD). FMT: Faecal microbiota transplantation.

Table 13 The short-chain fatty acids concentration in the faeces of the placebo group and the patients that received donor's faecs (faecal microbiota transplantation) at the baseline and 1 mo after faecal microbiota transplantation

Acids	Placebo				Active treated			
	Baseline		1 mo after FMT		Baseline		1 mo after FMT	
	Females	Males	Females	Males	Females	Males	Females	Males
Total SCFAs	72 ± 37	69 ± 23.	73 ± 37	69 ± 23	77 ± 40	72 ± 40	87 ± 42	89 ± 26
Acetic acid	42 ± 18	40 ± 15	41 ± 17	40 ± 14	44 ± 21	44 ± 20	46 ± 13	40.2 ± 15.0
Propionic acid	12 ± 8	11 ± 5	12 ± 8	11 ± 5	13 ± 10	13 ± 8	14 ± 4	11 ± 7
Iso-butyric acid	2 ± 2	1 ± 1	1 ± 2	1 ± 1	1 ± 1	1 ± 1	2 ± 2	1 ± 1
Butyric acid	14 ± 9	12 ± 6	13 ± 8	12 ± 6	11 ± 8	12 ± 9	18 ± 14	16 ± 10
Iso-valeric acid	2 ± 2	2 ± 1	2 ± 2	2 ± 1	2 ± 1	2 ± 2	2 ± 2	2 ± 1
Valeric acid	2 ± 2	1 ± 1	2 ± 2	1 ± 1	2 ± 2	2 ± 2	2 ± 1	2 ± 1
Iso-capronic acid	0.1 ± 0.04	0.01 ± 0.07	0.5 ± 0.7	0.4 ± 0.7	0.0 ± 0.0	0.02 ± 0.08	0.01 ± 0.04	0.0 ± 0.0
Capronic acid	0.5 ± 0.7	0.5 ± 0.7	0.01 ± 0.04	0.1 ± 0.08	0.7 ± 1.3	0.6 ± 0.8	0.6 ± 0.9	0.5 ± 0.9

The values were expressed as mmol/kg wet weight (mean ± SD). FMT: Faecal microbiota transplantation; SCFAs: Short-chain fatty acids.

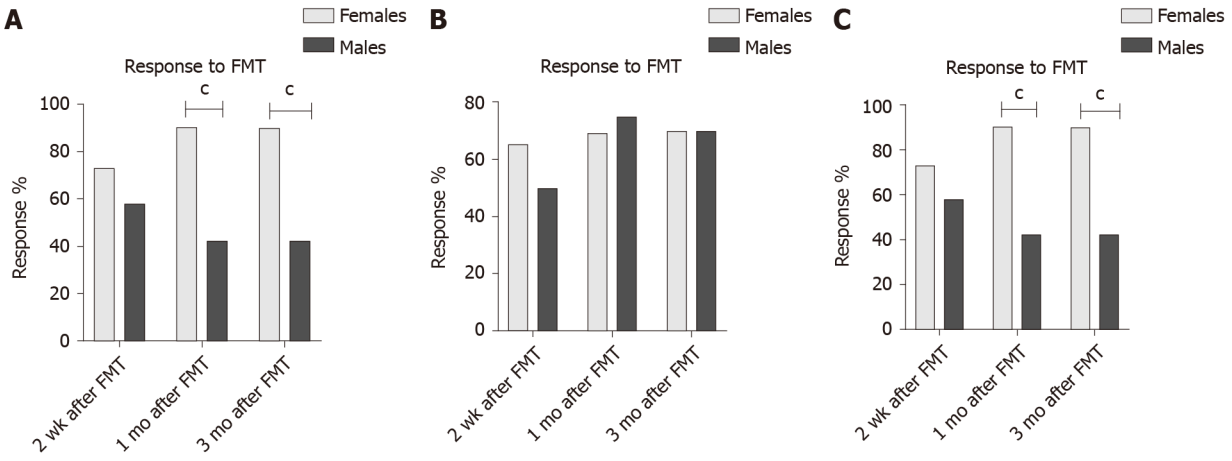

Figure 2 Response rates to faecal microbiota transplantation of female and male irritable bowel syndrome patients. A: Irritable bowel syndrome with diarrhoea-predominant; B: Irritable bowel syndrome with constipation-predominant; C: Irritable bowel syndrome with mixed diarrhoea and constipation. $^{c}P < 0.001$. FMT: Faecal microbiota transplantation.

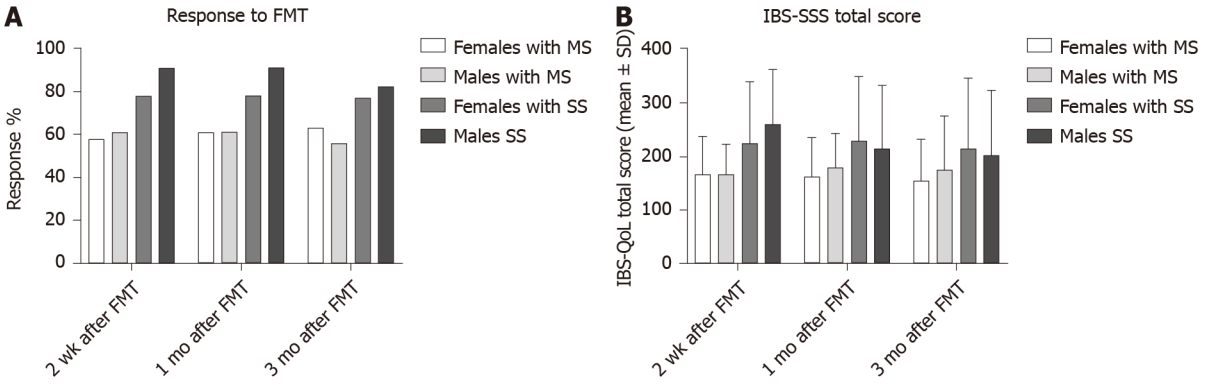

Figure 3 Response rates to faecal microbiota transplantation and the total irritable bowel syndrome-severity scoring system scores of irritable bowel syndrome patients with moderate irritable bowel syndrome symptoms (irritable bowel syndrome-severity scoring system total score between 175 and 300) and with severe irritable bowel syndrome symptoms (irritable bowel syndrome-severity scoring system total score of ≥ 300). A: Faecal microbiota transplantation; B: Irritable bowel syndrome severity scoring system total score. MS: Moderate irritable bowel syndrome symptoms; SS: Severe irritable bowel syndrome symptoms; FMT: Faecal microbiota transplantation.

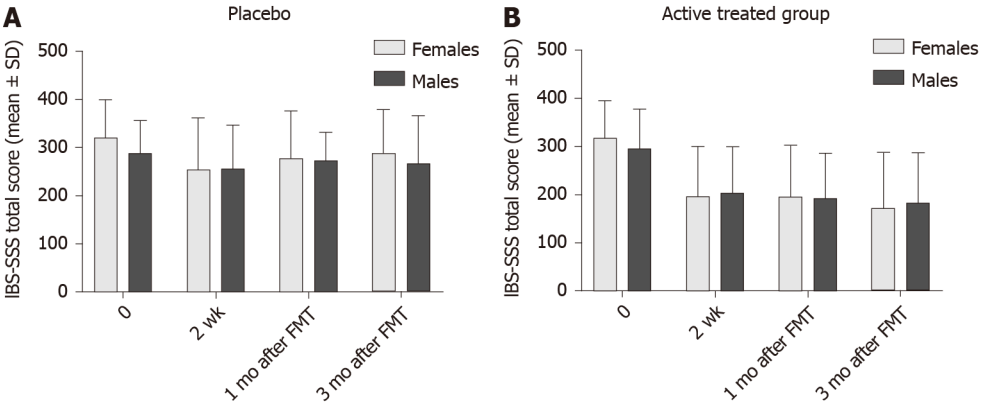

Figure 4 The total irritable bowel syndrome-severity scoring system scores in females and males. A: Placebo group; B: Active treated group. FMT: Faecal microbiota transplantation.

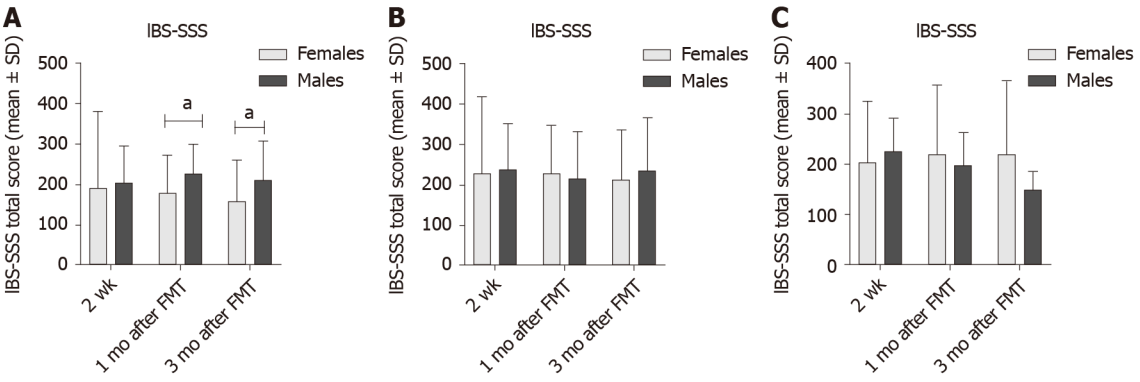

Figure 5 The total irritable bowel syndrome-severity scoring system scores in females and males. A: Irritable bowel syndrome with diarrhoea-predominant; B: Irritable bowel syndrome with constipation-predominant; C: Irritable bowel syndrome with mixed diarrhoea and constipation. $^{a}P < 0.05$. IBD-SSS: Irritable bowel syndrome-severity scoring system; FMT: Faecal microbiota transplantation.

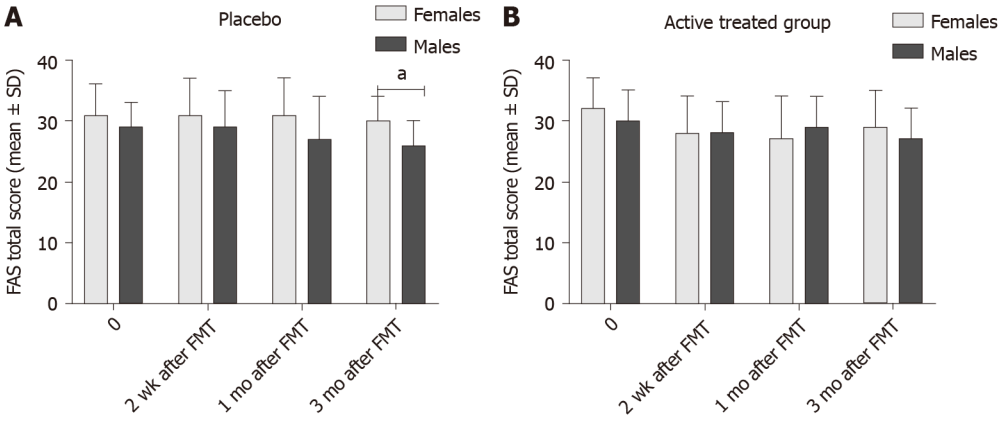

Figure 6 The total Fatigue Assessment Scale scores in female and male irritable bowel syndrome patients. A: Placebo group; B: Active treated group. [a]*P* < 0.05. FAS: Fatigue Assessment Scale; FMT: Faecal microbiota transplantation.

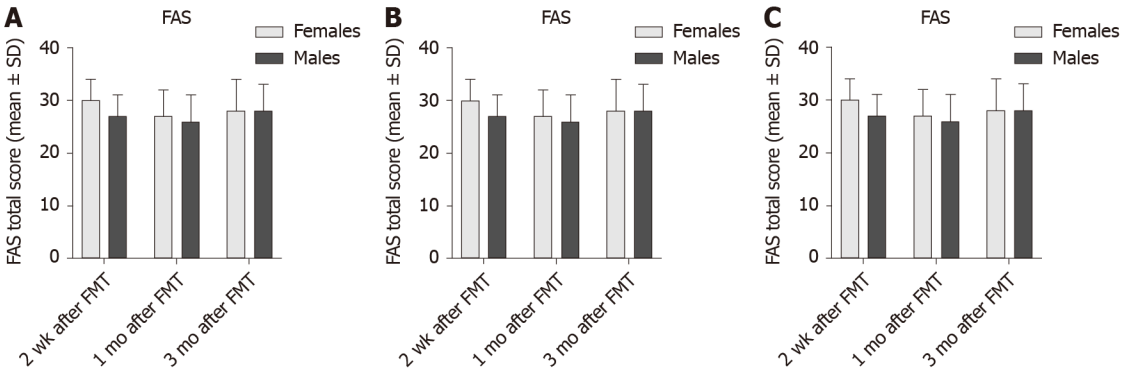

Figure 7 Total Fatigue Assessment Scale scores in female and male patients. A: Irritable bowel syndrome with diarrhoea-predominant; B: Irritable bowel syndrome with constipation-predominant; C: Irritable bowel syndrome with mixed diarrhoea and constipation. FAS: Fatigue Assessment Scale; FMT: Faecal microbiota transplantation.

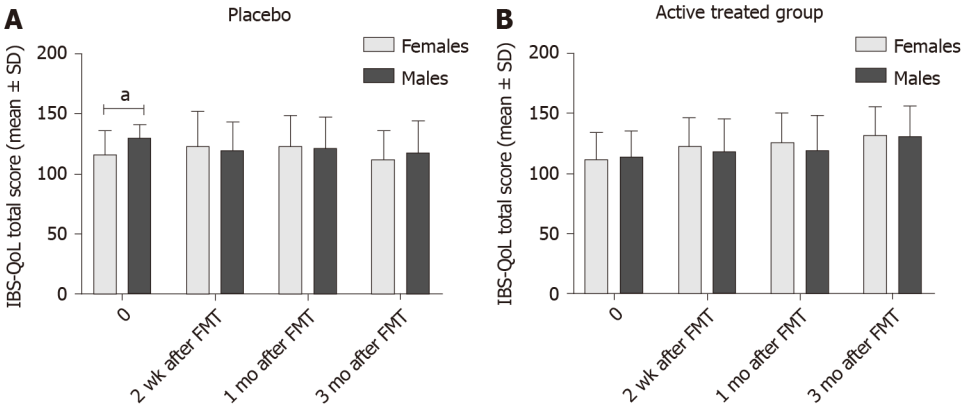

Figure 8 Total irritable bowel syndrome quality of life scale scores in female and male patients. A: Placebo group; B: Active treated group. [a]*P* < 0.05. IBS-QoL: Irritable bowel syndrome quality of life scale; FMT: Faecal microbiota transplantation.

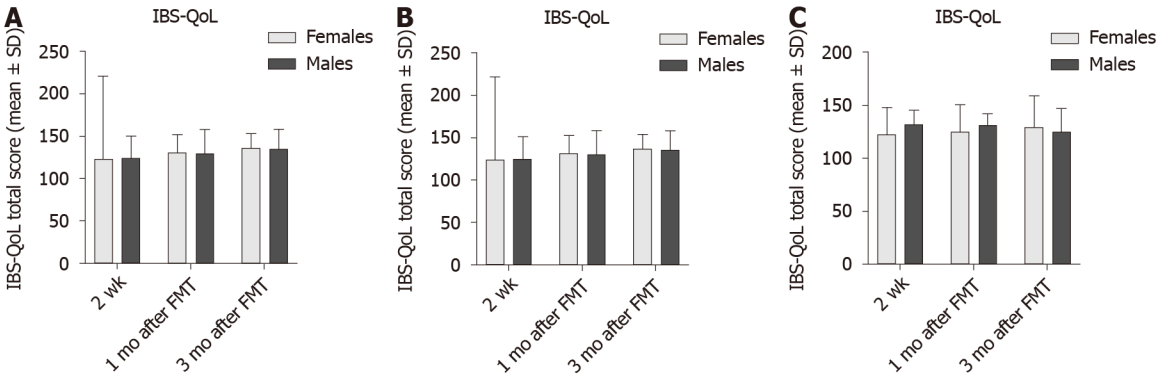

Figure 9 Total irritable bowel syndrome quality of life scale scores in females and males. A: Irritable bowel syndrome with diarrhoea-predominant; B: Irritable bowel syndrome with constipation-predominant; C: Irritable bowel syndrome with mixed diarrhoea and constipation. IBS-QoL: Irritable bowel syndrome quality of life scale; FMT: Faecal microbiota transplantation.

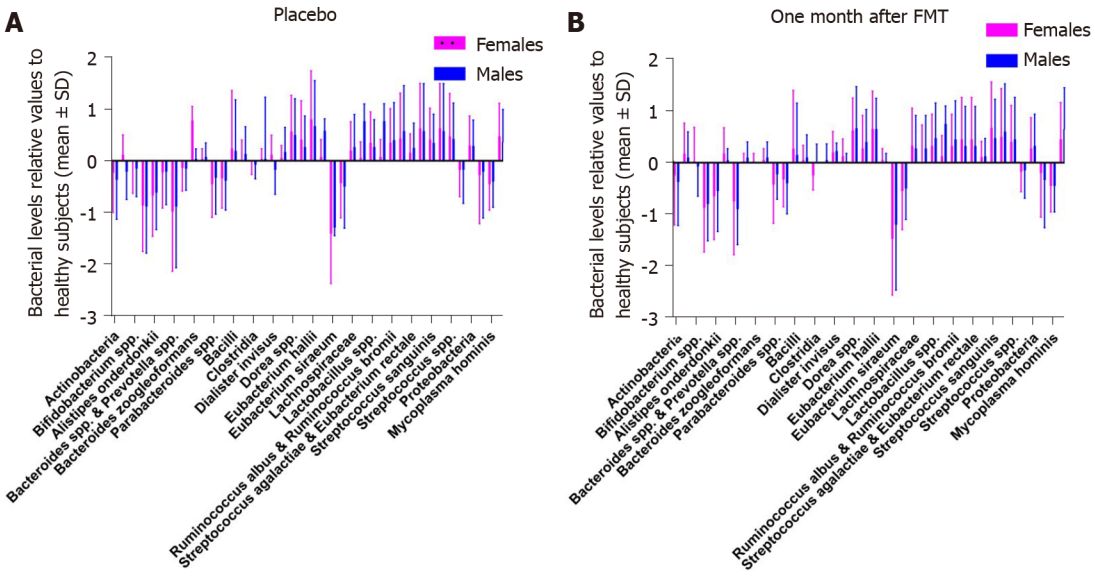

Figure 10 Bacteria levels in the faeces of female and male irritable bowel syndrome patients in the placebo group at the baseline and 1 mo after faecal microbiota transplantation. A: Baseline; B: 1 mo after faecal microbiota transplantation. The bacterial levels are relative values to a normobiotic microbiota profile of 165 healthy subjects. FMT: Faecal microbiota transplantation.

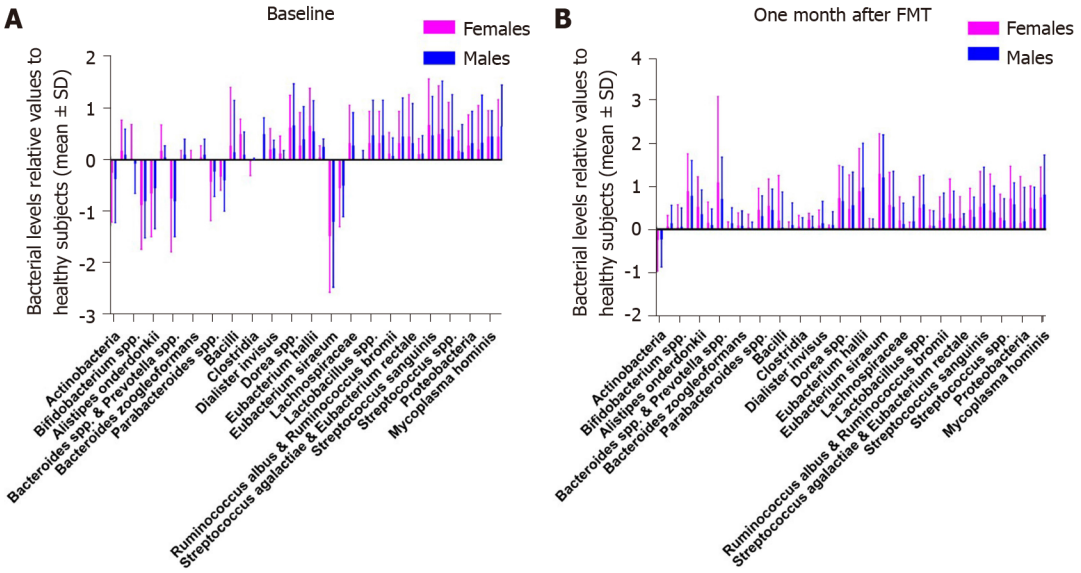

Figure 11 Faecal bacteria levels in the female and male irritable bowel syndrome patients in the active treated group at the baseline and 1 mo after faecal microbiota transplantation. A: Baseline; B: 1 mo after faecal microbiota transplantation. The bacterial levels are expressed as in Figure 10. FMT: Faecal microbiota transplantation.

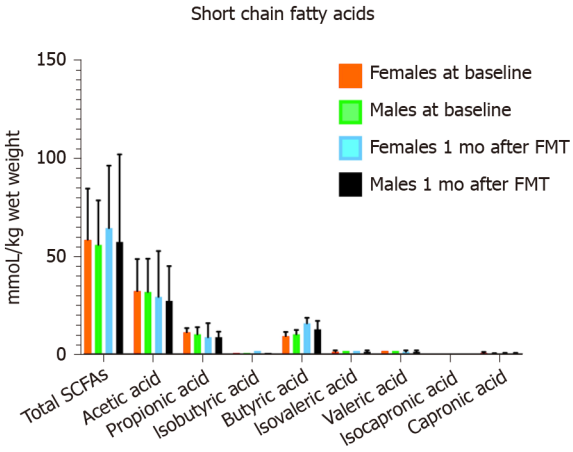

Figure 12 The faecal short chain fatty acids concentration of female and male patients in both the placebo and active treated groups at the baseline and 1 mo after faecal microbiota transplantation. The values were expressed as mmol/kg wet weight (mean ± SD).

ARTICLE HIGHLIGHTS

Research background

Irritable bowel syndrome (IBS) is a common chronic disorder, where intestinal microbiota plays a pivotal role in its pathophysiology. Faecal microbiota transplantation for IBS appears to be a promising treatment of IBS.

Research motivation

In Western countries, there is a female predominance in IBS with female:male ratio of 2:1. In a recent randomized double-blind placebo-controlled trial on faecal microbiota transplantation (FMT) in IBS females responded better to FMT than did males.

Research objectives

We aimed to investigate whether there is a sex difference in the response to FMT in terms of symptoms, dysbiosis, and bacteria and short-chain fatty acids (SCFAs) profiles in the same cohort of patients that we had investigated in our previous randomized controlled trial.

Research methods

This study included 164 patients who fulfilled the Rome IV criteria for the diagnosis of IBS. These patient's cohort included IBS diarrhoea-predominant (IBS-D), IBS-constipation predominant (IBS-C) and mixed diarrhoea and constipation (IBS-M) subtypes. They were randomized to placebo (own faeces), 30 g or 60 g donor's faeces at a ratio of 1:1:1. The faecal transplant was administered *via* gastroscope to the duodenum. Patients completed IBS severity scoring system (IBS-SSS), the Fatigue Assessment Scale (FAS) and the IBS quality of life scale (IBS-QoL) questionnaires at the baseline and 2 wk, 1 mo and 3 mo after FMT. They also provided faecal samples at the baseline and 1 mo after FMT. Response was defined as a decrease of ≥ 50 points in the IBS-SSS total score after FMT. The faecal bacteria profile and dysbiosis were determined by the GA-map Dysbiosis Test (Genetic Analysis, Oslo, Norway) using the 16S rRNA gene. The levels of faecal SCFAs were determined by gas chromatography.

Research results

There was no sex difference in the response to FMT either in the placebo group or active treated group. There was no difference between females and males in either the placebo group or actively treated groups in the total score on the IBS-SSS, FAS or IBS-QoL, in dysbiosis, or in the faecal bacteria or SCFA level. However, the response rate was significantly higher in females with IBS-D than that of males at 1 mo, and 3 mo after FMT. Moreover, IBS-SSS total score was significantly lower in female patients with IBS-D than that of male patients both 1 mo and 3 mo after FMT.

Research conclusions

There is no sex difference in the response to FMT in IBS patients with moderate-to-severe IBS symptoms belonging to the three of IBS subtypes of IBS-C and IBS-M in patients who did not responded to National Institute for Health and Care Excellence-modified diet. However, female patients with IBS-D had a significant higher response rate to FMT and lower IBS-SSS score after FMT than males.

Research perspectives

The present observation that female patients with IBS-D respond better to FMT than males raise several questions as to the cause of this difference. Further studies are needed to explore the difference in diet and life style between females and males as possible causes for this difference.

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