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Dietary intake of sulforaphane-rich broccoli sprouts decreases fecal calprotectin levels in patients with ulcerative colitis

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ABSTRACT

Background: Ulcerative colitis (UC), which is linked to chronic oxidative stress, has been on the rise in Japan, attributed to the recent Westernization of dietary habits. Sulforaphane, which is abundant in broccoli sprouts (BS), has been shown to enhance antioxidant activity by up-regulating nrf2-mediated antioxidant enzymes. We have previously shown that the dietary intake of sulforaphane-rich broccoli sprouts mitigates H.pylori-induced gastritis not only by enhancing nrf2-dependent anti-oxidant activity against oxidative stress from H.pylori infection, but also by directly inhibiting H.pylori activity (Cancer Prev Res 2:353-360,2009). In this study, we examined if the dietary approach with BS affects intestinal microbiota, thereby mitigating colonic inflammation in mesalazine-treated human UC patients.

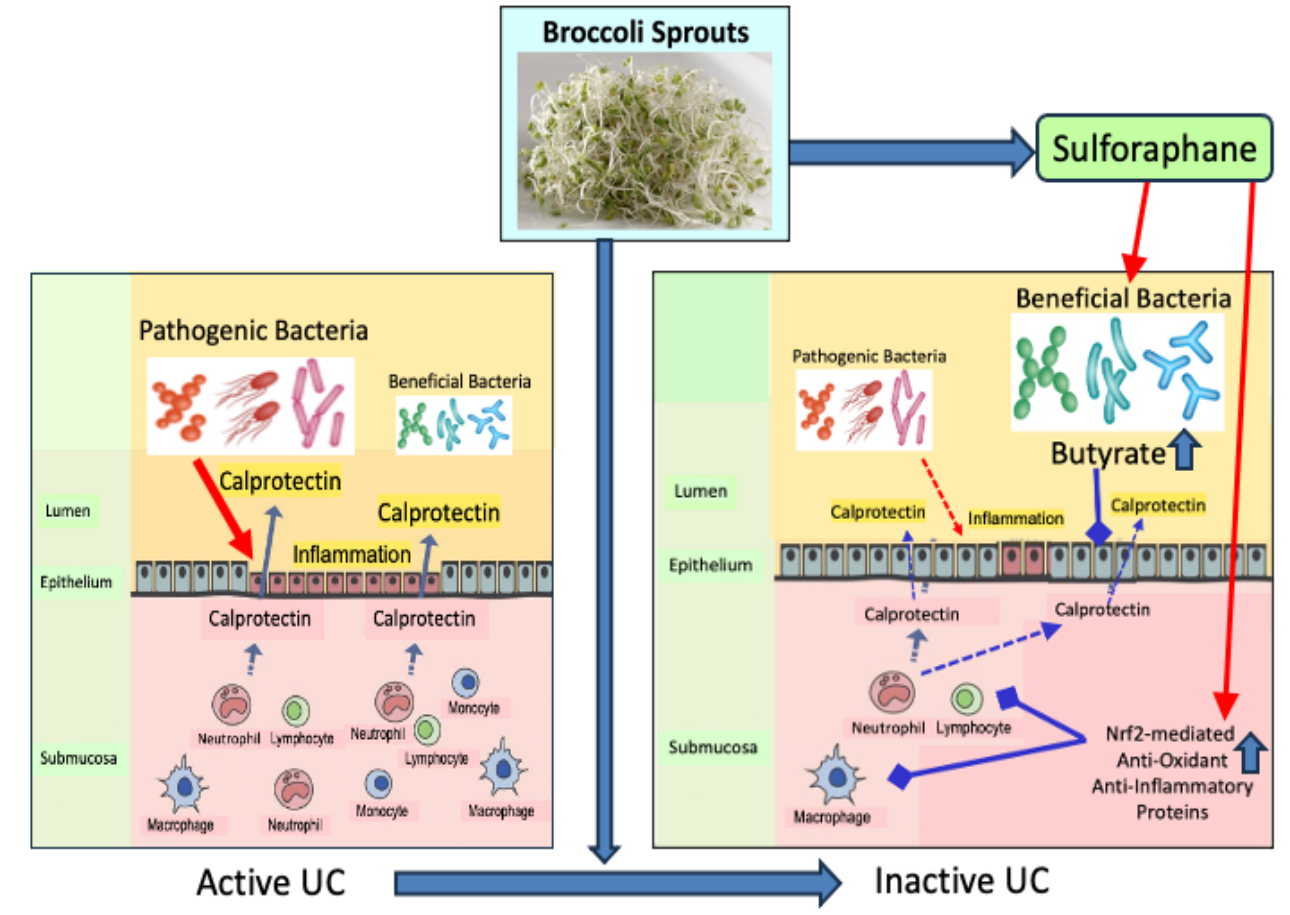
Objective: In this study, we examined if dietary intake of sulforaphane-rich broccoli sprouts decreases fecal calprotectin levels in patients with ulcerative colitis

Methods: This study was registered with the University hospital Medical Information Network (UMIN) in Japan under the Clinical Trial Registration Number, UMIN000041972. Twenty-eight mild UC patients treated with mesalazine were divided between the sulforaphane-rich BS group (n=14) or the sulforaphane-free alfalfa sprouts (AS) group (n=14).

These subjects were instructed to take 20 g of raw BS or AS daily for 8 weeks, with BS containing 4.4 mg/g glucoraphanin, a precursor of sulforaphane, and AS containing no glucoraphanin. Stool samples were obtained just before and after the 8 weeks sprouts treatment. Levels of fecal calprotectin were measured as the quantitative indices for colonic mucosal inflammation. We conducted an examination of gut bacteria through a method called terminal restriction fragment length polymorphism (TR-FLP) analysis of fecal samples.

Results: 1. Twelve patients from the BS group and 11 from the AS group completed the intervention study as requested. 2. Only the treatment with BS caused a significant decrease in the level of fecal calprotectin as well as an increase in the fecal component of Clostridium subcluster IV and XIVa, which have been reported to enhance butyrate production. 3. There was no change in the clinical activity index after intake of either the BS or the AS.

Conclusion: These results indicate that dietary approach with sulforaphane-rich BS mitigates colonic inflammation in mesalazine-treated UC patients. Our data also suggest that the beneficial effects of the BS may be related with the increase in butyrate-producing intestinal microbiota by dietary approach with sulforaphane.



Graphical Abstract: Dietary intake of sulforaphane-rich broccoli sprouts decreases fecal calprotectin levels in patients with ulcerative colitis

INTRODUCTION

Ulcerative colitis (UC) has been increasing in Japan due to the Westernization of the Japanese diet [1]. Most recent UC patients do not show severe symptoms, presumably due to development of several kinds of new drugs, especially biological drugs [2]. However, these new drugs sometimes cause severe adverse reactions, which significantly impair patients' quality of life [2]. On the other hand, mesalazine has long been recognized as safer than other drugs and has been widely used to control colonic inflammation in mild UC patients and to prevent relapse of UC [3]. However, some of the mesalazine-treated UC patients show some resistance or allergy to mesalazine [4]. Thus, it seems likely that mesalazine alone is not enough to control mild UC patients. Since the occurrence of UC is strongly associated with oxidative stress [5], it seems reasonable to assume that dietary approach with antioxidant food may be useful to mitigate colonic inflammation in UC patients [6]. Sulforaphane (SFN), an antioxidant-inducing compound rich in cruciferous vegetables, especially broccoli sprout (BS) [7], has been known to activate the nrf2-dependent antioxidant system [8]. Previously, we have shown that the dietary intake of sulforaphane-rich BS reduces gastritis in *H.pylori*-infected human patients [9] and normalizes bowel function in patients with chronic constipation [10]. These effects appear to be relieved in part by inducing antioxidant enzymes and/or by suppressing pathogenic bacteria in gastrointestinal tract [9-10]. Based on this background, we hypothesized that dietary approach with antioxidant inducer, SFN, may mitigate colonic inflammation and prevent UC relapse in mesalazine-treated UC patients. In this study, we examined if a dietary approach with SFN-rich BS mitigates colonic mucosal inflammation in mesalazine-treated UC patients.

Materials and Methods: To assess whether daily intake of BS reduces colonic mucosal inflammation in UC patients, we designed a placebo-controlled intervention trial with a semi-open design. Approved by the Hitachi General Hospital and the Tsukuba Gakuen Hospital ethical committees, with the respective Approval Numbers 2020-43 and 21-01, the study was then registered with the University Hospital Medical Information Network in Japan (U-Min: UMIN000041972). The trial was registered with the name: "Clinical trial on the effects of dietary intake of sulforaphane-rich broccoli sprouts on severity of ulcerative colitis."

Primary Endpoint: The primary endpoint of this study was fecal calprotectin level, which reflects degree of colonic mucosal inflammation [11]. The secondary endpoints were composition of fecal microbiota, and clinical disease activity, which were evaluated as stated below.

Determination of Sample Size: Sample size calculation for this clinical trial was conducted according to the method described by others [12]. In brief, we predicted that BS treatment causes approximately 20 % decreases in fecal calprotectin by the BS intervention, since our previous report showed BS treatment caused 20% decreased serum pepsinogen level, an inflammation marker of gastritis, in *H.pylori*-infected human subjects [9]. Using the average and standard deviation of the baseline fecal calprotectin levels across all participants, we determined that a minimum of 23 subjects per group was necessary to maintain a power of 0.80 with a one-

tailed significance level of 0.05. Considering possible dropouts, the target sample size was calculated as 23 subjects in each group, i.e. 50 subjects in the whole study. Since we were not able to recruit required number of patients during the 3 years initially designed for this study, we decided to analyze the preliminary data obtained from 32 participants.

Participant Recruitment: Subjects recruited from the outpatient clinic of each hospital participated in this study from October 2020 to March 2023. All subjects agreed to partake in the study after being informed and signed a consent form. All the subjects had been diagnosed with UC by a colonoscopy conducted within the past two years and were diagnosed as clinical remission based on the colonoscopy findings and clinical symptoms. Of the participants who showed clinical activity index (CAI) (or Rachmilewitz index) [13], less than 5 were eligible for enroll the present study. All the participants had been followed by prescription of mesalazine alone or mesalazine in combination with probiotics during the past 12 months. The patients who were treated with either steroids, biological drugs, immunomodulators, or other drugs, which may have affected colonic inflammation, were excluded in this study. The patients who had been treated with cytapheresis, such as leukocytapheresis or granulocyte monocyte apheresis were also excluded. Individuals with a history of gastrointestinal issues or conditions affecting other organs, including the liver, kidneys, and endocrine system, were excluded from the study. According to these criteria, we recruited 32 subjects with mild UC. Among the 32 subjects, 4 patients were excluded, since the fecal calprotectin levels were less than 50 μg/g. Finally, the remaining 28 subjects enrolled the sprouts intervention trial.

Protocol for Sprouts Intervention: This study used SFN-rich BS which contains 4.4 mg/g of glucoraphanin (or sulforaphane glucosinolate), a precursor of SFN. For the placebo control, alfalfa sprouts (AS) were used since they scarcely contain glucoraphanin (Table 1). We have confirmed in our previous reports that the BS group showed high amount of urinary level of dithiocarbamates, a metabolite of SFN, while AS group showed no dithiocarbamates in urine [9]), suggesting that most of the absorbed SFN in BS group were derived only from the assigned BS. These previous data also suggest that ordinary daily diet for the participants did not contain SFN.

Other substituents included in both sprouts are shown, in accordance with the Standard Tables of Food Composition in Japan (http://www.mext.go.jp/a_menu/syokuhinseibun/1365297.htm) (Table 1). Although this table shows some differences in the number of other substituents between BS and AS, this difference could be masked in this study, since the total amounts of these substituents included in 20 g of sprouts are far smaller than those included in their daily diet. In addition, both sprouts have similar appearances (Figure 1). All the sprouts were sourced from Murakami Farm Co. Ltd and delivered directly to participants' home or office twice a week during the treatment period.

Table 1. Major Nutrients in Broccoli Sprouts and Alfalfa Sprouts (/100g). Major difference between BS and AS was broccoli sprout contains high concentration of glucoraphanin, while alfalfa sprouts contain no glucoraphanin.

			Broccoli Sprouts	Alfalfa Sprouts
Glucoraphanin		mg	440	0
Energy		kcal	41	12
Water		g	87.4	96
Protein		g	4.7	1.6
Lipids		g	0.7	0.1
Carbohydrates		g	6.6	2
Minerals		g	0.6	0.3
Sodium		mg	3	7
Potassium		mg	105	43
Calcium		mg	66	14
Magnesium		mg	43	13
Phosphorus		mg	121	37
Iron		mg	1.1	0.5
Zinc		mg	0.6	0.4
Copper		mg	0.04	0.09
Manganese		mg	0.55	0.1
β-carotene		µg	930	56
Retinol		µg	78	5
Vitamins	D	µg	0	0
	E	mg	2.5	1.9
	K	µg	125	47
	B1	mg	0.16	0.07
	B2	mg	0.17	0.09
	Niacin	mg	2.6	0.2
	B6	mg	0.3	0.1
	B12	µg	0	0
	Folic Acid	µg	170	56
	Pantothenic Acid	mg	1.04	0.46
	C	mg	80	5
Dietary Fibers	Water Soluble	g	0.3	0.1
	Water Insoluble	g	1.8	1.3
	Total Amount	g	2.1	1.4

The 28 participants who satisfied the criteria described above were assigned to the SFN-rich BS group (n=14) or the SFN-free AS group (n=14) (Table 2A, Figure 1).

Participants were allocated to each group to prevent a significant difference in age, male/female ratio, clinical activity index, and fecal calprotectin by a gastroenterologist, who did not conduct this study (Table 2A). The results of the allocation were informed neither to the participants, nor to the doctors who conducted the intervention. Since the shape and the taste of the BS and the AS used in this study appear to be very similar, we believe that this placebo-controlled two arm study was conducted basically in a double-blinded fashion. Subjects were not informed of their assigned groups, and all were requested to eat 20 g of their designated 20 g of the raw sprouts without cooking with other foods basically at breakfast every day for 8 weeks. To minimize the impact of external factors on the data, participants were instructed to avoid consuming cruciferous vegetables, fermented foods, laxatives, probiotics, and antibiotics during the entire 8-week intervention and the 2-week pre-trial phase. During the 8 weeks, stool samples for fecal calprotectin assay and fecal microbiota were collected immediately before the treatment, and at the end of the 8-weeks (Figure 1).

Data was obtained from the initial allocation, ie. intention to treat base [A], and from only the patients, who completed the sprouts intervention study, as initially planned, ie per protocol base [B]. In both [A] and [B], there were no differences in age, male/female ratio, clinical activity index, and initial fecal calprotectin level between the broccoli sprouts and the alfalfa sprouts groups.

Table 2: Patient profile in the sprout intervention study

[A] Intention to Treat	Broccoli Sprouts	Alfalfa Sprouts
Number of Subjects	14	14
Sex (M:F)	9:5	9:5
Age (years)	51.3 ± 3.95	50.9 ± 5.16
Clinical Activity Index	0.71 ± 0.16	0.79 ± 0.19
Fecal Calprotectin (ug/g)	1,557 ± 489	1,688 ± 604
[B] Per Protocol	Broccoli Sprouts	Alfalfa Sprouts
Number of Subjects	12	11
Sex (M:F)	7:5	6:5
Age (years)	50.4 ± 4.44	55.8 ± 5.45
Clinical Activity Index	0.64 ± 0.24	0.73 ± 0.24
Fecal Calprotectin (ug/g)	1,575 ± 657	984 ± 513

Values are expressed as mean ±SD

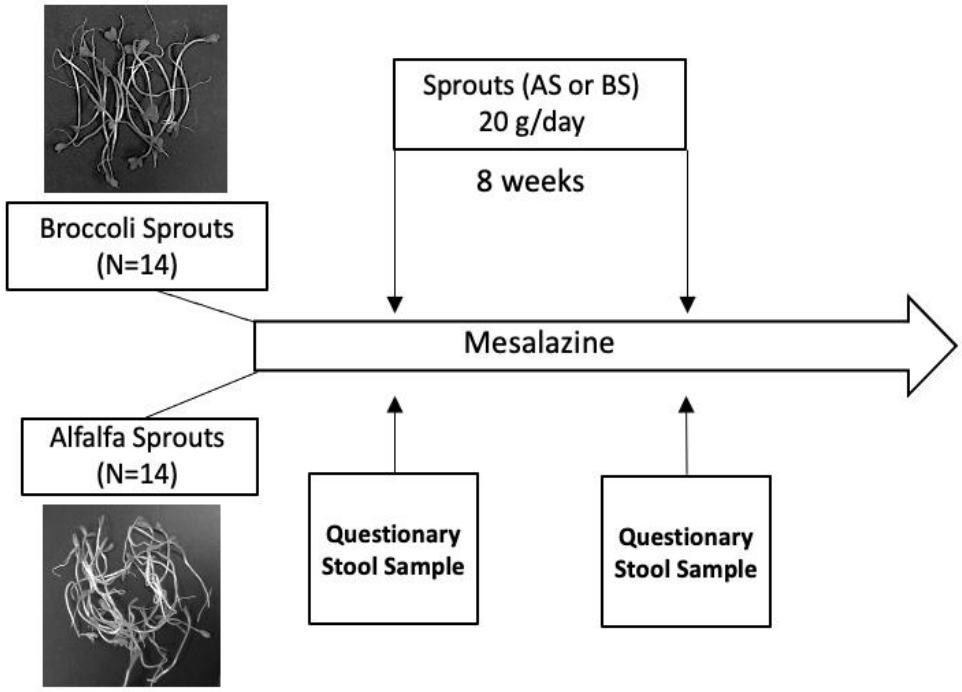


Figure 1. Protocol for the clinical trial on the effect of dietary BS or AS on mesalazine-treated UC.

Sample Analysis: The levels of calprotectin in the stool samples were measured using fluorescence enzyme immunoassay kit provided by SRL Co, Ltd Japan, as the quantitative indices of colonic mucosal inflammation [13]. The microbiota composition in the stool samples were evaluated via TR-FLP flora analysis at Techno Suruga Lab in Shizuoka, Japan [14], revealing the percentage of *Bifidobacterium, Lactobacillus, Bacteroides, Prevotella, Clostridium* [cluster IV, IX, XI, XIVa, XVIII], and other organisms.

Data Analysis: For continuous data that followed a normal distribution, we applied a Student's t-test. In cases where the continuous data did not adhere to a normal distribution, as well as for discrete data, we utilized non-parametric methods, specifically the Wilcoxon signed-rank test for comparisons. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

1. Clinical Trial Flow Diagram during the Sprouts Intervention: In this study, one patient from each group was excluded from the data analysis, because they did not eat the number of sprouts requested, for personal reasons. The rest of the participants completed the sprout intervention trial, as requested, without showing any subjective symptoms or adverse reactions during the 8 weeks intervention period. One participant from the BS group and two from the AS group were excluded from the final analysis due to insufficient stool samples for accurate calprotectin level measurement. As a result, changes in the paired data on the fecal calprotectin from 12 patients in the BS group, and from 11 patients in the AS group were assessed (Figure 2). There were no differences in age, male/female ratio, clinical activity index, and starting fecal calprotectin level between the BS and the AS group on the per protocol base (Table 2B).

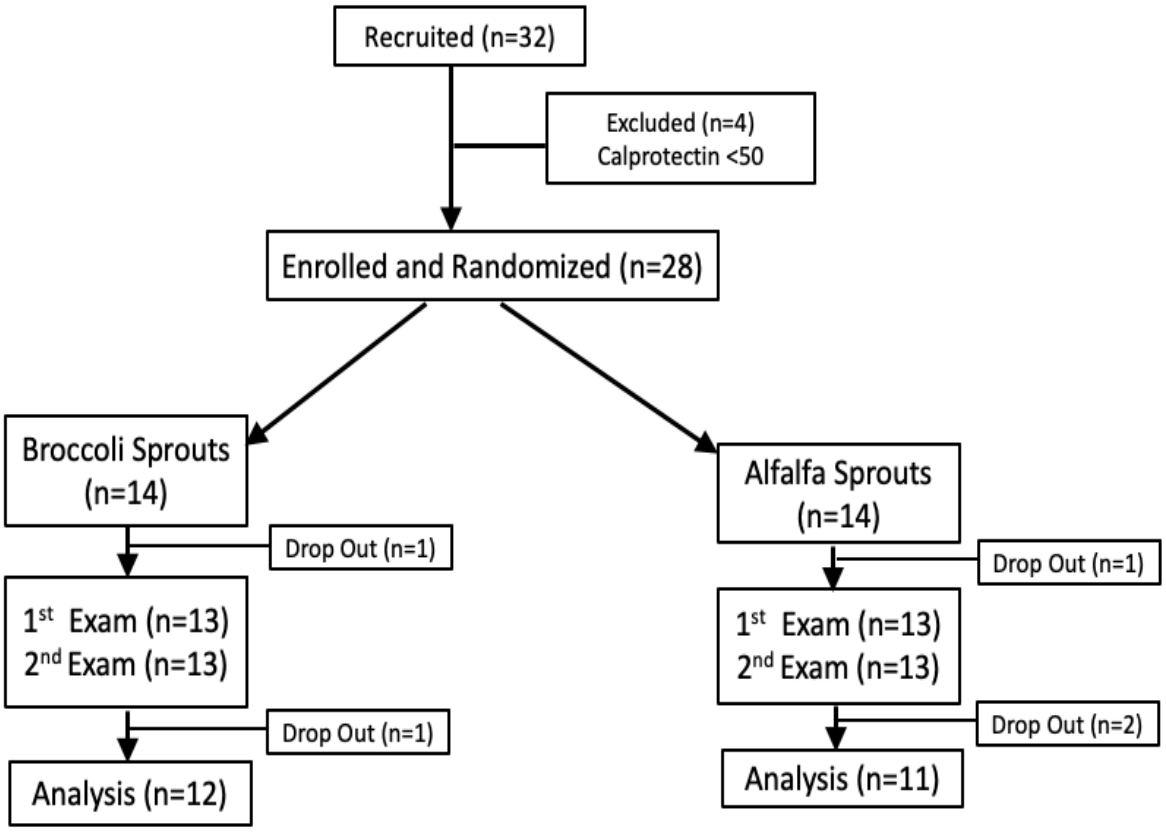


Figure 2. Flow diagram of the clinical trial examining the impact of a sprouts intervention on colonic inflammation and intestinal microbiota in patients with ulcerative colitis treated with mesalazine.

2. Changes in Clinical Activity Index after BS/AS intervention: No change in clinical activity index (CAI) was demonstrated after the intervention in both the BS and the AS groups. (Table 3)

Table 3: Changes in Clinical Activity Index after the sprout's intervention

	Sample #	Before	After
Broccoli Sprouts Group	12	0.67 ± 0.19	0.58 ± 0.26
Alfalfa Sprouts Group	11	0.73 ± 0.24	0.64 ± 0.31
Values are expressed as mean ±SD			

No change in clinical activity was demonstrated after intake of either the broccoli sprouts or the alfalfa sprouts.

3. Changes in the level of fecal calprotectin after BS/AS intervention: Levels of fecal calprotectin significantly decreased after the BS treatment, while no changes were demonstrated after the AS treatment (Figure 3). No significant difference was observed in the absolute fecal calprotectin levels between the BS and AS groups at the post-intervention period. However, the percentage of the fecal calprotectin level after the intervention, compared to the corresponding values before the treatment were 56.5 $\pm$ 17.0% in the BS group, and 664 $\pm$ 466% in the AS group, showing a significant difference between the BS and the AS group ($P<0.05$; mean $\pm$ SD).

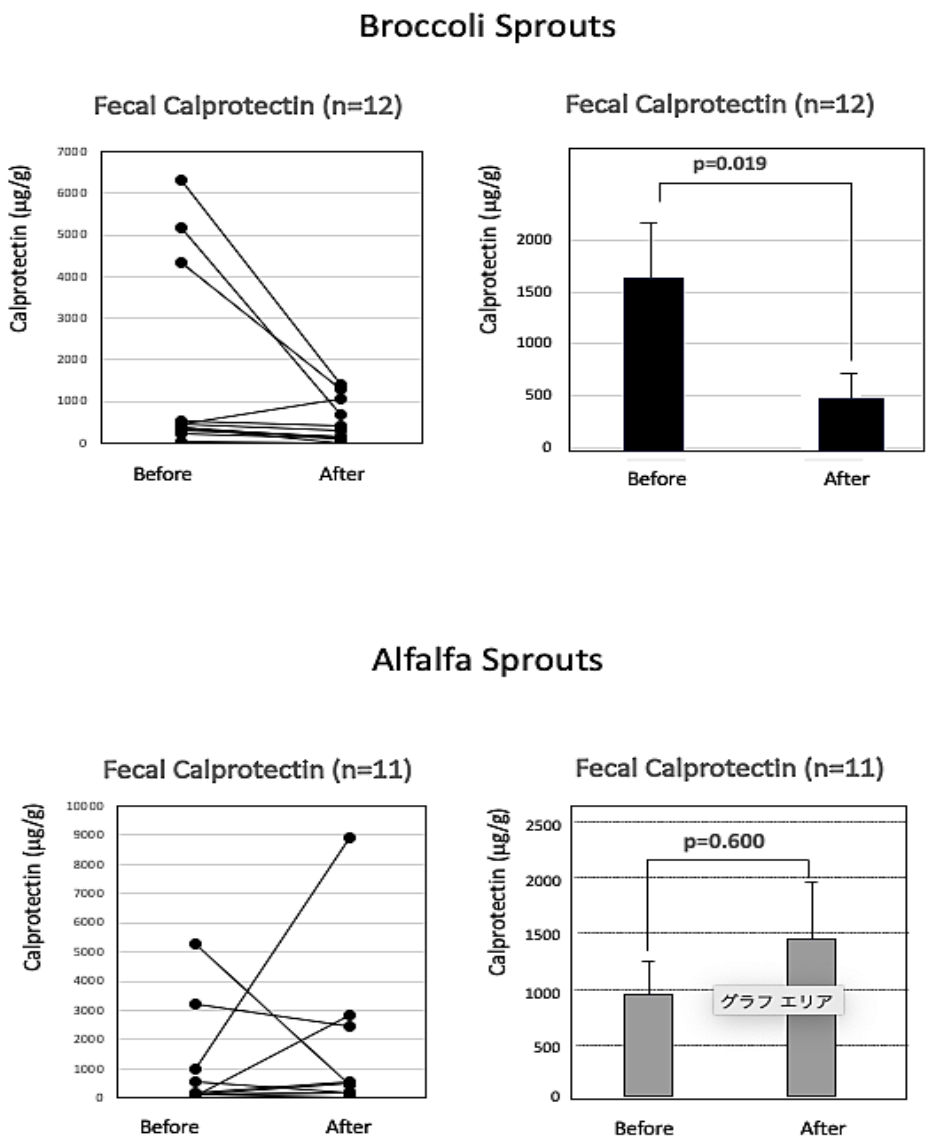


Figure 3: Changes in Fecal Calprotectin after Sprouts Intervention.

4. Changes in composition of fecal microbiota after BS/AS intervention: Composition of *Clostridium subcluster IV and XIVa* increased significantly after the BS treatment. In contrast, no changes were demonstrated after the AS treatment (Table 4, Figure 4). No significant difference was found in the absolute composition of Clostridium clusters IV and XIVa between the BS and AS groups at the post-intervention period. However, the changes observed in the composition of Clostridium cluster IV were +1.76 ± 3.21 in the BS group, compared to -1.08 ± 4.54 in the AS group, indicating a statistically significant difference (mean ± SD; $P < 0.05$). In contrast, the changes in Clostridium subcluster XIVa showed +2.80 ± 4.53 in the BS group and -2.80 ± 10.4 in the AS group, revealing no significant difference between the two groups (mean ± SD; $0.05 < P < 0.10$).

Table 4: Changes in Composition of the Colonic Microbiota after the sprout's intervention.

		Before	After
Broccoli Sprouts Group (n=12)			
Bacteria in Stool Samples			
	Bifidobacterium	8.57 ± 2.28	9.97± 2.35
	Lactobacillales	3.08 ± 0.89	2.59± 0.75
	Bacteroides	30.5 ± 4.32	28.1 ±3.28
	Prevotella	4.31 ± 2.36	3.77 ±2.35
	Clostridium cluster IV	**6.56 ±1.44**	**8.32 ± 1.87***
	Clostridium subcluster XIVa	**21.8 ±1.46**	**24.6 ± 1.77***
	Clostridium cluster IX	4.46 ±1.29	4.17 ± 1.24
	Clostridium cluster XI	0.37 ±0.074	0.32 ± 0.11
	Clostridium cluster XVIII	0.078 ±0.054	0.078 ±0.045
	Others	17.4 ± 1.61	16.1 ±1.41
Alfalfa Sprouts Group (n=11)			
Bacteria in Stool Samples			
	Bifidobacterium	5.83 ± 2.25	6.08± 1.87
	Lactobacillales	2.21 ± 1.12	2.35 ± 0.76
	Bacteroides	30.0 ± 3.08	27.7 ± 4.23
	Prevotella	4.52 ± 2.99	5.43 ± 3.55
	Clostridium cluster IV	6.76 ± 1.90	5.68 ± 1.66
	Clostridium subcluster XIVa	29.7 ± 3.05	26.9 ±3.20
	Clostridium cluster IX	3.76 ± 1.26	4.40 ± 1.35
	Clostridium cluster XI	0.61 ± 0.40	1.15 ± 0.62
	Clostridium cluster XVIII	0.76 ± 0.73	0.20 ± 0.17
	Others	14.4 ± 1.45	18.1 ± 2.09

Values are expressed as mean ±SD
** p<0.05; significant difference from the corresponding vale before the intervention.*

Decreases in composition of the *Clostridium cluster IV and subcluster XIVa* were demonstrated after the BS treatment, while no change in composition of the intestinal microbiota was demonstrated after the AS treatment.

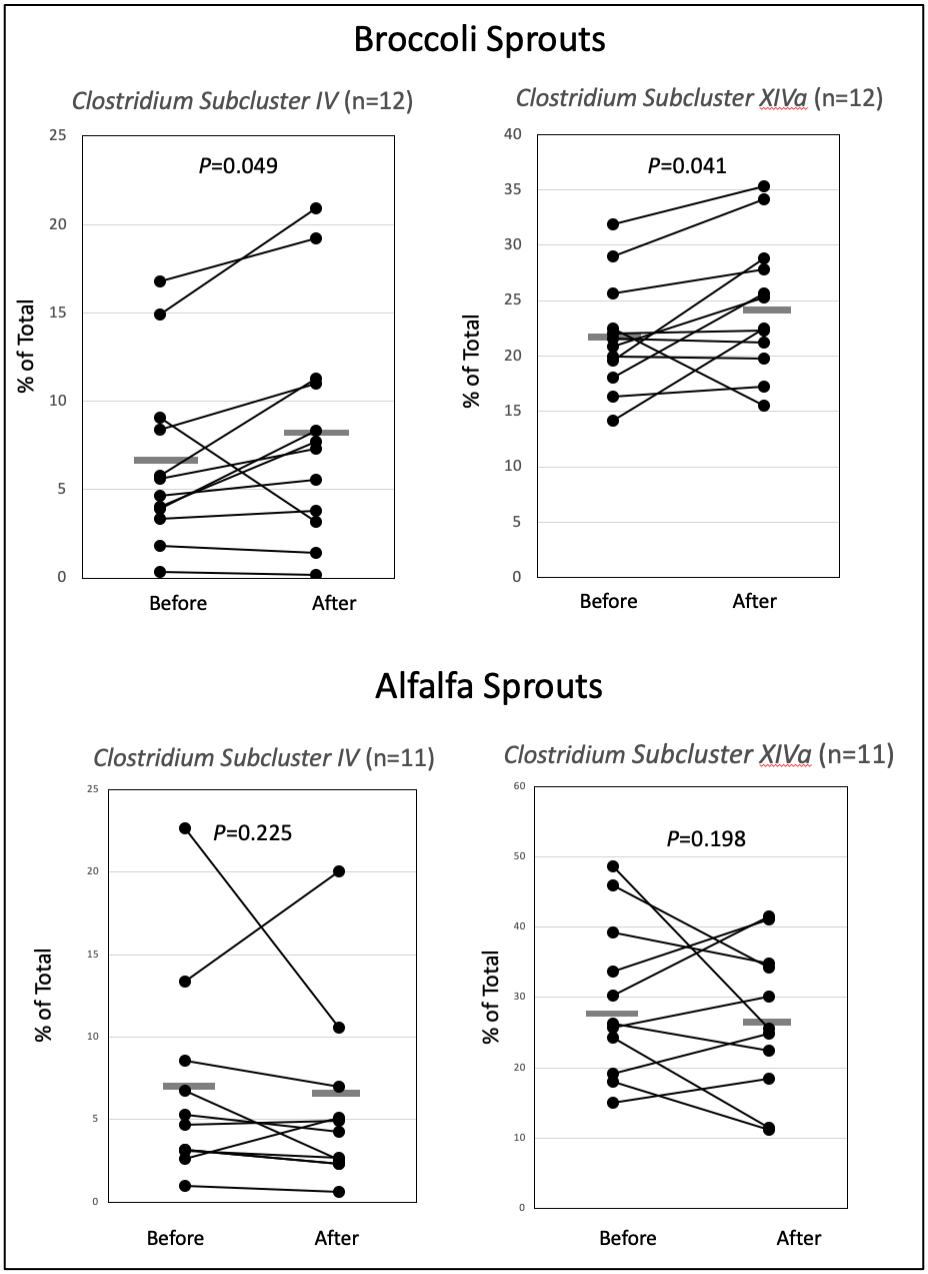


Figure 4; Changes in Butyrate Producing Microbiota after Alfalfa Sprouts Intake, Horizontal Bars show Means

DISCUSSION

We decided to use fecal calprotectin level as the index of colonic mucosal inflammation in mild UC patients since the average levels of fecal calprotectin of the patients (range between 1,500 and 1800 ug/g) were far greater than the cut off value of fecal calprotectin (50.0 ug/g [13]) even though all of these patients showed very low CAI values (less than 2). This data suggests that fecal calprotectin is a far more sensitive index than CAI for evaluating subtle changes in colonic mucosal inflammation after the sprout's intervention. In fact, our sprouts intervention trial in this study demonstrated that 8 weeks treatment with dietary BS significantly decreased fecal calprotectin levels in human UC patients, even though we did not observe significant decreases in CAI in both BS and AS group. These results strongly suggest that SFN-rich BS mitigates colonic mucosal inflammation in the mild UC patients who were

diagnosed as clinical remission, judged by endoscopic findings and clinical symptoms.

The exact mechanisms by which SFN ameliorates colonic mucosal inflammation in UC patients have not been clarified until recently. We assume that nrf2-mediated antioxidant effect of SFN [8] plays an important role in this protection, since numerous studies have shown that excessive amounts of free radicals are generated in UC patients by factors such as autoimmune abnormalities, changes in microbiota, and increasingly Western dietary habits [1,15-16]. We have previously reported that dietary SFN prevents chemically induced colon cancer in mice, and reduces the number of aberrant crypt foci, premalignant lesions in human patients with colonic adenoma [17]. Thus, it seems likely that the SFN included in BS activates antioxidant enzymes in colonic mucosa, thereby mitigating colonic mucosal inflammation in UC patients. Upregulation of antioxidant enzymes by dietary intake of SFN-rich BS has already been studied, and the administration of oral glucoraphanin, a precursor of SFN, mitigated gastrointestinal mucosal inflammation in mice and humans, accompanied by up-regulation of hemoxygenase-1 and decrease in myeloperoxidase activity [9].

In the present study, we also found that the composition of the *Clostridium subcluster IV and XIVa,* which are known to enhance butyrate production [18], increased significantly after the 8 weeks intake of BS, but not of AS, suggesting that SFN included in BS contributes to these changes. Recent studies have suggested that butyrate producing bacteria in colonic lumen has beneficial effects on colonic mucosa [19-20]. Thus, it seems reasonable to assume that increase in the butyrate producing microbiota by dietary intake of SFN-rich BS, also contributes to attenuation of colonic mucosal inflammation. Further studies are necessary to clarify the mechanisms by which dietary intake of BS increases butyrate level in colonic lumen in the mild UC patients. On the other hand, the antibacterial effect of SFN on *H.pylori* has been shown recently [21]. We have also reported in an earlier report that SFN decreases colonization of *H.pylori* in gastric mucosa [9] and suppresses the proliferation of pathogenic bacteria in small intestinal mucosa against nonsteroidal anti-inflammatory drugs in mice [22]. The alteration of colonic microbiota by SFN has been reported in DSS-treated mice elsewhere [23]. Based on the present data, we assume that the protective effects of SFN-rich BS on colonic mucosa in UC patients are mediated not only by a nrf2-dependent antioxidant effects of SFN, but also by stimulating proliferation of beneficial intestinal microbiota.

Instead of using glucoraphanin tablets, we used raw BS in our study. It has been reported that glucoraphanin, a precursor of SFN, included in BS, is biologically inactive [24]. However, once administered orally into the gastrointestinal tract, most of the glucoraphanin in BS is converted to biologically active SFN by myrosinase activity in the raw BS during chewing BS in the oral cavity [24]. The rest of the glucoraphanin is converted into biological active SFN by myrosinase activity in the intestinal microbiota. The oral intake of BS induces much higher concentrations of systemic SFN compared to taking the same amount of oral glucoraphanin tablet, which was confirmed by an increase in urinary levels of dithiocarbamate, a metabolite of SFN [9,25]. These results indicate that the better bioavailability of SFN can be obtained by chewing raw BS rather than taking glucoraphanin tablets orally. Furthermore, a recent study suggests that polysulfides, such as cysteine hydropersulfide and glutathione hydropersulfide, are included abundantly in BS and also demonstrate potent antioxidant effects via the nrf2-mediated system [26]. This supports our conclusion that taking raw BS orally provides more potent action than taking a glucoraphanin

tablet in terms of amelioration of chronic oxidative stress in the colonic mucosa in UC patients.

According to literatures, there have been no definition for the term, “functional foods” in the US until recently [27-29]. This situation has been the same in Japan. However, Martirosyan et al. from the Functional Food Center in the US, recently proposed a new definition for functional foods, as follows [27-29]. They proposed that functional foods are “natural or processed foods that contain biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms." We believe that this concept is very important when we consider developing new functional foods products, which show obvious benefit for human health in clinical practice. Regarding the previous studies on broccoli sprouts, numerous reports have shown that broccoli sprouts are natural foods that contains precursor of biologically active components, sulforaphane [7-8]. Furthermore, our data from the present study clearly show that dietary approach with non-toxic amounts of broccoli sprouts provides beneficial effects on colonic mucosal inflammation in UC patients, which is evaluated quantitatively by a specific biomarker, fecal calprotectin, induces better management of their symptoms, reduces risk of UC recurrence, and presumably prevents occurrence of colitis-associated colon cancer in the future. Taken together, we believe that our data provide strong evidence for broccoli sprouts as one of the functional foods, which clearly promotes human health.

Finally, we would like to emphasize that this is the first study to demonstrate that dietary intake of sulforaphane-rich broccoli sprouts mitigates colonic mucosal inflammation in patients with ulcerative colitis. The present study further suggests dietary approach with antioxidant food, such as sulforaphane-rich broccoli sprouts, not only mitigates colonic inflammation, but also contributes to keep remission in mesalazine-treated ulcerative colitis patients, indicating that dietary approaches by functional foods in combination with medical drugs could provide most beneficial effects on chronic inflammatory diseases.

Study Limitations: To start, the sample size in this trial fell short of the initially calculated requirement, which suggests that the data may still be preliminary. Consequently, we should consider planning another BS intervention study that involves a larger cohort of UC patients. Additionally, Second, while this study has suggested that BS increases the composition of the butyrate-producing microbiota, we need to examine if it specifically increases the butyrate level in the colonic lumen. Third, although this study was performed in a double-blinded fashion, BS and AS have minute differences that some participants may have been able to identify. Therefore, we must recognize that this study was not conducted in a completely double-blinded fashion. In addition, other dietary components may have affected the data during the trial period. Thus, another clinical trial using pure SFN, such as via glucoraphanin tablets, instead of using BS, must be conducted. However, most importantly, we were able to ameliorate colonic mucosal inflammation in UC patients by a dietary approach with SFN-rich BS.

Conclusion: This study demonstrated that a dietary approach with SFN-rich BS mitigates colonic inflammation in human UC patients treated with mesalazine. Our findings indicate that the positive effects of SFN-rich BS may be driven by the activation of the Nrf2-dependent antioxidant system, which helps combat chronic oxidative stress.

Abbreviations: AS: alfalfa sprouts**,** BS: broccoli sprouts**,** CAI: clinical activity index**,** SD: standard deviation, SFN:

sulforaphane**,** UC: ulcerative colitis

Authors Contribution: Akinori Yanaka designed the protocol of this study, obtained research funds, submitted proposal to ethical committees in the hospitals, analyzed the obtained data, and wrote the manuscript. Toshihide Ohmori, Masanori Ochi, and Hideo Suzuki checked the original protocol and the manuscript, and made some helpful comments. All the authors contributed to participants recruitment and conducted the clinical trial.

Competing Interests: Akinori Yanaka had been an endowed chair, supported by Hitachi Co. Ltd. between 2020 and 2021. Akinori Yanaka has been received consulting fee from Murakami farm Co. Ltd, Japan. Masanori Ochi has been an endowed assistant professor supported by Hitachi Co. Ltd., since 2021.

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