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Dietary Borage Oil Promotes Gamma-linoleic Acid Accumulation in Dextran Sulfate Sodium-treated Mice but Does not Manipulate the Severity of Colitis

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Abstract In this study, we examined the effect of borage oil (BO), which is rich in gamma-linolenic acid (GLA), on GLA accumulation in the bowel, and on the condition of dextran sulfate sodium (DSS)-induced colitis in mice. The results imply that dietary BO increased GLA accumulation in the liver and intestinal triglycerides (TG) and liver phospholipids (PL), whereas GLA accumulation in the intestinal PL was not significantly different between mice fed with BO and those with safflower oil diet. Although there were concerns about accumulation of arachidonic acid (AA) because it can be a substrate for the biosynthesis of proinflammatory eicosanoids, apparent AA accumulation in intestinal PL and exacerbation of colitis condition were not observed in BO groups. Taken together, it is considered that because of inefficient GLA accumulation in intestinal membrane, dietary BO did not manipulate DSS-induced colitis condition.

Keywords: *borage oil, colitis, dextran sulfate sodium, gamma linolenic acid, fatty acid composition*

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1. Introduction

Inflammatory bowel disease (IBD) is a collective term for conditions like ulcerative colitis and Crohn’s disease, which are responsible for chronic inflammation of the gastrointestinal tract. Although several studies correlate the cause of IBD with genetic and environmental factors that are associated with the disruption of the intestinal immune system [1], detailed mechanisms are still unclear. Therefore, IBD often becomes refractory; it relapses and results in the impairment of quality of life.

Dietary habit is considered one of the important factors for the onset of IBD. For instance, in Japan, an explosive increase in the number of patients with IBD was associated with the increase in intake of n-6 polyunsaturated fatty acid (PUFA) and animal protein [2]. On the other hand, cumulative data indicate that functional lipids such as docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA) have an anti-inflammatory effect and some of them are effective in the suppression of IBD development [3,4].

Gamma linolenic acid (GLA) is an n-6 PUFA designated as 18:3 and is rich in evening primrose oil and borage oil. GLA is one of the putative functional lipids for the prevention of inflammatory responses such as LPS response of macrophages [5]. Anti-inflammatory effect of GLA can be explained by the presence of 1-series prostaglandins (PG) and 3-series leukotrienes (LT), both of which are metabolites from DGLA, an elongated metabolite of GLA. On the other hand, additional 5-desaturated metabolites, like arachidonic acid (AA), have pro-inflammatory properties even under conditions of IBD [6,7]. In this study, we attempted to discern the effect of borage oil (BO) as a GLA-rich oil on the condition of dextran sulfate sodium (DSS)-induced colitis and accumulation of n-6 PUFAs in tissues. Although soybean oil is recommended for the preparation of AIN-93G formula, soybean oil contains ALA that may suppress IBD development. Therefore, we used safflower oil (SAF) that did not contain n-3 fatty acids as control to compare the effect of BO and SAF on IBD conditions.

2. Materials and Methods

2.1. Materials

SAF and BO were kindly provided by Nissin Oillio Co. Ltd, and DSM Japan Co. Ltd, respectively.

2.2. IBD Model and Feeding Condition

BALB/c mice (male, 8-wk-old) were purchased from Japan SLC (Hamamatsu, Japan) and acclimatized for a week. The mice were given free access to water and commercial pellet diet (MF pellet, Oriental Yeast, Tokyo, Japan). These mice were then divided into four groups: SAF (n = 4), borage oil control (BO) (n = 4), SAF + DSS (n = 12), and BO + DSS (n=11). Each of the groups was observed for 15 days. Diets were prepared according to AIN-93G, with modification in lipids (Table 1). In the BO

group, safflower oil was completely replaced with BO. Fatty acid composition of the diet is shown in Table 2. During these 15 days, DSS was not administered even in the DSS groups. On the 15th day, after the start of *ad libitum* feeding administration, water was replaced by 3% DSS solution in the SAF + DSS and BO + DSS groups. On the 24thday, DSS treatment was terminated, mice were euthanized and the organs were excised. These animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals at the University of Miyazaki, and conducted in compliance with the Law Concerning the Protection and Control of Animals (Japan Law No. 105), Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notification No. 88 of the Ministry of the Environment, Japan), and the Guidelines for Animal Experimentation (the Japanese Association for Laboratory Animal Science). Room temperature was maintained at 22–24°C with 12-hour light/12-hour dark cycle (0800-2000).

Table 1. Dietary composition of each experimental group

	SAF	BO
Ingredients (g/kg)		
Vitamin mix	10	10
Mineral mix	35	35
Choline Hydrogen Tartrate	2.5	2.5
L-Cystine	3	3
Safflower oil	70	-
Borage oil	-	70
Tertiary butyl hydroquinone	0.014	0.014
Sucrose	100	100
Casein	200	200
Cornstarch	397.5	397.5
Pregelatinized cornstarch	132	132
Cellulose	50	50

SAF; safflower oil, BO; borage oil.

Table 2. Fatty acid composition of the diet

	SAF	BO
Fatty acids (wt. %)		
C16:0	5.8	8.3
C16:1	0	0
C18:0	2.3	3.1
C18:1	16.8	15.5
C18:2	72.2	40.2
C18:3n6	0	22.5
C20:3n6	0	0.2
C20:4n6	0	0
C22:6n3	0	0

SAF; safflower oil, BO; borage oil.

2.3. Thin Layer Chromatography (TLC) and Gas Chromatography (GC) Analysis

Liver and colon tissues were homogenized with phosphate-buffered saline. The homogenate (1 mL) was mixed with 1 mL 0.1 mg/mL aqueous tripentadecanoin and 3 mL chloroform/methanol (2:1) solution, and the mixture was centrifuged at 600 × *g* for 5 min. The lower layer was collected and the upper layer was again mixed with 4 mL chloroform, followed by centrifugation at 600 × *g* for 5 min. The lower layer thus obtained was flushed with N_2 gas in a water bath maintained at 37°C. TLC plates (Silica gel $60F_{254}$; Analytical Chromatography.) were activated at 110°C for 1 h. After activation, extracted lipids were dissolved again in chloroform and spotted on the plate. Lipids were separated with the help of a development solvent (chloroform: methanol: water = 65:25:4) and detected by UV illumination. Bands for triglycerides (TG), phosphatidyl choline (PC), and phosphatidyl ethanolamine (PE) were collected and analyzed further by GC. Collected lipids were saponified with 0.5 M KOH/methanol solution at 100°C for 5 min. Next, the fatty acids were methylated with 0.4 mL 10% HCl/methanol solution at 100°C for 5 min. Methylated fatty acids were re-extracted using hexane. Fatty acid composition was analyzed by gas-liquid chromatography (GC-2014; Shimadzu), the instrument equipped with a Supelcowax-10 column (0.32mm × 60m, film thickness=0.25 μm; Supelco Inc., Bellefonte, PA). Column temperature was maintained at 150°C for 1 min and raised to 220°C at 2°C/min and kept constant for 40 min. The detector and injector temperatures were set at 250°C.

2.4. Severity of Bowel Inflammation

Severity of the IBD was evaluated by scoring the stool condition and bloody stool degree according to a previous report [8]. Briefly, stool consistency was scored on the following 4-point scale: 0, normal; 1, soft but still consistent; 2, very soft; 3: diarrhea. The blood score was as follows: 0, normal; 1, positive hemoccult; 2, hemoccult, blood traces in stool visible; 3, rectal bleeding.

2.5. Statistical Analysis

Data were first analyzed by the Turkey-Kramer test, and the differences were considered significant at $P < 0.05$.

3. Results and Discussion

Fatty acid compositions of the liver TG, PC, and PE are shown in Table 3. As for liver TG, accumulation of LA was significantly higher in the SO group. Two-way ANOVA showed that dietary lipids and DSS administration significantly affect GLA and DGLA levels, and significant interaction was observed between dietary lipid and DSS administration. Especially, DGLA level was significantly lower in the DSS-treated groups. No significant differences were detected in AA level. 6 desaturation index of the liver TG was significantly lower in the BO groups and no significant differences were detected in 5 desaturation index. As for liver PC and PE, no significant differences were observed in LA level, whereas GLA level was significantly higher in the BO groups (Table 3-2, Table 3-3). Notably, DSS treatment significantly increased DGLA level in liver PE. As is the case with liver TG, DSS and dietary lipids did not affect AA level in the liver PC and PE. In addition, a significant increase was detected in DHA level of liver PC. 6 desaturation indices of the liver PE and PC and 5 desaturation index in the liver PE were significantly lower in the BO groups.

Table 3-1. Fatty acid compositions of the liver triglycerides

	SAF	BO	SAF+DSS	BO+DSS
Fatty acids(wt. %)				
C16:0	20.1±1.0	18.5±1.2	20.6±1.0	20.3±2.1
C16:1	1.4±0.5	1.8±0.3	1.1±0.2	1.1±0.2
C18:0	2.8±0.1	5.5±1.9	6.6±1.5	5.1±0.6
C18:1	22.0±1.0	20.8±1.1	17.3±0.9	21.2±1.5
C18:2	31.9±1.2	20.0±0.3	30.9±1.8	20.0±1.4
C18:3n6	0.7±0.1[a]	3.2±0.1[b]	0.9±0.1[a]	2.2±0.2[c]
C20:3n6	1.9±0.1[a]	4.7±0.3[b]	1.0±0.1[c]	2.0±0.2[a]
C20:4n6	4.8±0.4	9.5±0.7	8.4±1.1	9.1±1.0
C22:6n3	2.7±0.2	3.7±0.2	2.5±0.3	2.9±0.2
Delta 5 index 20:4n6/(20:3n6+20:4n6)				
	2.5±0.3[ab]	2.1±0.3[a]	6.7±1.1[b]	5.0±0.7[ab]
Delta 6 index 18:3/(18:2+18:3n6)				
	1.0±0.0[a]	0.9±0.0[b]	1.0±0.0[a]	0.9±0.0[c]
Delta 9 index MUFA/MUFA+SFA				
	0.5±0.0	0.5±0.0	0.4±0.0	0.5±0.0

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$). MUFA, 16:1 and 18:1. SFA, 16:0 and 18:0.

Table 3-2. Fatty acid compositions of the liver phosphatidylethanolamine

	SAF	BO	SAF+DSS	BO+DSS
Fatty acids(wt. %)				
C16:0	12.0±2.0	13.8±1.5	12.3±2.1	13.5±2.4
C16:1	0.5±0.3	0.5±0.2	0.2±0.1	0.4±0.2
C18:0	13.4±4.7	17.8±1.0	20.2±1.7	15.4±1.9
C18:1	7.3±2.0[ab]	12.0±1.5[a]	6.3±1.0[b]	6.7±1.0[ab]
C18:2	8.2±4.0	8.4±2.5	9.1±3.9	4.3±1.0
C18:3n6	0.2±0.1	1.0±0.5	0.1±0.0	0.6±0.2
C20:3n6	6.6±2.6[ab]	6.8±3.3[a]	8.2±1.9[b]	9.8±2.5[ab]
C20:4n6	14.3±6.5	11.6±3.2	20.9±2.3	15.2±2.5
C22:6n3	6.6±2.6	6.8±3.3	8.2±1.9	9.8±2.5
Delta 5 index 20:4n6/(20:3n6+20:4n6)				
	0.9±0.0[ab]	0.8±0.1[a]	1.0±0.0[b]	0.9±0.0[ab]
Delta 6 index 18:3/(18:2+18:3n6)				
	1.0±0.0[a]	0.9±0.0[ab]	1.0±0.0[a]	0.9±0.0[b]
Delta 9 index MUFA/MUFA+SFA				
	0.2±0.1	0.3±0.0	0.2±0.0	0.2±0.0

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$). MUFA, 16:1 and 18:1. SFA, 16:0 and 18:0.

Table 3-3. Fatty acid compositions of the liver phosphatidylcholine

	SAF	BO	SAF+DSS	BO+DSS
Fatty acids(wt. %)				
C16:0	14.7±1.9[a]	13.0±2.3[a]	22.9±1.3[b]	18.6±1.2[ab]
C16:1	1.4±0.9[ab]	1.8±1.1[a]	0.2±0.1[b]	0.3±0.1[ab]
C18:0	15.4±2.0[ab]	11.3±2.1[a]	21.5±1.8[b]	19.4±0.8[b]
C18:1	6.8±2.0	4.3±1.2	3.5±0.2	4.1±0.8
C18:2	9.4±1.4	21,4±13.3	13.1±2.0	8.4±2.0
C18:3n6	0.3±0.2	0.8±0.4	0.2±0.1	1.0±0.1
C20:3n6	1.3±0.2	1.5±1.0	0.5±0.1	1.1±0.2
C20:4n6	24.7±7.3	8.4±5.8	21.7±3.0	23.2±4.2
C22:6n3	7.5±1.1[a]	1.4±1.4[b]	3.3±0.7[b]	5.1±0.8[ab]
Delta 5 index 20:4n6/(20:3n6+20:4n6)				
	0.9±0.0	0.8±0.2	1.0±0.0	0.8±0.1
Delta 6 index 18:3/(18:2+18:3n6)				
	1.0±0.0[a]	0.9±0.1[ab]	1.0±0.0[a]	0.8±0.1[b]
Delta 9 index MUFA/MUFA+SFA				
	0.2±0.1[a]	0.2±0.1[a]	0.1±0.0[b]	0.1±0.0[ab]

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$). MUFA, 16:1 and 18:1. SFA, 16:0 and 18:0.

Table 4-1. Fatty acid composition of the bowel triglycerides

	SAF	BO	SAF+DSS	BO+DSS
Fatty acids(wt. %)				
C16:0	25.8±3.4	11.8±3.8	20.7±3.5	17.9±1.7
C16:1	1.3±1.3	1.2±1.0	1.5±0.4	3.0±0.4
C18:0	17.2±9.1	9.0±3.8	11.0±2.0	8.4±1.2
C18:1	12.8±5.0	13.2±5.2	20.6±3.2	25.1±2.9
C18:2	22.3±11.5	7.9±2.5	21.3±2.4	14.6±1.9
C18:3n6	0.0±0.0[a]	0.7±0.5[b]	0.0±0.0[a]	2.4±0.4[c]
C20:3n6	0.9±0.7	0.5±0.3	0.4±0.2	1.0±0.2
C20:4n6	1.6±1.3	1.0±0.8	2.6±1.0	2.5±0.8
C22:6n3	0.1±1.3	1.0±0.8	2.6±1.0	2.5±0.8
Delta 5 index 20:4n6/(20:3n6+20:4n6)				
	0.4±0.2	0.3±0.2	0.7±0.1	0.7±0.1
Delta 6 index 18:3/(18:2+18:3n6)				
	1.0±0.0	0.9±0.0	1.0±0.0	0.9±0.0
Delta 9 index MUFA/MUFA+SFA				
	0.3±0.1	0.4±0.1	0.4±0.0	0.5±0.0

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$). MUFA, 16:1 and 18:1. SFA, 16:0 and 18:0.

Table 4-2. Fatty acid compositions of the bowel phosphatidylethanolamine

	SAF	BO	SAF+DSS	BO+DSS
Fatty acids(wt. %)				
C16:0	10.5±2.5	7.6±3.0	8.1±2.6	7.4±1.5
C16:1	0.0±0.0	0.4±0.4	0.6±0.3	0.2±0.1
C18:0	14.5±1.2	17.4±9.7	12.2±1.9	13.7±1.2
C18:1	12.7±3.4	5.0±3.9	6.7±1.9	6.7±1.2
C18:2	9.8±5.9	2.4±2.1	6.1±3.0	5.1±1.5
C18:3n6	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.1
C20:3n6	2.2±1.5	2.9±1.8	1.0±0.4	1.3±0.4
C20:4n6	15.7±9.1	10.0±3.4	16.2±2.6	19.1±1.9
C22:6n3	2.0±2.0	10.7±0.4	3.6±1.0	5.0±1.0
Delta 5 index 20:4n6/(20:3n6+20:4n6)				
	0.6±0.3[a]	0.8±0.1[ab]	1.0±0.0[b]	0.9±0.0[b]
Delta 6 index 18:3/(18:2+18:3n6)				
	1.0±0.0	0.6±0.3	0.9±0.1	0.7±0.1
Delta 9 index MUFA/MUFA+SFA				
	0.5±0.0	0.3±0.2	0.3±0.1	0.3±0.0

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$). MUFA, 16:1 and 18:1. SFA, 16:0 and 18:0.

Table 4-3. Fatty acid compositions of the bowel phosphatidylcholine

	SAF	BO	SAF+DSS	BO+DSS
Fatty acids(wt. %)				
C16:0	22.0±8.2	13.5±5.3	15.6±2.8	14.7±1.5
C16:1	0.7±0.7	2.3±0.7	1.2±0.4	2.9±1.7
C18:0	22.9±6.9	12.5±3.8	16.1±2.0	14.5±2.9
C18:1	8.7±1.1	6.0±3.6	6.3±1.0	7.4±1.0
C18:2	7.5±2.5	5.4±1.6	6.1±1.2	8.6±3.0
C18:3n6	0.0±0.0[a]	1.5±1.2[b]	0.1±0.1[a]	0.0±0.0[a]
C20:3n6	3.7±0.8	3.1±1.5	3.1±0.9	3.1±1.4
C20:4n6	15.6±5.6	14.7±7.4	17.9±3.4	9.8±2.9
C22:6n3	1.0±1.0	2.7±0.6	1.6±0.4	0.7±0.4
Delta 5 index 20:4n6/(20:3n6+20:4n6)				
	0.8±0.1	0.9±0.1	0.9±0.0	0.7±0.1
Delta 6 index 18:3/(18:2+18:3n6)				
	1.0±0.0	0.8±0.1	1.0±0.0	1.0±0.0
Delta 9 index MUFA/MUFA+SFA				
	0.2±0.0	0.3±0.1	0.2±0.0	0.3±0.0

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$). MUFA, 16:1 and 18:1. SFA, 16:0 and 18:0.

GLA level in bowel TG was higher in the BO groups and DSS treatment significantly increased GLA accumulation in these groups (Table 4-1). On the other hand, DSS treatment decreased GLA accumulation in bowel PE (Table 4-2). No significant differences were observed in DGLA and AA levels of bowel TG, PC and PE. Although Δ6 desaturation index of the bowel TG was significantly lower in the BO groups, no other significant differences in 6 desaturation indices were detected in the bowel TG, PC and PE.

Finally, severity of IBD condition was evaluated in terms of body weight decrease, stool condition, bloody stool degree, and bowel length. We preliminary studied the dose-dependent effect of 1, 2, 3% DSS on IBD severity. At 1 and 2%, IBD condition was not apparently observed within experimental period, therefore, we decided DSS dose at 3%. Although DSS treatment apparently induced these IBD conditions, no significant differences in any IBD conditions were detected between the four dietary groups (Table 5). It has been reported that GLA has an anti-inflammatory effect [9] and is a promising material for the prevention and alleviation of inflammation related-disorders. Essentially, anti-inflammatory dietary lipids such as EPA and DHA acid could alleviate the severity of experimental IBD [10]. Here, we examined the effect of dietary BO, which is rich in GLA, on GLA accumulation in inflammatory tissues and the severity of IBD condition, using a DSS-induced IBD model.

Table 5. Effect of dietary borage oil on the severity of the DSS-induced bowel inflammation

	SAF	BO	SAF+DSS	BO+DSS
		Weight		
0 day	25.5±0.5	25.5±0.7	25.8±0.3	26.3±0.3
2 day	25.6±0.5	25.7±0.9	25.0±0.3	25.9±0.3
4 day	26.0±0.5	26.2±0.8	25.0±0.4	25.8±0.3
6 day	26.2±0.5[a]	26.4±0.8[a]	23.9±0.3[b]	24.6±0.4[ab]
8 day	26.3±0.7[a]	26.0±1.5[ab]	23.5±0.4[b]	23.6±0.5[ab]
		Stool condition		
0 day	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2 day	0.0±0.0	0.0±0.0	0.7±0.1	0.5±0.2
4 day	0.0±0.0	0.0±0.0	0.4±0.1	0.9±0.2
6 day	0.0±0.0	0.0±0.0	0.8±0.2	1.1±0.3
8 day	0.0±0.0	0.0±0.0	1.2±0.3	1.5±0.2
		Bloody stool degree		
0 day	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2 day	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
4 day	0.0±0.0	0.0±0.0	0.1±0.3	0.4±0.2
6 day	0.0±0.0	0.0±0.0	0.7±0.3	0.5±0.2
8 day	0.0±0.0	0.0±0.0	1.1±0.3	0.6±0.2
		Colon length		
Colon (cm)	10.0±0.6[ab]	10.4±0.3[a]	8.5±0.4[b]	8.7±0.3[ab]

Data are means ± SE for 4 to 12 mice in each group. Means with the same letter are not significantly different from each other (Tukey–Kramer test, $P < 0.05$).

As shown in Table 3, dietary BO markedly increased GLA level not only in the liver phospholipids but also in the bowel TG irrespective of DSS treatment, whereas GLA accumulation in the intestinal PL was not apparent. These data suggest that dietary GLA distribution into the lipid classes was different between the liver and bowel. In addition, it is considered that bowel inflammation does not affect GLA accumulation. On the other hand, GLA accumulation in the intestinal PL of BO-fed mice was not apparent and not significant compared with that in the SO group. Accumulation of DHA and EPA was evaluated in a previous study using SMAD3-/- mice where 1.8 energy% of DHA+EPA was administered to the mice and approximately three times of DHA+EPA level was detected in bowel PL when compared with control mice. Here, although 8 energy% of dietary GLA was given to the mice, accumulation of GLA was not apparent, indicating low incorporation of GLA into the intestinal PL [11].

GLA is metabolized into DGLA and AA via elongation and Δ5 desaturation reactions. Membrane PL derived DGLA is a substrate for prostaglandin E_1 biosynthesis, which is an anti-inflammatory prostanoid and could reduce trinitrobenzene sulfonic acid-induced bowel damage [12]. The anti-inflammatory effect of GLA was at least in part attributed to PGE_1 synthesis. Table 4-1 shows that DGLA level in bowel TG was significantly higher in the BO group whereas DGLA level in PL was comparable among the dietary groups, indicating a homeostasis in DGLA level in the bowel PL at the elongation step. PL-derived AA can be a substrate for PGE_2, which is a representative pro-inflammatory cytokine. Dietary AA increased cycloxyganease-2 expression and exacerbated inflammatory condition in DSS-induced IBD model [6]. Results of the present study showed that dietary BO did not affect AA levels in the liver and bowel, suggesting a strict regulation of AA level by Δ5 desaturase as a rate-limiting enzyme even under conditions of IBD.

Finally, we compared the severity scores of IBD between the BO and the SO groups. In terms of body weight, condition of stool and blood stool, no apparent difference was observed between these dietary groups. These results are consistent with the fatty acid composition data in which bowel PL, DGLA and AA levels were strictly regulated and comparable among dietary groups. Here, we used a DSS model for the evaluation of IBD; this model might be insensitive to GLA administration. On the other hand, several reports indicate that increased dietary intake of n-6 PUFA can be a risk factor associated with the incidence of IBD [2,13].

4. Conclusion

In this study, we examined the effect of dietary GLA accumulation in the bowel and on the condition of DSS-induced colitis in mice. In addition, we used safflower oil for the preparation of control diet, and as shown in Table 2, safflower oil is rich in linoleic acid (18:2, n-6). As IBD is established on the basis of complicated factors (hereditary factor, dietary factor, intestinal microflora factor, etc), further evaluation of GLA on IBD incidence or condition is needed in various types of IBD models.

Conflict of Interest

We declare no conflicts of interest.

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The heading of the Acknowledgment section and the References section must not be numbered.

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