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# Beneficial Effects of *n*-9 Eicosatrienoic Acid on Experimental Bowel Lesions

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**Abstract**

***Purpose.*** Dietary fortification of *n*-9 polyunsaturated fatty acids (PUFA) or 5,8,11-eicosatrienoic acid (ETrA) as well as *n*-3 PUFA might contribute to the suppression of leukotriene B4 (LTB4) synthesis and thereby reduce inflammatory bowel lesions. As a result, the effect of an ETrA-enriched diet on experimental bowel lesions was examined in this study.

***Methods.*** In Expt. 1, rats were freely fed either an ETrA-enriched or a standard diet. After 7 days of feeding, acute bowel lesions were induced by the subcutaneous injection of 10 mg/kg indomethacin. In Expt. 2, chronic bowel lesions were made by performing subcutaneous injections of 7.5 mg/kg indomethacin twice. After the first injection, the rats were freely fed either an ETrA-enriched or a standard diet for 7 days.

***Results.*** In both experiments, the rats fed an ETrA-enriched diet showed increased levels of ETrA in the plasma and intestinal mucosa, and a decreased inflammation score. However, there was no significant decrease in plasma and intestinal mucosal LTB4 in the ETrA-enriched diet-fed rats.

***Conclusion.*** These results suggest that the dietary supplementation of ETrA may have both prophylactic and therapeutic effects on experimentally produced bowel lesions. Further investigations are necessary to clarify the effects of ETrA on bowel lesions and its mechanisms.

**Key words** Eicosatrienoic acid · Leukotriene B4 · Inflammatory bowel disease

## Introduction

It is well known that polyunsaturated fatty acids (PUFAs) have a number of important biological effects such as an anti-inflammatory response and immunosuppression. It has been reported that far more *n*-6 PUFAs are contained in most ordinary diets than are needed based on the essential requirements.

Arachidonic acid (AA) (20:4 *n*-6) is a dominant substrate for eicosanoid-forming enzymes, which is metabolized via the 5-lipoxygenase pathway to leukotriene B4 (LTB4), a potent inflammatory mediator. In addition, eicosapentaenoic acid (EPA) (20:5 *n*-3), a *n*-3 homologue of AA, has also been reported to compete for eicosanoid-forming enzymes, reduce LTB4 synthesis, and modulate inflammation.[1,2]

According to Sharon and Stenson, patients with inflammatory bowel disease tend to have significantly increased LTB4 levels, thus suggesting that LTB4 plays an important role as a mediator of intestinal inflammation.[3] They recommended dietary fortification with *n*-3 PUFA to both induce and maintain the remission of bowel lesions.

Another way to reduce LTB4 synthesis might be the restriction of essential fatty acids (EFA). Dietary EFA restriction is known to decrease AA and LTB4 synthesis and, furthermore, suppresses experimentally induced bowel lesions.[4,5]

5,8,11-Eicosatrienoic acid (ETrA) (20:3 *n*-9) is usually scarcely contained in plasma and tissues. It is known to increase only in the development of an EFA deficiency. For this reason, the ratio of plasma ETrA/AA (triene to tetraene ratio) has been used as an index of EFA deficiency. Stenson et al. showed that the decrease in LTB4 synthesis increased as the AA level decreased in EFA-deficient rats, thus indicating that ETrA contributes significantly to the inhibition of LTB4 synthesis.[4] Therefore, the dietary supplementation of ETrA might contribute to the suppression of

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LTB4 synthesis and thereby reduce the inflammatory response. Similarly, the supplementation of ETrA might have a beneficial effect in the suppression of inflammatory bowel lesions. However, the lack of a suitable biological source of ETrA has been a barrier to conducting investigations of the above issues.

Recently, ETrA-rich oil was developed coincidentally from cultures of a mutant of the fungus *Mortierella alpina*, and it has now become available for experimental use in rodents. The present study examined its effect on the occurrence of experimental bowel lesions in rats.

## Materials and Methods

ETrA-rich oil (*M. alpina* oil; triglyceride which contains 17% ETrA) was obtained from Suntory Limited, Osaka, Japan. AIN-76[6] was used as the standard rat diet, and the ETrA-enriched diet (SUNM-17) was made by replacing AIN76 corn oil with *M. alpina* oil (Table 1).

Fifty-three male Sprague-Dawley rats (Charles River, Japan) weighing 250–280 g were used in this study. All animals underwent acclimatization for 7 days before the experiment.

Inflammatory bowel lesions were produced by the subcutaneous injection of indomethacin (WAKO, Osaka, Japan) as previously described by Chen et al. who reported that one injection of indomethacin produces acute intestinal injury and inflammation, while two injections produce more extensive and chronic inflammation.[7,8]

### *Experiment 1*

The rats were randomly divided into two groups. The control group animals ($n = 8$) were fed the AIN-76 diet, while the experimental group animals ($n = 7$) were fed the SUNM-17 diet. Animals in both groups were freely fed these diets for 7 days, and were injected with 10 mg/kg indomethacin subcutaneously. One day after injection, all animals were killed.

### *Experiment 2*

The rats were randomly divided into two groups. The animals in both groups were injected with 7.5 mg/kg indomethacin subcutaneously, twice at an interval of 24 h. After the first injection, the control group animals ($n = 20$) were freely fed the AIN-76 diet, while the experimental group animals ($n = 18$) were freely fed the SUNM-17 diet. Two and 4 days after the first injection, 4 rats in each group were killed to assess the presence of bowel lesions. Seven days after the first injection, the remaining animals were killed. All of the above experimental procedures were conducted in accordance with Osaka University Medical School guidelines for the care and use of laboratory animals.

For the biochemical analysis, mucosal tissue of a 10-cm segment of the ileum was immediately scraped from the underlying muscular layer with a glass slide on an ice-cold surface. In both experiments, bowel lesions were assessed using as macroscopic inflammation score (Table 2),[8] and fatty acid compositions of plasma and intestinal mucosa were measured by gas chromatography. The LTB4 levels of plasma and intestinal mucosa were measured by an enzyme immunoassay (EIA) (Amersham, Bucks, UK).

The data are expressed as the mean ± standard deviation (SD). A statistical analysis was performed with *t*-tests. A $P$ value of less than 0.05 was considered to be statistically significant.

**Table 1.** Components of diets

| | AIN-76 (%) (standard diet) | SUNM-17 (%) (ETrA-enriched diet) |
|---|---|---|
| Casein | 20.0 | 20.0 |
| DL-Methionine | 0.3 | 0.3 |
| Corn starch | 15.0 | 15.0 |
| Sucrose | 50.0 | 50.0 |
| Fiber | 5.0 | 5.0 |
| Corn oil | 5.0 | |
| *M. alpina* oil | | 5.0 |
| AIN Mineral mix | 3.5 | 3.5 |
| AIN Vitamin mix | 1.0 | 1.0 |
| Choline bitartrate | 0.2 | 0.2 |
| Total | 100 | 100 |

AIN-76[6] was used as a standard rat diet. An ETrA-enriched diet (SUNM-17) was made by replacing corn oil of AIN-76 to *Mortierella* alpina oil (containing 17% ETrA)
ETrA, 5,8,11-eicosatrienoic acid

**Table 2.** Criteria for determining the macroscopic inflammation score[8]

| | |
|---|---|
| Adhesive lesions in the serosa and mesentery | |
| None | 0 |
| Minimal | 1 |
| Involving several loops | 2 |
| Wall thickness | |
| <1 mm | 0 |
| 1–3 mm | 1 |
| >3 mm | 2 |
| Hyperemia | |
| None | 0 |
| Recognized | 1 |
| Ulcers | |
| None | 0 |
| Single ulcer | 1 |
| Multiple (<10 cm) | 2 |
| Multiple (>10 cm) | 3 |

## Results

### *Experiment 1*

The experimental group animals showed a normal intestinal mucosa and muscular layer (Fig. 1), while the control group animals showed multiple superficial intestinal mucosal lesions (Fig. 2). The macroscopic inflammation score was 0.3 ± 0.8 in the experimental group, whereas it was 2.6 ± 1.9 in the control group ($P < 0.05$).

The fatty acid compositions of the plasma and intestinal mucosa in both groups are shown in Table 3. The plasma LTB4 level was 239 ± 54 pg/ml in the experimental group, whereas it was 118 ± 22 pg/ml in the control group ($P < 0.05$). Intestinal mucosal LTB4 was 14.5 ± 9.1 pg/mg in the experimental group, whereas it was 9.2 ± 6.2 pg/mg in the control group, with no significant difference.

### *Experiment 2*

The animals in both groups showed mutiple bowel lesions on day 2. The bowel lesions in the experimental group were attenuated by day 7 (Fig. 3). However, the control group showed linear ulcers and adhesions with the surrounding intestine on day 7 (Fig. 4). The macroscopic inflammation score on day 7 was 1.4 ± 1.5 in the experimental group, whereas it was 4.3 ± 3.2 in the control group ($P < 0.05$).

The fatty acid compositions of plasma and intestinal mucosa are shown in Table 4. Plasma LTB4 was 1550 ± 1030 pg/ml in the experimental group, whereas it was 1221 ± 944 pg/ml in the control group, with no significant difference. Intestinal mucosal LTB4 was 89.1 ± 72.0 pg/mg in the experimental group, whereas it was 32.4 ± 38.0 pg/mg in the control group ($P < 0.05$).

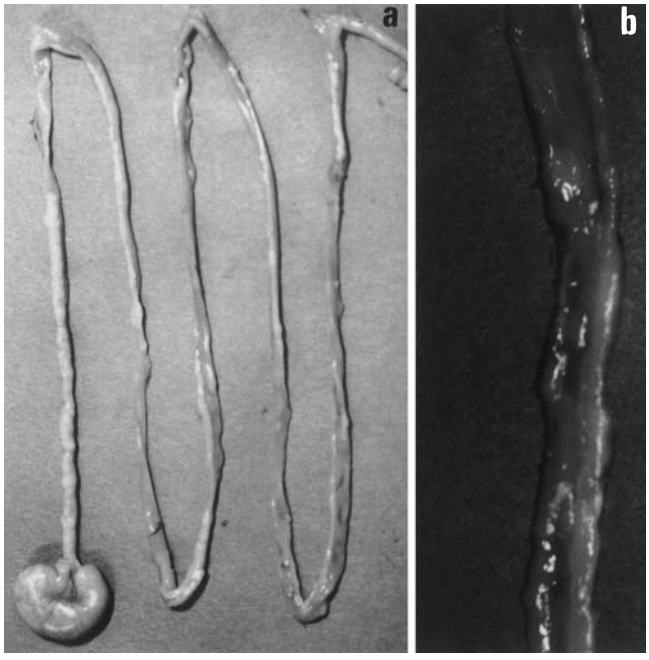


**Fig. 1a,b.** Experiment 1. The macroscopic findings of intestinal lesions in rats fed an eicosatrienoic acid (ETrA)-enriched diet demonstrate a normal intestinal mucosa and muscular layer. **b** High magnification

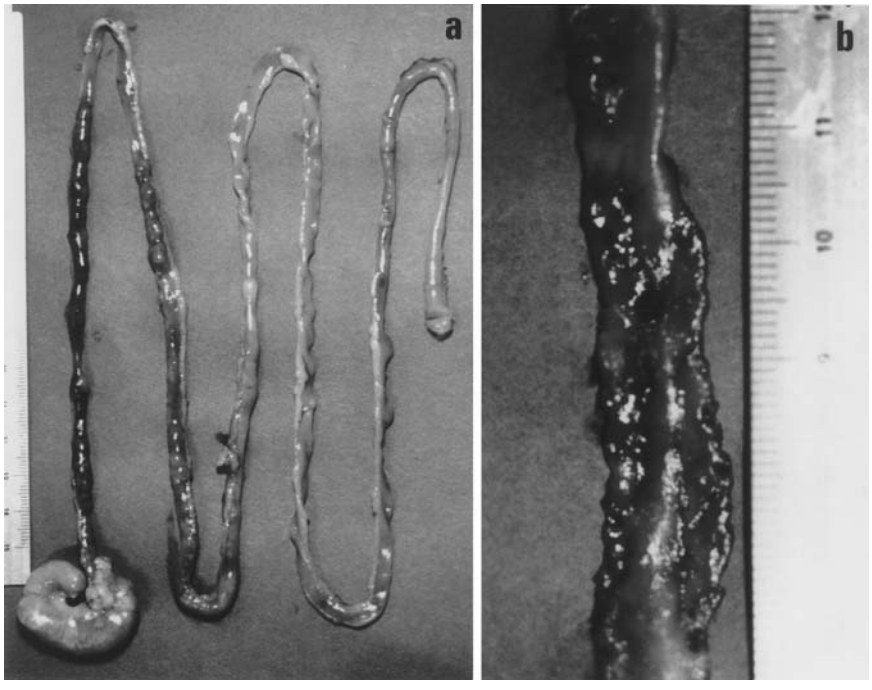


**Fig. 2a,b.** Experiment 1. The macroscopic findings of intestinal lesions in rats fed a standard diet demonstrate multiple intestinal ulcers and bleeding. **b** High magnification

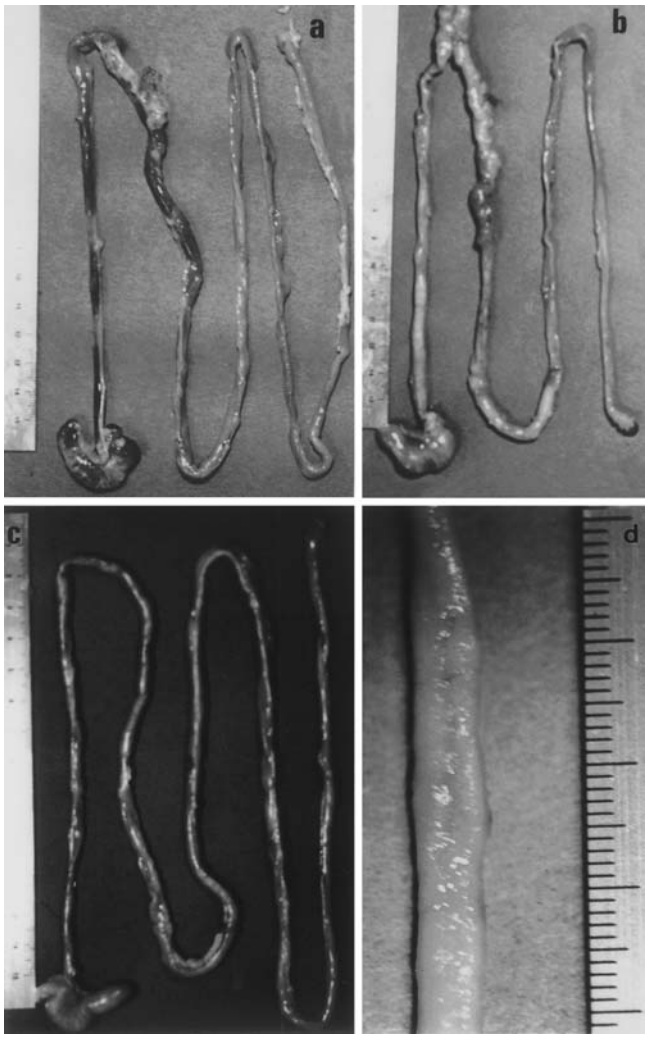


**Fig. 3a–d.** Experiment 2. The macroscopic findings of intestinal lesions in rats fed an ETrA-enriched diet demonstrate intestinal ulcers and bleeding on days 2 (**a**), 4 (**b**), and 7 (**c,d**) (**d** high magnification). An attenuation of the lesion during the time course is noted

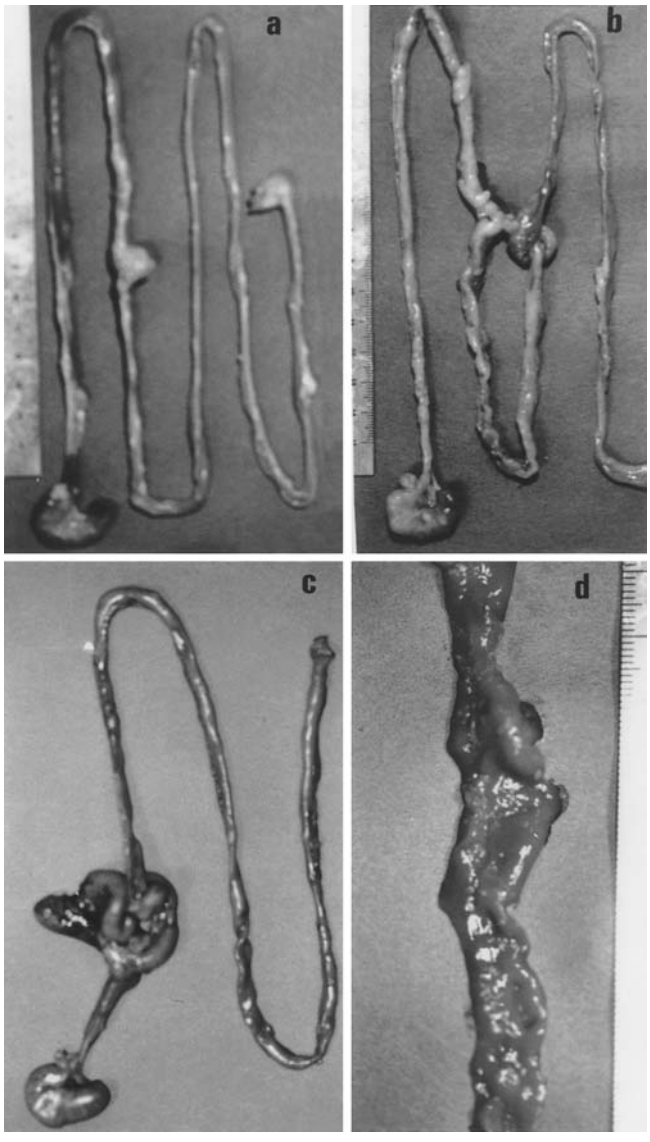


**Fig. 4a–d.** Experiment 2. The macroscopic findings of intestinal lesions in rats fed a standard diet demonstrate intestinal ulcers and bleeding on days 2 (**a**), 4 (**b**), and 7 (**c,d**) (**d** high magnification). Intestinal lesions are still observed to remain on day 7

**Table 3.** Fatty acid composition (Expt. 1)

| | Plasma | | Intestinal mucosa | |
|---|---|---|---|---|
| | Experimental group ($n$ = 7) (%) | Control ($n$ = 8) (%) | Experimental group ($n$ = 7) (%) | Control ($n$ = 8) (%) |
| C16:0 | 24.4 ± 1.6 | 23.9 ± 3.1 | 28.8 ± 0.9 | 26.5 ± 2.0 |
| C18:0 | 10.8 ± 1.9 | 11.5 ± 1.5 | 5.0 ± 0.6 | 5.2 ± 1.5 |
| C18:1 (*n*-9) | 24.0 ± 3.2 | 16.5 ± 3.8 | 33.4 ± 0.9 | 28.1 ± 2.2 |
| C18:2 (*n*-6) | 6.0 ± 2.3 | 19.2 ± 8.7 | 15.6 ± 1.2 | 26.6 ± 3.6 |
| C18:3 (*n*-3) | 0.2 ± 0.1 | 0.2 ± 0.1 | 1.0 ± 0.1 | 0.9 ± 0.2 |
| C20:3 (*n*-9) | 14.6 ± 1.3 | 0.2 ± 0.1 | 3.2 ± 0.3 | 0.1 ± 0.0 |
| C20:4 (*n*-6) | 9.8 ± 2.7 | 19.6 ± 2.9 | 1.3 ± 0.5 | 2.4 ± 1.4 |
| C20:5 (*n*-3) | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.0 ± 0.0 |
| C22:6 (*n*-3) | 2.4 ± 0.4 | 2.0 ± 0.4 | 0.3 ± 0.1 | 0.4 ± 0.2 |

**Table 4.** Fatty acid composition (Expt. 2)

| | Plasma | | Intestinal mucosa | |
|---|---|---|---|---|
| | Experimental group ($n$ = 7) (%) | Control ($n$ = 7) (%) | Experimental group ($n$ = 7) (%) | Control ($n$ = 7) (%) |
| C16:0 | 23.0 ± 2.2 | 22.3 ± 1.3 | 23.5 ± 1.3 | 22.6 ± 2.8 |
| C18:0 | 10.6 ± 1.3 | 10.4 ± 1.3 | 5.2 ± 1.1 | 5.9 ± 1.6 |
| C18:1 (*n*-9) | 21.4 ± 3.2 | 15.3 ± 2.4 | 31.3 ± 0.4 | 27.3 ± 1.1 |
| C18:2 (*n*-6) | 4.6 ± 1.7 | 20.7 ± 3.4 | 26.7 ± 1.6 | 31.4 ± 2.7 |
| C18:3 (*n*-3) | 0.2 ± 0.0 | 0.2 ± 0.0 | 1.4 ± 0.2 | 1.1 ± 0.2 |
| C20:3 (*n*-9) | 16.6 ± 1.5 | 0.2 ± 0.1 | 2.1 ± 0.4 | 0.1 ± 0.0 |
| C20:4 (*n*-6) | 12.6 ± 3.6 | 19.6 ± 3.2 | 1.5 ± 0.5 | 2.6 ± 1.0 |
| C20:5 (*n*-3) | 0.4 ± 0.1 | 0.2 ± 0.3 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| C22:6 (*n*-3) | 2.5 ± 0.5 | 3.2 ± 2.6 | 0.6 ± 0.1 | 0.8 ± 0.3 |

## Discussion

The anti-inflammatory effects of EFA deprivation have been reported in laboratory rodents fed EFA-deficient diets.[5,9] Regarding the anti-inflammatory effect of EFA deficiency, several actions, such as decreased macrophages and their migration to an inflammatory focus,[5] decreased macrophage spreading and adherence,[10] and reduced neutrophil LTB4 synthesis have been observed.[4,11]

The mechanism for decreased LTB4 in EFA deficiency may partly involve a decreased availability of the substrate, AA. However, AA is strongly conserved in EFA deficiency, even in the presence of a severe depletion of its precursor linoleic acid (LA).[12]

Cellular AA depletion is likely to be an important factor but not the only factor responsible for the anti-inflammatory effect of EFA deficiency. Theoretically, lowering cellular AA should reduce the degree of *n*-6 eicosanoid synthesis in inflammatory bowel lesions. A reduction in the amount of cellular AA through dietary avoidance of its *n*-6 precursor, LA, can be achieved in rats,[13] but this is not feasible for free-living human subjects.

Dietary ETrA supplements may confer some of the anti-inflammatory benefits of EFA deficiency if cellular ETrA is increased to an extent that it alters both eicosanoid synthesis and other properties. In the present study, the rats were fed an EFA-sufficient diet containing an ETrA-rich oil.

In Expt. 1, increased levels of ETrA in plasma and intestinal mucosa were observed in rats after consuming an ETrA-enriched diet for 7 days. Furthermore, in these rats the indomethacin-induced bowel lesions were significantly attenuated. This result suggests that an ETrA-enriched diet might have a preventive effect on bowel lesions.

In Expt. 2, a chronic bowel inflammation model was made by two injections of indomethacin, and in this model, an ETrA-enriched diet attenuated bowel lesions in the due course of time. This result suggests that an ETrA-enriched diet might have a therapeutic effect on the development of bowel lesions. However, contrary to other studies,[14,15] an ETrA-enriched diet did not decrease the LTB4 levels in this study. Nevertheless, bowel lesions were attenuated in these animals.

James et al.[14] used chemically synthesized ETrA or EPA in a mixture of vegetable oils. In our study, ETrA-rich oil and corn oil were used. Both oils contain little *n*-3 PUFAs, but ETrA-rich oil contains much ETrA and other *n*-9 fatty acid, less *n*-6 PUFAs than corn oil. This difference in the fatty acid composition might affect the production of LTB4. However, the amount of LTB4 derived from the oils which contain less *n*-6 PUFAs was expected to decrease.

ETrA and other *n*-9 fatty acids are less unsaturated than *n*-6 and *n*-3 PUFAs. Accordingly, they are more stable, and less easily oxidized. These properties of ETrA might thus be beneficial for reducing the inflammatory response.

PUFAs have been shown to have a variety of biological effects. Not only do they interact with the eicosanoid synthetic pathways, but they also have an effect on the membrane lipid composition and fluidity, the production and release of proinflammaory cytokines, interleukins, and the modulation of signaling pathways.[16,17] Studies on the mechanism of the anti-inflammatory effects of PUFAs have long been performed by comparing *n*-6 with *n*-3 PUFAs. In the history of lipid clinical research, the absence of any suitable biological source of ETrA has been a barrier to fully evaluating its effect on bowel lesions. Further investigations are necessary to clarify the effects of ETrA on the occurrence of intestinal inflammation and its mechanism of action.

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