

# Dietary n-3 polyunsaturated fatty acids ameliorate Crohn's disease in rats by modulating the expression of PPAR- $\gamma$ /NFAT

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**Abstract.** Dietary n-3 polyunsaturated fatty acid (PUFA) exerts anti-inflammatory and immunoregulatory effects via downregulation of the innate and adaptive immune responses. However, the effect of dietary n-3 PUFA on experimental Crohn's Disease (CD) in rats and the underlying mechanisms are unclear. The present study aimed to investigate the effects of n-3 PUFA on CD induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in rats, and to determine the underlying mechanisms, including the peroxisome proliferator-activated receptor (PPAR)- $\gamma$  and nuclear factor of activated T-cells (NFAT) pathway. Sprague-Dawley rats (n=90) were randomly assigned to the following groups: Control (intragastric distilled water); PUFA control (intragastric n-3 PUFA, 20 mg/kg/day); trans-fatty acid (TFA) control (intragastric TFA, 13 mg/kg/day); model (intragastric distilled water + TNBS); PUFA model (intragastric n-3 PUFA, 20 mg/kg/day + TNBS); and TFA model (intragastric TFA, 13 mg/kg/day + TNBS). The disease activity index (DAI), colon macroscopic damage index (CMDI) and tissue damage index (TDI) were evaluated. The expression of PPAR- $\gamma$ , NFAT, interleukin (IL)-4 and IL-2 mRNA in colonic tissues was determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and the serum levels of IL-6, IL-12, tumor necrosis factor- $\alpha$  and interferon- $\gamma$  were measured by ELISA. The results demonstrated that dietary n-3 PUFA markedly attenuated colonic inflammation compared with the model group, as indicated by reduced DAI, CMDI and TDI scores, amelioration in pathological evaluation and improvements in localized mucosal inflammation,

as indicated by the levels of cytokines associated with local mucosal immunity. Treatment with n-3 PUFA increased the gene expression of PPAR- $\gamma$  in TNBS-treated rats, and reduced the expression of NFAT, which ultimately reduced the release of IL-4 and IL-2 detected by RT-qPCR. A TFA-enriched diet was observed to increase DAI and TDI scores, aggravate pathological inflammation with epithelioid granulomas and enhance the release of proinflammatory cytokines, compared with the model group. In conclusion, the present study demonstrated that dietary n-3 PUFA may attenuate experimental CD induced by TNBS in rats by regulating the expression and activity of the PPAR- $\gamma$ /NFAT signaling pathway. These results provide a promising potential therapeutic method for the treatment of CD.

## Introduction

Inflammatory bowel disease (IBD) consists of two types: Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease is a chronic immune-mediated inflammatory disorder of the gastrointestinal tract. It is widely accepted that the key mechanism underlying the development of CD is a dysregulated immune response to commensal flora in a genetically susceptible host (1,2). However, the precise etiology remains to be determined, which leads to limitations of corresponding therapeutic methods.

Diet has been hypothesized to serve an important role in the pathogenesis and clinical course of CD, as the incidence of CD has risen markedly in countries where a western diet culture prevails (3,4). In particular, dietary fat may influence intestinal inflammation and regulate mucosal immunity, which is considered to be a core etiological factor and may be a promising therapeutic target for CD.

The fatty acids that are consumed as part of our daily diets are broadly classified as cis- and trans-fatty acids (TFAs). N-3 polyunsaturated fatty acids (PUFAs) belong to the group of cis PUFAs, and include docosahexaenoic acid and eicosapentaenoic acid. Numerous previous studies have demonstrated that TFA consumption was a risk factor, while n-3 PUFA consumption was a protective factor, for various diseases associated with systemic or localized inflammation, including diabetes and coronary heart disease (5,6). According to a retrospective study

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by Nanthakrishnan *et al* (5), which followed 170,805 women with IBD for 26 years, a high intake of dietary long-chain n-3 PUFA may be associated with a reduced risk of UC. By contrast, a high intake of TFA was reported to be associated with an increased risk of UC (7). Dietary n-3 PUFA competitively inhibits the formation of proinflammatory prostaglandins and leukotrienes via the arachidonic acid pathway (8), and inhibits angiogenesis and adaptive immune responses (9,10). However, the effect of dietary n-3 PUFA on CD and the underlying mechanisms remain unclear.

Peroxisome proliferator-activated receptor (PPAR)- $\gamma$  is a member of the nuclear receptor superfamily of ligand-dependent transcription factors, and is primarily expressed in adipose tissue and the colonic epithelium (11). Recently, its role in intestinal inflammation has emerged. Su *et al* (12) reported that the use of the PPAR- $\gamma$  synthetic agonist thiazolidinedione (TZD) markedly decreased colonic inflammation in mice with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. These results were subsequently confirmed in different animal models with various models of colitis induced by chemical compounds (13), ischaemia (14) or genetically modified animals (15,16). PPAR- $\gamma$  interferes with inflammatory pathways by antagonizing the activity of various transcription factors, such as nuclear factor of activated T-cells (NFAT). It is established that NFAT has an important function in the transcriptional induction of various proinflammatory genes, including interleukin (IL)-4 and IL-2, during T-cell activation (17,18). Thus, PPAR- $\gamma$  may serve a key role in the regulation of adipocyte differentiation and inflammation. In addition, fatty acids and their metabolites are able to modulate PPAR- $\gamma$ . The precise association between dietary fatty acids and the PPAR- $\gamma$ /NFAT pathway remains unknown.

In the current study, the primary objectives were to investigate the effect of long-term administration of an n-3 PUFA-enriched diet on CD rat models induced by TNBS, and to characterize the association between n-3 PUFA and PPAR- $\gamma$ /NFAT in intestinal inflammation. In addition, the current study aimed to determine whether n-3 PUFA may exhibit a potent anti-inflammatory effect in experimental CD rats, and whether the protective effect of this fatty acid involves the activation of PPAR- $\gamma$  and inhibition of NFAT.

## Materials and methods

**Ethics statement.** The experiments in the present study were performed in conformity with the National Institutes of Health (NIH) (19) and Animal Research: Reporting *in vivo* Experiments (ARRIVE) guidelines (20), and were approved by the Animal Care and Use Committee of Sun Yat-Sen University (approval no. 20161652118). All surgical and experimental procedures were performed according to the guidelines for the care and use of animals approved by the Sun Yat-Sen University and were in accordance with the code of Ethics of the EU Directive 2010/63/EU for animal experiments. All efforts were made to minimize animal suffering and to reduce the number of animals used.

**Animals.** Specific pathogen free male Sprague-Dawley rats (n=90; 6-week-old; weight, 180-220 g) were obtained from the Animal Experiment Center of the Sun Yat-Sen University.

Animals were maintained in controlled environment conditions with temperature (23 $\pm$ 1°C), humidity (60 $\pm$ 10%) and a 12-h light/dark cycle. They were housed in 15 cages each containing 6 rats and were allowed standard rat chow and water *ad libitum*.

**Experimental design.** Following 1-week acclimation, 90 Sprague-Dawley rats were randomly divided into the following 6 groups (n=15): Control, intragastric administration of distilled water; PUFA control, intragastric administration of n-3 PUFA (20 mg/kg/day); TFA control, intragastric administration of TFA (13 mg/kg/day); model, intragastric administration of distilled water + rectal TNBS administration; PUFA model, intragastric administration of n-3 PUFA (20 mg/kg/day) + rectal TNBS administration; and TFA model, intragastric administration of TFA (13 mg/kg/day) + rectal TNBS administration. CD rat models were induced by rectal administration of TNBS (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in ethanol (100 mg/kg TNBS + 50% ethanol; 0.25 ml) four times, with a 10 day interval between each TNBS treatment (days 0, 10, 20 and 30) (21). Rats in the control, PUFA and TFA control groups were given rectal administration of 0.9% NaCl solution instead of TNBS. TFA (13 mg/kg/day; Medicience Ltd., Yangzhou, China) and n-3 PUFA (20 mg/kg/day; Medicience Ltd.) were administered intragastrically every day from day 0 to day 60. We calculated the most suitable dose for rats according to the following formula: Dose for rats = (X mg/kg  $\times$  70 kg  $\times$  0.018)/0.2 kg = 6.3 X mg/kg. X denotes the effective dose for humans, 70 kg is the standard weight for humans, 0.018 is the ratio of the equivalent dose between humans and rats based on body surface area and 0.2 kg is the standard weight of a rat (22).

**Necropsy.** At the end of the experiment (day 60), rats were anesthetized and blood samples (2 ml from each rat) were collected into tubes by cardiac puncture. Colon tissue specimens were subjected to macroscopic assessment prior to being cut and fixed in 10% formaldehyde saline solution at room temperature for 24 h before histological analysis. Serum was collected following blood centrifugation at 550  $\times$  g for 10 min at 4°C. Tissue and serum samples were snap frozen in liquid nitrogen and stored at -80°C until further analysis.

**Blood fat and inflammatory marker detection.** Blood samples were collected in tubes at day 60 by cardiac puncture. Serum content of total cholesterol (TC), triglycerides (TGs), erythrocyte sedimentation rate (ESR) and high-sensitivity C-reactive protein (hs-CRP) was determined using the Olympus AU400 Clinical Chemistry analyzer (Olympus Corporation, Tokyo, Japan).

**Disease activity index (DAI) of rats.** Rats were examined daily by a blinded investigator to determine the DAI, which was achieved by scoring body weight loss, stool consistency and bleeding, as described previously (23,24), and scoring is described in Table I.

**Colon macroscopic damage index (CMDI) of rats.** The CMDI was the criteria for assessing macroscopic damage. The following numerical rating scores were employed: 0, no ulcer

Table I. Criteria for scoring disease activity index.

Score	Weight loss, %	Stool consistency	Occult blood or gross bleeding
0	0	Normal	Negative
1	1-5	Loose stool	Negative
2	5-10	Loose stool	Hemoccult positive
3	10-15	Diarrhea	Hemoccult positive
4	>15	Diarrhea	Gross bleeding

Disease activity index = (combined score of weight loss, stool consistency and bleeding)/3. Normal stools, well-formed pellets; loose stool, pasty stools that do not adhere to the anus; diarrhea, liquid stools that adhere to the anus.

or inflammation; 1, local hyperemia without ulcers; 2, ulceration without hyperemia; 3, ulceration and inflammation at one site only; 4, two or more sites of ulceration and inflammation extending >1 cm; and 5, ulceration extending >2 cm (24,25).

**Tissue damage index (TDI) of rats.** Formaldehyde-fixed colon specimens were obtained and cut into 4  $\mu$ m thick sections. The sections were then stained with 0.5% hematoxylin for 5 min, followed by 0.5% eosin for 1 min at room temperature. Hematoxylin and eosin staining was performed via a light microscope at x100 magnification. An expert pathologist evaluated the stained samples in a blind fashion and evaluated the TDI according to previously described criteria (26), which involves scores from 0-4 (0, no signs of inflammation; 1, low level of leukocyte infiltration; 2, moderate level of leukocyte infiltration; 3, high level of leukocyte infiltration, high vascular density and thickening of the colon wall; 4, in addition to points mentioned for score 3, the lesions exceed 50% of the specimens; 5, transmural infiltration, loss of goblet cells, high vascular density and thickening of the colon wall; and 6, in addition to points mentioned for score 5, the lesions exceed 50% of the specimens) (24).

**ELISA analysis.** The serum levels of cytokines associated with local mucosal immunity, including IL-6, IL-12, tumor necrosis factor (TNF)- $\alpha$  and interferon (IFN)- $\gamma$ , were measured using commercial ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocols. A standard curve was generated with each assay, with the limit of detection for IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  being 12.0, 5.5, 20.0 and 15.4 pg/ml, respectively. Each sample was performed in triplicate.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis.** All the rats in each group were sampled for RT-qPCR analysis. Total RNA was extracted from colon tissue using TRIzol (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to manufacturer protocol. The extracted RNA was diluted to a concentration of 1  $\mu$ g/ $\mu$ l, and aliquots were stored at -80°C. RT-qPCR was performed in triplicate for each specimen using SYBR-Green PCR Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). Total

RNA (500 ng) was used for cDNA synthesis and 1  $\mu$ l each reverse transcription product was added to a 9  $\mu$ l MasterMix reaction containing buffer, SYBR hydrolysis probes, Hot Start Taq polymerase and dNTPs. The reaction consisted of 0.2  $\mu$ l 25 mM dNTPs, 0.5  $\mu$ l 25  $\mu$ M corresponding primers and 9.3  $\mu$ l ddH<sub>2</sub>O for PCR amplification. qPCR cycling conditions were as follows: 10 min pre-denaturation at 95°C followed by 35 or 38 cycles of 10 sec denaturation at 95°C and 60 sec annealing at 52°C. Sequences of the primers used and each number of cycles are listed in Table II. Each experiment was performed in triplicate. The relative levels of the target mRNAs were normalized to the corresponding levels of GAPDH mRNA in the same cDNA sample using a standard curve method recommended in the LightCycler Software version 3.5 (Roche Molecular Diagnostics, Pleasanton, CA, USA).

**Statistical analysis.** Data are presented as the mean  $\pm$  standard deviation. Homoscedasticity tests and normal distribution were performed, and data were subsequently analyzed by one-way analysis of variance followed by the Bonferroni multiple comparisons test.  $P < 0.05$  was considered to indicate a statistically significant difference. All statistical analyses were performed using SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA).

## Results

**Serum fat and inflammatory indices.** The results in Table III demonstrated that TC in the TFA model group was significantly higher compared with other groups ( $P < 0.05$ ), which indicates that a TFA-enriched diet may disturb fat metabolism. However, no differences were observed in the TG content between all groups. Inflammatory indices, including ESR and hs-CRP, were significantly increased in all three model groups compared with the control groups, but the n-3 PUFA model group exhibited significantly reduced levels compared with the model and TFA model groups ( $P < 0.05$ ; Table III).

**DAI scores of rats.** DAI scores in the model, PUFA model and TFA model groups were significantly higher compared with those in control, PUFA control and TFA control groups ( $P < 0.001$  vs. all control groups; Fig. 1). No differences were observed in the DAI scores among the three control groups, which indicate that diet intervention did not have effects in healthy rats (Fig. 1). However, a significant decrease in DAI scores was observed in the PUFA model group ( $P < 0.001$  vs. model group) and an increase in the TFA model group ( $P < 0.01$  vs. model group; Fig. 1).

**CMDI and TDI.** CMDI and TDI indices in the model group were significantly higher compared with the three control groups ( $P < 0.001$ ). A high intake of n-3 PUFA significantly lowered both scores, exerting a protective effect on rats with CD ( $P < 0.01$  for CMDI and  $P < 0.001$  for TDI). A TFA-enriched diet significantly increased TDI scores ( $P < 0.001$ ) and CMDI scores were increased (without statistical significance;  $P > 0.05$ ) compared with the model group (Fig. 2).

**Pathological changes in rat colon tissue.** Colon tissue in TNBS-induced CD model rats was characterized by

Table II. Primers and product size for each target gene.

Gene	Primer sequence		Length, bp	Number of cycles	Annealing temperature, °C
	Forward	Reverse			
PPAR- $\gamma$	5'-ATTACGATAGCCGTTCC-3'	5'-TAATGCTATCGGCAATT-3'	190	35	52
NFAT	5'-GTACCGTACATGCGTTCA-3'	5'-CATGGCATGTACGCAAGT-3'	201	35	52
IL-2	5'-CGTATCATGCACTGCATA-3'	5'-GCATAGTACGTGACGTAT-3'	186	38	52
IL-4	5'-ATCCGATAACGTACCGTAC-3'	5'-TAGGCTATTGCATGGCATG-3'	173	35	52
GAPDH	5'-GAATCTGGTGGCTGTGGA-3'	5'-CCCTGAAAGGCTTGGTCT-3'	202	35	52

PPAR, peroxisome proliferator-activated receptor- $\gamma$ ; NFAT, nuclear factor of activated T-cells; IL, interleukin.

Table III. Serum fat and inflammatory index in rats in each group.

Group	Triglyceride, mmol/l	Total cholesterol, mmol/l	Erythrocyte sedimentation rate, mm/h	High-sensitivity C-reactive protein, mg/l
Control	1.21 $\pm$ 0.22	1.72 $\pm$ 0.42 <sup>a</sup>	12.00 $\pm$ 4.32 <sup>a-c</sup>	2.11 $\pm$ 0.28 <sup>a-c</sup>
PUFA control	1.32 $\pm$ 0.35	1.69 $\pm$ 0.61 <sup>a</sup>	13.41 $\pm$ 4.36 <sup>a-c</sup>	2.78 $\pm$ 0.34 <sup>a-c</sup>
TFA control	1.37 $\pm$ 0.28	2.14 $\pm$ 0.42 <sup>a</sup>	15.67 $\pm$ 4.84 <sup>a-c</sup>	2.65 $\pm$ 0.27 <sup>a-c</sup>
Model	1.17 $\pm$ 0.26	2.01 $\pm$ 0.53 <sup>a</sup>	23.07 $\pm$ 4.90 <sup>b</sup>	9.05 $\pm$ 2.50 <sup>b</sup>
PUFA model	1.39 $\pm$ 0.33	1.97 $\pm$ 0.45 <sup>a</sup>	19.34 $\pm$ 5.07 <sup>a,c</sup>	6.15 $\pm$ 1.75 <sup>a,c</sup>
TFA model	1.37 $\pm$ 0.30	3.01 $\pm$ 0.55	24.05 $\pm$ 4.82 <sup>b</sup>	8.98 $\pm$ 2.63 <sup>b</sup>

Data are presented as the mean  $\pm$  standard deviation, n=15 per group. <sup>a</sup>P<0.05 vs. TFA model group; <sup>b</sup>P<0.05 vs. PUFA model group; <sup>c</sup>P<0.05 vs. model group. PUFA, polyunsaturated fatty acid; TFA, trans-fatty acid.

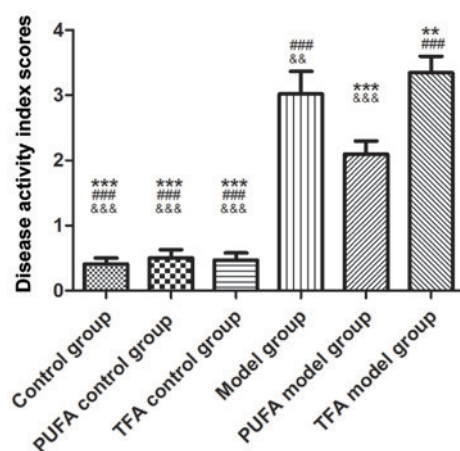


Figure 1. Disease activity index scores of rats in each group. Data in this figure represent results at day 60 only and are presented as the mean  $\pm$  standard deviation. PUFA, polyunsaturated fatty acid; TFA, trans fatty acid. \*\*P<0.01, \*\*\*P<0.001 vs. model group; ###P<0.001 vs. PUFA model group; &&P<0.01, &&&P<0.001 vs. TFA model group.

infiltration of the lamina propria with inflammatory cells and crypt abscesses (Fig. 3). Following intervention with n-3 PUFA treatment, a marked improvement in the level inflammation was observed (Fig. 3). However, TFA intervention appeared to aggravate inflammation with epithelioid granulomas, which are typical pathological changes of CD (Fig. 3).

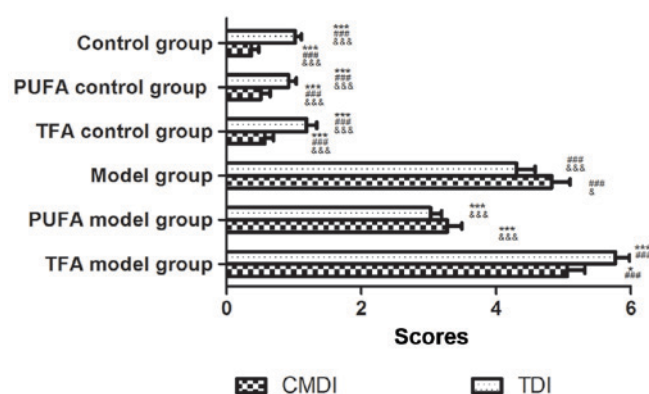


Figure 2. Evaluation of CMDI and TDI scores in each group. Data are presented as the mean  $\pm$  standard deviation. CMDI, colon macroscopic damage index; TDI, tissue damage index; PUFA, polyunsaturated fatty acid; TFA, trans fatty acid. \*P<0.05, \*\*\*P<0.001 vs. model group; ###P<0.001 vs. PUFA model group; &P<0.05, &&&P<0.001 vs. TFA model group.

Serum levels of cytokines in each group detected by ELISA. Marked increases in IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  serum levels were observed in the three model groups compared with the three control groups (Fig. 4), indicating that TNBS intervention successfully altered local mucosal immunity. IL-6, IL-12 and TNF- $\alpha$  concentrations were significantly decreased in the PUFA model group compared with the model group (P<0.05; Fig. 4). However, a TFA-enriched diet appeared to



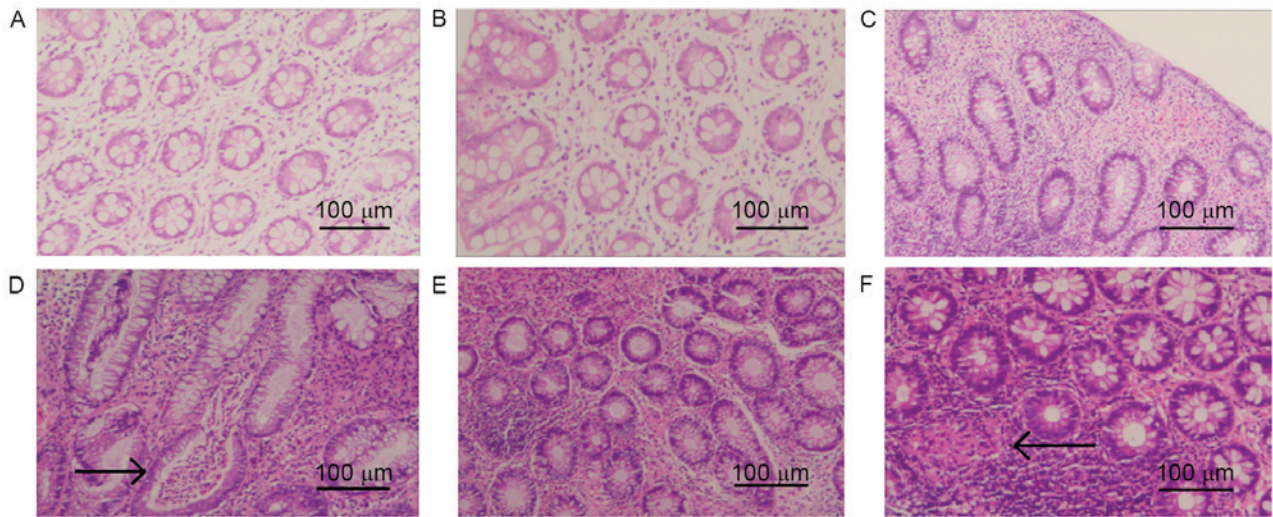


Figure 3. Pathological changes in the rat colon stained with hematoxylin and eosin in each group. (A) Colonic tissues in the control group with integrated architecture exhibiting no inflammation. Colonic tissues in the (B) n-3 PUFA control group and (C) TFA control group exhibiting mild or no inflammation. (D) Colonic tissues in 2,4,6-trinitrobenzene sulfonic acid-induced model group exhibiting increased inflammatory cell infiltration and typical crypt abscesses (arrow). (E) Colonic tissues in the n-3 PUFA model group exhibiting uneven distribution of inflammation. (F) Colonic tissues in the TFA model group exhibiting epithelioid granulomas in addition to inflammatory cell infiltration (arrow). Magnification, x100. PUFA, polyunsaturated fatty acid; TFA, trans fatty acid.

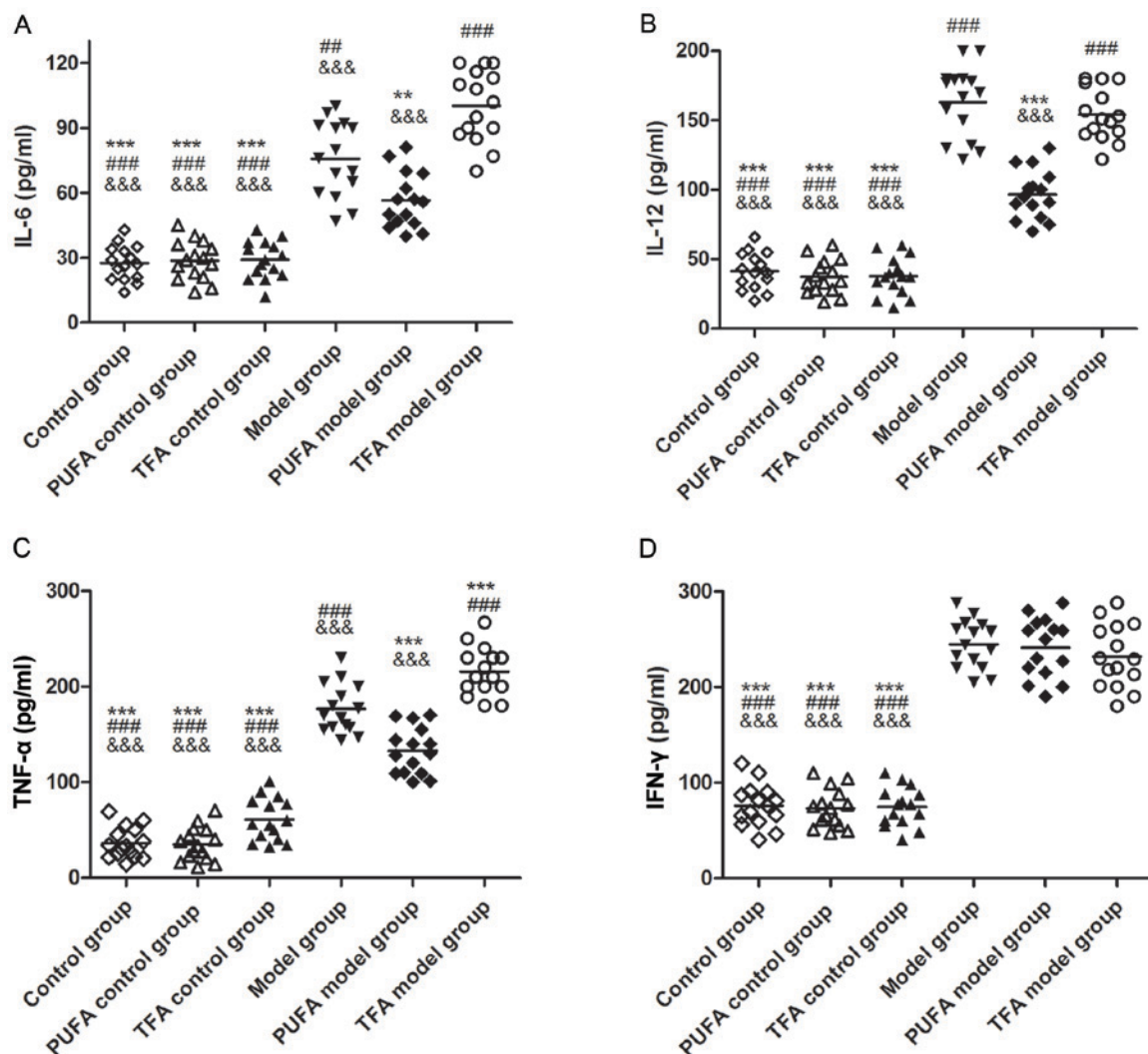


Figure 4. Concentration of cytokines in the serum detected by ELISA in each group. ELISA was performed to determine the levels of (A) IL-6, (B) IL-12, (C) TNF- $\alpha$  and (D) IFN- $\gamma$  in the serum of rats in each treatment group. Data points indicate the individual values of cytokines in single biopsies and horizontal bars represent the median value. IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; PUFA, polyunsaturated fatty acid; TFA, trans fatty acid. \*\* $P<0.01$ , \*\*\* $P<0.001$  vs. model group; ## $P<0.01$ , ### $P<0.001$  vs. PUFA model group; &&& $P<0.001$  vs. TFA model group.

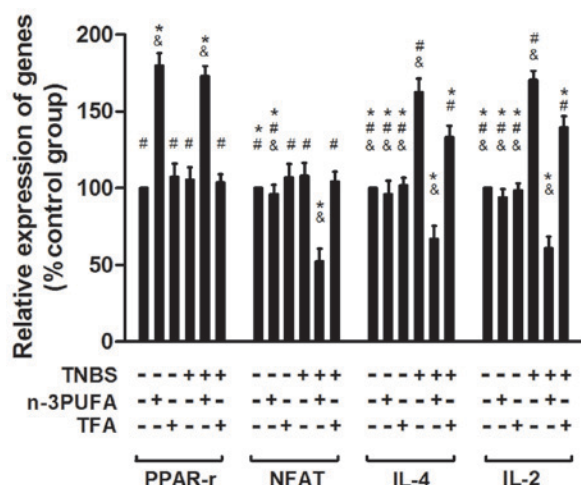


Figure 5. Relative mRNA expression of genes associated with the PPAR- $\gamma$ /NFAT pathway in each group. The relative mRNA expression of PPAR- $\gamma$ , NFAT, IL-4 and IL-2 was analyzed in colon samples taken from rats in each treatment group by reverse transcription-quantitative polymerase chain reaction, and expression was presented normalized to the control group. PPAR, peroxisome proliferator-activated receptor; NFAT, nuclear factor of activated T-cells; IL, interleukin; TNBS, 2,4,6-trinitrobenzene sulfonic acid; PUFA, polyunsaturated fatty acid; TFA, trans fatty acid. \* $P < 0.05$  vs. model group, # $P < 0.05$  vs. PUFA model group and & $P < 0.05$  vs. TFA model group.

worsen local mucosal immunity as levels of serum IL-6 and TNF- $\alpha$  were significantly increased in the TFA model group compared with the model group ( $P < 0.05$ ; Fig. 4).

**Expression of PPAR- $\gamma$ /NFAT-associated genes detected by RT-qPCR.** The mRNA expression of genes associated with the PPAR- $\gamma$ /NFAT pathway, including PPAR- $\gamma$ , NFAT, IL-2 and IL-4, was detected by RT-qPCR. A significant increase in the expression of the proinflammatory cytokines IL-2 and IL-4 was observed in the model and TFA model groups ( $P < 0.05$ ; Fig. 5) compared with the control group. However, IL-2 and IL-4 expression was reduced in the n-3 PUFA model group, indicating a potential anti-inflammatory effect of n-3 PUFA diet intervention (Fig. 5). Furthermore, the expression of PPAR- $\gamma$  was enhanced in the n-3 PUFA intervention group compared with model and TFA model groups (Fig. 5), which indicates that PPAR- $\gamma$  may be activated by an n-3 PUFA-enriched diet. In addition, the expression of NFAT was reduced in the n-3 PUFA model group compared with model and TFA model groups (Fig. 5), indicating that n-3 PUFA may inhibit NFAT, potentially via the activation of PPAR- $\gamma$  in TNBS-induced CD rats.

## Discussion

At present, no curative treatment is available for CD. Traditional treatments, including aminosalicylates, corticosteroids, immunomodulating drugs and biological agents, are associated with limitations due to adverse side effects (27-29). Therefore, the identification of alternative therapeutic strategies that are effective with reduced side effects is necessary. Previous studies have demonstrated the association between dietary fat and IBD. Borniquel *et al* (30) reported that dietary conjugated linoleic acid ameliorated colonic inflammation induced by dextran sulfate sodium in mice. In addition, another

study demonstrated the efficacy of short chain fatty acids in accelerating clinical disease in an IL-10 $^{-/-}$  mouse model (31).

Dietary n-3 PUFA is present in fish oil. It was reported to ameliorate intestinal inflammation in a colitis rat model. However, the underlying mechanisms were not determined (32). Based on this, the present study aimed to determine whether a similar protective effect of dietary n-3 PUFA may be observed in CD. Thus, TNBS-induced CD rat models were established, which were characterized as IL-12 driven and resembled the alterations observed in patients with CD (33). An n-3 PUFA diet was employed as an intervention strategy. The present study successfully established CD rat models with pathological changes such as massive transmural infiltration of inflammatory cells, typical epithelioid granulomas and crypt abscess formation. The results demonstrated that dietary supplementation with n-3 PUFA ameliorated the symptoms of experimental CD rats, with lower DAI, CDI and CMDI scores and milder changes demonstrated by pathological observation. In addition, the results indicated that the protective effects of n-3 PUFA may involve activation of the PPAR- $\gamma$ /NFAT pathway, which ultimately led to improvements in mucosal inflammation and amelioration of tissue damage. This study, to the best of our knowledge, was the first to report the protective effect of dietary n-3 PUFA on CD rats and to identify potential underlying mechanisms.

The role of PPAR- $\gamma$  in the etiology and treatment of colitis is a subject of great interest. Thiazolidinediones, PPAR- $\gamma$  ligands, have been previously reported to decrease the severity of UC in rats (34). Furthermore, PPAR- $\gamma^{+/-}$  heterozygous mice exhibited increased susceptibility to experimental-induced colitis, which further indicated a role for PPAR- $\gamma$  in the maintenance of gut homeostasis (35,36). Despite these observations, the role of PPAR- $\gamma$  in experimental CD rats and its association with dietary fatty acid intervention has not been previously reported. It is established that n-3 PUFAs and their metabolites are endogenous PPAR- $\gamma$  ligands (37). The results of the current study revealed that n-3 PUFA intake activated PPAR- $\gamma$  expression in healthy rats and TNBS-induced CD rats. Therefore, the protective effects of n-3 PUFA may be attributed to the activation and increased expression of PPAR- $\gamma$ , which was consistent with a previous study (30). PPAR- $\gamma$  usually exists as a heterodimer with retinoid X receptor, and has been reported to be associated with PPAR response elements in the regulation of NFAT, a transcription factor that binds to the IL-2 and IL-4 promoters and is necessary for gene transcription (18,38). The present study demonstrated that an n-3 PUFA-enriched diet inhibited the expression of NFAT in CD rats. In addition, the gene expression of the proinflammatory cytokines IL-2 and IL-4 was reduced, which may contribute to ameliorated mucosal localized inflammation in n-3 PUFA intervention CD rats. However, no difference in NFAT expression was observed between healthy rats with or without n-3 PUFA, which indicates that factors other than n-3 PUFA and PPAR- $\gamma$  may be involved in the modulation of NFAT expression, and further investigation is required.

TFAs are steric isomers of cis unsaturated fatty acids with distinct differences in biological effects. TFAs are primarily present in dairy products and meats, and are generated during the manufacturing process. Western-style diets usually contain high levels of TFAs (39). Recently, a number of reports have



demonstrated that TFA intake is a risk factor for diabetes mellitus, coronary heart disease and hyperlipemia (40,41). The present study demonstrated that TFA intake enhanced the risk of hypertriglyceridemia in CD rats, which was consistent with the results of a previous study (42). However, no differences in blood fat levels were observed in healthy rats despite the different diets, which indicated an association between intestinal inflammation and susceptibility to blood lipid disorders. Furthermore, the results of the current study revealed that a TFA-enriched diet enhanced the serum levels of IL-6 and TNF- $\alpha$ , exacerbated mucosal localized inflammation determined by pathological observation and increased the gene expression of IL-2 and IL-4. However, TFA exhibited no effect on the gene expression of PPAR- $\gamma$  and NFAT, indicating that TFA may worsen inflammatory in CD rats via pathways other than PPAR- $\gamma$ /NFAT.

In conclusion, the present study demonstrated a potent anti-inflammatory effect of dietary n-3 PUFA in experimental CD rats, and the protective effect of this fatty acid may involve the activation of PPAR- $\gamma$  and inhibition of NFAT. Therefore, an n-3 PUFA-enriched diet, with few adverse side effects and established mechanisms, may be a novel therapeutic approach for patients with CD. However, further investigation is required. An improved mechanistic understanding in combination with more comprehensive data from well-designed clinical studies will provide the basis for accelerating the development of nutrition-based therapies against CD.

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