Biochemical and Molecular Actions of Nutrients

Dietary Olive Oil Supplemented with Fish Oil, Rich in EPA and DHA (n-3) Polyunsaturated Fatty Acids, Attenuates Colonic Inflammation in Rats with DSS-Induced Colitis[1]

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ABSTRACT Previous studies proposed a protective role of the dietary intake of (n-3) PUFA in human inflammatory bowel disease (IBD), but almost no studies have been performed using olive oil. The aims of the present study were to test the beneficial effects of an olive oil–based diet with or without fish oil, rich in (n-3) PUFA, in the dextran sodium sulfate (DSS) model of rat colitis and to elucidate the mechanisms involved in their potential beneficial effects, with special attention to the production of some of the mediators involved in the intestinal inflammatory response, such as leukotriene B_4 (LTB_4), tumor necrosis factor α (TNFα) and nitric oxide (NO). Rats were fed the different diets for 2 wk before colitis induction and thereafter until colonic evaluation 15 d later. Colitic rats fed the olive oil–based diet had a lower colonic inflammatory response than those fed the soybean oil diet, and this beneficial effect was increased by the dietary incorporation of (n-3) PUFA. A restoration of colonic glutathione levels and lower colonic NO synthase expression occurred in all colitic rats fed an olive oil diet compared with the control colitic group that consumed the soybean oil diet. However, (n-3) PUFA incorporation into an olive oil diet significantly decreased colonic TNFα and LTB_4 levels compared with colitic rats that were not supplemented with fish oil. These results affirm the benefits of an olive oil diet in the management of IBD, which are further enhanced by the addition of (n-3) PUFA. J. Nutr. 135: 687–694, 2005.

KEY WORDS: • *rat experimental colitis* • *leukotriene B_4* • *nitric oxide synthase* • *(n-3) polyunsaturated fatty acid* • *tumor necrosis factor*

Inflammatory bowel disease (IBD)[4] is a chronic disease of the digestive tract, and usually refers to 2 related conditions, i.e., ulcerative colitis (UC) and Crohn's disease (CD), characterized by chronic and spontaneously relapsing inflammation. Although the etiology of IBD remains unknown, it is believed that the generation of an exaggerated intestinal immune response to otherwise innocuous stimuli plays a key role in the pathophysiology of these intestinal disorders (1). As a result, the synthesis and release of different proinflammatory mediators, including reactive oxygen and nitrogen metabolites, eicosanoids, platelet-activating factor (PAF), and cytokines, are upregulated (2). All of these mediators actively contribute to the pathogenic cascade that initiates and perpetuates the inflammatory response of the gut. At this time, a specific causal treatment for IBD is not available, and the best strategy to effectively downregulate the exacerbated immune response that characterizes IBD may be to interfere with multiple stages of the inflammatory cascade, preferably with a single drug treatment (3). In fact, the drugs currently used for the management of human IBD, i.e., 5-aminosalicylic acid derivatives and systemic or local glucocorticoids, exert their beneficial effects through a combination of different mechanisms (4,5). Unfortunately, these drugs are not devoid of potentially serious side effects, thus limiting their use (6,7). Consequently, dietary management of IBD may be an attractive alternative to drug therapy if it proves to be effective without adverse effects.

Several epidemiologic studies in Eskimos revealed a low incidence of IBD compared with Western populations, thus supporting the protective role of the dietary intake of (n-3) PUFA (8). In addition, patients with chronic intestinal disorders, such as IBD, had lower plasma levels of (n-3) PUFA than normal subjects (9). In fact, (n-3) PUFA may exert a beneficial effect in these intestinal conditions by competing with (n-6) PUFA for the production of lipid inflammatory

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[4] Abbreviations used: AP, alkaline phosphatase; CD, Crohn's disease; DAI, disease activity index; DHA, docosahexaenoic acid; DSS, dextran sodium sulfate; EPA, eicosapentaenoic acid; GSH, glutathione; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; LTB_4, leukotriene B_4; MPO, myeloperoxidase; NF, nuclear factor; NO, nitric oxide; NOS, nitric oxide synthase; PAF, platelet-activating factor; TNBS, trinitrobenzenesulphonic acid; TNFα, tumor necrosis factor α; UC, ulcerative colitis.

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mediators. Their anti-inflammatory properties are associated with the well-known ability of these fatty acids to inhibit the production of various proinflammatory mediators, including eicosanoids such as leukotriene B_4 (LTB_4), thromboxane A_2, or prostaglandin E_2, and cytokines (10). Although the clinical studies dealing with the use of (n-3) PUFA in IBD have yielded conflicting results, probably related to discrepancies in patient selection or the formulations and dosages used in the different protocols (8), most indicate a potential effectiveness of these fatty acids in the therapy of UC and CD. This is supported by the beneficial effects shown by (n-3) PUFA administration in different experimental models of rat intestinal inflammation, induced by acetic acid (11), trinitrobenzenesulfonic acid (TNBS) (12–14), or dextran sodium sulfate (DSS) (15). In most of these studies, the reported intestinal anti-inflammatory effect of these fatty acids was associated with a reduction in LTB_4 production (11–13,15,16). LTB_4 is a potent neutrophil chemotactic agent, which induces neutrophil adherence to the vascular wall and potentiates the effects of other mediators such as PAF in promoting neutrophil migration across the endothelial monolayer as well as in increasing mesenteric vascular permeability (17). In fact, LTB_4 synthesis was shown to be enhanced in the colonic mucosa of patients with UC and CD (18), and it was proposed that inhibition of LTB_4 synthesis may contribute to the therapeutic effect exerted by different drugs such as sulfasalazine and 5-ASA used in the treatment of IBD (4). Although it was reported that (n-3) PUFA supplementation to patients with proctocolitis resulted in the suppression of immune reactivity associated with a reduction in disease activity (19), little information is available concerning the in vivo effects of these fatty acids on proinflammatory mediators such as cytokines or nitric oxide (NO), which are also involved in the exacerbated immune response in these intestinal conditions. Thus, a (n-3) fatty acid–rich diet was reported to modulate cytokine production in patients with colorectal cancer (20) and to decrease mucosal interleukin 6 secretion in the TNBS model of enteritis (14).

On the other hand, olive oil is widely consumed in Mediterranean regions and may have health benefits that include modification of the immune and inflammatory responses. It was suggested that these effects may be derived from the antioxidant properties attributed to its components, including the monounsaturated fatty acid, oleic acid (21,22). However, little is known about the role of olive oil in intestinal inflammation, although it was reported recently that oleic acid protected human intestinal smooth muscle cells isolated from patients with CD from the oxidative stress that characterizes this intestinal condition (23).

The aim of the present study was to test the intestinal anti-inflammatory effects of dietary administration of olive oil, with or without supplemented of fish oil–derived (n-3) PUFA, in the DSS model of rat colitis, an experimental model of intestinal inflammation that has some histological and biochemical features of the human disease (24,25). Special attention was paid to the effects exerted by these lipid manipulations on the production of some of the mediators involved in the inflammatory response, i.e., LTB_4, NO, and tumor necrosis factor α (TNFα).

MATERIALS AND METHODS

This study was carried out in accordance with NIH guidelines and approved by the Animal Research and Ethic Committee of the University of Granada (Spain).

Reagents and composition of diets. All chemicals were obtained from Sigma Chemical, unless otherwise stated. Glutathione (GSH) reductase was provided by Boehringer Mannheim. The different semisynthetic diets used in the present study were provided by Harlan Interfauna Iberica S.A (#2014). The only difference among the diets was the source of fat included, which was the 4% (wt/wt) of the diet in all cases. The diets were designated as SO (crude soybean oil), OO (virgin olive oil), and FO (fat constituted by 96% virgin olive oil and 4% refined fish oil). Diets were composed of 14.5% crude protein, 4% crude oil (as previously described), 4.5% crude fiber (in the form of α-cellulose) and 63.9% carbohydrates (which included 44.5% starch and 5.7% sucrose among others). Other components such as ash and the mineral and vitamin mix represented 4.7 and 0.8%, respectively. The digestible energy of each diet was 3.2 kcal/g (13.39 kJ/g).

Experimental design. Female Wistar rats (180–200 g) obtained from the Laboratory Animal Service of the University of Granada (Granada, Spain) were housed individually in Makrolon® cages and maintained in an air-conditioned atmosphere with a 12-h light:dark cycle; they had free access to tap water and food. The rats were randomly assigned to 4 groups ($n = 10$); 2 groups (noncolitic and control colitic groups) were fed the SO diet, 1 was fed the OO diet, and the remaining group was fed the FO diet. Two weeks after the start of the experiment, 3 groups of rats (SO control colitic, OO, and FO groups) were made colitic as described previously (26) by replacing normal drinking water with distilled water containing 5% DSS (wt/v, prepared daily, mol wt 36,000–50,000) for 5 d. DSS was decreased to 2% (wt/v) for the next 10 d. A noncolitic group was also included for reference. All rats were killed by cervical dislocation 29 d after the start of the experiments.

Assessment of colonic damage. Animal body weight, the presence of gross blood in the feces, and stool consistency were recorded daily for each rat by an observer who was unaware of the treatment. These parameters were each assigned a score according to the criteria proposed by Cooper et al. (27) (**Table 1**), which was used to calculate

TABLE 1

Scoring of DAI and criteria for assessment of microscopic colonic damage[1]

	DAI		
DAI score	Weight loss	Stool consistency	Rectal bleeding
	%		
0	0	Normal	Normal
1	1–5		
2	5–10	Loose stools	
3	10–20		
4	>20	Diarrhea	Gross bleeding

Assessment criteria[2]

Mucosal epithelium
- Ulceration: none (0); mild surface (1); moderate (2); extensive-full thickness (3)

Crypts
- Mitotic activity: lower third (0); mild mid-third (1); moderate mid-third (2); upper third (3)
- Mucus depletion: none (0); mild (1); moderate (2); severe (3)

Lamina propria
- Mononuclear infiltrate: none (0); mild (1); moderate (2); severe (3)
- Granulocyte infiltrate: none (0); mild (1); moderate (2); severe (3)
- Vascularity: none (0); mild (1); moderate (2); severe (3)

Submucosal
- Mononuclear infiltrate: none (0); mild (1); moderate (2); severe (3)
- Granulocyte infiltrate: none (0); mild (1); moderate (2); severe (3)
- Edema: none (0); mild (1); moderate (2); severe (3)

[1] The DAI represents the combined scores of weight loss, stool consistency, and bleeding divided by 3. Adapted from Cooper et al. (27).

[2] Maximum score: 27. Modified from Stucchi et al. (26).

a mean daily disease activity index (DAI) for each rat. Food and water consumption was also recorded daily throughout the duration of the study. After the rats were killed, their colons were immediately removed and rinsed with ice-cold PBS. Each specimen was weighed and its length measured under a constant load (2 g). The colon was opened longitudinally and a cross section from the distal diseased area was immediately fixed in 4% formaldehyde and embedded in paraffin for histological analysis. It was subsequently divided into 5 longitudinal segments for biochemical determinations: 3 fragments were frozen at −80°C for myeloperoxidase (MPO) and alkaline phosphatase (AP) activities and for nitric oxide synthase (NOS) expression; another sample was weighed and frozen in 1 mL of 50 g/L trichloroacetic acid for determination of total GSH. The remaining sample was processed immediately for measurement of TNFα and LTB_4 levels. All biochemical measurements were completed within 1 wk from the time of sample collection and were performed in duplicate.

MPO activity was measured according to the technique described by Krawisz et al. (28); the results were expressed as MPO units/g wet tissue; 1 unit of MPO activity was defined as that degrading 1 μmol hydrogen peroxide/min at 25°C. AP activity was determined spectrophotometrically using disodium *p*-nitrophenylphosphate as a substrate (29), and the results expressed as mU/mg protein. Total GSH was quantified with the recycling assay described by Anderson (30), and the results were expressed as nmol/g wet tissue. Colonic samples for TNFα and LTB_4 determinations were immediately weighed and processed as previously described (29). TNFα was quantified by an ELISA (Amersham Pharmacia Biotech) and the results were expressed as pg/g wet tissue. LTB_4 was determined by enzyme-immunoassay (Amersham Pharmacia Biotech) and the results expressed as ng/g wet tissue.

Colonic inducible nitric oxide synthase (iNOS) expression was determined by Western blotting as described previously (29). Control of protein loading and transfer was conducted by detection of the β-actin levels.

Fatty acids profile in plasma, liver and colonic tissue. A total plasma fatty acid profile was determined as previously described (31). After homogenization in distilled water (1:2) of diets and colon samples, the total fatty acid profile was determined in the same way. Briefly, GC analyses were performed in an Agilent Technologies 6890N gas chromatograph equipped with a flame ionization detector and provided with a split-splitless injection port. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The separation of the compounds was performed using a Supelco SP2380 column, 60 m × 0.25 mm i.d., 0.25 μm film thickness. The oven temperature was programmed from 80°C (3.0 min) to 175°C at 10°C/min (10 min) and to 250°C at 3.3°C/min (4.0 min). The detector temperature was set at 280°C and the injector port temperature at 250°C. Analyses were performed in split mode (50%) using a Supelco 4.0 mm i.d. inlet liner.

Histological analysis. Colonic full-thickness (5 μm) sections were stained with hematoxylin and eosin and graded by two pathologists (A.N. and A.C., who were unaware of the experimental groups) according to the criteria described previously by Stucchi et al. (26), with modifications (Table 1). Microphotographs were taken with a Leika DM 5000B microscope.

Statistics. All results are expressed as means ± SEM. Differences between means were tested for significance using 1-way ANOVA and post hoc least significance difference tests. In all cases, the Shapiro-Wilk test allowed the assumption of normal distribution of the data. All statistical analyses were conducted with the Statgraphics 5.0 software package (STSC), with differences considered significant at $P < 0.05$.

RESULTS

The main differences among the 3 diets were the contents of oleic acid [18:1(n-9)], linoleic acid [18:2(n-6)], linolenic acid [18:3(n-3)], eicosapentaenoic acid (EPA) [20:5(n-3)], and docosahexaenoic acid (DHA) [22:6(n-3)] (**Table 2**). Levels of SFA did not differ among the diets. Linoleic acid concentrations were much lower, and oleic acid concentrations much greater, in the OO and FO diets than in the control diet.

TABLE 2

Fatty acid composition of the diets

Fatty acid	SO diet	OO diet	FO diet
	g/100 g fatty acids		
14:0	0.0	0.0	0.4
16:0	12.5	11.8	12.4
16:1(n-7)	0.0	0.9	1.3
18:0	2.1	3.0	3.0
18:1(n-9)	22.0	73.2	70.7
18:1(n-7)	1.1	2.3	2.4
18:2(n-6)	58.0	7.5	8.0
18:3(n-3)	3.7	0.5	0.6
20:4(n-6)	0.0	0.0	0.0
20:5(n-3)	0.0	0.0	0.8
22:6(n-3)	0.0	0.0	0.4
Σ Saturated	14.6	14.9	15.8
Σ Monounsaturated	23.0	76.3	74.4
Σ PUFA	61.7	8.0	9.8
Σ (n-3)	3.7	0.5	1.8
Σ (n-6)	58.0	7.5	8.0
Σ (n-9)	22.0	73.2	70.7
(n-6)/(n-9) ratio	2.6	0.1	0.1
(n-6)/(n-3) ratio	15.7	14.0	4.5

Similar to the reduction in (n-6) linoleic acid in the olive oil–based diets, substituting oleic acid reduced the soy-derived (n-3) linolenic acid in both diets. This replacement of both linoleic and linolenic acid altered the (n-6):(n-9) ratio in the olive oil–based diets, but the (n-6):(n-3) ratio did not differ between the SO and OO diets. The FO diet was enriched in EPA and DHA (n-3) fatty acids. Although EPA and DHA were not detectable in the SO and OO diets, they were present (0.5–0.7%) in the FO diet. In the FO diet, the change in the (n-6):(n-9) ratio was similar to that in the OO diet compared with the SO diet. However, although the (n-6):(n-3) ratio was similar (~15) in both the SO and OO diets, it was 4.5 in the FO diet as a consequence of the incorporation of fish oil–derived (n-3) PUFA (Table 2).

The lipid profile in plasma, liver, and colon did not change due to the induction of the colitic process (data not shown), although consumption of the different diets did affect the lipid profile (**Table 3**). The differences in plasma and colon samples paralleled the differences in the composition of fatty acids in the diet, thus confirming the in vivo incorporation of dietary lipids into different body compartments. The same effects were present in liver samples (data not shown). Compared with the SO-fed control rats, the reduction in the linoleic acid content in OO- and FO-fed rats, due to the substitution by oleic acid, was evident in all of the tissues analyzed. This reduction in linoleic acid suggests that total PUFA amounts were also reduced, especially in the (n-6) PUFA pools, thereby changing the (n-6):(n-9) ratio. Moreover, there was an increase in EPA and DHA in the tissues of FO-fed rats compared with both SO- and OO-fed rats. This increase in the (n-3) EPA and DHA led to an increase in the total (n-3) PUFA and a modification of the (n-6):(n-3) ratio, although it did not compensate completely for the reduction in total PUFA (Table 3).

Exposure to DSS in the drinking water induced a colonic inflammatory status, with features similar to those described previously (25,26). Half of the rats developed loose stools after d 1 of DSS administration, which turned to diarrhea in the vast majority of the rats (90–100%) at 4–5 d. After 6–7 d, gross rectal bleeding was evident in 70% of the colitic control

TABLE 3

Fatty acid composition in plasma and colon from DSS-induced colitic rats fed the SO, OO, or FO diets[1]

	Plasma			Colon		
Fatty acid	SO diet	OO diet	FO diet	SO diet	OO diet	FO diet
			g/100 g fatty acid			
16:0	21.1 ± 0.8	18.8 ± 0.8	20.3 ± 0.9	22.5 ± 0.5	21.8 ± 0.5	22.6 ± 0.6
18:0	16.3 ± 1.1	18.5 ± 0.8	19.8 ± 1.4	11.8 ± 1.5	12.5 ± 1.0	11.6 ± 1.3
18:1(n-9)	10.6 ± 0.6^a	22.2 ± 2.5^b	19.1 ± 1.4^b	19.5 ± 1.6^b	27.4 ± 1.4^a	27.3 ± 2.1^a
18:2(n-6)	23.9 ± 2.0^a	5.4 ± 1.3^b	7.4 ± 0.5^b	18.5 ± 1.9^a	10.0 ± 1.0^b	9.5 ± 1.4^b
18:3(n-3)	0.9 ± 0.7	1.0 ± 0.6	0.2 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	0.6 ± 0.1
20:4(n-6)	18.0 ± 2.1	21.4 ± 1.9	20.3 ± 2.7	10.9 ± 1.7	11.3 ± 1.4	9.7 ± 2.1
20:5(n-3)	0.2 ± 0.1^b	0.3 ± 0.1^b	1.2 ± 0.2^a	0.2 ± 0.1^b	0.1 ± 0.0^b	0.4 ± 0.0^a
22:6(n-3)	3.2 ± 0.2^b	2.4 ± 0.1^b	4.7 ± 0.2^a	1.4 ± 0.2^b	1.3 ± 1.3^b	2.5 ± 0.2^a
Σ Saturated	38.4 ± 1.9	38.5 ± 0.7	41.0 ± 2.3	36.1 ± 1.3	35.9 ± 1.0	36.2 ± 1.3
Σ Monounsaturated	13.7 ± 0.9^b	27.7 ± 3.3^a	24.0 ± 2.0^a	27.0 ± 1.9^b	35.7 ± 1.6^a	37.7 ± 2.5^a
Σ PUFA	46.4 ± 2.6^a	30.5 ± 0.8^b	34.2 ± 3.0^b	33.3 ± 0.6^a	24.7 ± 0.9^b	24.0 ± 1.6^b
Σ (n-3)	4.3 ± 0.7^a	3.7 ± 0.6^a	3.2 ± 0.1^b	2.6 ± 0.3^b	2.4 ± 0.5^b	4.0 ± 0.2^a
Σ (n-6)	42.1 ± 2.7^a	26.8 ± 0.9^b	28.0 ± 3.0^b	30.3 ± 0.5^a	22.0 ± 0.8^b	19.8 ± 1.7^b
Σ (n-9)	11.3 ± 0.8^b	22.7 ± 2.3^a	19.5 ± 1.5^a	19.5 ± 1.6^b	28.0 ± 1.4^a	30.6 ± 2.3^a
(n-6)/(n-9) ratio	3.9 ± 0.4^a	1.3 ± 0.1^b	1.6 ± 0.2^b	1.6 ± 0.2^a	0.8 ± 0.1^b	0.7 ± 0.1^b
(n-6)/(n-3) ratio	11.4 ± 1.3^a	8.8 ± 1.2^a	4.5 ± 0.5^b	12.8 ± 1.7^a	11.7 ± 2.2^a	5.2 ± 0.6^b

[1] Values are means ± SEM, $n \geq 8$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

rats, which persisted until the end of the experiment. The inflammatory process resulted in a progressive loss of weight in all colitic rats, which was associated with a reduction in food intake compared with noncolitic rats. The weight loss was evident 1 d after the start of DSS administration, and increased progressively until the day the percentage of DSS was changed, i.e., 6 d after the beginning of the experiments. From that time point, weight did not change significantly in these colitic rats. As a consequence of the inflammatory process, DAI was increased in control colitic rats from d 1 to 5. From d 6 to 15, when the DSS percentage in drinking water was reduced from 5 to 2%, DAI remained nearly constant in this group of rats (**Fig. 1**). Water consumption did not differ among the colitic groups. Daily water consumption was 18.5 ± 0.7 mL/rat, which was lower than that of noncolitic rats (22.5 ± 0.2 mL/rat, $P < 0.05$).

Macroscopic examination of the colonic specimens after 15 d of DSS treatment revealed bowel wall thickening with a significant increase in the colonic weight:length ratio compared with noncolitic rats (100.6 ± 6.0 vs. 64.9 ± 1.6 mg/cm; $P < 0.01$). The histological examination of colonic sections also assessed the intestinal inflammatory status; microscopically, the samples from control colitic rats showed typical inflammatory changes in the colonic architecture, i.e., ulceration, crypt dilation, goblet cell depletion, as well as mixed cell infiltration, composed mainly of mononuclear cells (macrophages, lymphocytes, and plasma cells), and granulocytes (**Fig. 2**). Using the criteria in Table 1, the tissue damage score assigned to these rats was 15.1 ± 1.1. The biochemical determinations revealed that DSS administration significantly increased colonic MPO and AP activities (**Table 4**). In addition, the colonic inflammatory process was also associated with an increase in colonic LTB_4 and TNFα, as well as a depletion of GSH, as a consequence of the colonic oxidative stress induced by the inflammatory process in this model of experimental colitis (26). The inflammatory status was associated with an increased expression of colonic iNOS compared with noncolitic rats (**Fig. 3**), which was corroborated after densitometry of the corresponding bands (289 ± 24 vs. 31 ± 6 arbitrary units; $P < 0.01$).

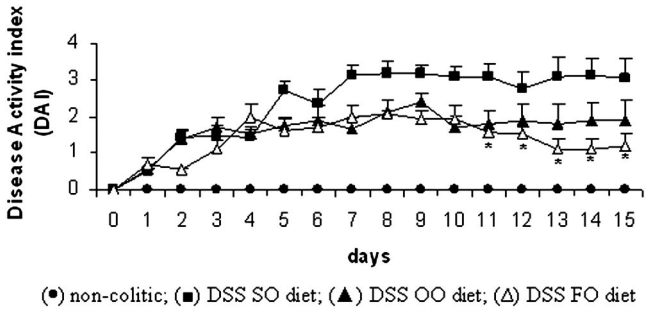

FIGURE 1 Time course of changes in the DAI in noncolitic rats fed the SO diet, and DSS-induced colitic rats fed the SO, OO, or FO diets based on the criteria shown in Table 1. *Different from the DSS control colitic group, $P < 0.05$.

Rats fed either the OO or FO diets had an overall lower level of DSS-induced colonic damage compared with the untreated control group. Colitic rats fed the OO or FO diets had an attenuated response compared with the group of rats fed the SO diet as demonstrated by the decrease in DAI from d 11 to 15 in FO-fed rats ($P < 0.05$; Fig. 1), whereas in OO fed rats, the DAI tended ($P = 0.15$) to decrease. The reduction was due to a decreased incidence of diarrhea and presence of blood in feces, as well as the smaller weight loss in treated colitic rats. After the rats were killed, macroscopic evaluation of the colonic segments from rats fed both olive oil–based diets showed that the weight:length ratio was significantly reduced compared with SO-fed colitic rats (84.2 ± 5.7 mg/cm in the OO-fed group and 81.7 ± 3.6 mg/cm in the FO-fed group vs. 100.6 ± 6.0 mg/cm in the SO-fed control group; $P < 0.05$). Histological analysis revealed that FO intake significantly improved the colonic architecture of colitic rats compared with control colitic rats fed the SO diet (Fig. 2), and they were assigned a lower tissue damage score (6.7 ± 1.3) than that assigned to the control colitic rats (15.1 ± 1.1; $P < 0.05$)

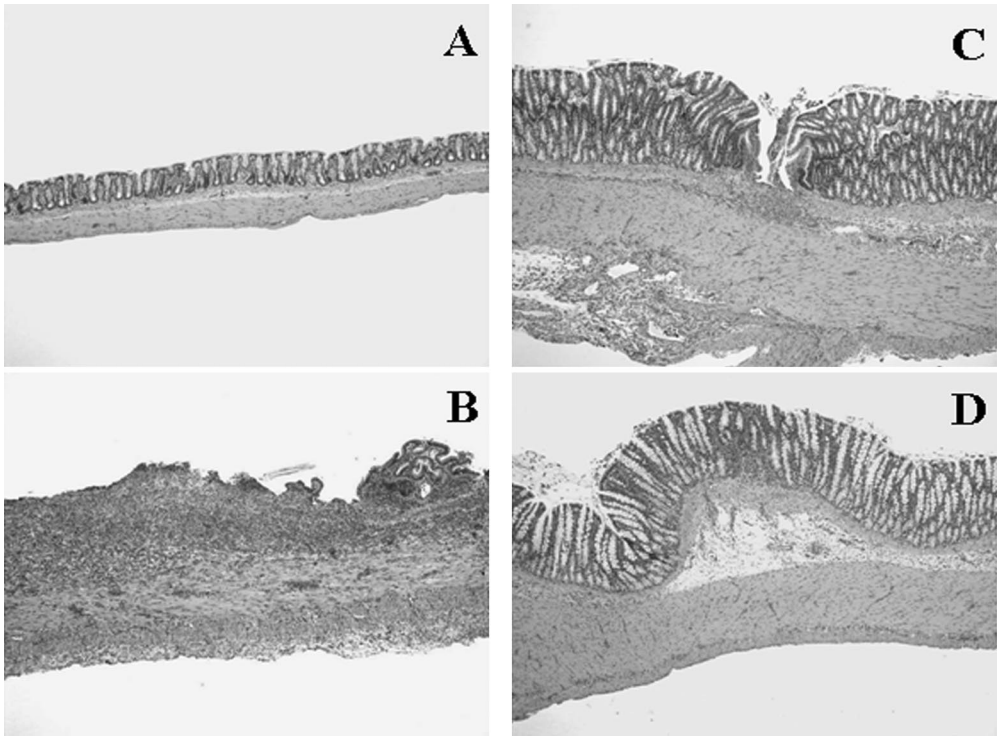

FIGURE 2 Histological sections of colonic mucosa from noncolitic rats fed the SO diet, and DSS-induced colitic rats fed the SO, OO, or FO diets stained with hematoxylin and eosin showing the anti-inflammatory effects of FO diet administration. (*A*) Noncolitic rat showing the normal histology of the colon (X50). (*B*) DSS control rat fed the SO diet showing extensive intestinal ulceration with a severe inflammatory cell infiltrate in the lamina propria and submucosa (X50). (*C*) OO-fed rat showing a focal infiltrate of inflammatory cells (X50). (*D*) FO fed rat showing a total recovery from the inflammatory process without ulceration of the intestinal mucosa; only an edema in the submucosa can be observed (X50).

(**Table 5**); however, the SO and OO groups did not differ with a tissue damage score of 10.3 ± 2.4 (Table 5). Thus, in the group of rats fed the OO diet, only 2 of 10 rats had ulceration of the mucosa affecting ~50% of the mucosal surface; this was accompanied by a substantial cell infiltrate, composed mainly of macrophages, granulocytes, and, to a lesser extent, lymphocytes. The rest of rats had an almost complete restoration of the damaged colon displaying a moderate inflammatory infiltrate with a focal distribution; some crypt dilation and goblet cell depletion remained (Fig. 2). The analysis of the samples from colitic rats fed the FO diet revealed that most of the rats (7/10) had a reduction in the areas of ulcerated colonic mucosa, <25% of the surface. The reduction in the leukocyte infiltrate was evident in most of the samples from this group. The crypts had a nearly normal architecture and goblet cell component, accompanied by mucin replenishment, compared with control colitic rats (Fig. 2).

Both colonic TNFα and LTB_4 levels were reduced significantly in colitic rats fed FO compared with control SO colitic rats (Table 4). Moreover, the intake of FO diet by colitic rats also significantly decreased colonic AP and MPO activities (Table 4). The latter suggests a reduction in the infiltration of neutrophils into the colonic mucosa because MPO activity is a biochemical marker of neutrophil infiltration (28), confirming the histological observations in this group of rats. In addition, colonic GSH content was normal in both OO- and FO-fed colitic rats, in contrast with the GSH depletion noted earlier in colitic control rats (Table 4). Both olive oil–based diets (OO and FO) reduced colonic iNOS expression, which was increased as a result of the inflammatory process induced by DSS in the control group of rats fed the SO diet (**Fig. 3**) (33 ± 8 and 41 ± 5 arbitrary units in OO- and FO-fed colitic rats, respectively, vs. 289 ± 24 arbitrary units in the SO-fed group; $P < 0.01$).

DISCUSSION

A series of studies demonstrated that (n-3) EPA and DHA PUFAs have many biological effects, ranging from decreasing the levels of serum triacylglycerols (32) and reducing blood pressure (33) to inhibiting the growth of tumor cells (34) and modulating symptoms in patients with autoimmune and in-

TABLE 4

MPO and AP activities and GSH, TNFα, and LTB_4 levels in the colon of rats fed the SO diet, and DSS-induced colitic rats fed the SO, OO, or FO diets[1]

Group	MPO activity[2]	AP	GSH	TNFα	LTB_4
	U MPO/g	*mU/mg protein*	*nmol/g*	*pmol/g*	*ng/g*
Noncolitic	15.1 ± 4.0[c]	5.82 ± 0.72[b]	2202 ± 31[a]	191.2 ± 27.4[b]	3.66 ± 0.38[b]
DSS SO diet	130.9 ± 6.7[a]	15.46 ± 1.77[a]	1694 ± 66[b]	846.1 ± 93.5[a]	6.80 ± 0.77[a]
DSS OO diet	115.3 ± 21.1[a]	11.43 ± 2.11[a]	1985 ± 84[a]	596.9 ± 74.3[a]	5.98 ± 0.63[a]
DSS FO diet	102.1 ± 9.8[b]	7.22 ± 0.57[b]	1958 ± 59[a]	408.3 ± 52.4[b]	4.48 ± 0.33[b]

[1] Values are means ± SEM, $n = 10$. Means in a column without a common letter differ, $P < 0.05$.
[2] One unit of MPO activity was defined as that degrading 1 μmol hydrogen peroxide/min at 25°C.

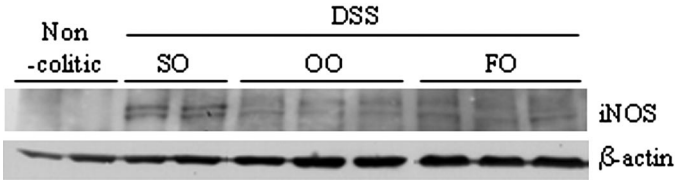

FIGURE 3 Western blots showing iNOS expression in colonic protein samples from noncolitic rats fed the SO diet, and DSS-induced colitic rats fed the SO, OO, or FO diets.

flammatory diseases (35,36). These findings confirm the importance of (n-3) EPA and DHA essential fatty acids in the diet. However, it is not the amount of (n-3) EPA and DHA that is directly involved in this beneficial effect, but their influence on the (n-6):(n-3) ratio because both PUFA types compete with the same enzymes to produce different inflammatory lipid mediators (10). In fact, it is currently recommended by nutritional authorities that the human diet return to a more balanced (n-6):(n-3) ratio of ~4:1 rather than the ratio of 20–15:1 provided by the current Western diet.

The aim of this work was to analyze the effects of an intervention involving the composition of lipids in the diet on the progression or induction of an inflammatory process such as an experimental model of colitis in rats. We compared a control Western-type diet (SO) rich in (n-6) fatty acids with a diet with lower amounts of (n-6) linoleic acid after its substitution with oleic acid (OO), without modifying the (n-6):(n-3) ratio, as well as with the same diet supplemented with a small quantity of fish oil (FO), thus modifying the (n-6):(n-3) ratio.

We decided to incorporate a low amount of fish oil (5% of the total fat) in the diet because it is an affordable supplementation to dietary manipulation. Although this amount is lower than the amounts used in some previous studies (12), it is enough to change the (n-6):(n-3) ratio in both diets and rat tissues. Moreover, this lower level of supplementation would also eliminate the need to incorporate antioxidants such as α-tocopherol in the diet (12); antioxidants prevent the decrease in the colonic antioxidant defense system that these (n-3) PUFA may induce.

We showed for the first time a positive effect of olive oil and also confirmed the therapeutic efficacy of (n-3) PUFA dietary supplementation in intestinal inflammation as was suggested previously both in humans (8,19) and in experimental models of colitis (11–15). Furthermore, our results demonstrate the importance of modulating the (n-6):(n-3) ratio in obtaining this beneficial effect rather than simply reducing the (n-6) PUFA levels. Similar observations were made after administration of parenteral lipid emulsions enriched with different proportions of (n-3) fatty acids in rats with acetic acid–induced colitis (11). Thus, in the present study, when colitic rats were fed a (n-3) PUFA–enriched diet incorporated into olive oil, the attenuation in the colonic damage induced by DSS was more evident than that observed after reducing only the (n-6) content (OO group).

The DSS model of experimental colitis is a well-characterized model with a predictable disease progression; it shares numerous clinical, biochemical, and histological features with human UC (24,25). The protocol used in the present study was modified from that originally described (26) because rats were given 5% (wt/v) DSS dissolved in the drinking water for 5 d, and then the DSS percentage was reduced to 2% (wt/v) for the next 10 d, in an attempt to sustain the colonic damage over time.

The beneficial effects exerted by olive oil and (n-3) PUFA dietary supplementation were evidenced both histologically, with a reduction in the weight:length ratio of colonic tissue and an improvement of the altered intestinal architecture, and also biochemically, by a decrease in colonic MPO and AP activities. MPO activity has been widely used to detect and follow intestinal inflammation, and a reduction in the activity of this enzyme can be interpreted as a manifestation of the anti-inflammatory activity of a given compound (37). Similarly, and based on our previous studies performed in 2 other models of experimental colitis (TNBS and acetic acid), AP activity can be a sensitive marker of inflammation in the intestine because the activity is invariably augmented under these experimental conditions (29,38).

The inflammatory process was not associated with significant changes in the lipid profile in plasma, liver, or colon, in comparison with noncolitic rats, indicating that lipid absorption is not compromised as a consequence of the colonic insult. This is likely because this process takes place in the small intestine, supporting the possibility of a dietary manipulation with fatty acids in these intestinal conditions. However, it is important to note that the preventive effect shown by the lipid dietary intervention in colitic rats was correlated with a significant change in the lipid profile in both plasma and colonic tissue with both diets, thus indicating the absorption and tissue incorporation of the corresponding fatty acids present in the diets administered.

The intestinal anti-inflammatory effects showed by dietary manipulation in this model of experimental colitis can be explained by the participation of different mechanisms that may synergistically act to ameliorate the colonic damage induced by DSS. These include antioxidant activity as well as inhibition in the production and/or release of proinflammatory mediators including eicosanoids, NO, and cytokines. Dietary fatty acid incorporation into membrane phospholipid pools was suggested not only to influence the production of eicosanoids but also to modulate lipid-related intracellular signaling events including actions above different second messengers or transduction pathways, such as peroxisome proliferator-activated receptor, liver X receptor, mitogen-activated protein kinase, and modifying gene expression (39), thus modulating the inflammatory response.

There is ample experimental and clinical evidence to suggest that the inflamed colon undergoes substantial oxidative stress, derived from overproduction of reactive oxygen and nitrogen metabolites in the colon, two proinflammatory mediators that contribute to gastrointestinal immunopathology during the chronic inflammatory events that take place in IBD (40,41). The present study shows that the inflammatory status induced by DSS is associated with GSH depletion and in-

TABLE 5

Effects of OO and FO diet administration on colonic microscopic score in rats with DSS-induced colitis fed the SO, OO, or FO diets[1]

Diet	Mucosal epithelium	Crypts	Lamina propria	Submucosa
SO	1.7 ± 0.3[a]	4.7 ± 0.7[a]	4.1 ± 0.7	4.7 ± 0.5[a]
OO	0.7 ± 0.4[a]	3.9 ± 0.7[a]	3.6 ± 0.5	2.1 ± 0.5[b]
FO	0.3 ± 0.1[b]	2.1 ± 0.4[b]	3.1 ± 0.5	1.1 ± 0.3[b]

[1] Values are means ± SEM, $n = 10$. Means in a column without a common letter differ, $P < 0.05$.

creased iNOS expression; this was also reported to occur in both human IBD and experimental models of colitis (42–44). As a result, the restoration of GSH levels achieved after administration of the 2 olive oil–based diets (FO and OO) to colitic rats demonstrates the antioxidant properties attributed to the different components of olive oil (12,21,22). In addition, fish oil (n-3) fatty acids may possess antioxidant properties, thus justifying the decrease in oxidative stress in patients with UC administered these fatty acids plus sulfasalazine (45). Similarly, both diets (OO and FO) promoted a reduction in iNOS expression in colitic rats compared with the SO-fed control group. This effect may also be associated with the above-mentioned antioxidant properties given the relation previously established between oxidative stress and upregulation of iNOS expression (46). The final consequence of this dietary intervention would be the downregulation of the production and release of 2 important proinflammatory mediators in IBD, i.e., reactive oxygen and nitrogen metabolites.

However, additional mechanisms may be at work in the anti-inflammatory effect obtained after (n-3) PUFA incorporation because the 2 diets, OO and FO, differed in their effects on the other proinflammatory mediators assayed. It is important to note that some (n-3) PUFA, such EPA and DHA, appear to be more effective than others, such as α-linoleic acid, in inhibiting inflammation (47), thus partially explaining the superiority of the FO diet compared with the OO diet. The intake of (n-3) PUFA (DHA and EPA) by colitic rats was associated with a significant decrease in colonic LTB_4 levels, confirming previous observations in both humans and experimental models of rat colitis (12,17,48). Because these (n-3) PUFA induce PGE_3 and LTB_5 production, they have anti-inflammatory effects. The eicosanoids induced have a lower potency as pro-inflammatory mediators than PGE_2 and LTB_4, which alter the absorptive and secretory functions of the intestine as well as cellular immunology, and lead to cell damage (10). Moreover, LTB_4 facilitates neutrophil accumulation within the mucosa with subsequent tissue damage as a result of cellular activity of this type of leukocyte (49). Consequently, a direct inhibitory effect on LTB_4 synthesis and/or release could be involved in the beneficial effect exerted by dietary intake of (n-3) PUFA in this model of experimental colitis, similar to the action of different drugs used in the treatment of IBD such as sulfasalazine and 5-ASA (4).

The beneficial effect exerted by FO intake in colitic rats was also associated with an inhibition of the production of colonic TNFα, a proinflammatory cytokine that is considered to play a key role in these intestinal conditions; its synthesis and release are upregulated in different cell types residing in the inflamed mucosa, especially macrophages (50). In fact, it was shown by immunofluorescence studies performed by our group in intestinal specimens from rats with DSS-induced colitis that macrophages constitute the predominant cell type in the inflamed areas of the intestine (29). In vitro studies consistently demonstrated that the anti-inflammatory properties attributed to fish oil emulsions rich in (n-3) PUFA are exerted primarily through their effects on the macrophage component of the inflammatory response (51). The ability of these emulsions to inhibit lipopolysaccharide-mediated TNFα expression in macrophages was reported. The effect is likely related to the inhibition of the nuclear factor (NF)-κB signal transduction cascade that plays an important role in controlling inflammatory gene activation (52). Similarly, because a relation was established between NF-κB activation and upregulation of iNOS expression (53), the inhibition of this or other signaling cascades may also explain the inhibitory effect on colonic iNOS expression observed in rats with DSS-induced colitis and fed the FO diet.

In conclusion, all of these results support the beneficial role of olive oil diet supplementation to rats with DSS-induced colitis. This effect is probably due to the antioxidant properties of the olive oil and to a reduction in the expression of iNOS. It is important to note that this anti-inflammatory effect is enhanced after (n-3) PUFA incorporation into the olive oil–based diet, thus modifying the (n-6):(n-3) PUFA ratio in colonic tissue. This dietary manipulation could result in a synergistic effect in the management of IBD because other proinflammatory mediators such as LTB_4 and TNFα are also downregulated.

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