

# Thermally Processed Oil Exaggerates Colonic Inflammation and Colitis-Associated Colon Tumorigenesis in Mice



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## Abstract

Frying in vegetable oil is a popular cooking and food processing method worldwide; as a result, the oils used for frying are widely consumed by the general public and it is of practical importance to better understand their health impacts. To date, the effects of frying oil consumption on human health are inconclusive, making it difficult to establish dietary recommendations or guidelines. Here we show that dietary administration of frying oil, which was prepared under the conditions of good commercial practice, exaggerated dextran sodium sulfate (DSS)-induced colitis and azoxymethane (AOM)/DSS-induced colon tumorigenesis in mice. In addition, dietary administration of frying oil impaired

intestinal barrier function, enhanced translocation of lipopolysaccharide (LPS) and bacteria from the gut into the systemic circulation, and increased tissue inflammation. Finally, to explore the potential compounds involved in the actions of the frying oil, we isolated polar compounds from the frying oil and found that administration of the polar compounds exacerbated DSS-induced colitis in mice. Together, our results showed that dietary administration of frying oil exaggerated development of inflammatory bowel disease (IBD) and IBD-associated colon tumorigenesis in mice, and these effects could be mediated by the polar compounds in the frying oil.

## Introduction

Frying in vegetable oil (e.g., canola, soybean, and corn oils) is a popular cooking and food processing method worldwide. As a result, the frying oils or frying food are widely consumed by the general public. The Nurses' Health Study (NHS) showed that approximately 14% women and approximately 23% men consume fried food 4–6 times per week (1). In the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC) study, it was estimated that the average daily consumption of frying food is as high as 123 grams,

with 14 grams of frying oils (2). During the frying process, the vegetable oils undergo an array of chemical reactions, resulting in formation of various oil degradation-derived products (3, 4). Notably, vegetable oils are rich in polyunsaturated fatty acids such as linoleic acid (LA, 18:2), which are susceptible to oxidation, and previous studies have shown that the oils used for frying contain many LA oxidation-derived compounds, such as 2,4-decadienal, 4-hydroxynonenal (4-HNE), and malondialdehyde (5, 6). In addition, the frying oils could also have other types of oil degradation compounds, including free fatty acids derived from breakdown of triglycerides, dimeric and polymerized triglycerides from polymerization reaction, and acrylamide from Maillard reaction (5, 6). Because frying oils are widely consumed by the general public, it is of practical importance to better understand their impact on human health.

To date, the effects of frying oil consumption on human health are inconclusive, making it difficult to establish dietary recommendations or guidelines (3, 7). Early animal experiments showed that treatment with frying oils could cause adverse effects in test animals; however, many of these studies used extremely heated oils that have limited relevance with human consumption (3). Recent studies generally support that the oral toxicity of frying oils, which are prepared under the

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conditions of good commercial practice, is low (3, 7). Nolen and colleagues performed a 2-year feeding study in rats and found that dietary administration of frying oils, which were prepared under practical restaurant-type frying conditions, induced no pathologic disorders (8). The results from this study are supported by many other animal experiments, although there are inconsistent results (see review papers in refs. 3, 7). Some human studies have shown that frequent consumption of frying food or frying oil is associated with increased risks of human disorders (1, 9, 10). It remains unclear, however, whether frying oil consumption is causally involved in the elevated risks of these diseases. With a lack of definitive toxicology data, currently there are no governmentally or industrially established limits to regulate the use of frying oils in food preparation. A better understanding of the health impacts of frying oils could lead to significant impact for public health and regulatory policy.

Most previous studies were performed to investigate the effects of frying oils on disease initiation in healthy animals, the effects of frying oil on disease development are not well understood (3, 7). Notably, a recent study showed that treatment with frying oil accelerated tumor metastasis in a late-stage breast cancer model in mice (11), suggesting potential promoting effects of frying oil on tumorigenesis. After oral consumption of frying oils or frying food, they have direct interactions with gastrointestinal tract in particular the colon tissues; therefore, it is of practical importance to study the effects of frying oils on gut health. To date, the effects of frying oil consumption on colonic inflammation and colon cancer are unknown. To better understand the health impacts of frying oil, here we treated mice with a frying oil sample, and studied its effects on development of dextran sulfate sodium (DSS)-induced colonic inflammation and azoxymethane (AOM)/DSS-induced colon tumorigenesis in mice.

## Materials and Methods

### Collection and characterization of frying oil

A sample of frying canola oil, which was used for one week to fry falafel (a deep-fried ball made from ground chickpeas, fava beans, or both) in a standard commercial fryer, was collected from UMass-Amherst Dining Commons (see Supplementary Fig. S1). The fryolator could hold 50 pounds of oil. The oil was fried about 6–8 times per day, and the total number of frying hours was approximately 8 hours per day. The oil was fried with a temperature of 325F (163°C). We performed the following assays to characterize the frying oil: (i) the oxidative status of the oil was determined using a lipid peroxide assay, as described (12), (ii) the fatty acid profiles of the oils were analyzed by GC-MS (13), and (iii) the levels of free fatty acids in the oil were determined using titration (14).

### Preparation of control fresh oil

Because many commercial canola oil options were already oxidized with varied degrees of lipid oxidation, we purchased a freshly opened commercial canola oil from a local market in Hadley, MA and purified it using a silicic acid-activated charcoal chromatography, as described previously (14). This is a well-established technique to remove oxidized compounds from the oils. Briefly, three layers, including 400 g silicic acid (Clarkson Chromatography Products), 100 g activated charcoal (Sigma-Aldrich), and another 400 g silicic acid, were sequentially packed into a column (3-inch internal diameter × 18-inch height, 2,000 mL reservoir capacity) using hexane as the mobile phase. One liter of the canola oil dissolved in the same volume of hexane was loaded onto the column and eluted with hexane. The oxidized compounds are more polar and were thus absorbed onto the column, and the unoxidized triglycerides were eluted out by hexane and evaporated to dryness using a rotary vacuum evaporator. The obtained unoxidized oil was fortified with 500 ppm tert-butylhydroquinone (TBHQ, Sigma-Aldrich) as an antioxidant to prevent oxidation, then stored at –80°C for the animal experiment. The fatty acid profiles, levels of free fatty acids, and the concentrations of lipid peroxides of the prepared fresh oil were analyzed, as described above.

### Isolation of polar compounds from the frying oil

The frying oil sample (500 g) dissolved in 500 mL hexane was loaded onto a silica gel column, then eluted with a mixed solvent of ethyl acetate and hexane (see scheme of experiment in Supplementary Information; Supplementary Fig. S3A). The obtained fractions were pooled based on thin-layer chromatography (TLC) analysis using a mobile phase of hexane: ethyl acetate = 15:1 (vol/vol). The polar compounds were combined, evaporated to dryness using a vacuum rotary evaporator, and used for animal experiment.

### Animal experiments

All animal experiments were conducted in accordance with the protocols approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst (Amherst, MA).

The mice were maintained in a specific pathogen free (SPF) facility of the University of Massachusetts (Amherst, MA). At the end of the experiment, the mice were sacrificed by CO<sub>2</sub> overdose and the tissues were collected in a clean operation room located in the SPF facility.

### Animal experiment 1: effects of frying oil on DSS-induced colitis

C57BL/6 male mice (Charles River) were randomly assigned to two groups, and treated with: (i) a diet containing 10 wt/wt % fresh oil that was fortified with 500 ppm TBHQ, and (ii) a diet containing 4% frying oil (fortified

with 500 ppm TBHQ) and 6% fresh oil (fortified with 500 ppm TBHQ), throughout the whole experiment. The composition of the experimental diet is shown in Supplementary Table S1. The diets were prepared and changed every other day, to prevent oxidation during the animal feeding. After 3 weeks, the mice were stimulated with 2 wt/vol % DSS (MP Biomedicals) in drinking water for 1 week to induce colitis, then the mice were sacrificed for biochemical analyses.

#### Animal experiment 2: effects of frying oil on AOM/DSS-induced colon cancer

C57BL/6 male mice were randomly assigned to two groups, and treated with: (i) a diet containing 10 wt/wt % fresh oil (fortified with 500 ppm TBHQ), and (ii) a diet containing 4% frying oil (with 500 ppm TBHQ) and 6% fresh oil (with 500 ppm TBHQ), throughout the whole experiment. After 2 weeks, the mice were treated with 10 mg/kg AOM (Sigma-Aldrich) via intraperitoneal injection; after 1 week, the mice were stimulated with 2 wt/vol % DSS in drinking water for 1 week. At week 10 post AOM injection, the mice were sacrificed for analysis. For analysis of colon tumors, the colon tissues were cut open longitudinally, washed in PBS buffer, and inspected under a dissection microscope. The size of the tumor was determined by the following formula: tumor size =  $\pi \times D^2/4$  ( $D$  is the diameter of the tumor).

#### Animal experiment 3: effects of frying oil-derived polar compounds on DSS-induced colitis

C57BL/6 male mice were randomly assigned to two groups and treated with a diet with or without 0.1 wt/wt % of frying oil-derived polar compounds (see composition of the experimental diet in Supplementary Table S2) throughout the whole experiment. The diets were prepared and changed every other day, to prevent further oxidation during the animal treatment. After 3 weeks, the mice were stimulated with 2% DSS in drinking water for 1 week to induce colitis. At end of the experiment, the mice were sacrificed for analyses.

#### Flow cytometry quantification of immune cell infiltration in tissues

The distal colon tissues were dissected, cleaned, and digested using Hank-balanced salt solution (Lonza) supplemented with 1 mmol/L dithiothreitol (DTT) and 5 mmol/L EDTA overnight at 4°C, then filtered through 70- $\mu$ m cell strainer (BD Biosciences) to afford single-cell suspensions. The cells were stained with FITC-conjugated anti-mouse CD45 antibody, PerCP/Cy5.5-conjugated anti-mouse F4/80 antibody, PE/Cy7-conjugated anti-mouse Ly-6G/Ly-6C (GR-1) antibody, isotype control antibody, and Zombie Violet dye according to the manufacturer's instructions (BioLegend). The stained cells were analyzed using BD LSRFortessa cell analyzer (BD

Biosciences) and data were analyzed using FlowJo software (FlowJo LLC).

#### qRT-PCR analysis

Tissues were ground after freezing in liquid nitrogen, and total rRNA was isolated using TRIzol reagent (Ambion). The concentration of RNA was measured by NanoDrop spectrophotometer (Thermo Fisher Scientific). The RNA was reverse transcribed into cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to manufacturer's instructions. qRT-PCR was carried out with a DNA Engine Opticon system (Bio-Rad Laboratories) with Maxima SYBR-green Master Mix (Thermo Fisher Scientific). The sequences of mouse-specific primer (Thermo Fisher Scientific) are listed in Supplementary Table S3. The results were normalized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) using the  $2^{-\Delta\Delta C_t}$  method.

#### qRT-PCR analysis of 16S rRNA to quantify bacterial load in tissues

Bacterial load in tissues was analyzed as described previously (15, 16). Total DNA was extracted from the tissues using QIAamp DNAeasy Blood & Tissue Kit (Qiagen), following the manufacturer's instructions with the addition of a bead-beating step. The quality of the extracted DNA was measured using a NanoDrop Spectrophotometer (Thermo Fisher Scientific) and qRT-PCR was performed using the same amount of DNA (5 ng/ $\mu$ L) in a DNA Engine Opticon system with Maxima SYBR-Green Master Mix. The sequences of mouse-specific primer (Thermo Fisher Scientific) are listed in Supplementary Table S3.

#### ELISA analysis of cytokines and lipopolysaccharides in plasma

Blood samples were collected via cardiac puncture and the plasma samples were obtained by centrifugation of the blood samples at 1,500  $\times$  g for 10 minutes at 4°C. The concentration of cytokines in plasma was determined using a CBA Mouse Inflammation Kit (BD Biosciences) and the concentration of LPS in plasma were determined using an ELISA kit (MyBioSource), according to the manufacturer's instructions.

#### Histologic analysis

The dissected colon tissues were fixed in 10% neutral buffered formalin (Thermo Fisher Scientific) for 48 hours. After dehydration, the tissues were embedded in paraffin and sliced (5  $\mu$ m) by Rotary Microtome (Thermo Fisher Scientific). The slices were dewaxed in serial xylene and rehydrated through ethanol solutions, stained with hematoxylin and eosin (Sigma-Aldrich), and examined with a light microscopy (Nikon Instruments). The histologic scores were evaluated by a blinded observer according to the following measures: crypt architecture, degree of inflammatory cell infiltration, muscle thickening, goblet

cell depletion, and crypt abscess. The histologic damage score is the sum of each individual score.

### IHC

Formalin-fixed tissue was embedded in paraffin (Thermo Fisher Scientific), sliced to 5- $\mu$ m sections, and dewaxed in serial xylene (Thermo Fisher Scientific), rehydrated through graded ethanol solutions (Pharmco-Aaper). Antigen retrieval was performed by heating the sections in 0.01 mol/L citrate buffer (pH 6.0) to 95°C for 10 minutes. Samples were incubated with primary antibodies against occludin and proliferating cell nuclear antigen (PCNA) overnight at 4°C. Horseradish peroxidase (HRP)-conjugated secondary antibodies were then applied to the sections, followed by chromogen 4-diaminobenzidine staining according to the instructions of HRP/DAB (ABC) Detection IHC Kit (Abcam). Sections were then counterstained with hematoxylin for 1 minute and observed under a light microscope (Nikon Instruments).

### Statistical analysis

All data are expressed as means  $\pm$  SEM. Shapiro-Wilk test was used to verify the normality of data and Levene mean test was used to assess equal variance of data. Statistical comparison of two groups was performed using Student *t* test, or Wilcoxon-Mann-Whitney test (when normality test fails). The corresponding *P* values were calculated on the basis of the false discovery rate (FDR) using the Benjamini-Hochberg procedure. The statistical analyses were performed using SAS statistical software (SAS Institute). See both raw *P* value and FDR *P* value in Supplementary Table S4.

## Results

### Collection and characterization of frying oil

To study the effects of frying oil on gut health, we collected a "real-life" frying oil sample, which has been used for 1 week to prepare Falafel, from the Dining Commons of UMass-Amherst (Amherst, MA). We analyzed the oxidative status of the frying oil: the concentration of lipid hydroperoxide (a marker of fat oxidation) is  $1.03 \pm 0.26$  mEq/kg for the frying oil versus  $0.08 \pm 0.01$  mEq/kg for the control fresh oil, and the level of free fatty acid (a marker of triglyceride breakdown) was  $0.17\% \pm 0.00\%$  for the frying oil versus  $0.07\% \pm 0.00\%$  for the fresh oil (Table 1). These results demonstrate enhanced fat oxidation and degradation in the frying oil. We have to point out that the concentration of lipid hydroperoxide is not a reliable marker of oxidative status, since the lipid peroxides could decompose (17). Consistent with the increased oxidative status of the frying oil, GC-MS analysis showed that compared with the control fresh oil, the frying oil had lower levels of polyunsaturated fatty acids: the level of LA (18:2 $\omega$ -6) in the frying oil is  $14.20\% \pm 0.11\%$  versus  $19.06\% \pm 0.09\%$  in the fresh oil, and the level of  $\alpha$ -linoleic

**Table 1.** Fatty acid profile and oxidation status of fresh oil and frying oil

	Fresh oil	Frying oil	FDR <i>P</i>
	Mean (SEM)	Mean (SEM)	
<b>Fatty acid profile (%)<sup>a</sup></b>			
C16:0	4.37 (0.03)	4.14 (0.02)	0.024
C18:0	1.95 (0.03)	2.00 (0.03)	0.345
C18:1 ( $\omega$ -9)	62.48 (0.19)	72.50 (0.15)	0.001
C18:1 ( $\omega$ -7)	3.49 (0.02)	3.48 (0.01)	0.816
C18:2 ( $\omega$ -6)	19.06 (0.09)	14.20 (0.11)	0.001
C18:3 ( $\omega$ -3)	6.82 (0.09)	1.82 (0.03)	0.001
C20:0	0.60 (0.02)	0.62 (0.02)	0.716
C20:1	1.24 (0.03)	1.24 (0.02)	0.786
<b>Oxidation status</b>			
Lipid hydroperoxide (mM/kg oil)	0.08 (0.01)	1.03 (0.26)	0.055
Free fatty acid (%)	0.07 (0.00)	0.17 (0.00)	0.001

<sup>a</sup>The results are expressed as % of individual fatty acid to total fatty acids, assessed by GC-MS analysis.

acid ( $\alpha$ -LA, 18:3 $\omega$ -3) in the frying oil is  $1.82\% \pm 0.03\%$  versus  $6.82\% \pm 0.09\%$  in the fresh oil (Table 1).

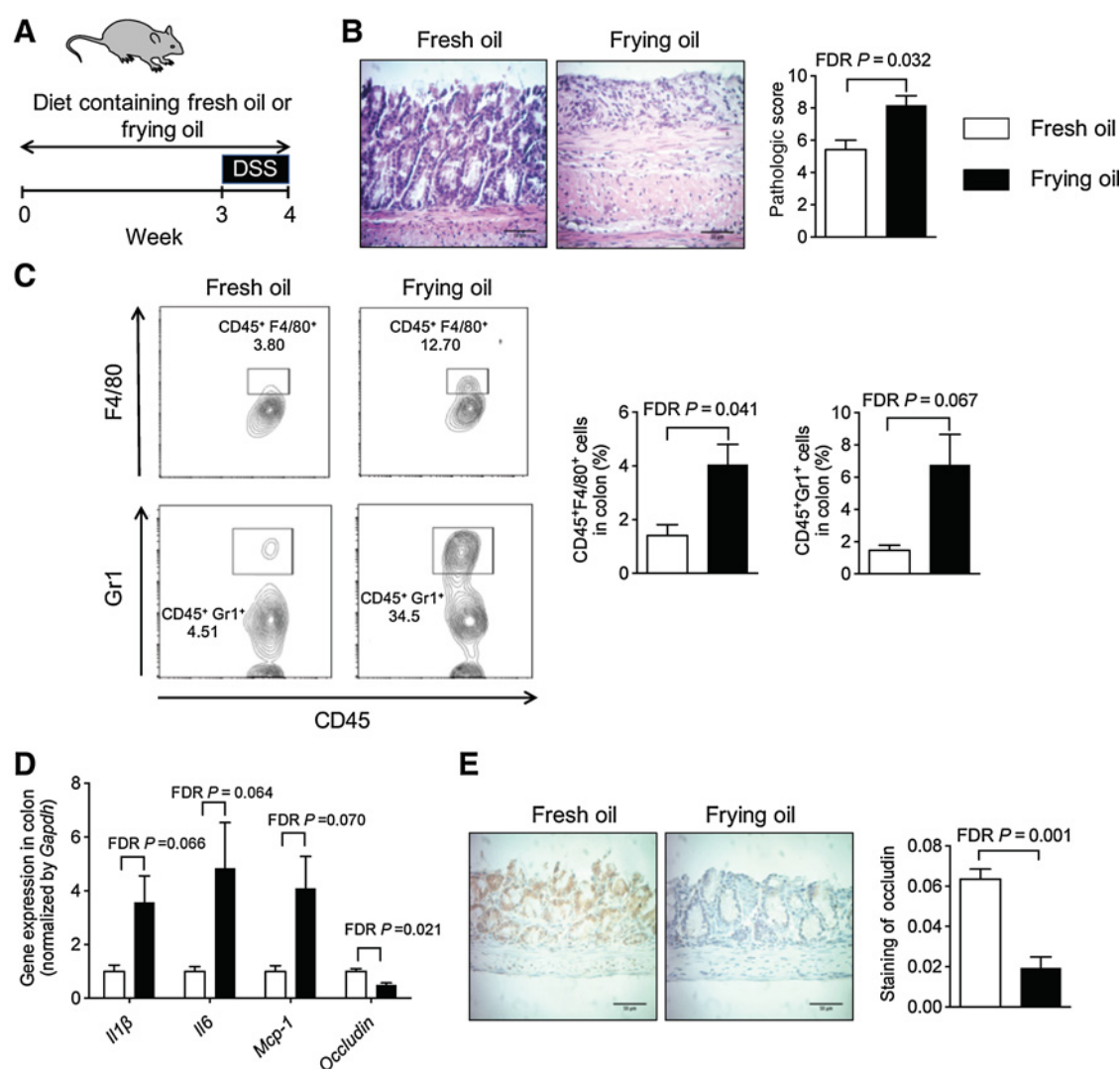
### Effects of frying oil on DSS-induced colitis

We studied the effect of the frying oil on colonic inflammation, using a well-established DSS-induced colitis model in C57BL/6 mice (see scheme of animal experiment in Fig. 1A). We treated mice with a completely defined isocaloric diet containing 10% (wt/wt) fresh oil, or a combination of 4% frying oil and 6% fresh oil. We determined the level of frying oil in the experimental diet (4 wt/wt %) to mimic human consumption: a previous study estimated that the average intake of fried foods (containing ~10% wt/wt frying oil) is 0–817 g per person per day, leading to a calculated intake of frying oil to be approximately 0%–4% wt/wt of the diet (2).

We found that dietary administration of the frying oil enhanced DSS-induced colitis. Compared with the DSS mice treated with the fresh oil, the DSS mice treated with the frying oil had exaggerated crypt damage (Fig. 1B), enhanced colonic infiltration of CD45<sup>+</sup> F4/80<sup>+</sup> and CD45<sup>+</sup> Gr1<sup>+</sup> immune cells (Fig. 1C), increased colonic expression of proinflammatory cytokines (*Il1 $\beta$* , *Il6*, and *Mcp-1*; Fig. 1D), and attenuated colonic expression of a tight-junction protein occludin (Fig. 1D and E). Together, these results demonstrate that dietary administration of frying oil exaggerated DSS-induced colitis in mice.

### Effects of frying oil on AOM/DSS-induced colon tumorigenesis

We studied the effects of frying oil on colon tumorigenesis, using a well-established AOM/DSS-induced colon cancer model in C57BL/6 mice (see scheme of animal experiment in Fig. 2A). All the AOM/DSS-stimulated mice developed colon tumors, and we found that dietary administration of frying oil enhanced AOM/DSS-induced colon tumorigenesis, with increased average tumor size and total tumor size, while the tumor number per mouse was not changed (Fig. 2B). Furthermore, compared with the mice treated with the fresh oil, the mice treated with the frying oil had enlarged spleen tissue (Fig. 2C), higher colonic

**Figure 1.**

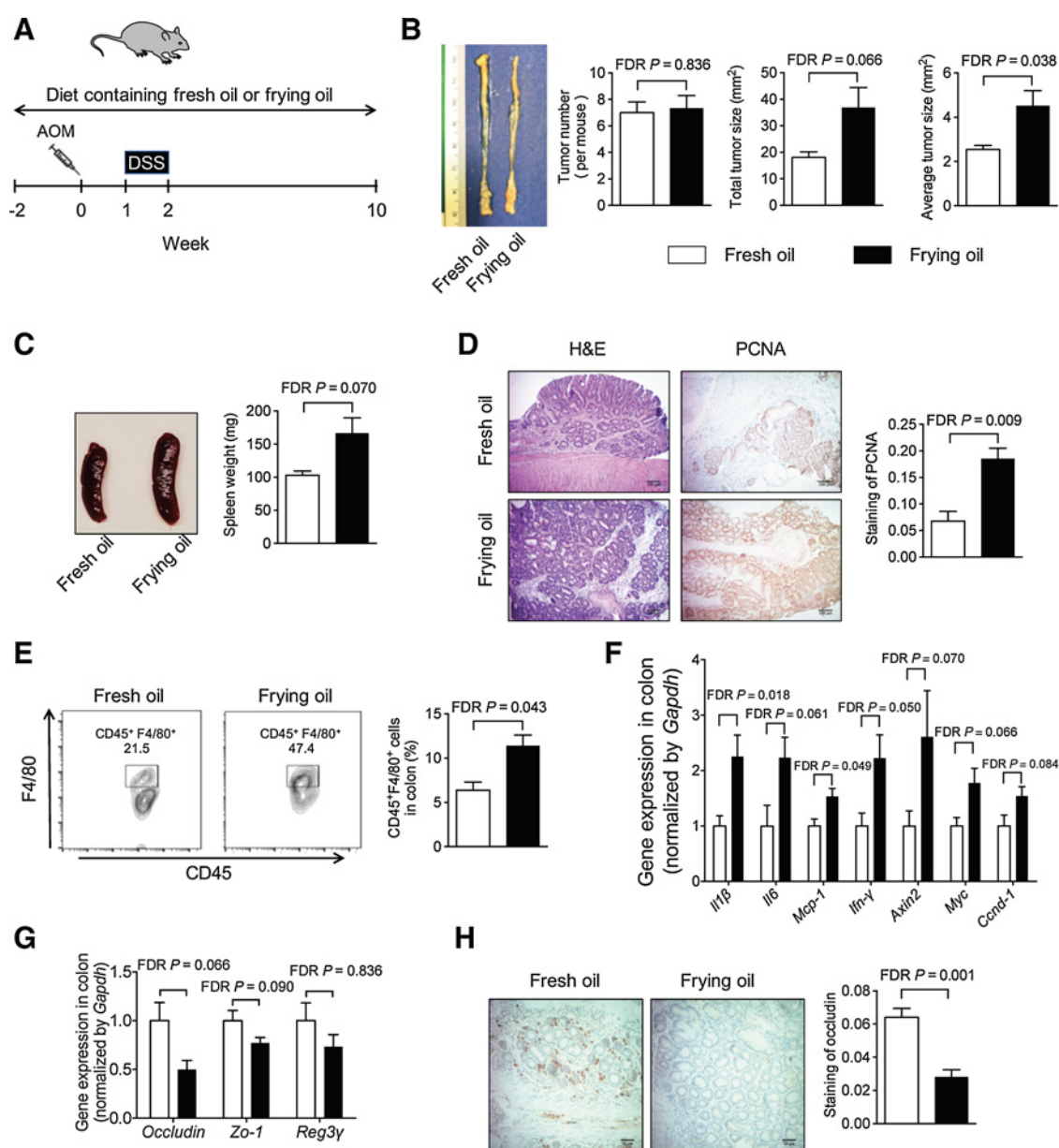
Dietary administration of frying oil exaggerates DSS-induced colitis in mice. **A**, Scheme of animal experiment. **B**, Left, representative images of H&E staining of the colon (magnification 600 $\times$ ; scale bar, 50  $\mu$ m). Right, quantification of H&E staining. **C**, FACS quantification of immune cell infiltration in the colon. **D**, Gene expression in the colon. **E**, Left, representative staining of occludin in the colon (magnification 600 $\times$ , scale bar = 50  $\mu$ m). Right, quantification of occludin staining. The data are mean  $\pm$  SEM,  $n = 7$  mice per group.

expression of PCNA (Fig. 2D), increased colonic infiltration of CD45<sup>+</sup> F4/80<sup>+</sup> immune cells (Fig. 2E, the levels of CD45<sup>+</sup> Gr1<sup>+</sup> immune cells were not changed, see Supplementary Fig. S2), and enhanced gene expression of proinflammatory cytokines (*Il1b*, *Il6*, *Mcp-1*, and *Ifn $\gamma$* ) and protumorigenic markers (*Axin2*, *Myc*, and *Ccnd-1*) in the colon (Fig. 2F). In addition, GC-MS analysis showed that consistent with the fatty acid profiles of the experimental oils (see Table 1), the colon tissues from the mice treated with the frying oil had lower levels of LA (8.75%  $\pm$  0.27% in frying oil group vs. 10.34%  $\pm$  0.57% in fresh oil group), and lower level of  $\alpha$ -linolenic (0.66%  $\pm$  0.16% in frying oil group vs. 1.73%  $\pm$  0.16% in fresh oil group; Supplementary Table S5). Together, these results demonstrate that

dietary administration of frying oil exaggerated AOM/DSS-induced colon tumorigenesis in mice.

#### Effects of frying oil on colonic expression of tight-junction proteins

Intestinal barrier function plays a critical role in the pathogenesis of many human disorders including colon cancer (18). We studied the effects of frying oil on colonic expression of tight-junction proteins, which play critical roles in regulating the intestinal barrier function (18). In the AOM/DSS model, qRT-PCR analysis showed that dietary administration of frying oil reduced colonic expression of *Occludin*, while had little effects on other tight-junction proteins such as *Zo-1* and *Reg3 $\gamma$*  (Fig. 2G). This



**Figure 2.**

Dietary administration of frying oil exaggerates AOM/DSS-induced colon tumorigenesis in mice. **A**, Scheme of animal experiment. **B**, Tumor number, total tumor size, and average tumor size in mice. **C**, Spleen weight. **D**, Left panel: representative images of H&E and IHC staining of PCNA of the colon (magnification 150 ×, scale bar = 100 μm). Right, quantification of PCNA staining. **E**, FACS quantification of immune cell infiltration in the colon. **F** and **G**, Gene expression in the colon. **H**, Left, representative IHC staining of occludin in the colon (magnification 600 ×, scale bar = 50 μm). Right, quantification of occludin staining. The data are mean ± SEM,  $n = 8$  mice in fresh oil group, and  $n = 10$  mice in frying oil group.

result is further validated by IHC staining, which showed that frying oil reduced colonic expression of occludin at the protein level (Fig. 2H).

#### Effects of frying oil on LPS/bacterial translocation

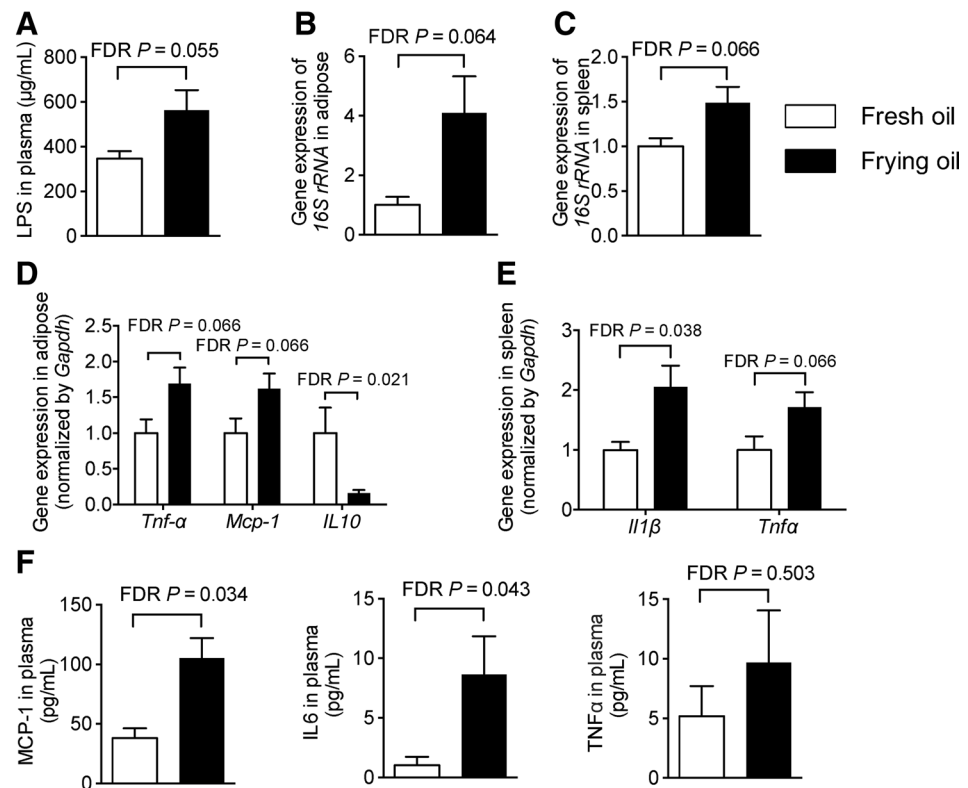
Defective intestinal barrier function is associated with enhanced translocation of LPS and bacteria from the gut into systemic circulation (18). We studied the effects of frying oil on LPS/bacterial translocation. ELISA analysis

showed that compared with the mice treated with the fresh oil, the mice treated with the frying oil had higher concentration of LPS in the plasma (Fig. 3A). In addition, qRT-PCR analysis of *16S rRNA* gene showed that the adipose and spleen tissues from the frying oil-treated mice had higher abundance of bacterial DNA (Fig. 3B and C), illustrating enhanced bacterial invasion into these tissues. Together, these results showed that dietary administration of frying oil enhanced translocation of



**Figure 3.**

Dietary administration of frying oil enhances LPS/bacterial translocation and exaggerates tissue inflammation. The mice were fed with a diet containing fresh oil or frying oil, then stimulated with AOM/DSS to induce colon tumorigenesis. **A**, LPS concentration in plasma. **B** and **C**, Gene expression of *16S rRNA* gene in gonadal adipose tissues (**B**) and spleen tissues (**C**). **D** and **E**, Gene expression of cytokines in gonadal adipose tissues (**D**) and spleen tissues (**E**). **F**, Concentrations of proinflammatory cytokines in plasma. The data are mean  $\pm$  SEM,  $n = 8$  mice in fresh oil group, and  $n = 10$  mice in frying oil group.



LPS and/or bacteria into the systemic circulation and various tissues.

#### Effects of frying oil on tissue inflammation

Bacterial invasion in tissue is associated with tissue inflammation (18). We studied the effects of frying oil on inflammatory responses in the adipose and spleen tissues. qRT-PCR analysis showed that compared with the AOM/DSS mice treated with the fresh oil, the adipose tissues from the AOM/DSS mice treated with the frying oil had higher expression of proinflammatory cytokines (*Tnfa* and *Mcp-1*) and lower expression of an anti-inflammatory cytokine *Il10* (Fig. 3D), illustrating enhanced adipose inflammation. A similar result was also observed in the spleen tissue (Fig. 3E). These results are further supported by ELISA analysis, which showed that treatment with frying oil increased concentrations of proinflammatory cytokines in plasma (Fig. 3F). Together, these results showed that dietary administration of frying oil enhanced bacterial translocation into tissues, resulting in exaggerated tissue inflammation.

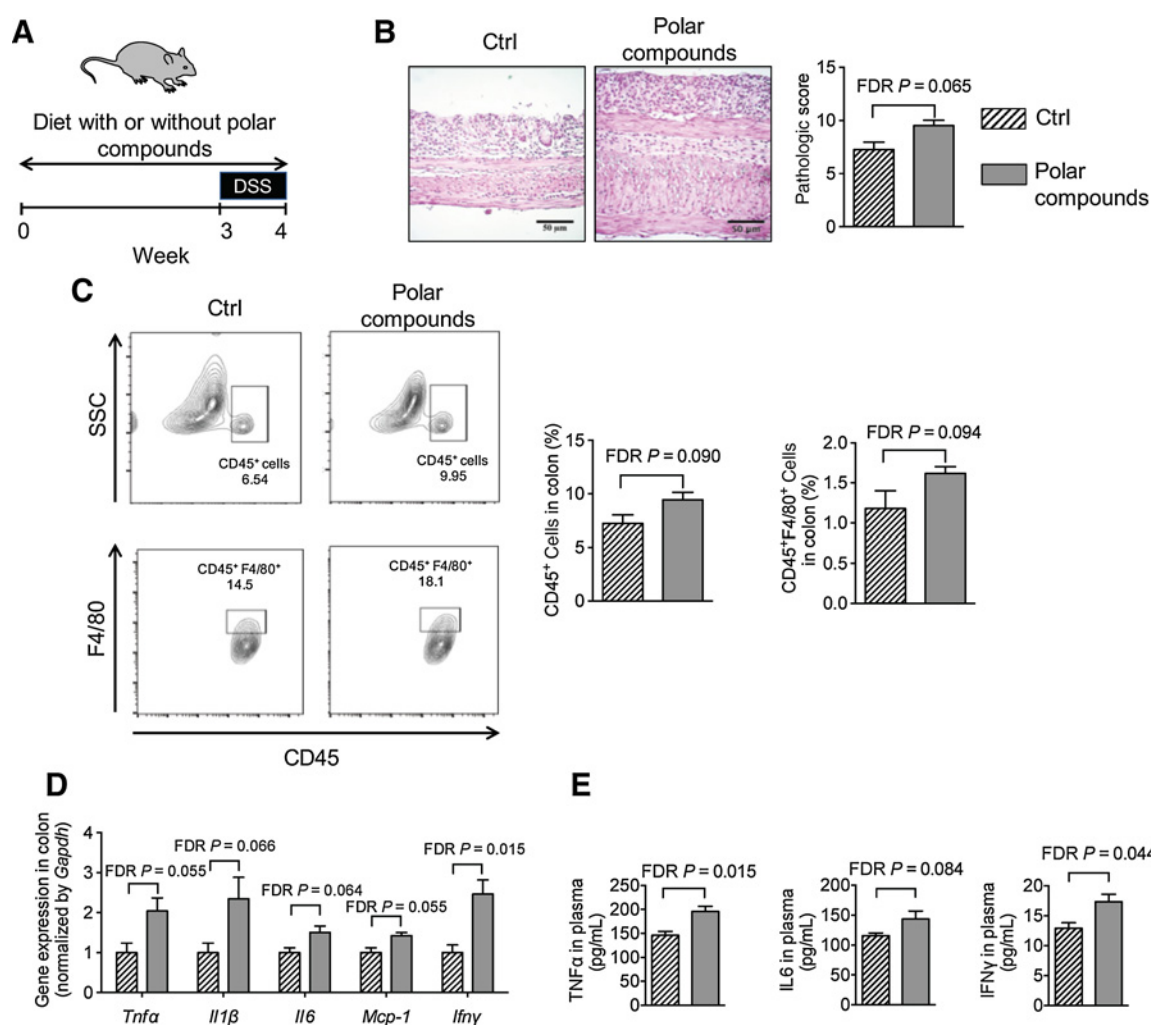
#### Isolation and characterization of polar compounds from frying oil

To determine the extent to which the observed biological effects of the frying oil are mediated by lipid oxidation-derived compounds, we isolated the polar compounds from the frying oil. The rationale of this approach is that

during lipid oxidation, the lipids are derivatized with polar moieties (e.g. hydroxyl, keto, or epoxide groups), resulting in formation of lipid oxidation-derived compounds that are generally more polar than the unoxidized lipids. Using a silica gel-based column chromatography, we isolated the polar compounds from the frying oil (see scheme of experiment in Supplementary Fig. S3A). The isolated polar compounds represent approximately 2.6 wt/wt % of the frying oil. Compared with the frying oil, the isolated polar compounds have higher concentrations of lipid oxidation-derived compounds: the concentration of lipid hydroperoxides was  $10.02 \pm 0.08$  mEq/kg for the isolated polar compounds versus  $1.03 \pm 0.26$  mEq/kg for the frying oil (Supplementary Fig. S3B).

#### Effects of frying oil-derived polar compounds on DSS-induced colitis

To study the biological actions of the frying oil-derived polar compounds, we treated the mice with a diet with or without 0.1 wt/wt % of the isolated polar compounds, then stimulated the mice with DSS to induce colitis (see scheme of animal experiment in Fig. 4A). We have determined the level of the polar compounds in the diet (0.1 wt/wt %), because the experimental diet with 4% frying oil (containing  $\sim 2.6\%$  polar compounds) as described above in Figs. 1–4 contains similar amounts of the polar compounds, allowing us to study the roles of the polar compounds in the actions of the frying oil.



**Figure 4.**

Dietary administration of frying oil-derived polar compounds exacerbates DSS-induced colitis in mice. **A**, Scheme of animal experiment. **B**, Left, representative images of H&E staining of the colon tissues (magnification 600 ×, scale bar = 50 μm). Right, histologic score. **C**, FACS quantification of immune cells in the colon. **D**, Gene expression of proinflammatory cytokines (*Tnfa*, *Il1b*, *Il6*, *Mcp-1*, and *Ifnγ*) in the colon. **E**, Concentration of proinflammatory cytokines (TNFα, IL6, and IFNγ) in the plasma. The data are mean ± SEM,  $n = 10$  mice in ctrl group, and  $n = 8$  mice in polar compounds group.

We found that dietary administration of the polar compounds exaggerated DSS-induced colitis in mice. Compared with the control DSS-stimulated mice, the DSS-stimulated mice treated with the polar compounds had exaggerated crypt damage (Fig. 4B), enhanced colonic infiltration of CD45<sup>+</sup> and CD45<sup>+</sup> F4/80<sup>+</sup> immune cells (Fig. 4C), increased expression of proinflammatory genes (*Tnfa*, *Il1b*, *Il6*, *Mcp-1*, and *Ifnγ*) in the colon (Fig. 4D), and increased concentrations of proinflammatory cytokines (TNFα and IFNγ) in the plasma (Fig. 4E). Together, these results demonstrate that polar compounds, which are enriched with lipid oxidation-derived compounds, have similar procolitis effects as the frying oil, supporting that these compounds could contribute to the observed biological actions of the frying oil.

## Discussion

The incidence and prevalence of IBD have risen dramatically in recent decades in the United States and other countries (19). The rapidity of the development suggests that environmental and dietary factors, which remain to be identified, could be primarily responsible for the elevated risks of IBD. It is of practical importance to identify novel risk factors of IBD, which could lead to significant impact for public health and regulatory policy. Here our central finding is that dietary administration of a frying oil sample exaggerated DSS-induced IBD and AOM/DSS-induced IBD-associated colon tumorigenesis in mice. Overall, these results support that the frying oils could be potential dietary risk factors of IBD and associated colon cancer. In our study, we studied the effects of frying canola oil; it is expected that other types



of frying oils (e.g., soybean oil) could induce similar adverse effects since they also have high levels of LA that is prone to lipid oxidation. On the basis of our finding, the individuals with or prone to IBD and colon cancer could be susceptible to the adverse effects of frying oils and may need to reduce the dietary intake of frying oils or fried foods, although more studies are needed to validate the impact of frying oils on gut health before any dietary recommendations could be established.

In our experiment, we used a frying oil sample, which has been used for 1 week to prepare falafel, from UMass-Amherst Dining Commons. The objective was to get an oil that truly represented oxidized oil that is consumed by the general public. Commercial deep fat fryers are designed to handle large volumes of food meaning that they must be able to maintain a constant temperature. This is accomplished by having a large oil reservoir and a heating apparatus with a large surface area for rapid heat transfer. These conditions are difficult to mimic in a laboratory setting even with small household frying appliances. Because the initial heating of the fryer takes so long, fryer is left on all day in a restaurant setting. Therefore, restaurant fryers undergo periods where they are held at high temperature without frying meaning that they are close to anaerobic followed by periods where foods are cooked resulting in aeration and large amounts of oxygenation. These conditions as well as the large volume of food cooked in a restaurant are also difficult to accurately reproduce in a laboratory setting. Thus, we felt that to study an oil that represents dietary fat from frying, it was critical to use actual food service oil. To date, the accurate assessment of exposure and absorption of frying oil in human populations is under-studied. In our study, we treated mice with a diet containing 4 wt/wt % of the frying oil sample, because a previous study estimated that the average intake of fried foods (containing ~10% frying oil) is 0–817 g per person per day, leading to a calculated intake of frying oil to be approximately 0%–4% wt/wt of the diet (2). Together, these results support that the procolitis and proneoplastic effects of frying oils in mouse models, and more studies are needed to characterize their impacts on human health.

During the frying process, several different types of chemical reactions, including breakdown of triglycerides to generate free fatty acids, lipid oxidation, and reactions between lipids with other food components such as food proteins, could happen, resulting in formation of an array of complicated products (5). Our results showed that dietary inclusion of "polar compounds" isolated from the frying oil, which had high concentrations of lipid peroxides, exaggerated DSS-induced colitis in mice, supporting that lipid oxidation–derived compounds could contribute to the pro-IBD effects of frying vegetable oils. Consistent with this notion, our recent study showed that systemic treatment (via intraperitoneal injection) with 4-HNE, a

lipid oxidation–derived compound, exaggerated DSS-induced IBD in mice (16). To date, the biological fates of the lipid oxidation–derived compounds in systemic circulation are controversial (20). It is feasible that some of these compounds could locally interact with the gut cells or microbiota, resulting in exaggerated colonic inflammation and colon tumorigenesis. It should be noted that the body has a battery of antioxidant systems (e.g. glutathione, catalase, and superoxide dismutase), to counteract the potential adverse effects of lipid oxidation–derived compounds. However, previous studies have shown that the mice and human patients with IBD have significantly elevated tissue levels of reactive oxygen species and enhanced severity of oxidative status, suggesting that the antioxidant protective system could be compromised in the patients with IBD (21–23). These results suggest that these individuals could be highly sensitive to the adverse effects of oxidized lipids, which are commonly present in frying oils.

In summary, here our results showed that dietary intake of frying vegetable oils, as well as frying oil–derived polar compounds, increased the severity of experimental colitis in mice, supporting that the frying oils could be novel risk factors of IBD and associated diseases. More studies are needed to better characterize the impacts of frying oils on gut health and determine the specific compounds involved, and these efforts could lead to significant impact for public health and regulatory policy.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** E.A. Decker, G. Zhang

**Development of methodology:** J. Zhang, X. Chen, Q. Ma, E.A. Decker

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** J. Zhang, R. Yang, W. Qi, K.Z. Sanidad

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** J. Zhang, X. Chen, W. Qi, K.Z. Sanidad, D. Kim, G. Zhang

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**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** J. Zhang, Q. Ma, K.Z. Sanidad, Y. Park, G. Zhang

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