Research Article

Phase II Study of the Effects of Ginger Root Extract on Eicosanoids in Colon Mucosa in People at Normal Risk for Colorectal Cancer

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Abstract

Inhibitors of COX indicate that upregulation of inflammatory eicosanoids produced by COX, and in particular prostaglandin E2 (PGE2), are early events in the development of colorectal cancer (CRC). Ginger has shown downregulation of COX in vitro and decreased incidence/multiplicity of adenomas in rats. This study was conducted to determine if 2.0 g/d of ginger could decrease the levels of PGE2, 13-hydroxyoctadecadienoic acids, and 5-, 12-, and 15-hydroxyeicosatetraenoic acid (5-, 12-, and 15-HETE), in the colon mucosa of healthy volunteers. To investigate this aim, we randomized 30 subjects to 2.0 g/d ginger or placebo for 28 days. Flexible sigmoidoscopy at baseline and day 28 was used to obtain colon biopsies. A liquid chromatography mass spectrometry method was used to determine eicosanoid levels in the biopsies, and levels were expressed per protein or per free arachidonic acid. There were no significant differences in mean percent change between baseline and day 28 for any of the eicosanoids, when normalized to protein. There was a significant decrease in mean percent change in PGE2 ($P = 0.05$) and 5-HETE ($P = 0.04$), and a trend toward significant decreases in 12-HETE ($P = 0.09$) and 15-HETE ($P = 0.06$) normalized to free arachidonic acid. There was no difference between the groups in terms of total adverse events ($P = 0.55$).

On the basis of these results, it seems that ginger has the potential to decrease eicosanoid levels, perhaps by inhibiting their synthesis from arachidonic acid. Ginger also seemed to be tolerable and safe. Further investigation in people at high risk for CRC seems warranted.

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Introduction

Anti-inflammatory agents such as aspirin and related nonsteroidal anti-inflammatory drugs (NSAID), which inhibit COX enzymes and decrease the levels of the inflammatory prostaglandin E2 (PGE2) seem to be promising colorectal cancer (CRC) chemopreventive agents (1). Aspirin and related NSAIDs have been shown to prevent the development of adenomas and CRC in both animal models of CRC and in numerous epidemiologic studies (1). Although aspirin and related NSAIDs are encouraging chemopreventive agents, there is some speculation that the inhibition of COX enzymes could cause the shunting of arachidonic acid (AA), the substrate for COX, toward the production of other inflammatory eicosanoids (2).

The lipoxygenase (LOX) enzymes also use AA as a substrate to produce eicosanoids. Eicosanoids products of 5-, 12-, and 15-lipoxygenase (5-, 12-, and 15-LOX) are 5-, 12-, and 15-hydroxyeicosatetraenoic acid (HETE; ref. 3). There is some evidence that the simultaneous inhibition of COX-2 and 5-LOX causes greater inhibition of tumor growth and decreased concentrations of PGE2 compared with inhibition of COX-2 or 5-LOX alone (4). While evidence is strongest for the role of PGE2 in colon tumor initiation and progression 5-HETE and 12-HETE have also been implicated in the development of CRC (5). Soumaoro and colleagues showed that 5-LOX expression and 5-HETE concentrations are upregulated in human colorectal cancer specimens, and are correlated with tumor size, depth, and vessel invasion (6, 7). 12-HETE was found to stimulate the proliferation of both HT-29 and HCF-15 human colon carcinoma cells (8). Increased production of 5-HETE and 12-HETE has also been reported in the mucosa of colon cancer patients (9).

Complicating this picture is the production of putative antitumor/anti-inflammatory eicosanoids in colorectal tumorigenesis such as 15-HETE and 13-hydroxy-octadecadienoic (13-HODE) acids (10, 11). Although both 15-HETE...
and 13-HODE are catalyzed from different substrates (AA for 15-HETE and linoleic acid for 13-HODE) by 15-LOX, they are produced by different isoforms of 15-LOX, with 15-LOX-1 metabolizing linoleic acid and 15-LOX-2 metabolizing AA (see Fig. 1) (3, 10). Consequently, an attractive colorectal chemopreventive agent would impact not just the COX enzymes, but the balance of products from differing eicosanoid enzymes, potentially shifting the eicosanoid system toward a local anti-inflammatory state.

Ginger root (*Zingiber officinale* Roscoe, Zingiberaceae) is one of the most heavily consumed dietary substances in the world and is one of the top selling dietary supplements in the United States (12, 13). Ginger's mechanism of anticarcinogenesis action is not entirely known, but seems to be associated with the antioxidant and anti-inflammatory actions of its nonvolatile pungent components the gingerols and shogaols (12). Ginger inhibits 5-LOX (14–17) and COX-1 and -2; (14, 18–20) decreases inflammation in various murine models (12, 16, 21–23), and reduces serum concentrations of PGE2 in rats (23). Several studies of chemically induced colon carcinogenesis have shown that ginger is preventive (24–26). When ginger was administered in the postinitiation stage, however, it did not suppress aberrant crypt foci formation nor did ginger significantly change the proliferative or apoptotic indexes of the colonic crypt (27).

The purpose of this study was to examine the effect of 2.0 g of ginger taken daily for 28 days compared with placebo on eicosanoids in the colon mucosa of people at normal risk for developing colorectal cancer. Secondary objectives were to evaluate the safety, tolerability, adherence, and blinding success of ginger given orally for 28 days.

**Methods**

**Participants**

A total of 33 participants were recruited from the surrounding community through fliers or word-of-mouth between April 2007 and May 2008. To be eligible for the

![Diagram of Metabolism of Linoleic and Arachidonic Acid](https://example.com/diagram.png)
Ginger’s Effect on Eicosanoids in the Colon of Volunteers

study, participants had to be 18 years or older and in good health as defined by an unremarkable medical history, physical, and screening blood work (chemistry screen, complete blood count) within 60 days of study entry. No chronic medication use was allowed and participants could not have taken aspirin or related NSAIDs during the study or 14 days before the first dose of the study medication. Participants also had to be classified as being at normal-risk for developing colorectal cancer. Normal-risk was defined as having: no history of familial colorectal cancer syndromes; no first-degree relatives with colon cancer diagnosed before the age of 60; no personal history of colorectal cancer and no adenomas more than 1 cm in size or containing carcinoma in situ. Exclusion criteria for the study included: (i) a history of peptic ulcer disease, gastrointestinal bleeding from gastric or duodenal ulcers, or gastrin secreting tumors; (ii) pregnant or lactating women; (iii) history of cardiovascular disease; (iv) lactose intolerance; and (v) an allergy to ginger. Participants were asked to avoid all foods containing ginger within the 14 days prior to drug administration. This was confirmed by having participants complete a food checklist to verify that they were not consuming any ginger-rich foods such as ginger ale or Japanese food. All of the participants were reimbursed for their time. All study procedures were administered at the University of Michigan Clinical Research Unit (MCRU) after the participant gave written, informed consent. The study was approved by the University of Michigan Institutional Review Board.

Drug

The ginger product used in this study was manufactured by Pure Encapsulations. Pure Encapsulation’s ginger (Z. officinale) powder was processed using Good Manufacturing Procedures (GMP). Each capsule contained 250 mg dry extract of ginger root [10:1 (v/v) extraction solvent (ethanol 50%): root] normalized to 15 mg (5%) of total gingerols. On the basis of high performance liquid chromatography (HPLC) analysis, a 250 mg capsule of ginger extract (from both batches) contained 5.38 mg (2.15%) 6-gingerol, 1.80 mg (0.72%) 8-gingerol, 4.19 mg (1.78%) 10-gingerol, and 0.92 mg (0.37%) 6-shogaol. Gingerol and shogaol content was verified by an independent laboratory using appropriate HPLC techniques (Integrated Biomolecule Corporation). The study was conducted using 2 batches (Z0/06006 and Z0/07006) of ginger powder extract, both of which were prepared for the procedure with any enemas. Participants were asked to evacuate their rectum within 24 hours of the agreed upon time, twice daily. Participants were classified as adherent if the adherence monitoring suggested that 80% or more of the doses were taken as prescribed.

Blinding was assessed by asking the participants during their final visit which treatment they believed they received ("ginger," "placebo," or "do not know"). Participants were also asked the reason for their response, for example, "Was it the way the capsule smelled?"

Toxicity assessment

Participants were assessed for toxicity by direct questioning in person, by email or by telephone at weekly intervals. The National Cancer Institute (NCI) Common Toxicity Scale V 3.0 (Regulatory Affairs Branch, Cancer Therapy Evaluation program, Division of Cancer Treatment, Diagnosis, and Centers, NCI; ref. 29) was used to quantify toxicity.

Flexible sigmoidoscopy and tissue collection

Participants underwent 2 flexible sigmoidoscopies, one before drug treatment and the second 28 days after ginger extract treatment commenced. The second procedure was done at a time as close as possible to 24 hours after the participant took the final ginger dose. The participants were not prepared for the procedure with any enemas. Participants were, however, asked to evacuate their rectum within 12 hours of the procedure, but to not take any laxatives to enhance evacuation.

Participants were placed in a left lateral decubitus position and a flexible sigmoidoscope was passed to 20 to 25 cm from the anal sphincter. Four tissue samples were taken by opening and pressing the biopsy forceps perpendicular to the mucosal surface with mild pressure. Each biopsy specimen was taken approximately 2 cm or more from other biopsy sites in distal sigmoid colon mucosa that had no visual appearance of trauma or recent biopsy.
Tissue handling and disposition
Biopsy samples were placed into a sterile 1.5-ml Eppendorf tube and frozen in liquid nitrogen at exactly 50 seconds after the time the biopsy forceps were closed. The specimens were stored at −70°C until immediately before analysis.

Frozen biopsy samples weighed approximately 5 mg and yielded between 400 and 600 μg protein. Triplicate assays for the eicosanoids required approximately 10 to 20 μg of colon tissue. The remaining frozen tissue samples were stored at −70°C for future use.

Analytical methods eicosanoids (PGE2, 5-HETE, 12-HETE, 15-HETE, and 13-HODE)
All eicosanoids and deuterated internal standards used in this study were purchased from Cayman Chemical Co. Arachidonate, butylated hydroxytoluene (BHT), citric acid, and EDTA were obtained from Sigma Chemical Co. All Burdick and Jackson brand HPLC-grade solvents were purchased from Fisher Scientific Co.

Reverse-phase LC electrospray ionization mass spectrometry (LC/MS/MS) analyses were used for quantitation of PGE2, 5-HETE, 12-HETE, 15-HETE, and 13-HODE as described previously (30, 31). Two frozen colonic biopsy specimens from the same participant and time point were removed from the freezer, combined and ground to a fine powder using a liquid-nitrogen–cooled mortar. Samples were then transferred to sealed microcentrifuge tubes, and 3 volumes of ice-cold PBS buffer containing 0.1% BHT and 1 mmol/L EDTA were added. The samples were then homogenized by an Ultrasonic Processor (Misonix) at 0°C for 3 minutes. A 100-μL aliquot of the homogenate was transferred to a glass tube (13 × 100 mm) for extraction of eicosanoids. Briefly, 20-μL aliquots of 1 N citric acid and 10 μL of deuterated PGE2, 5-, 12-, or 15-HETE; or 13-HODE (100 ng/mL) were added to the samples. Eicosanoids were then extracted with 1 ml of hexane:ethyl acetate (1:1, v/v) and vortexed for 2 minutes. All extraction procedures were done at minimum light levels under cold conditions (4°C). Samples were centrifuged at 1800 g for 10 minutes at 4°C. The upper organic layer was collected, and the organic phases from 3 extractions were combined before drying under a stream of nitrogen at room temperature. Samples were then reconstituted in 100 μL of methanol:ammonium acetate buffer (10 mmol/L at pH 8.5; 70:30, v/v) before LC/MS/MS analysis. The protein concentration in the homogenate was determined by a Bradford protein assay (Bio-Rad).

LC/MS/MS analyses were done using a Quattro Ultima tandem mass spectrometer (Micromass) equipped with an Agilent HP 1100 binary pump HPLC inlet. Eicosanoids were separated using a Luna 3 μm Phenyl-Hexyl 2 × 150 mm LC column (Phenomenex). The mobile phase consisted of 10 mmol/L ammonium acetate buffer (pH 8.5) and methanol. For the analysis of PGE2, HETEs, and 13-HODE, the separation was achieved using a linear methanol gradient from 40% to 60% more than 18 minutes followed by a methanol flush. The flow rate was 250 μL/min with a column temperature of 50°C. The sample injection volume was 25 μL. Samples were kept at 4°C during the analysis. All eicosanoids were detected using electrospray negative ionization and multi-reaction monitoring of the transition ions for the metabolites and their internal standards (32).

The mass spectrometer (Thermo Finnigan TSQ Quantum) was operated in the electrospray negative ion mode with a cone voltage of 2,300 V, a cone gas flow rate of 117 L/h, and a collision gas flow rate of 998 L/h. The temperature of the desolvation region was 350°C, and the temperature of the source region was 120°C. Fragmentation for all compounds was done using argon as the collision gas at a collision cell pressure of 2.10 × 10−3 Torr. The collision energy ranged from 16 to 31 V depending on the analyte. The results were either expressed as nanogram (ng) of eicosanoid per milligram (mg) of protein or as ng of eicosanoid per microgram (μg) of free AA. All of the biopsy samples from a given individual were assayed in the same batch to eliminate any batch effects on changes over time. Four batches were assayed for this study. The within-day coefficients of variation (CV) of the assay, based on 3 injections of the same sample on the same day, for PGE2, 5-, 12-, 15-HETE, 13-HODE, and AA were 3.8%, 13.2%, 15.4%, 13.2%, 12.2%, and 2.5%, respectively and the between day CV for PGE2, 5-, 12-, 15-HETE, 13-HODE, and AA were 5.3%, 18.9%, 16.0%, 34.2%, 28.4, and 7.0%, respectively.

Statistical methods and sample size
Statistical analyses were conducted using SPSS software version 18.0. Baseline characteristics were analyzed, stratified by treatment group, using means and SDs for continuous variables, and counts and percentages for categorical variables. Balance between treatment groups on baseline characteristics was tested using independent sample t tests for continuous variables and Pearson’s χ2 and Fisher’s exact tests, as appropriate, for categorical variables.

Mean percent change from baseline to day 28 for each treatment group for PGE2, 5-HETE, 12-HETE, 15-HETE, and 13-HODE was calculated [e.g., (PGE2 at day 28—PGE2 at baseline)/PGE2 at baseline]. Also, given the large batch-to-batch variability of the eicosanoid assays, we investigated the effect of batch on mean percent change using general linear models. A Kolmogorov-Smirnov-Lilliefors’ (KSL) test for normality was conducted to determine if either treatment groups were normally distributed. Depending on the results of the KSL test, independent sample t tests were used to calculate the differences between treatment groups for mean percent change when normally distributed and the Mann–Whitney U test was used when not normally distributed. Results are reported as means ± SD. Adverse events (AE), blinding, and adherence between groups were analyzed by Pearson’s χ2 or Fisher’s exact test as appropriate. A P ≤ 0.05 was considered statistically significant.

The sample size needed for the study was determined using published data on PGE2 levels (33). PGE2 concentration in human colon tissue at baseline had a mean and SD of 11.7 pg/mg ± 1.7 pg/mg. On the basis of this PGE2...
level, we calculated that a sample size of 15/treatment group would have better than 80% power to detect a reduction in PGE₂ level of at least 25%. A post-hoc power analysis was also done for PGE₂. The analysis was based on the observed data using a 2-sample t test of percent change for PGE₂ to determine the sample size needed for 80% power.

**Results**

**Subjects and toxicity**

We screened 50 people between January 2007 to June 2008, of whom 33 met all eligibility criteria and were randomized: 17 to placebo and 16 to 2.0 g ginger, for 28 days. Figure 2 documents the numbers of participants, reasons for exclusions and reasons for discontinuing the intervention. There was no significant difference between treatment groups for any demographic or clinical characteristic. Less than one-half of the participants were male (n = 16, 48.5%) with mean (±SD) age of 33.9 ± 11.5 (range 20–59 years), and more than one-half (n = 21, 63.6%) of the participants were Caucasian. Less than one-fifth were African American (n = 6, 18.2%) and only 3% (n = 1) of participants reported being of Hispanic ethnicity. The mean body mass index (BMI) was 25.9 ± 5.0 (range 18.2–39.3).

All toxicities reported are shown in Table 1. No toxicities greater than NCI Common Toxicity Criteria (v. 3.0) Grade 1 was reported (29). There was no difference between the groups in terms of total AEs (P = 0.55) or specific types of AEs such as gastrointestinal (GI) toxicities (P = 0.71).

**Eicosanoids (PGE₂, 5-HETE, 12-HETE, 15-HETE, and 13-HODE)**

The baseline values of PGE₂, 5-HETE, 12-HETE, 15-HETE, and 13-HODE in colon biopsies across both groups were 10.8 ± 10.3, 0.7 ± 0.5, 0.8 ± 0.6, 7.8 ±
5.0, and 27.1 ± 19.1 pg/μg protein, respectively (mean ± SD, n = 30). Table 2, presents all continuous outcomes (PGE2, 5-, 12-, 15-HETE, and 13-HODE, AA), and mean percent change from baseline to day 28 of PGE2, 5-, 12-, 15-HETE, 13-HODE, and AA. Table 2 presents eicosanoid concentrations normalized by protein mass, the, the conventional method used to report ELISA results, and normalize by the mass of AA. The LC/MS/MS method also detects and quantifies AA. The mean percent change in PGE2 in the colon mucosa after 28 days, as compared with placebo, was significantly lower (–28% vs. +26%, P = 0.05), as shown in Table 2. When PGE2 was normalized to protein the results were not significant (P = 0.16), but there was a trend suggesting that ginger decreased PGE2 compared with placebo (7% vs. +32%, Table 2).

Changes in the other eicosanoids also were more evident when normalized to AA levels. There were no significant differences in mean percent change between baseline and day 28 for any of the other eicosanoids (HETE-5, -12, -15, and 13-HODE), when normalized to protein. In contrast, there was significant decrease in 5-HETE (P = 0.04) compared with placebo and trends toward significant decreases in 12-HETE (P = 0.09) and 15-HETE (P = 0.06) when eicosanoid concentrations were normalized to AA. There was no significant effect of batch (P = 0.47–0.95) on mean

### Table 1. Adverse events reported by person

<table>
<thead>
<tr>
<th>AE</th>
<th>Ginger (n = 14)</th>
<th>Placebo (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants with any AE, no. (%)</td>
<td>5 (35.7)</td>
<td>7 (43.8)</td>
<td>0.55</td>
</tr>
<tr>
<td>GI</td>
<td>5 (35.7)</td>
<td>6 (37.5)</td>
<td>0.71</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (7.14)</td>
<td>1 (6.25)</td>
<td>0.83</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0 (0)</td>
<td>2 (12.5)</td>
<td>0.47</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
<td>2 (12.5)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

**Note:** All AEs are NCI grade 1.

*P*: χ² or Fisher's exact test as appropriate.

**G** symptoms include: bloating, gas, nausea, heartburn, mouth burning, rectal itching, stomach pain, IBS.

**Other** includes: flu symptoms, pain in leg.

### Table 2. Eicosanoids levels in normal mucosa in participants at normal risk for colorectal cancer [mean (SD)]

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Baseline 28-day follow-up</th>
<th>Mean % change (SD) BTWN baseline and week 4 ⁴</th>
<th>Baseline 28-day follow-up</th>
<th>Mean % change (SD) BTWN baseline and week 4 ⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized to protein (pg/μg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE₂</td>
<td>9.1 (10.5)</td>
<td>8.6 (6.9)</td>
<td>31.9 (89.8)</td>
<td>12.9 (10.1)</td>
</tr>
<tr>
<td>5-HETE</td>
<td>0.6 (0.4)</td>
<td>0.9 (1.1)</td>
<td>54.9 (190.5)</td>
<td>0.8 (0.7)</td>
</tr>
<tr>
<td>12-HETE</td>
<td>0.8 (0.7)</td>
<td>1.5 (2.8)</td>
<td>71.5 (158.8)</td>
<td>0.8 (0.6)</td>
</tr>
<tr>
<td>15-HETE</td>
<td>7.4 (5.0)</td>
<td>12.7 (22.0)</td>
<td>63.3 (171.0)</td>
<td>8.2 (5.1)</td>
</tr>
<tr>
<td>13-HODE</td>
<td>22.1 (15.4)</td>
<td>22.4 (15.3)</td>
<td>17.7 (75.0)</td>
<td>32.8 (21.8)</td>
</tr>
<tr>
<td>AA (ng/pg)</td>
<td>0.7 (0.4)</td>
<td>0.8 (0.6)</td>
<td>24.1 (84.7)</td>
<td>0.7 (0.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Baseline 28-day follow-up</th>
<th>Mean % change (SD) BTWN baseline and week 4 ⁴</th>
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<th>Mean % change (SD) BTWN baseline and week 4 ⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized to AA (ng/μg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE₂</td>
<td>13.1 (11.5)</td>
<td>11.5 (7.6)</td>
<td>26.4 (96.0)</td>
<td>18.2 (12.1)</td>
</tr>
<tr>
<td>5-HETE</td>
<td>1.0 (1.0)</td>
<td>1.0 (0.6)</td>
<td>21.5 (58.5)</td>
<td>1.2 (0.8)</td>
</tr>
<tr>
<td>12-HETE</td>
<td>1.2 (1.0)</td>
<td>1.4 (1.0)</td>
<td>41.0 (57.8)</td>
<td>1.2 (0.8)</td>
</tr>
<tr>
<td>15-HETE</td>
<td>9.3 (5.9)</td>
<td>11.2 (7.9)</td>
<td>26.7 (61.7)</td>
<td>11.0 (6.1)</td>
</tr>
<tr>
<td>13-HODE</td>
<td>37.1 (34.2)</td>
<td>32.5 (20.8)</td>
<td>9.7 (69.8)</td>
<td>50.6 (34.4)</td>
</tr>
</tbody>
</table>

*Independent t test or

**Mann-Whitney U test, as appropriate, on the difference between mean percent change between baseline and day 28.

**Mean percent change between baseline and week 4 is calculated as [(eicosanoid at time 2/eicosanoid at time 1)/eicosanoid at time 1] per participant and then an average is obtained. Mean percent change may not seem reflective of change in baseline and 28-day follow-up mean values. This is due to the large amount of variability in the baseline measures.
percent change for any eicosanoid whether normalized to protein or free AA.

Blinding and adherence

Participants were able to determine whether or not they had received ginger compared with placebo ($P = 0.02$). Participants who were randomized to placebo were unable to correctly guess their group assignment (44% guessed they were taking ginger). In contrast, participants who received ginger correctly guessed “ginger” 86% of the time. We also asked participants, "Was it the way the capsule worked, tasted, looked or smelled?" that helped you guess what you were taking? Only the way in which the capsule tasted was significantly different ($P = 0.01$) between treatment groups.

All participants were adherent per our definition of taking at least 80% of their capsules. Participants on average took $100 \pm 9.9\%$ of their capsules and there was no difference between study groups ($P = 0.15$).

Discussion

We found a significant effect of a ginger root extract, in the dose and formulation used, to decrease our primary endpoint, the mean percent change in PGE$_2$ levels in colon biopsies from subjects at normal risk for developing colorectal cancer when normalized to free AA. We did not, however, find a significant difference in PGE$_2$ concentrations when normalized to protein. Similarly, we found no difference in the concentrations of 5-, 12-, 15-HETE, or 13-HODE when normalized per protein. However, when normalized per free AA there was a significance decrease in 5-HETE and decreases in both 12- and 15-HETE approached significance. Eicosanoid levels per amount of protein reflect absolute concentrations of eicosanoids in the tissue; however, eicosanoid levels per amount of free AA could reflect enzymatic activity of the COX and LOX enzymes. In essence, when the catalytic enzymes, that is, COX are blocked, less substrate is metabolized increasing the amount of AA and decreasing the eicosanoids. This may possibly imply some inhibition of COX-1, LOX-5, -12, and LOX-15-2 enzymes by ginger extract. However, rigorous kinetic experiments assessing COX and LOX enzymatic activity would need to be conducted to confirm this hypothesis. Linoleic acid was not quantified, making interpretation of 13-HODE levels difficult.

This study observed a 28.0% mean decrease in PGE$_2$ normalized to free AA and a roughly 7% decrease when normalized to protein from baseline colon mucosal levels. To date, how much PGE$_2$ concentration needs to be decreased in human colonic mucosa to prevent the occurrence of adenomas is unknown. While aspirin has been shown to both prevent adenomas and decrease colonic mucosal PGE$_2$, no studies have combined these endpoints. Several studies have examined the effect of aspirin on production of PGE$_2$ in human colonic mucosa showing anywhere from no reduction to an 85% decrease in PGE$_2$ in colonic mucosa (33–37). Unlike aspirin, a study examining sulindac, another NSAID, did examine the effect on mucosal prostanoids and polyp occurrence in patients with genotypically affected familial adenomatous polyposis. On average, PGE$_2$ concentrations in rectal biopsies in participants that received sulindac decreased significantly by 19.2% from baseline levels when taken for 48 months at doses of either 75 to 150 mg daily (38). In the sulindac arm, those participants that did not develop an adenoma had a 33.9% mean reduction in baseline PGE$_2$ rectal mucosal concentrations compared with baseline levels, while those who received sulindac and developed a polyp had a slight increase of 2.4% from baseline PGE$_2$ levels (38). In contrast, taking difluoromethylornithine/sulindac for 3 years resulted in a 70% to 90% reduction in the recurrence of colorectal adenomas but this was not correlated with reductions in mucosal PGE$_2$, although higher baseline levels of PGE$_2$ were associated with higher recurrence rates (39). Our results are more modest than those observed for aspirin, but only slightly lower than those observed for sulindac. Our results are most likely attenuated by lower baseline PGE$_2$ levels because of our healthy normal risk for CRC sample and relatively short study duration of 28 days. Longer-term use in high-risk patients could possibly maximize the effect of ginger.

Previous to this study, ginger and ginger constituents’ anti-inflammatory effects on COX and LOX enzymes and their products had only been observed ex vivo (40). The only exception is in one study of rats where decreased serum levels of PGE$_2$ were observed with ginger treatment (22). The present study indicates that oral ginger could have inhibitory effects on colon tissue COX and LOX enzymes in humans.

This study had several limitations. We had a small sample size of only 30 participants and this study was intended as a pilot to determine whether a larger study with ginger extract was warranted. Also, our results had much larger SD for all of the eicosanoids than anticipated, and as such we had inadequate sample size to detect meaningful changes in colon eicosanoid concentrations in several instances, especially when normalized to protein. The sample size of this study was based on the mean and SD of PGE$_2$ concentrations in human colon tissue determined by our group’s previous study using ELISA (33). The ELISA assay results indicated SD of around 10% of the mean. In contrast, the LC/MS/MS assay, employed in our study had SD that exceeded 100% of the mean. With this SD, a post-hoc sample size analysis indicated that 61 subjects would be needed to detect a significant difference in PGE$_2$ levels normalized to protein.

Despite the variability of the LC/MS/MS assay it provided several advantages over an ELISA. Mainly, with LC/MS/MS we could measure numerous eicosanoids and free AA simultaneously. The LC/MS/MS method is also more specific for a given analyte than ELISA as it avoids cross-reactivity issues inherent in ELISAs. Importantly, however, we did determine that our mean baseline PGE$_2$, 12-, 15-HETE, and 13-HODE concentrations per protein derived from LC/MS/MS were similar to other studies (11, 32, 36), which used other methods to determine eicosanoid concentrations. Other studies using ELISA and gas chromatography-mass spectrometry also found high amounts of
variability in colonic PGE$_2$ concentrations, not dissimilar to our results (38, 41). One explanation for the high level of variability in our eicosanoid assays is more than 15% between-day CV (42) for all the eicosanoids except PGE$_2$ and AA. However, assay batch had no significant effect on mean percent change for any eicosanoid when examined in linear models, and was thus not added to the final analysis. Another source of variability is the considerable dissimilarity of eicosanoids at different locations of the colon both between and within people (41). To help address this, we combined 2 biopsies from the same participant at the same time point, but it was in the same section of the colon.

Participants reported a high level of adherence in this study with an average intake of 100% of study medication, making it an unlikely source of variability. A recent study has also found that adenoma risk was not significantly associated with genetic variation in PGE$_2$ synthase and prostaglandin dehydrogenase, however, genetic variations in these key enzymes and associations with variation in levels of PGE$_2$ were not examined (43). Similarly, no significant associations were found between age, BMI, percentage of body fat, NSAID drug use, history of adenomas, and family history of colon cancer with either baseline levels or at the length of time form the last dose of ginger extract due to the fast half-lives (between 1 to 3 hours) and clearance of 10-gingerols glucuronide and sulfate within 24 hours of the last dose of ingesting 2.0 g of ginger extract for 28 days. Presence of gingerols in tissue were affected by the length of time form the last dose of ginger extract due to the fast half-lives (between 1 to 3 hours) and clearance of the gingerols and shogaol in humans (44). These findings argue for large sample sizes, careful recording of when ginger was last consumed and the use of colonic biopsies taken at multiple time points to help draw meaningful conclusions that would otherwise be masked by the considerable variability in this marker.

Future studies of ginger root extract should focus on examining the mechanisms of action by which ginger extract is affecting the COX and LOX enzymes involved in the production of both the inflammatory and anti-inflammatory eicosanoids. In addition, the effect of ginger on microsomal prostaglandin E$_2$ synthase-1 (mPGES-1) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) should be considered as the role of both of these enzymes in PGE$_2$ production and degradation are being recognized as increasingly important to governing tissue concentrations of PGE$_2$ (45).

Subsequent studies should also examine the effect of ginger extract in people at high risk for CRC to determine if there is a differential or similar effect between normal and high-risk populations.

In conclusion, ginger seemed to be well tolerated. There were no differences between placebo and ginger for total AEs or in common AE categories including fatigue, gastrointestinal effects, or headaches. Participants reported a high level of adherence with all participants reporting taking at least 80% of their study medication. Ginger extract had no significant effect on colon concentrations of AA, PGE$_2$, 5-, 12-, and 15-HETE or 13-HODE normalized to protein when compared with the placebo group. However, ginger extract did seem to have an inhibitory effect on COX and LOX-5, 12-, and 15-2 enzymes as observed by significant or close to significant decreases in the mean percent change in PGE$_2$, 5-, 12-, and 15-HETE normalized to AA. Consequently, it would seem that ginger extract has an anti-inflammatory effect in the colon of persons at normal risk for CRC and warrants further study.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**


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