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Colonic Saturated Fatty Acid Concentrations and Expression of COX-1, but not Diet, Predict Prostaglandin E_2 in Normal Human Colon Tissue

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Abstract

Prostaglandin E_2 (PGE_2) in the colon is a pro-inflammatory mediator that is associated with increased risk of colon cancer. In this study, expression of genes in the PGE_2 pathway were quantified in colon biopsies from a trial of a Mediterranean versus a Healthy Eating diet in 113 individuals at high risk for colon cancer. Colon biopsies were obtained before and after 6 months of intervention. Quantitative, real-time PCR was used to measure mRNA expression of prostaglandin H synthases (PTGS1and 2), prostaglandin E synthases (PTGES-1 and 3), prostaglandin dehydrogenase (HPGD) and PGE_2 receptors (PTGER2, PTGER4). The most highly expressed genes were HPGD and PTGS1. In multivariate linear regression models of baseline data, both colon saturated fatty acid concentrations and PTGS1 expression were significant, positive predictors of colon PGE_2 concentrations after controlling for non-steroidal anti-inflammatory drug use, gender, age, and smoking status. The effects of dietary intervention on gene expression were minimal with small increases in expression noted for PTGES3 in both arms and in PTGER4 in the Mediterranean arm. These results indicate that short-term dietary change had little effect on enzymes in the prostaglandin pathway in the colon and other factors, such as differences in fatty acid metabolism, might be more influential.

Keywords

Prostaglandin E_2; colon biopsy; gene expression; dietary intervention

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Introduction

Colorectal cancer is the third most common type of cancer and the third leading cause of death in both men and women in the United States (1). Approximately 60-70% of colon cancer cases are thought to be sporadic, pointing to the potential role of modifiable risk factors in prevention of this disease. These factors include lifestyle choices such regular physical activity, smoking and diet (2).

One important mechanism by which diet could reduce risk of colon cancer is by modification of prostaglandin E_2 (PGE_2) concentrations in the colon. Colon cancer risk is strongly associated with increased levels of prostaglandin E_2 (PGE_2), a pro-inflammatory mediator in the colon that promotes colon cancer development (3). PGE_2 is synthesized from the n-6 fatty acid arachidonic acid (AA) by the action of constitutive COX-1 (gene PTGS1) and inducible COX-2 (gene PTGS2) to form an unstable endoperoxide intermediate prostaglandin H_2 (PGH_2). PGH_2 isomerization via prostaglandin E synthases (PGES) subsequently results in formation of PGE_2. There are three forms of PGES: cytosolic PGES (cPGES, gene PTGES3) and microsomal PGES-1 and 2 (mPGES1 and 2, genes PTGES2 and PTGES2, respectively). PTGES2 has an important role in colon tumor formation (4, 5).

In this study, we analyzed normal colon tissue biopsies from healthy individuals. We quantified expression of PTGES3, that appears to be a constitutive enzyme (6), and PTGES1, that is inducible in response to inflammatory stimuli (4). We also quantified expression 15-prostaglandin dehydrogenase (15-PGDH, gene name HPGD) that catabolizes PGE_2 and is down-regulated during tumorigenesis (7). Finally, we quantified expression of PGE_2 receptor genes, PTGER2 and PTGER4, for the receptors EP2 and EP4 that are expressed in colon and have well-defined roles in colon inflammation (8).

In addition to the role of enzymatic pathways on production and degradation of PGE_2, there is a potential for dietary fatty acids in controlling PGE_2 levels. Higher intakes of dietary n-6 fatty acids can increase the COX substrate, arachidonic acid (AA, n-6), in colon tissue (9). On the other hand, increased dietary intake of eicosapentaenoic acid (EPA, n-3) increases the ratio of EPA to AA in cells, which in turn decreases PGE_2 levels due to the inhibition of COX-1 activity by EPA (10). Similarly, increased intakes of monounsaturated fatty acids (MUFA) have been shown to decrease AA in the colon and to decrease PGE_2 synthesis (11). Both monounsaturated fats and n-3 fats have been associated with lower risks of colon cancer (12). In contrast, saturated fatty acids (SFA) increase formation of PGE_2, induce COX-2 expression and increase inflammation through activation of toll-like receptors (13). This may be one mechanism by which a Western diet increases risk of colon cancer.

The traditional Mediterranean diet, on the other hand, is high in fruits, vegetables, fish, olive oil and whole grains, and has been associated with lower risk of chronic diseases and most types of cancers, including colon cancer (14). In addition to the effects of dietary fat discussed above, the antioxidant effects of higher consumption of fruits and vegetable also may work to reduce production of inflammatory mediators (15). Despite this, we previously reported that a Mediterranean intervention did not significantly affect colon PGE_2 (16). PGE_2 concentrations at the time of biopsy, however, might not reflect the capacity of the

tissue to produce PGE_2 since PGE_2 can be rapidly formed and degraded in cells. Here we evaluated relationships between colonic expression of genes in the PGE_2 pathway and PGE_2 concentrations in 113 individuals at increased risk of colon cancer. We also evaluated if six months of intervention with a Mediterranean or Healthy Eating diet would affect gene expression in the colon.

Materials and Methods

Study participants, design and dietary intervention

The Healthy Eating Study a randomized dietary intervention trial that was approved by the University of Michigan Institutional Review Board (HUM00007622). Informed, written consent was obtained from all study participants. Detailed information on the recruitments, eligibility criteria, dietary assessment and intervention was previously described (17, 18). In brief, the study recruited 120 individuals with a family history of colon cancer, or a personal history of adenomatous polyps or early stage colon cancer. Other inclusion criteria included being in good general health, being at least 21 years old, and having a body mass index (BMI) of ≥ 18.5 and < 35 kg/m^2. A study questionnaire captured medical history, demographic, behavioral factors, and medication use as well as colon cancer risk factors. Dietary eligibility was assessed using two days of written records and one unannounced 24-hour recall.

Eligible subjects were randomized into one of two dietary interventions, a Healthy Eating diet or a Mediterranean diet, for 6 months. Both diet arms were designed to increase the intakes of fruit, vegetable and whole grain; however, goals for fat intake differed in each diet arm. The Healthy Eating diet, which was based on the U.S. Healthy People 2010 recommendations, limited saturated fat (SFA) intake to 10% of an individuals' total energy intake while the Mediterranean diet goals sought to decrease PUFA intake by 50%. The Mediterranean group was also asked to consume foods high in n-3 fatty acids, at least twice a week and to increase monounsaturated fat intake from plant sources by 50%. These diet goals resulted in a reduction in saturated fat intakes in both arms, as previously reported (18).

Medication use

Medications that were reported by study participants were evaluated for effects on the biomarkers quantified in colon biopsies. The most prevalent medication was regular use of non-steroidal anti-inflammatory drug (NSAID) by 24 subjects (aspirin with a dose of 81 mg/day or 325 mg every other day) for cardiovascular disease prevention. Cholesterol medications were used by 19 subjects and this included Crestor, Ezetimibe, Lipitor, Lovastatin, Mevacor, Simvastatin, Vytorin, and Welchol. Lastly, blood pressure medications were used by 21 subjects and these included Acebutolol, Atenolol, Hydrochlorothiazide, Losartan, Lisinopril and Metoprolol or combinations (Table 1). Occasional use of other medications was not analyzed.

Flexible sigmoidoscopy and tissue collection

A flexible sigmoidoscopy procedure without prior preparation of the bowels was performed to obtain colon tissue biopsies. Eight mucosal tissue biopsy specimens from each individual were collected in the distal sigmoid colonic mucosa at each time point. Of these, six biopsies were immediately placed in liquid nitrogen exactly 20 seconds after excision from the individual. Biopsies were stored at −70°C until biomarker analysis and gene expression could be quantified. The other two biopsies were submerged in ice-cold, phosphate-buffered saline (pH 7.4) and fixed in formalin (10% formaliomega-90% phosphate buffered saline pH 7.4). Biopsies were kept for 18-24 hours in formalin before being transferred to 70% ethanol.

RNA extraction and reverse transcription (cDNA synthesis)

One frozen biopsy of approximately 5 mg tissue from each participant at each time point was used for RNA extraction. The tissue was placed in 100 μL of RNA later-ice overnight at −20°C. Tissues were pulverized in a liquid-nitrogen-cooled mortar. RNA was extracted using 1 ml TRIzol following the manufacturers' protocol (Invitrogen, Waltham, MA). The tissue was homogenized on ice using an Ultrasonic Processor (Misonix, Farmingdale, NJ). The tissue homogenate was placed in a sterile 1.5 ml tube containing isopropanol 100% (0.5 mL) and 50 μl of glycogen for RNA precipitation with 0.5 mL of isopropanol.

The RNA pellet was washed three times with 75% ethanol, dried, and then resuspended in 20μl RNAse free water. RNA concentration and purity were determined using a ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE, USA). cDNA constructs were made for samples that showed a ratio of 260/280 of greater than 1.7. Samples were diluted as follows: one μg RNA diluted in 9.9 μl of RNase free water. cDNA was synthesized using the Reverse Transcription System following the manufacturer protocol (Promega, Madison, WI). The cDNA samples were placed in a thermal cycler (BIO-RAD, cat. T100), for 1 minute at 25°C, 1.5 hours at 42°C, 5 seconds at 95°C, and left at 4°C until cool.

Quantitative real-time PCR (RT-qPCR)

Real-Time PCR was performed by using TaqMan® Environmental Master Mix 2.0 (Life Tech, Grand Island, NY). A mixture of cDNA from the sample pool was used to construct a standard curve for each gene. All samples and standard curves were run in duplicate in the real-time PCR reactions. The primers and probes used for real time PCR were purchased from Applied Biosystems (Foster City, CA, USA). The primers used were as follows: COX-1 (PTGS1), Hs00377726_ml), COX-2 (PTGS2, Hs00153133_m1), mPGES1 (PTGES1, Hs01115610_ml), cPGES (PTGES3, Hs00832847_gH), 15 HPGD (HPGD, Hs00168359_ml), EP2 (PTGER2, Hs04183523_m1), and EP4 (PTGER4, Hs00168761_m1). Cytokeratin 20 Krt20, a marker of colonic epithelial cell mass, was used as an internal control for normalization (Hs00300643_m1) (19). The real-time PCR thermal conditions were: 50°C 2 min, 95°C 10 min followed by 40 cycles of 95°C 15 sec and 60°C 1 min.

The mean efficiency for the PCR standard curve for each primer was: PTGS1 (95%), PTGS2 (90.4%), PTGES (105%), PTGES3 (97.2%), HPGD (99%), PTGER2 (93%), PTGER4

(94%), and Krt20 (94%). For quantification, the standard curve method was used, and the average amount of each target mRNA expression and Krt20 mRNA expression was established from the standard curve. Each target gene expression was then normalized by Krt20 expression.

Immunohistochemical staining for protein quantification

Formalin-fixed, paraffin-embedded tissue blocks of colon biopsies were used to construct a tissue microarray (20). A total 114 of 212 tissue samples from the trial were available for protein quantification to validate the qrtPCR results. Each sample was represented by a single 1 mm diameter core and 32 slides were made from each TMA. The first and last slides were stained with hematoxylin and eosin. Immunohistochemical (IHC) staining was conducted using slides that had the largest number of full-length crypts, as predicted from the H&E stained slides. The PTGES1 and PTGS2 proteins are known to be expressed in very low or undetectable levels in normal colon tissue, and therefore were not subjected to IHC. The samples available with full-length crypts for IHC quantification were HPGD (n=46), PTGS1 (n=43), PTGES3 (n=24), PTGER2 (n=32) and PTGER4 (n=35).

IHC staining was performed on a DAKO Autostainer (DAKO, Carpinteria, CA) using DAKO LSAB+ or Envision+ and diaminobenzidine (DAB) as the chromogen. De-paraffinized TMA sections were labeled with antibodies at ambient temperature. Microwave epitope retrieval was used prior to staining. Appropriate negative (no primary antibody) and positive controls (tumor tissue) were stained in parallel with each set of antibodies studied. A light counterstain with H&E was used (Table 1s). Figure 1s shows representative sections of colon stained for each protein.

The slides were imaged in a microscope with a high resolution Leica Biosystems scanner to generate whole-slide digital scans of all TMA slides. Images were transferred to files for quantification of staining with Aperio ePathology image analysis software (Leica Biosystems). Although some tissue samples did not show full-length crypts due the direction of cutting or amount of tissue available, those slides were still quantified for the whole tissue reading. For each tissue sample, the whole tissue, the mucosal layer, and the submucosa under the epithelium (using a thickness equivalent to the thickness of the mucosa) were quantified for positive and negative staining. Aperio Image analysis algorithms based on color partitions and intensity of positive staining were used to quantity the proteins of interest. The percentage of the total positive staining for all three areas of the tissue was analyzed for each gene.

PGE_2 and fatty acids in biopsies

Reverse-phase liquid chromatography with tandem mass spectral detection (LC-MS-MS) was used to quantify PGE_2 and PGE_3 as previously reported (21). In short, tissue homogenates were prepared from two frozen colon biopsies. Eicosanoids were extracted with ether prior to LC-MS-MS analysis. Deuterated internal standards (Cayman Chemical, Ann Arbor, MI) and a Luna Phenyl-Hexyl analytical column (2 × 150 mm, 3 μm particle size, Phenomenex, Torrance, CA) were used. Because deuterated internal standard was not available for PGE_3, both were quantified using PGE_2-d_4. Protein content of the homogenate

was determined by the Bradford assay. Eicosanoids were expressed as nanogram (ng) of PGE_2 or PGE_3 per milligram (mg) of protein.

GC-MS analysis was used to measure fatty acids from the same colon homogenates using methods we previously published (22). Briefly, 5 mg of pulverized colon tissue biopsy was added to a tube containing 150 μl of ice-cold phosphate buffered saline with 0.1% BHT and 1mM EDTA. Tubes were placed in an ultrasonic processor for two intervals of 30 seconds. A ratio of 1:1 chloroform and methanol was used for extraction of lipids. Prior to GC-MS analysis, methyl esters were prepared with METH-PREP II derivatization reagent (Alltech, Deerfield, IL) (23).

Statistical analysis

Statistical analyses were conducted using IBM SPSS software version 22 (PASW Statistics, IBM Corporation, Armonk, New York). Variables were checked for normality of the distribution before analyses and transformed as needed. Natural log transformation was used for normalizing gene expression of enzymes and receptors in the PGE_2 pathway, and the square root was used for concentrations of PGE_2 and arachidonic acid.

To evaluate the effects of the baseline factors on PGE_2 concentrations, two-sample t-tests and partial correlations were used. Spearman correlation coefficients were used to determine the associations between PGE_2, gene expression and colon tissue. Generalized linear regression (GLM) models were used to determine the predictors of colon PGE_2 concentrations. Although known demographic risk factors for colon cancer, namely age, gender and smoking status, did not show significant effects on colon PGE_2 concentrations, they were nonetheless entered as covariates in the models.

Linear mixed models including a random intercept for subjects were used to determine changes in expression of genes in PGE_2 pathway over six months. This analysis utilized log-transformed gene expression variables. Finally, to confirm and validate RNA expression, protein expression of the enzymes and receptors in the PGE_2 pathway were correlated using Spearman correlation coefficients.

Results

Effect of the demographic factors and medication use on the PGE_2 pathway

Biopsy samples were available from 113 of the 120 subjects who participated in the study. The characteristics of these study subjects at baseline were similar to that reported for the full study group (18): mean age 52 (range 21-81 years), body mass index (BMI) of 27 (range 18-34 kg/m^2), 70% female, 87% Caucasian, and 11% current smoker. None of these demographic factors had significant relationships with colon PGE_2 concentrations or gene expression (not shown). There were significant effects of medication use on gene expression as shown in Table 1. NSAID use decreased PGE_2 and expression of PTGS2, cholesterol medication use decreased PTGES1 and blood pressure medication use decreased PTGS2 expression.

Subgroup analyses were conducted excluding those who used more than one type of medication (data not shown). The significant difference in PGE_2 remained for users of NSAIDs (n=11) versus non-users of any medications (n=69) but the effects on PTGS2 was no longer significant. Effects of cholesterol medications use only (n=8) and blood pressure medications use only (n=11) also were no longer significant, although there was a trend for lower PTGS1 (p=0.06) with use of blood pressure medications.

Gene Expression of enzymes and receptors in the PGE_2 pathway

In Figure 1, the highest relative mean mRNA expression in colon biopsies was 1.4 for HPGD and 1.15 for PTGS1. PTGS1 expression was more than five-fold higher in comparison to PTGS2 expression. PTGES3 mRNA expression was more than three-fold higher than PTGES1 expression. Lastly, PTGER4 was expressed almost three fold higher than PTGER2 (Figure 1).

Associations of colon tissue fatty acids with PGE_2 concentrations and gene expression

Table 2 shows Spearman correlations between colon PGE_2 concentrations and gene expression. Correlations with PGE_3 were not significant in any case, even with tissue EPA. Colon PGE_2 concentration was significantly correlated with PTGS1 expression only. Expression of PTGS1 was significantly correlated with expression of PTGS2, PTGES1 and both receptors. Expression of PTGES3 was not correlated with any of the other enzymes or colon PGE_2. Expression of HPGD was correlated with PTGS2 and PTGES1. The PGE_2 receptors correlated with expression of PTGES2, PTGES1, and HPGD.

Table 2 also shows correlations of colon nutrient concentrations with gene expression. There was a significant positive association between PGE_2 and colon tissue saturated fatty acids (SFA). Colon MUFA tended to be negatively correlated with enzyme expression while EPA unexpectedly displayed positive correlations. Colon AA concentrations were not correlated with PGE_2 or gene expression.

Predictors of baseline PGE_2 levels in linear regression models

Linear regression models were utilized to identify factors that account for inter-individual variation in colon PGE_2 concentrations (Table 3). Colon PGE_2 concentration was square root transformed to achieve normality. Interestingly, SFA and PTGS1 were both significant in the model as predictors for PGE_2, and both remained significant predictors of PGE_2 in the final model that included NSAID use, age, current smoking status and gender. This model accounted for 23% of the variability in colon PGE_2. The beta coefficients indicated that higher PGE_2 was predicted by higher colon SFA, higher PTGS1 expression, not using NSAIDs, current smoking, being younger and being male. Adding BMI to the model did not improve its predictive value. Colon PGE_2 concentrations were not correlated with dietary intakes of fat (neither total fat nor subtypes of fat), fiber, carotenoids or glycemic load (not shown).

Changes in gene expression after dietary intervention

Changes in mRNA expression of genes in the PGE_2 pathway were evaluated over six months of dietary intervention (Table 4). Linear mixed models were used consistent with intention to

treat principles. Gene expression variables were log transformed to achieve normality, but the data in Table 4 shows simple means of all available data for ease of interpretation. The ANOVA models included regular use of NSAIDs and age as covariates. Including BMI and current smoking yielded essentially the same results (not shown). Since NSAID use showed significant effects on PTGS2 gene expression at baseline, a subgroup analysis limited to NSAID non-users was conducted as well with essentially the same as for the entire study group (data not shown). PTGES3 was the only gene in the PGE_2 pathway that showed a significant increase in RNA expression in both dietary arms over the 6 month period, and PTGER4 increased in the Mediterranean arm (Table 4).

Validation of RNA gene expression with protein expression by IHC

To validate mRNA expression of genes in the PGE2 pathway, we quantified protein expression by IHC for genes that showed the highest mRNA expression in colon tissue. Those genes were HPGD, PTGS1, PTGES3, PTGER 2 and PTGER 4. The relative RNA expression pattern of each gene was similar to that of protein expression in the colon tissue as a whole, and in the sub-mucosa and whole tissue (Figure 2). Correlations between protein and gene expression was also done and are shown in supplemental Table 2s. HPGD and PTGS1 RNA expression showed significant associations with protein expression all three histological areas: whole tissue, mucosa, and submucosa. PTGES3 RNA expression showed a strong positive association with whole tissue protein expression, but not when evaluated in the sub regions. Stornger correlations would be expected for whole tissue staining since RNA expression as determined on whole biopsy homogenates. RNA expression of both PGE_2 receptors (PTGER2 and 4) showed significant associations with their respective protein expression extent in all areas of the colon tissue. Sections of IHC-stained colon tissue with high expression and low expression of proteins are shown in supplementary Figure 1s.

Discussion

Epidemiological and clinical studies have emphasized the usefulness of surrogate end point biomarkers in understanding both colon cancer development and prevention. Elevated PGE_2 is a well-recognized biomarker of increased colon cancer risk that promotes a pro-inflammatory state in the colon. However, few studies have investigated expression of genes in its metabolic and signaling pathways. In colon biopsies, our study confirmed that expression of both HPGD and PTGS1 was the highest, as could be expected for normal tissue, while gene expression of PTGS2 and PTGES1 were the lowest (Figure 1). Both of the latter two enzymes are overexpressed during carcinogenesis, but in normal tissue, expression is quite low (24, 25).

High inter-individual variability in gene expression was found among subjects, especially for HPGD (Figure 1). Published data supports the finding that HPGD gene expression varies between individuals but remains stable within the colon of each individual over time, regardless of where in the colon the biopsy is obtained (26). Variability of these biomarkers among high-risk subjects indicates a potential to categorize individuals with regard to risk based on differences in expression of genes in the PGE_2 pathway. For example, a recent

study found that higher expression HPGD expression in normal colon tissue was a potential predictor of the efficacy of aspirin treatment for colon cancer prevention (27).

Of all the demographic factors examined at baseline, medication use was the only factor that significantly affected expression of genes in the PGE_2 pathway. NSAIDs are known to lower colon cancer risk by decreasing PGE_2 levels via inhibition of the cyclooxygenase activity of both PTGS1 and PTGS2 (28). Here we found that NSAID use reduced PTGS2, but not PTGS1 mRNA expression levels (Table 1). These findings indicate that PTGS2 may contribute at least partially to PGE_2 production in the normal colon since NSAID use significantly decreased colon PGE_2 concentrations in biopsy samples from this study, as we published previously (29). Cholesterol medication use was significantly associated with reduced mRNA expression of PTGES1, and PTGES1 is the synthase that associates with PTGS2 (Table 1). This significant reduction in PTGES1 is consistent with data suggesting use of statins as a potential pharmacological approach for colon cancer prevention (30). Previous research also has indicated that combining NSAID use with cholesterol medications may be useful for CRC prevention and treatment (31).

Although the results with NSAID and cholesterol medication use indicate a role of PTGS2 in governing PGE_2 concentrations (Table 1), PTGS1 but not PTGS2 showed a significant correlation with colon PGE_2 concentration (Table 2). This finding is possibly due to the higher expression level of PTGS1 versus PTGS2. Therefore, PTGS1 may be seen as an additional potential target for colon cancer prevention among those individuals at high-risk. This is consistent with results of an intervention trial of ginger supplementation that found protein levels of PTGS1 in colon of high-risk subjects was two-fold higher in comparison to colon PTGS1 from the normal risk group (32). That trial did not quantify PTGS2 protein expression since the low expression of this enzyme makes it hard to measure protein expression in normal colon tissue (24).

There were also interesting associations of gene expression with colon fatty acid concentrations. The negative association between colon tissue monounsaturated fatty acids (MUFA) with both PTGS2 and PGE_2 is consistent with the anti-inflammatory effects of MUFA found in olive oil (33). In contrast, the positive relationship between saturated fatty acids (SFA) and PGE_2 concentration (Table 2) is consistent with the finding that high dietary intake of SFA increases colon cancer risk (34). The mechanism invoked has been activation of inflammatory pathways by SFA through toll-like receptors (TLRs) (35). Activation of TLRs in turn can induce COX-2 expression, although some reports indicate that COX-1 can be induced as well (36). Alternatively, SFA have been shown to allosterically stimulate the cyclooxygenase activity of PTGS2 to increase the production of PGE_2 (37).

We next evaluated whether a Mediterranean or Healthy Eating diet intervention would affect expression of genes in the PGE_2 pathway in the colon biopsies. Both diets increased intakes of whole grains, fruit and vegetables but fat intakes differed with an increase in monounsaturated fat in the Mediterranean arm and a decrease in saturated fat in the Healthy Eating arm (18). Although Mediterranean diets increase longevity and lower the risk for most chronic diseases, including colon cancer (14, 38, 39), little research has been conducted to understand the mechanisms involved. We hypothesized that the Mediterranean

diet would act to reduce capacity for production of PGE_2 in the colon. However, there were few effects of dietary intervention on gene expression, as shown in Table 4. The reasons why PTGES3 might increase are not clear, although we cannot exclude chance findings. During colon carcinogenesis, PGE_2 has a promoting role (3). In normal tissues, however, this is not entirely clear. In the immune cells, for example, PGE_2 has a role in dampening and resolving inflammatory responses to infection (40).

In summary, the findings from this study indicated that mRNA expression of genes in the PGE_2 pathway is highly variable among individuals. Colon PTGS1 expression and SFA concentrations were identified as important factors affecting inter-individual differences in colon PGE_2 concentrations. These results are consistent with the view that PTGS1 in normal colon is a potential target for colon cancer prevention among high-risk individuals (32). Although both the Healthy Eating and Mediterranean interventions reduced dietary intakes of saturated fats (18), these interventions had little effect on expression of genes in the PGE_2 pathway. Since most individuals required 2-3 months to make all the requested changes, they were only following the intervention diets for 3-4 months before the final biopsy was obtained. An intervention duration longer than six months therefore may be needed to allow enough time for the diet to affect stores of fat-soluble nutrients (41). Another factor could be the role of genetic factors in controlling fatty acid metabolism and gene expression, which would limit the effect of diet on fatty acid changes. Future research should evaluate this and other factors that govern production of PGE_2 in normal colon tissue to better understand how preventive strategies can be derived.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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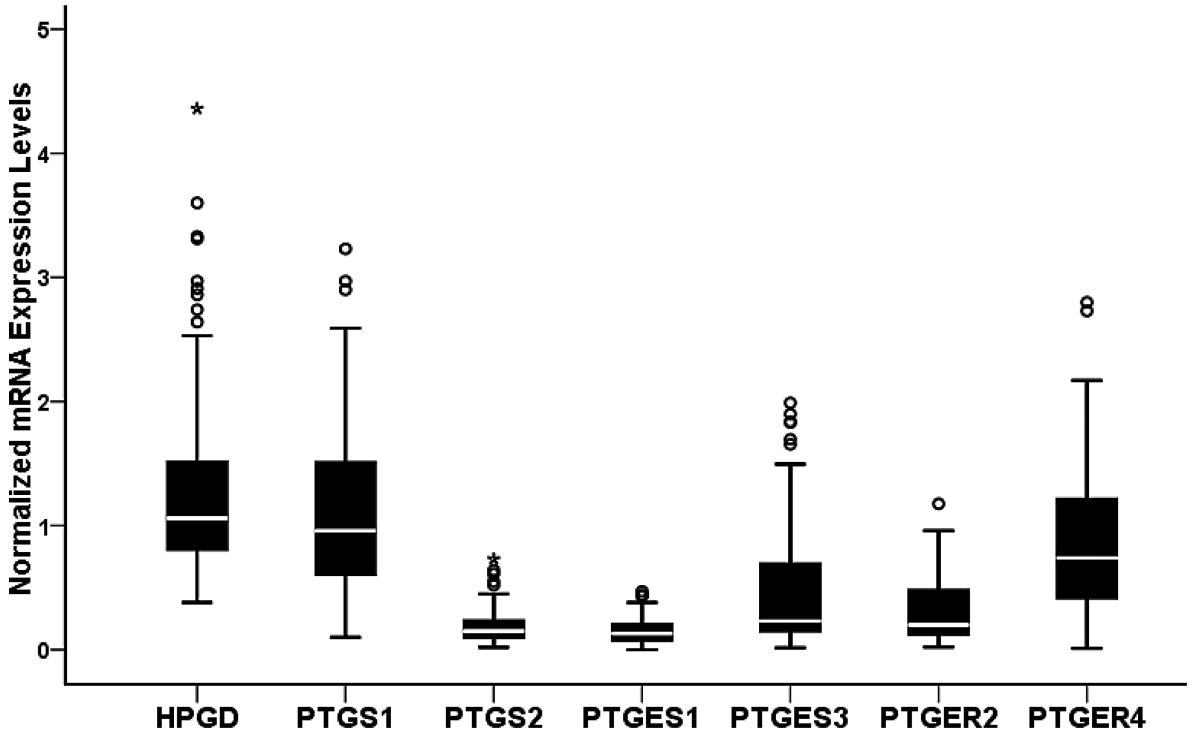

FIGURE 1.

Normalized mRNA expression of enzymes and receptors in the PGE_2 pathway for all study participants at baseline (n=113). The boxes show median, 25^{th} and 75^{th} percentiles, and the whiskers show 1.5 times the inter-quartile range. Outliers are shown as individual values as either circles (outlier) or stars (extreme outlier).

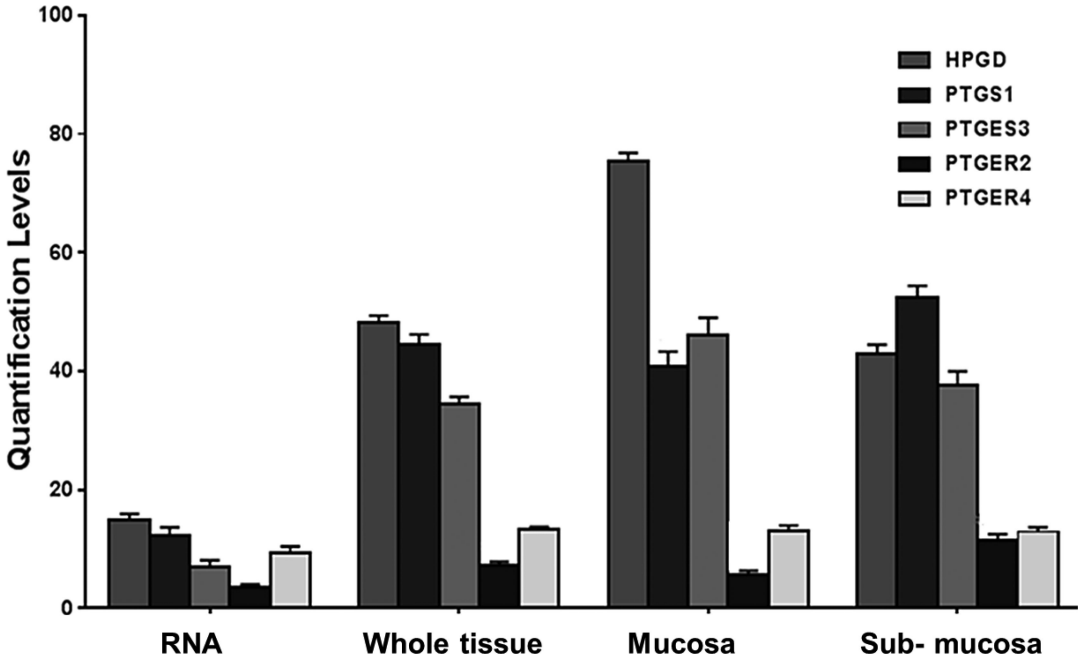

FIGURE 2.

Normalized mRNA expression (first set of bars) and protein expression (in whole tissue, mucosa and sub-mucosa) of genes in the PGE_2 Pathway at baseline in 113 individuals.

TABLE 1

PGE_2 concentrations and relative expression of enzymes and receptors in PGE_2 pathway by medication use in 113 high risk individuals for colon cancer at baseline. Data are given as mean, SD.

Demographic Factors	N	Relative Gene Expression							
		PGE_2	**PTGS1**	**PTGS2**	**PTGES1**	**PTGES3**	**HPGD**	**PTGER2**	**PTGER4**
NSAID Users[b]									
Yes	24	**11, 9** [a]	1.08 0.73	**0.16, 0.10** [a]	0.15, 0.12	0.57, 0.60	1.74, 1.35	0.34, 0.33	0.86, 0.51
No	89	**20, 14**	1.13, 0.67	**0.23, 0.17**	0.18, 0.14	0.57, 0.90	1.32, 0.78	0.34, 0.29	0.94, 0.77
Cholesterol Medications[c]									
Yes	19	17, 15	1.03, 0.64	0.16, 0.15	**0.11, 0.08** [a]	0.54, 0.65	1.32, 0.81	0.28, 0.31	0.84, 0.65
No	94	18, 14	1.14, 0.69	0.22, 0.16	**0.19, 0.16**	0.58, 0.88	1.42, 0.97	0.35, 0.30	0.94,0.73
Blood Pressure Medications[d]									
Yes	21	14, 11	0.90, 0.61	**0.16, 0.11** [a]	0.16, 0.12	0.46, 0.46	1.60, 1.15	0.25, 0.21	0.78, 0.62
No	92	19, 14	1.17, 0.69	**0.22, 0.17**	0.19, 0.16	0.59, 0.90	1.36, 0.89	0.37, 0.31	0.96, 0.74

[a] Bolded pairs differ significantly by medication use ($p<0.05$) using the 2-sample t-test.

[b] Regular use of aspirin daily or every other day.

[c] Cholesterol medications were Rosuvastatin, Ezetimibe, Atorvastatin, Lovastatin and Simvastatin.

[d] Blood pressure medications were Acebutolol, Atenolol, Hydrochlorothiazide, Losartan, Lisinopril and Metoprolol or combinations.

TABLE 2

Spearman correlation coefficients for colon PGE_2 concentrations, normalized mRNA expression levels of enzymes and receptors in the PGE_2 pathway and colon tissue fatty acid concentrations at baseline (n=113).

Colon Markers	PGE_2	PTGS1	PTGS2	PTGES1	PTGES3	HPGD	PTGER2	PTGER4
PTGS1	**0.29**[a]	1.00						
PTGS2	0.16	**0.50**[a]	1.00					
PTGES1	0.13	**0.60** [a]	**0.62**[a]	1.00				
PTGES3	0.05	−0.01	−0.02	−0.19	1.00			
HPGD	0.08	0.15	**0.33**[a]	**0.32**[a]	0.11	1.00		
PTGER2	0.04	**0.28**[a]	**0.58**[a]	**0.50**[a]	0.07	**0.55**[a]	1.00	
PTGER4	0.16	**0.52**[a]	**0.50**[a]	**0.46**[a]	0.08	**0.34**[a]	**0.53**[a]	1.00
AA	0.11	0.16	0.22	0.12	0.10	0.21	0.22	**0.43**[a]
EPA	0.19	**0.25**[a]	0.06	0.21	0.01	0.20	0.03	**0.38**[a]
MUFA	−0.11	−0.19	**−0.30**[a]	−0.15	−0.03	−0.18	−0.23	**−0.37**[a]
SFA	**0.31**[a]	0.11	−0.02	0.02	0.06	0.06	−0.01	0.10

[a]Correlation is significant at the p<0.01 level (2-tailed).

TABLE 3

Predictors of colon PGE_2 concentrations in linear regression models at baseline.

Model	Adjusted R Square	AIC [a]	P-value for F Change
NSAID use + Age+ Smoking status + Gender	0.104	441	0.002
NSAID use + Age+ Smoking status + Gender **+ PTGS1 expression** [b]	0.181	426	<0.001
NSAID use + Age+ Smoking status + Gender **+ Colon SFA**	0.163	434	<0.001
NSAID use + Age+ Smoking status + Gender **+ Colon SFA + PTGS1 expression**	0.230[c]	420	<0.001

[a] AIC, Akaike Information Criterion.

[b] Bolded predictors were significant in the linear regression model with (square root of) colon PGE_2 as the outcome.

[c] The beta coefficients in the final model for each predictor were: NSAID use $\beta = -0.68$ ($p = 0.060$); female gender $\beta = 0.64$ (females had lower PGE_2, $p = 0.033$); age $\beta = -0.02$ ($p = 0.109$); current smoker $\beta = 0.88$ (non-smokers had higher PGE_2, $p = 0.044$); PTGS1 expression, $\beta = 0.65$ ($p = 0.001$); colon SFA, $\beta = 0.10$ ($p = 0.004$).

TABLE 4

Relative expression of genes in colonic mucosa before and after 6 months of intervention [a].

Markers	Healthy Eating Diet		Mediterranean Diet	
	Baseline N = 57	Six months N = 45	Baseline N = 56	Six months N = 45
PTGS1	1.14, 0.67	1.16, 0.85	1.09, 0.70	1.25, 0.93
PTGS2	0.21, 0.15	0.24, 0.26	0.21, 0.17	0.22, 0.17
PTGES1	0.19, 0.18	0.16, 0.17	0.17, 0.12	0.23, 0.23
PTGES3	0.65, 0.97	0.86, 1.22[b]	0.49, 0.70	0.65, 0.96[b]
HPGD	1.35, 0.94	1.30, 0.98	1.46, 0.95	1.52, 1.08
PTGER2	0.35, 0.31	0.41, 0.52	0.34, 0.29	0.41, 0.46
PTGER4	0.94, 0.77	1.05, 0.87	0.90, 0.66	1.17, 0.71[b]

[a] Data shown is mean and SD.

[b] Significantly different than baseline for that diet group, $p < 0.05$ from mixed models ANOVA for log-transformed gene expression values.