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Plasma Lipoxin A_4 and Resolvin D1 are Not Associated with Reduced Adenoma Risk in a Randomized Trial of Aspirin to Prevent Colon Adenomas

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Abstract

Inflammation plays a major role in colon carcinogenesis. Endogenously produced specialized proresolving lipid mediators (SPMs) play a central role in inflammation and tissue homeostasis, and have been implicated in carcinogenesis. We studied the associations of plasma levels of two SPMs [lipoxin A_4 (LXA_4) and resolvin D1(RvD1)] with risk for recurrent adenoma. In this pilot study, we used data and biosamples from an adenoma chemoprevention study investigating the

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Conflict of interest

Dr. Baron and Dartmouth College hold a use patent for the chemopreventive use of aspirin, currently not licensed.

Clinicaltrials.gov **identifier**: NCT00272324

effects of aspirin and/or folic acid on the occurrence of colorectal adenomas. In the parent study, 1,121 participants with a recent adenoma were randomized to study agents to be taken until the next surveillance colonoscopy about 3 years later. In this pilot study, LXA_4 and RvD1 from samples taken near the end of study treatment were measured in a randomly selected sub-set of 200 participants. Commercially available ELISA kits to assay the analytes were validated using a metabololipidomic LC-MS/MS assay. Poisson regression with a robust error variance was used to calculate risk ratios and 95% confidence intervals. Plasma LXA_4 and RvD1 were not associated with the risk of adenoma occurrence. LXA_4 at the end of study follow-up was 32% (P=0.01) proportionately higher in women compared to men. A similar non-significant trend towards higher levels among women was observed for RvD1. Our preliminary findings provided no evidence that plasma LXA_4 or RvD1 are associated with reduced risk of colorectal adenoma occurrence, but suggest LXA_4 may differ among men and women. Future studies focusing on SPM's local effects and levels in the colon are needed.

Keywords (based on MeSH terms)

Aspirin; lipoxin A_4; resolvin D1; colorectal neoplasms; randomized controlled trial

INTRODUCTION

It is well established that inflammation plays a major role in colon carcinogenesis (1, 2). Correspondingly, anti-inflammatory factors appear to suppress carcinogenesis in the large bowel. For example, aspirin, one of the most commonly used drugs in the world, is an anti-inflammatory agent that is emerging as a potential chemopreventive agent against colorectal cancer (CRC) (2, 3).

Lipoxins are endogenous eicosanoids, lipoxygenase pathway products of arachidonic acid, that have the potential to exert anti-neoplastic effects (4). Lipoxins have recently been shown to mediate resolution of inflammation (4, 5), and may also enhance epithelial integrity in various mucosal tissues (6). Aspirin has a unique ability to initiate the formation of some lipoxins. Aspirin-acetylated COX-2 metabolizes arachidonic acid (AA) to 15R-hydroxyeicosatetraenoic acid (15R-HETE). This, in turn, is metabolized by 5-lipoxygenase in polymorphonuclear neutrophils to the carbon 15 epimers of the native lipoxins (LX), specifically locally acting 15-epi-lipoxin A_4 and 15-epi-lipoxin B_4 [15-epi-LXA_4 and 15-epi-LXB_4, the so-called "aspirin-triggered lipoxins" (ATLs)].(7) In animal models and *in vitro*, both native and the aspirin-induced lipoxins promote apoptosis, affect tumor-associated macrophage activity profile, inhibit NFκB activity, cell proliferation, angiogenesis, and tumor progression (8–16).

Similar to lipoxins, resolvins, are anti-inflammatory and pro-resolving endogenous lipid mediators derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by lipoxygenase, P450 and cyclooxygenase enzymes (4). Though the anti-inflammatory and pro-resolving actions of resolvins are well documented (17, 18), their potential roles in carcinogenesis have only recently begun to emerge. In addition to inhibition of inflammation/NFκB activity and promotion of repair of damaged tissue, these are likely to

include modulation of transforming growth factor-β (TGF-β) signaling (19–22), an important pathway in colorectal carcinogenesis and tumor progression (23).

Therefore, to begin to understand the role of some specialized anti-inflammatory and pro-resolving lipid mediators in colorectal carcinogenesis, we conducted secondary analyses in the Aspirin/Folate Polyp Prevention Study (AFPPS) (24, 25), a clinical trial of aspirin and/or folic acid for the prevention of occurrence of colorectal adenomas. Our goals were to obtain preliminary data on the associations of native plasma LXA_4 and resolvin D1 (RvD1) with adenoma recurrence diagnosed during treatment.

MATERIALS AND METHODS

Design

These data were collected as part of the AFPPS, a randomized, double-blind, placebo-controlled, three-by-two factorial trial testing whether oral aspirin (81 or 325 mg daily) or folic acid (1 mg daily) reduces the risk of new colorectal adenomas (clinicaltrials.gov identifier: NCT00272324) (24, 25).

Recruitment, randomization, treatment, and follow-up

Details of subject eligibility, recruitment, randomization, treatment follow-up, and study outcomes have been previously described (24, 25). Briefly, patients with a recent history of colorectal adenomas were recruited between 1994–1998 from nine clinical centers in the US and Canada. Eligible subjects were between 21 and 80 years of age, in good health, had a complete colonoscopy within 3 months before enrollment with no known polyps left in the bowel, and had received a recommendation for a 3-year follow-up colonoscopy by their endoscopists.

At enrollment, eligible subjects completed a questionnaire regarding demographics, medical history, and lifestyle habits. All participants were asked to avoid the use of aspirin, NSAIDs, and supplements containing folate for the duration of active treatment. Each subject underwent a three-month, single-blind run-in period on 325 mg of aspirin per day. Only subjects with at least 80% compliance and no adverse effects of aspirin were randomized to placebo, low-dose aspirin (81 mg/d), or high-dose aspirin (325 mg/d), and to receive folate placebo or folate (1 mg/d). A total of 1,021 full factorial subjects were randomized, as well as 100 “aspirin-only” subjects who were recruited before the folic acid (1 mg/d) component of the study was added.

By protocol, participants were to remain on study treatment until their anticipated surveillance colonoscopy, about three years after the qualifying exam. Every 4 months during study treatment subjects completed a questionnaire regarding compliance with study agents, use of medications and supplements, large bowel endoscopy, and medical events. Baseline and year 3 blood samples were collected, handled and stored according to a standardized protocol. Briefly, blood was drawn from a peripheral vein into three 7 ml pre-chilled Vacutainer tubes containing liquid K3EDTA, and immediately thrust into ice and shielded from light. The blood was then immediately taken to clinical laboratory and processed within two hours. Plasma was aliquotted and then immediately placed into a

−75°C (or colder) freezer for storage. Samples were shipped to the Dartmouth Biospecimen Storage Facility in styrofoam insulated boxes and packed in dry ice. Self-reported compliance in the initial 3-year treatment period was excellent: 87% to 95% of subjects took study pills at least 6 days per week during those three years. All study aspirin treatment ended on September 28, 2001.

For this pilot study, we randomly selected participants from each treatment group (69 assigned placebo, 63 assigned 81 mg, and 68 assigned 325 mg), and, within each treatment group, about the same number of participants from each outcome category (no recurrence, small tubular adenoma, and advanced adenoma). Participants were further restricted to those who were at least 50% compliant with study tablets at the time of blood draw, and had at least 1 mL of plasma stored in the biobank.

Study outcomes

The primary outcome — adenoma occurrence — was determined by colonoscopy and confirmed by pathology review. All lesions removed from the large bowels of study subjects were reviewed by a single study pathologist. Polyps were classified as adenomatous, hyperplastic, serrated or other findings; the degree of dysplasia was recorded. Low-risk adenomas were defined as solitary adenomatous polyps < 1 cm in greatest diameter with tubular histology (“small tubular adenoma”). Advanced adenomas were defined as adenomatous polyps with an estimated diameter of ≥1 cm, or at least 25% villous component, any high-grade dysplasia, or invasive cancer.

Laboratory Measurements

The study was initially intended to investigate the aspirin-triggered 15(R)-epimeric form of LXA_4 [15(R)-epi-LXA_4] and resolvin D1 (RvD1) plasma concentrations, using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Neogen, Lexington, KY, and Cayman Chemical Co., Ann Arbor, MI, respectively) in the Advanced Analytics Core of the Center for Gastrointestinal Biology and Disease (CGIBD) at the University of North Carolina (UNC). We conducted a validation of the ELISA assays using a validated metabololipidomic liquid chromatography-tandem mass spectrometry (LC-MS/MS) (26, 27) with 10 samples (8 from samples used in the study and 2 others).

The validation study yielded results consistent with what would be expected if the 15(R)-epi-LXA_4 ELISA assay largely measured LXA_4. The ELISA 15(R)-epi-LXA_4 measurements were only moderately correlated with those for LC-MS/MS 15(R)-epi-LXA_4 ($r = 0.47$), but strongly correlated with those for LC-MS/MS LXA_4 ($r = 0.91$) and the sum of LC-MS/MS 15(R)-epi-LXA_4 and LXA_4 ($r = 0.88$). At the same time, the correlation of LC-MS/MS LXA_4 with LC-MS/MS 15(R)-epi-LXA_4 was 0.59 and that with the sum of LC-MS/MS 15(R)-epi-LXA_4 and LXA_4 was 0.88. (Thus, the correlation of the ELISA assay with LC-MS/MS 15(R)-epi-LXA_4 simply seemed to reflect that of LC-MS/MS 15(R)-epi-LXA_4 with LC-MS/MS LXA_4.) The antibody used in the RvD1 assay recognized 17(S)-resolvin D1. In our validation study, the ELISA/LC-MS-MS correlation for RvD1 was 0.93.

Intra-plate coefficients of correlation (CVs) were 12.6% and 11.8% for LXA_4 and RvD1, respectively. Laboratory staff were blinded to the treatment group assignment and adenoma

recurrence status of study participants, and treated all samples identically. Samples from each treatment and adenoma recurrence status group were randomly included in every batch. As this was a pilot study, LXA_4 and RvD1 were not measured at blood samples from study entry. However, the end-of-treatment measurements reflect the subjects' environment during the period when the adenomas were forming.

Statistical Analyses

Levels of plasma biomarkers were natural log transformed to improve normality. Values below limit of detection (LOD) were replaced by LOD/2 [n = 1 (0.5%) for LXA_4]. Fisher's exact tests (for categorical variables) and analysis of covariance (ANCOVA; for continuous variables with adjustment for age, sex, batch, and aspirin and/or folic acid treatment group where appropriate) were used to compare randomized treatment groups at baseline. Correlations between biomarkers were calculated using Spearman's correlation coefficient (ρ). Analysis of covariance was used to examine geometric mean differences in LXA_4 and RvD1 plasma concentrations by treatment group, sex, and statin use [which increases biosynthesis of resolvins (28)] in models with age, sex, aspirin treatment group and batch where appropriate.

We used modified Poisson regression with a robust variance estimate to calculate the risk ratios (RR) and 95% confidence intervals (CIs) of having at least one adenoma (29). In these analyses, the LXA_4 and RvD1 plasma concentrations were categorized as above or below sex-specific median biomarker value among all study participants. Since RvD1 was analyzed in two batches (of 73 and 127 participants), we used batch- and sex-specific median values. Other methods to adjust for batch (including batch in the model and batch-standardizing by dividing each value by the batch-specific mean) yielded similar results. Covariates in multivariable models included age (continuous), sex, clinical center, number of baseline adenomas (continuous), follow-up time (continuous), and aspirin treatment group assignment (placebo, 81 mg/d, or 325 mg/d). An interaction by aspirin treatment assignment (placebo, 81 mg/d, or 325 mg/d) or sex was assessed by including the cross product of the treatment/sex variable and biomarker, and evaluated using the Wald test. In secondary analyses, further adjustment for randomized folic acid treatment, first degree family history of colorectal cancer (yes/no), body mass index (<25, $25 – 30$, ≥ 30 kg/m^2), alcohol use (yes/no), statin use (yes/no), and smoking status (never/former/current) did not substantially change the results; therefore, only the more parsimonious models are presented. All statistical tests were two-sided, and *P* values of less than 0.05 were considered statistically significant. All statistical analyses were conducted using SAS version 9.4 (SAS Institute, Inc.).

RESULTS

The baseline characteristics of the study participants selected for this analysis are shown in Table 1. There were no statistically significant differences among the three arms with regard to demographic and lifestyle factors at the study entry. The three treatment arms were also similar with regard to the percentage of subjects who were randomly assigned to folic acid

treatment. The baseline characteristics of the study participants were similar to those of the trial participants as a whole.(24)

Among all study participants, peripheral blood plasma LXA_4 was strongly correlated with RvD1 (adjusted for batch, partial $\rho = 0.77$, $P < 0.001$). Similar correlations were found in each treatment group (data not shown). No significant correlations were found between either biomarker and body mass index (BMI) or age (all $|\rho| < 0.07$, all $P > 0.40$). Neither LXA_4 nor RvD1 differed by statin use status (all $P > 0.60$), smoking status (all $P > 0.20$), and folic acid treatment (all $P > 0.71$).

Aspirin treatment was not associated with plasma LXA_4 or RvD1 obtained from peripheral blood. Interestingly, plasma levels of LXA_4 and RvD1 were proportionately 32% ($P = 0.01$) and 24% ($P = 0.18$) higher in women compared to men in all treatment groups combined (Table 2). Higher LXA_4 levels among women were observed within each treatment group (Supplementary Table 1). However, those for RvD1 were higher among women only in the placebo group (77%, $P = 0.03$, although the sex/RvD1 differences did not differ significantly over aspirin treatments (Supplementary Table 1).

There was no evidence that either plasma LXA_4 or RvD1 differed by adenoma outcome regardless of aspirin treatment group (Supplementary Table 2), nor were these measures associated with risk for any adenoma or with one or more advanced adenomas (Table 3). Although there were no significant associations of plasma LXA_4 or RvD1 with risk and no statistically significant interaction by sex (all $P > 0.12$), estimates of the risk ratios for advanced adenomas were greater than 1.0- and substantial for women [≥ median compared to < median, RR = 2.0 for LXA_4 and RR = 2.89 for RvD1]. Neither the non-significant positive associations seen among women, nor the small non-significant negative associations observed among men, supported a reduction in adenoma risk associated with high plasma LXA_4 or RvD1.

DISCUSSION

In this analysis of individuals participating in a randomized clinical trial, neither LXA_4 nor RvD1 was statistically significantly associated with any measure of adenoma risk. Among men, the reduction in risk was very small, and among women, the biomarkers were positively associated with risk, though non-significantly so. Aspirin treatment was not associated with higher levels of either LXA_4 or RvD1 obtained from peripheral blood. However, women had statistically significantly higher concentrations of LXA_4 compared to men after adjustment for age, and aspirin treatment group. Taken together, these findings do not support the hypothesis that LXA_4 and RvD1 found in peripheral blood are inversely associated with adenoma occurrence, despite their anti-inflammatory and pro-resolving (and therefore potentially anti-neoplastic) local actions.

It is not clear why our women would have higher post-aspirin concentrations of LXA_4 compared with men. In a small randomized clinical trial (n = 128), low-dose aspirin (81 mg/d) increased 15-epi-LXA_4 in a gender- and age-specific manner (30). Among women, the largest increase in 15-epi-LXA_4 after aspirin supplementation was observed in the oldest

group (>55 years old); whereas among men, an opposite trend was noted: 15-epi-LXA_4 increased most in the youngest group (30). We were not able to conduct a similar analysis in our trial as we did not measure the biomarkers at baseline. Nonetheless, our data were supportive of gender-specific findings, as post-treatment concentrations of LXA_4 were higher in women compared with men. Furthermore, our study suggested that the association between LXA_4 and colorectal adenoma occurrence might differ by sex so that higher levels of LXA_4 are associated with lower risk among men, and with higher risk for colorectal adenomas occurrence among women; however, our study did not have sufficient power to determine whether these findings are due to chance.

This study is the first human study to investigate the association between plasma pro-resolving lipid mediators [LXA_4 and RvD1] and risk of colorectal adenomas. The strengths of the study include the high follow-up rate, the systemic collection of risk factor information at baseline and follow-up intervals as well as outcomes at the end of treatment; and the validation of lipid mediator measurements using the current state of the art assessment by LC-MS/MS profiling (27).

This study has several limitations. First, the fact that the 15-epi-LXA_4 ELISA kits we received did not measure the aspirin-triggered form of LXA_4 was not known at the beginning of our study but has now been demonstrated by our LC-MS/MS validation. Therefore, we were unable to assess the effect of daily aspirin intake on 15-epi-LXA_4, as planned initially. Second, participants in this analysis had a previous history of at least one colorectal adenoma, potentially limiting the generalizability of the data. Third, we did not measure the local colon tissue at any time, or plasma eicosanoid concentrations at the study entry. However, the on-treatment measurements do reflect the subjects' metabolic milieu during the period when the adenomas were forming. Fourth, we had only a limited sample size to investigate risk of advanced adenoma occurrence, and conduct stratified analyses, such as those by sex. Fifth, the biomarker measurements had relatively high CVs (<13%), were subject to batch effects [only RvD1], and could not be divided into more than 2 categories to study adenoma outcomes due to small sample size. Finally, additional local specialized pro-resolving lipid mediators such as protectin D1, maresins, or the E-series (resolvin E1) and other D-series resolvins (RvD2, RvD3, and RvD4) were not studied herein and remain of interest (31–33).

In conclusion, our preliminary findings do not support an association between plasma levels of the two pro-resolving lipid mediators LXA_4 and RvD1 and reduced risk of colorectal adenoma occurrence, though local actions in the colorectal mucosa remain to be evaluated. Finally, our results suggested that LXA_4 concentrations may differ among men and women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

COX	cyclooxygenase enzyme
AA	arachidonic acid
EPA	eicosapentaenoic acid
DHA	docohexaenoic acid
15R-HETE	15R-hydroxyeicosatetraenoic acid
15-epi-LXA$_4$	15-epi-Lipoxin A$_4$
LX	lipoxins
ATLs	aspirin-triggered lipoxins
SPMs	specialized pro-resolving lipid mediators
AFPPS	Aspirin/Folate Polyp Prevention Study
LOD	limit of detection
ANCOVA	analysis of covariance
RR	risk ratios
CIs	confidence intervals

References

1. Ullman TA, Itzkowitz SH. Intestinal inflammation and cancer. Gastroenterology. 2011; 140:1807–16. [PubMed: 21530747]
2. Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007; 369:1603–13. [PubMed: 17499602]
3. Chan AT, Arber N, Burn J, Chia WK, Elwood P, Hull MA, et al. Aspirin in the chemoprevention of colorectal neoplasia: an overview. Cancer Prev Res (Phila). 2012; 5:164–78. [PubMed: 22084361]
4. Greene ER, Huang S, Serhan CN, Panigrahy D. Regulation of inflammation in cancer by eicosanoids. Prostaglandins Other Lipid Mediat. 2011; 96:27–36. [PubMed: 21864702]
5. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. Semin Immunol. 2015; 27:200–15. [PubMed: 25857211]
6. Wallace JL, Fiorucci S. A magic bullet for mucosal protection…and aspirin is the trigger! Trends Pharmacol Sci. 2003; 24:323–6. [PubMed: 12871661]
7. Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol. 2007; 25:101–37. [PubMed: 17090225]

8. Claria J, Lee MH, Serhan CN. Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. Mol Med. 1996; 2:583–96. [PubMed: 8898374]
9. Chen Y, Hao H, He S, Cai L, Li Y, Hu S, et al. Lipoxin A4 and its analogue suppress the tumor growth of transplanted H22 in mice: the role of antiangiogenesis. Mol Cancer Ther. 2010; 9:2164–74. [PubMed: 20682645]
10. Hao H, Liu M, Wu P, Cai L, Tang K, Yi P, et al. Lipoxin A4 and its analog suppress hepatocellular carcinoma via remodeling tumor microenvironment. Cancer Lett. 2011; 309:85–94. [PubMed: 21683517]
11. Zhou XY, Li YS, Wu P, Wang HM, Cai ZY, Xu FY, et al. Lipoxin A(4) inhibited hepatocyte growth factor-induced invasion of human hepatoma cells. Hepatol Res. 2009; 39:921–30. [PubMed: 19456898]
12. Poorani R, Bhatt AN, Dwarakanath BS, Das UN. COX-2, aspirin and metabolism of arachidonic, eicosapentaenoic and docosahexaenoic acids and their physiological and clinical significance. Eur J Pharmacol. 2016; 785:116–32. [PubMed: 26335394]
13. Marginean A, Sharma-Walia N. Lipoxins exert antiangiogenic and anti-inflammatory effects on Kaposi's sarcoma cells. Transl Res. 2015; 166:111–33. [PubMed: 25814167]
14. Hao H, Xu F, Hao J, He YQ, Zhou XY, Dai H, et al. Lipoxin A4 suppresses lipopolysaccharide-induced hela cell proliferation and migration via NF-kappaB pathway. Inflammation. 2015; 38:400–8. [PubMed: 25348861]
15. Zong L, Li J, Chen X, Chen K, Li W, Li X, et al. Lipoxin A4 Attenuates Cell Invasion by Inhibiting ROS/ERK/MMP Pathway in Pancreatic Cancer. Oxid Med Cell Longev. 2016; 2016:6815727. [PubMed: 26649143]
16. Simoes RL, De-Brito NM, Cunha-Costa H, Morandi V, Fierro IM, Roitt IM, et al. Lipoxin A4 selectively programs the profile of M2 tumor-associated macrophages which favour control of tumor progression. Int J Cancer. 2017; 140:346–57. [PubMed: 27615282]
17. Ji RR, Xu ZZ, Strichartz G, Serhan CN. Emerging roles of resolvins in the resolution of inflammation and pain. Trends Neurosci. 2011; 34:599–609. [PubMed: 21963090]
18. Recchiuti A, Serhan CN. Pro-Resolving Lipid Mediators (SPMs) and Their Actions in Regulating miRNA in Novel Resolution Circuits in Inflammation. Front Immunol. 2012; 3
19. Lee HJ, Park MK, Lee EJ, Lee CH. Resolvin D1 inhibits TGF-beta1-induced epithelial mesenchymal transition of A549 lung cancer cells via lipoxin A4 receptor/formyl peptide receptor 2 and GPR32. Int J Biochem Cell Biol. 2013; 45:2801–7. [PubMed: 24120851]
20. Chiu LC, Tong KF, Ooi VE. Cytostatic and cytotoxic effects of cyclooxygenase inhibitors and their synergy with docosahexaenoic acid on the growth of human skin melanoma A-375 cells. Biomed Pharmacother. 2005; 59(Suppl 2):S293–7. [PubMed: 16507396]
21. Kuang H, Hua X, Zhou J, Yang R. Resolvin D1 and E1 alleviate the progress of hepatitis toward liver cancer in long-term concanavalin A-induced mice through inhibition of NF-kappaB activity. Oncol Rep. 2016; 35:307–17. [PubMed: 26531230]
22. Halder RC, Almasi A, Sagong B, Leung J, Jewett A, Fiala M. Curcuminoids and omega-3 fatty acids with anti-oxidants potentiate cytotoxicity of natural killer cells against pancreatic ductal adenocarcinoma cells and inhibit interferon gamma production. Front Physiol. 2015; 6:129. [PubMed: 26052286]
23. Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. Nat Rev Cancer. 2010; 10:415–24. [PubMed: 20495575]
24. Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med. 2003; 348:891–9. [PubMed: 12621133]
25. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA. 2007; 297:2351–9. [PubMed: 17551129]
26. Gronert K, Clish CB, Romano M, Serhan CN. Transcellular regulation of eicosanoid biosynthesis. Methods Mol Biol. 1999; 120:119–44. [PubMed: 10343315]

27. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol. 2014; 307:C39–54. [PubMed: 24696140]

28. Dalli J, Chiang N, Serhan CN. Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. Nat Med. 2015; 21:1071–5. [PubMed: 26236990]

29. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004; 159:702–6. [PubMed: 15033648]

30. Chiang N, Hurwitz S, Ridker PM, Serhan CN. Aspirin has a gender-dependent impact on antiinflammatory 15-epi-lipoxin A4 formation: a randomized human trial. Arterioscler Thromb Vasc Biol. 2006; 26:e14–7. [PubMed: 16293793]

31. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med. 2000; 192:1197–204. [PubMed: 11034610]

32. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, et al. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med. 2002; 196:1025–37. [PubMed: 12391014]

33. Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxins are endogenous components of antiinflammation: emergence of the counterregulatory side. Arch Immunol Ther Exp (Warsz). 2001; 49:177–88. [PubMed: 11478391]

Table 1

Baseline selected characteristics of the study participants.

Baseline characteristic	Placebo (N = 69)	81 mg/d aspirin (N = 63)	325 mg/d aspirin (N = 68)
Age, yrs, mean (SD)	58.7 (8.4)	58.0 (9.1)	58.3 (8.3)
Male sex, no. (%)	48 (69.6)	45 (71.4)	45 (66.2)
White, no. (%)	61 (88.4)	52 (82.5)	57 (83.8)
BMI, kg/m^2, mean (SD)	27.4 (4.1)	27.0 (3.6)	27.8 (4.4)
Overweight, no. (%)	35 (50.7)	33 (52.4)	37 (54.4)
Obese, no. (%)	14 (20.3)	12 (19.1)	15 (22.1)
Current smoker, no. (%)	6 (8.7)	12 (19.1)	10 (14.7)
Alcohol drinker, no. (%)	50 (72.5)	49 (77.8)	54 (79.4)
Multivitamin use, no. (%)	24 (34.8)	22 (35.5)	19 (27.9)
Statin use, no. (%)	4 (5.8)	7 (11.1)	6 (8.8)
Family history of colorectal cancer, no. (%)#	25 (44.6)	18 (34.6)	18 (34.0)
Family history of colorectal polyps, no. (%)#	16 (28.6)	14 (26.9)	17 (32.1)
Baseline adenoma characteristics *			
Number, mean (SD)	1.7 (0.9)	1.9 (1.4)	1.7 (1.0)
Advanced adenomas, no. (%) **	18 (35.3)	20 (37.0)	24 (42.1)
Proximal location, no. (%) ***	35 (55.6)	29 (58.0)	39 (63.9)
Folate treatment group, no. (%)&	37 (53.6)	28 (44.4)	29 (42.7)

Data available for 56, 52, and 53 participants in the placebo, aspirin 81 mg/d, and aspirin 325 mg/d treatment groups, respectively.

* Data available for 66, 58, and 64 participants in the placebo, aspirin 81 mg/d, and aspirin 325 mg/d treatment groups, respectively.

** Advanced adenomas include large adenomas (>1cm) and those with >25% villous histology; data available for 51, 54, and 57 in the placebo, aspirin 81 mg/d, and aspirin 325 mg/d treatment groups, respectively.

*** Data available for 63, 50 and 61 participants in the placebo, aspirin 81 mg/d, and aspirin 325 mg/d treatment groups, respectively.

& 22 participants were randomized to aspirin only (4, 11 and 7 in the placebo, aspirin 81 mg/d, and aspirin 325 mg/d treatment groups)

Table 2

Plasma Lipoxin A_4 and Resolvin D1 concentrations by aspirin treatment group and sex, at the end of study treatment period, the Aspirin/Folate Polyp Prevention Study (AFPPS).

Biomarker/Group	N	Geometric* mean	(95% CI)	Diff (%)**	*P* (vs placebo)	*P* (vs 81 mg)	*P* (sex)&
Lipoxin A_4, ng/mL							
By treatment group							
Placebo	69	13.40	(11.39–15.77)				
81 mg aspirin	63	13.46	(11.31–16.01)	0.4	*0.97*		
325 mg aspirin	68	12.42	(10.56–14.60)	−7	*0.50*	*0.49*	
By sex							
Men	138	11.38	(10.18–12.72)				
Women	62	15.04	(12.68–17.85)	32			*0.01*
Resolvin D1, pg/mL							
By treatment group							
Placebo	69	738	(574–949)				
81 mg aspirin	63	714	(546–935)	−3	*0.58*		
325 mg aspirin	68	813	(633–1044)	10	*0.86*	*0.47*	
By sex							
Men	138	677	(570–805)				
Women	62	839	(644–1093)	24			*0.18*

*Age-, sex-, aspirin treatment group-, and batch-adjusted where appropriate.

**The proportional difference in geometric means was calculated as ([treatment group – placebo group]/placebo group)*100% or as ([women – men]/men)*100%.

&*P*-value for difference by sex.

Table 3

Association of recurrent adenoma with plasma Lipoxin A_4 and Resolvin D1 concentrations at the end of study treatment period, the Aspirin/Folate Polyp Prevention Study (AFPPS).

Biomarker/Sex-specific median**	Any Adenoma			Advanced adenoma		
	Cases/total N	RR (95% CI)*	P_{sex}#	Cases/total N	RR (95% CI)*	P_{sex}#
Lipoxin A_4, ng/mL						
All participants						
<median	65/100	ref		33/100	ref	
≥median	68/100	1.02 (0.71–1.45)		31/100	1.02 (0.61–1.72)	
per 10 ng/mL	133/200	1.01 (0.86–1.19)		64/200	1.00 (0.79–1.26)	
Men			*0.19*			*0.12*
<median	50/69	ref		27/69	ref	
≥median	45/69	0.88 (0.57–1.35)		20/69	0.80 (0.43–1.49)	
per 10 ng/mL	95/138	0.94 (0.75–1.17)		47/138	0.87 (0.63–1.20)	
Women						
<median	15/31	ref		6/31	ref	
≥median	23/31	1.64 (0.78–3.46)		11/31	2.00 (0.67–5.88)	
per 10 ng/mL	38/62	1.17 (0.88–1.56)		17/62	1.27 (0.83–1.95)	
Resolvin D1, pg/mL						
< median$	63/100	ref		30/100	ref	
≥ median$	70/100	1.05 (0.73–1.52)		34/100	1.27 (0.75–2.17)	
per 1,000 pg/mL&	133/200	1.02 (0.95–1.08)		64/200	0.97 (0.85–1.11)	
Men			*0.41*			*0.25*
< median$	47/69	ref		24/69	ref	
≥ median$	48/69	0.94 (0.61–1.47)		23/69	1.06 (0.57–2.00)	
per 1,000 pg/mL&	95/138	1.01 (0.93–1.09)		47/138	0.94 (0.78–1.13)	
Women						
< median$	16/31	ref		6/31	ref	
≥ median$	22/31	1.41 (0.68–2.94)		11/31	2.86 (0.83–10.00)	

Biomarker/Sex-specific median**	Any Adenoma			Advanced adenoma		
	Cases/total N	RR (95% CI)*	P_{sex}#	Cases/total N	RR (95% CI)*	P_{sex}#
per 1,000 pg/mL&	38/62	1.19 (0.94–1.50)		17/62	1.20 (0.83–1.74)	

*Adjusted for age (continuous), sex, center (categorical), number of baseline adenomas (continuous), follow-up time (continuous) and aspirin treatment group (placebo, 81 mg, 325 mg).

**Lipoxin A_4 median for men = 10.61 ng/mL, and women = 15.92 ng/mL; Resolvin D1, men batch 1 = 943.993 pg/mL, batch 2 = 403.85 pg/mL; Resolvin D1, women batch 1 = 1668.52 pg/mL, batch 2 = 524.2 pg/mL.

#*P*-value for interaction by sex.

$Sex- and batch-specific.

&Additionally adjusted for batch in the model.