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Circulating 27-hydroxycholesterol and risk of colorectal adenomas and serrated polyps

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Abstract

The oxysterol 27-hydroxycholesterol (27-OHC) is an endogenous selective estrogen receptor modulator implicated in breast cancer etiology. It is unknown whether circulating 27-OHC is associated with colorectal neoplasia risk. Circulating 27-OHC was measured using liquid chromatography/mass spectrometry in fasting plasma collected at baseline from participants of the Vitamin D/Calcium Polyp Prevention Study, a completed randomized clinical trial. Participants were between 45 and 75 years old, recently diagnosed with 1 colorectal adenoma, and followed for new colorectal polyps during colonoscopic surveillance. Adjusted risk ratios (RR) with 95% confidence intervals (CI) of new colorectal polyps were estimated for quartiles of circulating 27-OHC using log-linear regression for repeated outcomes. Polyp phenotypes included any adenomas, advanced adenomas, hyperplastic polyps, and sessile serrated adenomas/polyps. Circulating 27-OHC was measured at baseline for 1,246 participants. Compared with participants with circulating 27-OHC below the first quartile (<138 ng/mL), those with circulating 27-OHC at or above the fourth quartile (≥201 ng/mL) had 24% higher risk of adenomas (RR, 1.24; 95% CI, 1.05–1.47) and 89% higher risk of advanced adenomas (RR, 1.89; 95% CI, 1.17–3.06). Stronger associations were observed among participants with advanced adenomas at baseline. Circulating 27-OHC was not associated with risk of hyperplastic polyps (RR, 0.90; 95% CI, 0.66–1.22) or

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Together with the Trustees of Dartmouth College, J. A. Baron holds a patent, currently not licensed, for the chemopreventive use of calcium for colorectal cancer. All other authors declare no potential conflicts of interest.

sessile serrated adenomas/polyps (RR, 1.02; 95% CI, 0.50–2.07). Circulating 27-OHC may be a risk factor for colorectal adenomas but not serrated polyps.

Keywords

27-hydroxycholesterol; oxysterols; colonoscopy; colorectal adenomas; colorectal cancer; colorectal serrated polyp

Introduction

Oxysterols are ubiquitous cholesterol metabolites that regulate lipid homeostasis (1,2). A role for oxysterols in carcinogenesis is emerging as these metabolites are involved in cellular adhesion, proliferation, apoptosis, inflammation, and DNA repair (3,4). 27-hydroxycholesterol (27-OHC) is the most abundant oxysterol detectable in circulation, though typically at concentrations approximately 4–5 orders of magnitude lower than total cholesterol (2). Oxysterols such as 27-OHC are ligands to liver X receptors (LXR α and LXR β) (5,6), nuclear receptors that serve as transcription factors for target genes involved in lipid and glucose metabolism (7,8). 27-OHC can also bind to estrogen receptors (ER α and ER β) and has been classified as an endogenous selective estrogen receptor modulator (9,10).

Several preclinical studies implicate 27-OHC in the promotion and progression of ER α positive (ER+) breast cancer (11–13). In contrast, an epidemiologic study suggested circulating 27-OHC was inversely associated with the risk of ER+ and ER- breast cancer among women who were postmenopausal at blood collection (14). There is limited evidence that 27-OHC plays a role in the development of colorectal cancer, which arises from colorectal adenomas and sessile serrated adenomas/polyps (15). An LXR-mediated anti-neoplastic pathway has been suggested from preclinical studies, as LXR α and LXR β expression in the colorectal epithelium are associated with decreased cellular proliferation (16,17). Alternatively, an ER-mediated pathway is also plausible, given the role of estrogens in the suppression of colorectal neoplasia possibly through ER β expression, which generally exerts anti-neoplastic effects (18). To test whether circulating 27-OHC is associated with the development of colorectal neoplasia, we assessed the association of plasma 27-OHC with adenoma and serrated polyp occurrence among participants of the Vitamin D/Calcium Polyp Prevention Study, a completed adenoma prevention trial (19).

Materials and Methods

Study design

The Vitamin D/Calcium Polyp Prevention Study is a multicenter, double-blind, placebo-controlled, randomized clinical trial of 1,000 IU/day of vitamin D₃ and 1,200 mg/day of calcium as carbonate for the prevention of new colorectal adenomas among those recently diagnosed with these lesions ([NCT00153816](#)). Study methods have been documented previously (19). Eligible participants were between 45 and 75 years of age and diagnosed with 1 adenoma resected within 4 months of recruitment. Those with a history of colorectal cancer, inflammatory bowel disease, or familial polyposis syndromes were ineligible, as

were those with medical conditions that could be treated or exacerbated by study agents. Between July 2004 and July 2008, participants were enrolled from 11 academic medical centers in the United States. All potential participants provided a blood specimen prior to receiving a randomized treatment assignment to confirm that circulating concentrations of calcium, creatinine, and 25-hydroxyvitamin D (25(OH)D) were within allowable ranges. A total of 2,259 participants were randomly assigned to treatment groups. The study employed a partial 2×2 factorial design, permitting women to elect to be randomly assigned to only to vitamin D or placebo while receiving calcium (2-group randomization). All other participants were randomly assigned to vitamin D only, calcium only, both vitamin D and calcium, or placebo (full-factorial randomization). In accordance with the ethical guidelines of the U.S. Common Rule, participants provided written informed consent, and the study protocol was approved by institutional review boards at all clinical centers.

Baseline assessment

Baseline data included sex, age, race/ethnicity, height, weight, smoking status, alcohol consumption, history of diabetes mellitus, family history of colorectal cancer, use in the past 4 months of aspirin or non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs), use in the past 12 months of HMG-CoA reductase inhibitors (statins), and any use of postmenopausal hormone therapy. Alcohol consumption was ascertained from a semi-quantitative food frequency questionnaire (NutritionQuest; Berkeley, CA). Diabetes mellitus status was based on self-report of a physician diagnosis of diabetes (excluding gestational diabetes) or self-report of use in the past 12 months of prescription antidiabetic medications including insulin, metformin, sulfonylureas, and thiazolidinediones. Statin dose was not collected, and participants who reported less than daily use of statins (<5% of those who reported any use in past 12 months) were classified as non-users.

Follow-up

Study treatment continued until a follow-up colonoscopy scheduled for 3 or 5 years after the initial colonoscopy qualifying for study eligibility, as determined by each participant's gastroenterologist. After the treatment period ended, participants reconsented to be followed observationally. Participants were contacted every 6 months while on treatment and annually during the post-treatment period. Medical records of gastrointestinal exams were obtained for central blinded adjudication by study physicians. All participants completed treatment by August 2013 and post-treatment follow-up ended in June 2016. As reported previously, no beneficial treatment effects were observed for colorectal adenoma endpoints (19,20), but there was suggestive evidence of post-treatment adverse effects for risk of serrated polyps (21).

Pathologic classification of colorectal polyps

Colorectal tissue slides collected from baseline and follow-up colonoscopies underwent a uniform blinded pathology review by a single pathologist (DCS). The primary endpoint was the occurrence of 1 colorectal adenoma after the first year after random treatment assignment and up to 6 months after the conclusion of the 3- or 5-year colonoscopic surveillance interval. This included diagnoses made during any interim colonoscopies occurring before the planned surveillance exams. Secondary endpoints were advanced

adenomas and adenomas classified by anatomic location. Advanced adenomas were those diagnosed as tubulovillous, villous, 1 cm in diameter, with high-grade dysplasia, or invasive adenocarcinoma (15). Serrated colorectal polyps were distinguished from conventional adenomas and categorized as hyperplastic polyps, traditional serrated adenomas, and sessile serrated adenomas/polyps with or without cytologic dysplasia (22). The proximal colon was defined as the cecum up to, but not including, the splenic flexure. The distal colon/rectum was defined as the splenic flexure, descending colon, sigmoid colon, and rectum.

27-OHC measurement

Total 27-OHC (free plus esterified) was measured from baseline plasma for participants who reported fasting at least 8 hours at the time of blood draw. Excluded from 27-OHC measurement were participants who did not consent for use of blood for research purposes (n=45), did not report fasting status at the time of blood draw (n=2), reported fasting <8 hours at the time of blood draw (n=620), had an insufficient volume of archived plasma (n=7), or did not complete a follow-up colonoscopy (n=145). Plasma specimens from 1,440 participants were sent frozen on dry ice to the University of Texas Southwestern Medical Center for 27-OHC quantification by high-performance liquid chromatography/mass spectrometry (LC/MS) according to an established protocol (23).

Lipids were hydrolyzed with a solution of 0.5M KOH in MeOH (methanolic KOH), after addition of deuterated standards (Avanti Polar Lipids, Alabaster, AL) then extracted using a modified Bligh-Dyer procedure and purified using aminopropyl solid-phase extraction (Biotage, Charlotte, NC, USA). 27-OHC was quantitatively analyzed using a Shimadzu LC-20XR high-performance LC system (Shimadzu Scientific Instruments, Columbia, MD), coupled to a Sciex API-5000 triple quadrupole MS equipped with Turbo V ESI source (Framingham, MA), and resolved with a binary solvent gradient, using a Kinetex C18 high-performance LC column (150 2.1 mm, 1.7-mm particle size; Phenomenex, Torrance, CA). Analyte identification used the LIPIDMAPS standard for 27-OHC (LMST01010057) (24).

We selected a random sample of 144 (10%) participants to serve as blinded duplicates. Half of duplicates were aliquoted from the participant's primary archived baseline plasma specimen (n=72), and half of duplicates were aliquoted from one of the participant's alternative archived baseline plasma specimens (n=72). The within-subject coefficient of variation and intra-class correlation coefficient for 27-OHC were 10% and 0.87, respectively, and did not meaningfully differ for same- and different-specimen duplicates.

Statistical analysis

Premenopausal women (n=111) and postmenopausal women using hormone replacement therapy at baseline (n=83) were excluded from all analyses, leaving 1,246 participants with available circulating 27-OHC measurements. Risk ratios (RR) with 95% confidence intervals (CI) were estimated from log-linear Poisson regression with robust standard errors (25) using generalized estimating equations (GEE) to account for repeated outcome data during the treatment and post-treatment follow-up periods. RRs were preferred over odds ratios because odds ratios differ substantially from RRs for common events, as

metachronous adenomas are in this population. RRs for adenoma occurrence were contrasted with absence of any adenomas during follow-up. Separately, we estimated RRs for the occurrence of hyperplastic polyps and sessile serrated adenomas/polyps, each contrasted with absence of serrated polyps, regardless of other diagnosed adenomas.

RRs for quartiles of circulating 27-OHC were adjusted for potential confounding variables, all measured at baseline, including sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, diabetes mellitus, first-degree family history of colorectal cancer, aspirin use, non-aspirin NSAID use, statin use, and former use of postmenopausal hormone therapy. RRs were also calculated in analyses stratified by sex using separate regression models and sex-specific quartiles for circulating 27-OHC. P-values for linear trend were calculated from regression models that included continuous circulating 27-OHC. Circulating 27-OHC was modestly right-skewed (Supplemental Figure 1). For analyses using continuous values of circulating 27-OHC, we applied the natural logarithm to minimize the influence of extreme values and report geometric means. To assess the appropriateness of modelling assumptions in primary analyses, we evaluated several different parameterizations of log-transformed circulating 27-OHC in regression models using: 1) quartiles; 2) a linear term; 3) linear and quadratic terms; 4) linear, quadratic, and cubic terms; and 5) a linear spline with knots at the quartile cutoffs (Supplemental Figure 2).

Subgroup analyses considered associations specific to advanced adenoma status at baseline (1 vs. none), follow-up period of polyp diagnosis (treatment period vs. post-treatment period), statin use at baseline (yes vs. no), and treatment assignment (separately for full-factorial and 2-group randomization for the primary endpoint). Heterogeneity of associations was evaluated using a Wald test for a multiplicative interaction term between the subgroup variable and log-transformed circulating 27-OHC. We also assessed the Pearson correlation (r) between circulating 27-OHC and season-adjusted 25(OH)D measured at baseline (both log-transformed). Analyses were performed using R version 3.5.2 with $P < 0.05$ considered statistically significant.

Results

Baseline characteristics of 1,246 participants in the analysis are summarized in Table 1. Most participants were men, white, and the mean age at enrollment was 59 years. The distributions of these characteristics were similar to those of the full study cohort of 2,259 participants (Supplemental Table 1). Geometric mean circulating 27HC at baseline was 167 ng/mL (95% CI, 164 to 169), and was lower among postmenopausal women (geometric mean, 148 ng/mL; 95% CI, 144 to 153) than men (geometric mean, 175 ng/mL; 95% CI, 172 to 178). Circulating 27-OHC was also lower among white participants, never smokers, those consuming <1 alcoholic drink/day, those with diabetes mellitus, and statin users (Supplemental Table 2). Circulating 27-OHC was uncorrelated with circulating 25(OH)D at baseline (mean, 24 ng/mL; SD, 8 ng/mL; $r = 0.02$) and did not substantially differ by year or season of blood collection, fasting time at blood collection, or geographic location of each study center (Supplemental Table 3).

In total, 569 (46%) of 1,246 participants were diagnosed with 1 colorectal adenoma during the treatment period, including 130 (10%) with advanced adenomas. A total of 1,180 (95%) participants agreed to be followed after the treatment period; of these, 664 (56%) had available colonoscopy outcomes during the post-treatment period. In total, 348 (52%) of the 664 participants were diagnosed with 1 colorectal adenoma during the post-treatment period, including 69 (10%) with advanced adenomas.

Higher circulating 27-OHC was associated with higher colorectal adenoma risk (Table 2). Compared with the first quartile (<138 ng/mL) of circulating 27-OHC, participants in the fourth quartile (≥201 ng/mL) had 24% higher risk of adenomas (RR, 1.24; 95% CI, 1.05–1.47) and 89% higher risk of advanced adenomas (RR, 1.89; 95% CI, 1.17–3.06). Associations were stronger among 233 participants with advanced adenomas at baseline compared with 993 participants without advanced adenomas at baseline (*P* for interaction=0.05 for risk of any adenoma; Table 3). Associations were of similar magnitude according to sex (Table 4). There was also no evidence of heterogeneity of the association between circulating 27-OHC and adenoma risk according to anatomic location of adenomas (Supplemental Table 4), follow-up period (Supplemental Table 5), statin use at baseline (Supplemental Table 6), and vitamin D or calcium treatment assignment (Supplemental Table 7). Circulating 27-OHC was not associated with risk of hyperplastic polyps or sessile serrated adenomas/polyps (Table 5).

Discussion

We found higher circulating 27-OHC measured at the time of an initial colorectal adenoma diagnosis is modestly associated with higher risk of subsequent adenomas. Stronger associations were observed for risk of advanced adenomas and among those with advanced adenomas at baseline. Associations were of similar magnitude for men and postmenopausal women. Circulating 27-OHC was not a risk factor for hyperplastic polyps or sessile serrated adenomas/polyps after an initial adenoma diagnosis.

Oxysterols such as 27-OHC are the natural ligands to LXRs (5,6). LXR β is expressed throughout the body, whereas LXR α is expressed primarily in the liver, gut, adipose tissue, and macrophages (26). In the small intestine, LXR activation is thought to promote transintestinal cholesterol excretion (27,28). A protective role for LXRs in colorectal carcinogenesis has been suggested from preclinical studies. Experiments in APC^{Min/+} mice suggest LXR α suppresses mucosal proliferation in the colon (16). A study of LXR $\alpha\beta^{-/-}$ mice found both LXR α and LXR β may be necessary to observe decreased colonic cell growth in response to an LXR agonist (17). In contrast to the direction of association found in our study for circulating 27-OHC, local exposure to 27-OHC in the colorectal mucosa may protect against colorectal cancer by activating LXRs. Indeed, direct treatment of colorectal cancer cells *in vitro* with concentrations of 27-OHC above 10 μ M appears to decrease cellular proliferation (29). The fact that our findings for circulating 27-OHC seem unsupported by these previous experimental studies of LXR agonism points to potentially greater relevance of ER-mediated pathways. Although 27-OHC has not been as carefully investigated in mechanistic studies of colorectal cancer (predominated by ER β) relative to

breast cancer (predominated by ER α) (30), it appears that 27-OHC does not have major differences in affinity to ER α and ER β (9,10).

Our findings are in the opposite direction as those reported from a breast cancer case-control study nested within the Heidelberg site of the European Prospective Investigation on Cancer (EPIC) cohort study, in which 2-fold higher circulating 27-OHC was associated with about 50% lower odds of developing breast cancer among postmenopausal women who did not previously use hormone replacement therapy (14). This association for breast cancer risk is itself in the opposite direction from that expected from animal and *in vitro* studies of breast cancer, which suggest 27-OHC promotes ER-mediated tumor growth and LXR-mediated metastasis in mammary tissue (11–13). That our results for colorectal adenoma risk and those of EPIC-Heidelberg for breast cancer risk (14) both appear inconsistent with the respective preclinical evidence suggests circulating 27-OHC may poorly reflect tissue-specific 27-OHC activity. However, Nelson et al. treated transgenic mice with early-onset mammary cancer with 27-OHC by systemic injection at a daily dose sufficient to increase circulating 27-OHC by 40-fold and found this induced within-tumor expression of several LXR-target genes (11).

Some evidence supports the different role for 27-OHC in the breast and colon. 27-OHC was deemed a selective estrogen receptor modulator partly because of its opposing effects on ER activity in breast cancer cells (MCF-7) and colon cancer cells (Caco-2) (9). Unlike for breast cancer (31), use of postmenopausal hormone therapy can reduce colorectal cancer risk (32), especially for tumors that express ER β (33). Observational studies of postmenopausal hormone therapy use have typically reported a similar magnitude risk reduction for adenomas as in studies of colorectal cancer (34,35). Woodson et al. investigated metachronous adenomas specifically, concluding that postmenopausal hormone therapy use may reduce risk for younger women (36). Several studies have found exogenous selective estrogen receptor modulators, such as tamoxifen and raloxifene used as breast cancer therapy, increase risk of colorectal cancer after breast cancer (37–39).

Sources of variation in circulating 27-OHC are not fully understood. Our baseline data suggest smoking and alcohol consumption may be associated with higher circulating 27-OHC, consistent with an analysis of women from EPIC-Heidelberg (40). We also found evidence that use of statins, aspirin, and antidiabetic medication may be associated with lower circulating 27-OHC. A few small clinical studies also report statin use may lower circulating 27-OHC (41,42). In contrast, there was no association between cholesterol-lowering drugs use and circulating 27-OHC in EPIC-Heidelberg, but the overall prevalence of use of these medications in that study (4%) was substantially lower than the overall prevalence of statin use in our study (30%) (40). We also identified differences in circulating 27-OHC according to race/ethnicity that warrant further study.

The enzyme directly responsible for 27-OHC formation from cholesterol, sterol 27-hydroxylase (CYP27A1), may be overexpressed in colorectal aberrant crypt foci and adenoma tissue (43), but another study comparing expression in invasive colorectal cancer tissue to normal mucosa did not detect meaningful differences (44). Derivation of 27-OHC from cholesterol by CYP27A1 is an initial step of the alternative (acidic) pathway of bile

acid synthesis, and the direction of association we found for circulating 27-OHC and adenoma risk is consistent with the association of circulating concentrations of certain conjugated bile acids and higher risk of colorectal cancer (45). This raises the possibility that circulating 27-OHC may be a biomarker of bile acid synthesis. However, more than a dozen additional enzymatic steps are needed to convert 27-OHC into bile acids in the liver (46), and it is unclear whether circulating 27-OHC is correlated with the specific circulating conjugated bile acids found to be associated with colorectal cancer risk (45).

Two separate studies using different laboratories than our study reported lower mean values of circulating 27-OHC (14,47). Specifically, a case-control study nested within the Women's Health Initiative investigating cardiovascular disease reported a median plasma 27-OHC among controls of <20 ng/mL (47), and the EPIC-Heidelberg study investigating breast cancer reported a median serum 27-OHC among controls of 200 nM (81 ng/mL) (14). It is possible that our evaluation of those recently diagnosed with colorectal adenomas resulted in higher circulating 27-OHC overall (mean of 167 ng/mL among men and women). However, colorectal adenomas are relatively common in older populations (48) and the distribution of circulating 27-OHC in our study was similar to that of the Dallas Heart Study (mean of 158 ng/mL among men and women) (2) and an analysis of bone fracture risk from the Women's Health Initiative (mean of 149 ng/mL among women in the placebo group without bone fracture) (49), both of which were not restricted to participants with premalignant conditions and used the same laboratory and oxysterol quantification methods as our study (23). Furthermore, fasting status at blood collection does not appear to account for these differences. Alternatively, it is possible that the lower concentrations in other studies are the result of different laboratory methods. Approximately 90% of circulating 27-OHC in humans is esterified (50) and lower 27-OHC concentrations can result from incomplete hydrolysis, limiting quantification mostly to free 27-OHC.

Our analysis of prospectively-collected specimens from the Vitamin D/Calcium Polyp Prevention Study has several strengths. The detailed characterization of participants allowed us to control for numerous potential confounding factors and detailed polyp classification allowed us to contrast multiple lesion phenotypes. The clinical applicability of these findings is enhanced by the fact that circulating 27-OHC was measured from a blood specimen collected near the time of an initial adenoma diagnosis and follow-up was timed according to colonoscopic surveillance intervals. A previous study of circulating 27-OHC measurements repeated about 1 year apart suggests good reproducibility (51), although longer-term biological variability is unknown and was not assessed in our study.

A few study limitations warrant discussion. Without measuring circulating 27-OHC after baseline we could not evaluate whether vitamin D or calcium supplementation altered circulating 27-OHC over 3 or 5 years of use. A small pilot study of 29 breast cancer patients randomly assigned to low-dose (400 IU/day) or high-dose (10,000 IU/day) vitamin D₃ found mean serum 27-OHC was lowered by 12 ng/mL among women in the high-dose group during up to 6 weeks of supplementation (52). At baseline in our study, circulating 27-OHC was uncorrelated with circulating 25(OH)D, consistent with a previous report (2).

We did not consider diet or measurements of circulating lipids or estrogens. It is unclear whether direct ingestion of oxidized cholesterol, which can arise from food preparation and storage (53), can appreciably alter circulating 27-OHC concentrations. Circulating 27-OHC is positively correlated with blood measurements of total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol and negatively correlated with high-density lipoprotein (HDL) cholesterol (2). Circulating cholesterol may be modestly associated with adenoma risk (54–56) and statin use has been linked to reduced risk of developing adenomas, although studies have been inconsistent (57). Our analyses adjusted for statin use at baseline which may provide some indication of latent cholesterol levels under the assumption that those on statins likely have circulating cholesterol controlled to be near target levels for cardiovascular disease prevention, while those not on statins likely have circulating cholesterol below the recommended threshold for initiating therapy. Consistent with the ER-binding properties of 27-OHC, a study of postmenopausal women reported that circulating 27-OHC was not associated with hip fracture risk, but a higher ratio of circulating 27-OHC to bioavailable estradiol was positively associated with risk (49). Evaluation of circulating 27-OHC in the context of circulating estrogens may be useful to consider in future studies of colorectal neoplasia risk.

We found circulating 27-OHC was associated with incidence of conventional adenomas, but not with hyperplastic polyps or sessile serrated adenomas/polyps, suggesting circulating 27-OHC may be more relevant to the traditional adenoma-carcinoma pathway to colorectal cancer (58), rather than the more recently recognized serrated pathway characterized by CpG island methylation and microsatellite instability (59). Statistical power was limited, however, to assess associations with sessile serrated adenomas/polyps given their lower incidence in this population. We evaluated the risk of new colorectal polyps among those followed after an initial adenoma diagnosis, and believe our findings are generalizable to the broader population at risk of a first adenoma because risk factors for first adenomas are generally similar to those for metachronous adenomas (60). Furthermore, because colorectal adenomas are relatively common in asymptomatic individuals (48), participants of our study likely represent a large portion of the general U.S. adult population. Although advanced adenomas are frequently used as surrogate endpoints for colorectal cancer, prospective studies that evaluate circulating 27-OHC in association with risk of invasive colorectal cancer, rather than precursor lesions, should be conducted.

The strength of associations we observed between circulating 27-OHC and colorectal adenoma risk were generally modest and thus our findings are unlikely to have immediate clinical utility. Mechanistic studies are needed to understand where 27-OHC fits in the complex landscape of other lipid signaling molecules thought to be colorectal cancer biomarkers, including various sphingolipids and eicosanoids (61). Natural and synthetic LXR agonists that promote reverse cholesterol transport are being pursued as alternatives to statin therapy for cardiovascular disease prevention (62). However, an early-phase clinical trial of an LXR agonist for the secondary prevention of atherosclerosis raised safety concerns (63). Agents that selectively activate LXR in the small intestine (64) have been identified as promising candidates for colorectal cancer treatment (65), but have yet to be evaluated in clinical trials. Our results suggest studies of novel therapies resulting in

increased blood levels of 27-OHC should also consider potential long-term effects on colorectal cancer development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Prevention Relevance

This study found that plasma concentration of 27-hydroxycholesterol, a metabolite of cholesterol that regulates lipid metabolism and acts as a selective estrogen receptor modulator, is associated with the risk of developing precursor lesions for colorectal cancer.

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Table 1.

Baseline characteristics for Vitamin D/Calcium Polyp Prevention Study participants with circulating 27-hydroxycholesterol (27-OHC) measured at baseline according to treatment assignment.

Baseline characteristic ^a	Total (N=1,246)
Men, no. (%)	894 (72%)
Age, y, mean (SD)	59 (7)
Race/ethnicity, no. (%)	
White	1,008 (81%)
Black	122 (10%)
Asian or Pacific Islander	34 (3%)
Other/unknown	82 (7%)
Body mass index, kg/m ² , mean (SD)	29 (5)
Smoking status, no. (%)	
Never	655 (53%)
Former	475 (38%)
Current	116 (9%)
Alcohol consumption, 1 drink/day, no. (%)	358 (29%)
Diabetes mellitus, no. (%)	133 (11%)
First-degree family history of colorectal cancer, no. (%)	209 (18%)
Aspirin use, 4 days/week, no. (%)	489 (39%)
Non-aspirin NSAID use, 4 days/week, no. (%)	126 (10%)
Statin use, no. (%)	413 (33%)
Postmenopausal hormone therapy use ^b , no. (%)	
Never	188 (53%)
Former	164 (47%)
1 advanced adenoma at baseline, no. (%)	233 (19%)
Treatment assignment, no. (%) ^c	
Full-factorial randomization	
Placebo + Placebo	241 (24%)
Calcium + Placebo	249 (25%)
Placebo + Vitamin D	249 (25%)
Calcium + Vitamin D	247 (25%)
2-group randomization	
Calcium + Placebo	129 (50%)
Calcium + Vitamin D	131 (50%)

Abbreviations: 27-OHC, 27-hydroxycholesterol; NSAID, nonsteroidal anti-inflammatory drug; SD, standard deviation.

^aCirculating 27-OHC was measured for 1,246 of 2,259 participants of the Vitamin D/Calcium Polyp Prevention Study, excluding premenopausal women and postmenopausal women currently using postmenopausal hormone therapy. One participant was missing body mass index, 93 participants were missing alcohol consumption, 85 participants were missing family history of colorectal cancer, and 20 participants were missing advanced adenoma status at baseline.

^bPercent calculated among postmenopausal women.

^cPercent calculated among those with the same randomization type (full-factorial or 2-group randomization). Of 260 postmenopausal women who elected to take calcium, 129 were randomly assigned to placebo for vitamin D and 131 were randomly assigned to vitamin D.

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Table 2.

Association of circulating 27-hydroxycholesterol (27-OHC) at baseline with risk of colorectal adenomas and advanced colorectal adenomas.

Circulating 27-OHC at baseline, quartiles (N at baseline)	Any adenoma			Advanced adenoma		
	N (%) with any adenoma ^a			N (%) with advanced adenoma ^a		
	Treatment period	Post-treatment period	RR ^b (95% CI)	Treatment period	Post-treatment period	RR ^b (95% CI)
<138 ng/mL (N=309)	138 (45%)	75 (48%)	1 (Ref)	27 (9%)	15 (10%)	1 (Ref)
138 to <166 ng/mL (N=314)	134 (43%)	83 (48%)	1.00 (0.85, 1.18)	36 (11%)	20 (11%)	1.44 (0.93, 2.24)
166 to <201 ng/mL (N=311)	143 (46%)	95 (57%)	1.14 (0.97, 1.33)	31 (10%)	10 (6%)	1.17 (0.70, 1.93)
201 ng/mL (N=312)	154 (49%)	95 (57%)	1.24 (1.05, 1.47)	36 (12%)	24 (14%)	1.89 (1.17, 3.06)
<i>P</i> for trend ^c			0.003			<0.001

Abbreviations: 27-OHC, 27-hydroxycholesterol; CI, confidence interval; RR, risk ratio.

^a A total of 582 participants did not undergo follow-up examination during the post-treatment period. Additionally, 5 participants were missing advanced adenoma status during the post-treatment period.

^b Risk ratio averaged across treatment and post-treatment periods using a generalized estimating equations log-linear regression model adjusted for sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, diabetes mellitus, first-degree family history of colorectal cancer, aspirin use, non-aspirin nonsteroidal anti-inflammatory drug use, statin use, and former use of postmenopausal hormone therapy.

^c *P* for linear trend in adjusted risk ratio for continuous log-transformed circulating 27-OHC.

Table 3.

Association of circulating 27-hydroxycholesterol (27-OHC) at baseline with risk of colorectal adenomas and advanced colorectal adenomas according to advanced adenoma status at baseline.

Circulating 27-OHC at baseline, quartiles (N at baseline)	Any adenoma			Advanced adenoma		
	N (%) with any adenoma ^a			N (%) with advanced adenoma ^a		
	Treatment period	Post-treatment period	RR ^b (95% CI)	Treatment period	Post-treatment period	RR ^b (95% CI)
No advanced adenoma at baseline						
<138 ng/mL (N=256)	116 (45%)	60 (47%)	1 (Ref)	22 (9%)	13 (10%)	1 (Ref)
138 to <166 ng/mL (N=245)	105 (43%)	65 (50%)	1.01 (0.84, 1.20)	26 (11%)	16 (12%)	1.33 (0.83, 2.15)
166 to <201 ng/mL (N=248)	110 (44%)	75 (57%)	1.07 (0.89, 1.27)	22 (9%)	8 (6%)	0.98 (0.56, 1.71)
201 ng/mL (N=244)	118 (48%)	64 (53%)	1.18 (0.98, 1.41)	26 (11%)	12 (10%)	1.43 (0.83, 2.44)
<i>P</i> for trend ^c			0.07			0.06
1 advanced adenoma at baseline						
<138 ng/mL (N=48)	18 (38%)	14 (56%)	1 (Ref)	5 (10%)	2 (8%)	1 (Ref)
138 to <166 ng/mL (N=66)	27 (41%)	18 (42%)	1.07 (0.71, 1.63)	9 (15%)	4 (9%)	2.26 (0.86, 5.99)
166 to <201 ng/mL (N=57)	29 (51%)	18 (56%)	1.57 (1.07, 2.31)	9 (16%)	2 (6%)	2.66 (0.93, 7.57)
201 ng/mL (N=62)	32 (48%)	29 (64%)	1.59 (1.08, 2.34)	9 (15%)	10 (22%)	4.34 (1.71, 11.01)
<i>P</i> for trend ^c			0.001			0.001
<i>P</i> for interaction ^d			0.05			0.10

Abbreviations: 27-OHC, 27-hydroxycholesterol; CI, confidence interval; RR, risk ratio.

^a A total of 20 participants with adenoma outcomes during the treatment period were missing advanced adenoma status at baseline. A total of 582 participants (482 without advanced adenoma at baseline, 88 with advanced adenoma at baseline, and 12 with missing advanced adenoma status at baseline) did not undergo follow-up examination during the post-treatment period. Additionally, 5 participants (5 without advanced adenoma at baseline) were missing advanced adenoma status during the post-treatment period.

^b Risk ratio averaged across treatment and post-treatment periods using a generalized estimating equations log-linear regression model adjusted for sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, diabetes mellitus, first-degree family history of colorectal cancer, aspirin use, non-aspirin nonsteroidal anti-inflammatory drug use, statin use, former use of postmenopausal hormone therapy, and advanced adenoma status at baseline with a multiplicative interaction term between circulating 27-OHC and advanced adenoma status at baseline.

^c *P* for linear trend in adjusted risk ratio for continuous log-transformed circulating 27-OHC.

^d *P* for interaction between continuous log-transformed circulating 27-OHC and advanced adenoma status at baseline.

Table 4.

Association of circulating 27-hydroxycholesterol (27-OHC) at baseline with risk of colorectal adenomas and advanced colorectal adenomas according to sex.

Circulating 27-OHC at baseline, sex-specific quartiles (N at baseline)	Any adenoma			Advanced adenoma		
	N (%) with any adenoma ^a			N (%) with advanced adenoma ^a		
	Treatment period	Post- treatment period	RR ^b (95% CI)	Treatment period	Post- treatment period	RR ^b (95% CI)
Men						
<147 ng/mL (N=222)	105 (47%)	70 (58%)	1 (Ref)	19 (9%)	13 (11%)	1 (Ref)
147 to <175 ng/mL (N=222)	105 (47%)	64 (51%)	0.98 (0.82, 1.16)	29 (13%)	12 (9%)	1.27 (0.75, 2.13)
175 to <209 ng/mL (N=226)	106 (47%)	71 (60%)	1.12 (0.94, 1.32)	20 (9%)	10 (8%)	1.31 (0.76, 2.26)
209 ng/mL (N=224)	113 (50%)	72 (58%)	1.18 (0.99, 1.40)	27 (12%)	18 (15%)	1.74 (1.03, 2.95)
<i>P</i> for trend ^c			0.03			0.004
Postmenopausal women						
<127 ng/mL (N=89)	33 (37%)	14 (33%)	1 (Ref)	5 (6%)	3 (7%)	1 (Ref)
127 to <147 ng/mL (N=90)	40 (44%)	19 (45%)	1.43 (1.01, 2.04)	11 (12%)	3 (7%)	2.91 (1.08, 7.84)
147 to <174 ng/mL (N=82)	27 (33%)	20 (43%)	1.21 (0.83, 1.76)	8 (10%)	6 (13%)	2.67 (1.00, 7.11)
174 ng/mL (N=91)	40 (44%)	18 (41%)	1.43 (0.96, 2.11)	11 (12%)	4 (9%)	2.90 (0.93, 8.97)
<i>P</i> for trend ^c			0.07			0.04
<i>P</i> for interaction ^d			0.93			0.88

Abbreviations: 27-OHC, 27-hydroxycholesterol; CI, confidence interval; RR, risk ratio.

^a A total of 582 participants (404 men and 178 postmenopausal women) did not undergo follow-up examination during the post-treatment period. Additionally, 5 men were missing advanced adenoma status during the post-treatment period.

^b Risk ratios from separate regression models for men and postmenopausal women averaged across treatment and post-treatment periods using a generalized estimating equations log-linear regression model adjusted for age, race/ethnicity, body mass index, smoking status, alcohol consumption, diabetes mellitus, first-degree family history of colorectal cancer, aspirin use, non-aspirin nonsteroidal anti-inflammatory drug use, statin use, and former use of postmenopausal hormone therapy (among postmenopausal women). Estimates are shown for sex-specific quartiles of circulating 27-OHC.

^c *P* for linear trend in adjusted risk ratio for continuous log-transformed circulating 27-OHC.

^d *P* for interaction between continuous log-transformed circulating 27-OHC and sex from regression model adjusted for the same covariates and sex with a multiplicative interaction term between continuous log-transformed circulating 27-OHC and sex.

Table 5.

Association of circulating 27-hydroxycholesterol (27-OHC) at baseline with risk of colorectal hyperplastic polyps and colorectal sessile serrated adenomas/polyps.

Circulating 27-OHC at baseline, quartiles (N at baseline)	Any hyperplastic polyp			Any sessile serrated adenomas/polyps		
	N (%) with any hyperplastic polyp ^a			N (%) with any sessile serrated adenomas/polyps ^a		
	Treatment period	Post-treatment period	RR ^b (95% CI)	Treatment period	Post-treatment period	RR ^b (95% CI)
<138 ng/mL (N=309)	75 (24%)	33 (22%)	1 (Ref)	12 (4%)	8 (5%)	1 (Ref)
138 to <166 ng/mL (N=314)	80 (26%)	48 (28%)	1.13 (0.86, 1.48)	17 (6%)	13 (8%)	1.34 (0.73, 2.45)
166 to <201 ng/mL (N=311)	72 (23%)	45 (28%)	1.18 (0.90, 1.54)	19 (6%)	14 (9%)	1.64 (0.90, 2.96)
201 ng/mL (N=312)	74 (24%)	37 (22%)	0.90 (0.66, 1.22)	18 (6%)	7 (4%)	1.02 (0.50, 2.07)
<i>P</i> for trend ^c			0.43			0.48

Abbreviations: 27-OHC, 27-hydroxycholesterol; CI, confidence interval; RR, risk ratio.

^a A total of 582 participants did not undergo follow-up examination during the post-treatment period. A total of 10 participants were missing hyperplastic polyp outcomes and 22 participants were missing sessile serrated adenoma/polyp outcomes during the treatment period. A total of 14 participants were missing hyperplastic polyp outcomes and 22 participants were missing sessile serrated adenoma/polyp outcomes during the post-treatment period.

^b Risk ratio averaged across treatment and post-treatment periods using a generalized estimating equations log-linear regression model adjusted for sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, diabetes mellitus, first-degree family history of colorectal cancer, aspirin use, non-aspirin nonsteroidal anti-inflammatory drug use, statin use, and former use of postmenopausal hormone therapy.

^c *P* for linear trend in adjusted risk ratio for continuous log-transformed circulating 27-OHC.