Research Article

Cancer Prevention Research

Association between Circulating Vitamin D Metabolites and Fecal Bile Acid Concentrations

Elizabeth T. Jacobs[1,2], Mark R. Haussler[3], David S. Alberts[2], Lindsay N. Kohler[1], Peter Lance[1], María Elena Martínez[4], Denise J. Roe[1,2], and Peter W. Jurutka[2,3,5]

Abstract

Although hydrophobic bile acids have been demonstrated to exhibit cytotoxic and carcinogenic effects in the colorectum, ursodeoxycholic acid (UDCA) has been investigated as a potential chemopreventive agent. Vitamin D has been shown to play a role in both bile acid metabolism and in the development of colorectal neoplasia. Using a cross-sectional design, we sought to determine whether baseline circulating concentrations of the vitamin D metabolites 25(OH)D and $1,25(OH)_2D$ were associated with baseline fecal bile acid concentrations in a trial of UDCA for the prevention of colorectal adenoma recurrence. We also prospectively evaluated whether vitamin D metabolite concentrations modified the effect of UDCA on adenoma recurrence. After adjustment for age, sex, BMI, physical activity, and calcium intake, adequate concentrations of 25(OH)D ($\geq$30 ng/mL) were statistically significantly associated with reduced odds for high levels of total [OR, 0.61; 95% confidence interval (CI), 0.38–0.97], and primary (OR, 0.61; 95% CI, 0.38–0.96) bile acids, as well as individually with chenodeoxycholic acid (OR, 0.39; 95% CI, 0.24–0.63) and cholic acid (OR, 0.56; 95% CI, 0.36–0.90). No significant associations were observed for $1,25(OH)_2D$ and high versus low fecal bile acid concentrations. In addition, neither 25(OH)D nor $1,25(OH)_2D$ modified the effect of UDCA on colorectal adenoma recurrence. In conclusion, this is the first study to demonstrate an inverse relationship between circulating levels of 25(OH)D and primary fecal bile acid concentrations. These results support prior data demonstrating that vitamin D plays a key role in bile acid metabolism, and suggest a potential mechanism of action for 25(OH)D in colorectal cancer prevention. *Cancer Prev Res; 9(7); 589–97. ©2016 AACR.*

Introduction

It is estimated that there will be 134,000 new cases and 49,200 deaths from colorectal cancer in the United States in 2016 (1). Although colonoscopy is an effective preventive measure for detection and removal of colorectal adenomas, the precursor lesions for colorectal cancer, screening rates remain well below targeted levels (2). Therefore, the identification of modifiable risk factors and potential pathway targets for colorectal carcinogenesis remains critical for prevention and treatment of this disease. Bile acids (BA) represent one such avenue of investigation. As requisite components of lipid metabolism, BAs are found in the gastrointestinal tract after hepatic synthesis of the primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA) from cholesterol (3). The majority of BAs are reabsorbed into the small intestine, but approximately 5% of the BA pool reaches the colon, at which time they are further metabolized by colonic bacteria flora to secondary bile acids such as lithocholic acid (LCA) and deoxycholic acid (DCA; ref. 4).

Although it is clear that bile acids play a key physiologic role in normal fat metabolism, prolonged exposure of the colon to hydrophobic BAs, particularly secondary BAs such as LCA and DCA, has been hypothesized to be carcinogenic (4, 5). Conversely, the hydrophilic BA ursodeoxycholic acid (UDCA) has been identified as a potential agent for the chemoprevention of colorectal cancer (6–11), although this remains a subject of continuing debate (10, 11). A large, randomized, double-blind trial of 8 to 10 mg/kg/d UDCA for prevention of colorectal adenoma recurrence conducted by our group revealed no overall effect on the development of recurrent colorectal neoplasia; however, a statistically significant reduction in the formation of lesions with high-grade dysplasia was observed (6). Further analyses of the same trial revealed that there were sex differences in response to UDCA treatment, with men, but not women, who received UDCA having a significantly reduced risk of developing advanced lesions (12). Effect modification of UDCA treatment by genetic variation in *cholesterol 7α-hydroxylase* (*CYP7A1*), the expression of which is controlled primarily by the farnesoid X receptor (FXR) and liver X receptor (LXR), was also reported (13).

The vitamin D receptor (VDR) also has been identified as a bile acid sensor (14), primarily through binding of secondary BAs directly to VDR followed by receptor activation. Moreover, prior work has demonstrated that vitamin D specifically inhibits the expression of CYP7A1, which in turn reduces the synthesis of bile acids (15). Vitamin D has also been demonstrated to upregulate CYP3A4 (16–18), an enzyme with a key role in bile acid detoxification (19). However, little is known about the potential role of circulating concentrations of the vitamin D metabolites 25(OH)D or $1,25(OH)_2D$ on bile acid metabolism, *in vivo*. Thus, although

[1]Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, Arizona. [2]University of Arizona Cancer Center, Tucson, Arizona. [3]Department of Basic Medical Sciences, University of Arizona College of Medicine, Phoenix, Arizona. [4]Department of Family and Preventive Medicine, University of California San Diego, La Jolla, California. [5]School of Mathematical and Natural Sciences, Arizona State University, Phoenix, Arizona.

Corresponding Author: Elizabeth T. Jacobs, University of Arizona, 1515 North Campbell Avenue, Tucson, AZ 85724-5024. Phone: 520-626-0341; Fax: 520-626-9274; E-mail: jacobse@email.arizona.edu

doi: 10.1158/1940-6207.CAPR-16-0033

patient characteristics that may influence response to UDCA have been identified, including those associated with vitamin D, a further understanding of genetic or lifestyle factors associated with response may aid in identification of targeted populations who may benefit from UDCA. The goal of the current study was to determine whether circulating concentrations of vitamin D metabolites were associated with fecal bile acid levels at baseline, as well as whether they modified the effect of UDCA treatment on colorectal adenoma recurrence.

Materials and Methods

Study population

The current study was performed with participant data from a randomized, double blind, placebo-controlled phase III trial of UDCA for adenoma prevention that was conducted at the University of Arizona Cancer Center and which has been described in detail previously (6). Briefly, eligible participants were male and female Arizona residents ($n = 1{,}192$) ranging in age from 40 to 80 years who had one or more colorectal adenomas removed by colonoscopy within 6 months before entering the study. The goal of the trial was to compare the effect of UDCA (8–10 mg/kg of body weight/day) versus placebo on the risk of recurrent colorectal adenomas (6). Primary analyses demonstrated no effect of UDCA treatment (6). Of the 1,192 participants in the UDCA trial, complete data for recurrent adenomas and circulating 25(OH)D and $1,25(OH)_2D$ concentrations were available for 1,150 participants. These study subjects were included in the analysis of heterogeneity of the treatment effect of UDCA on adenoma recurrence by vitamin D metabolite levels. A total of 651 of these participants also had data for baseline fecal bile acid concentrations and were included in the cross-sectional analysis of the association between vitamin D metabolite levels and fecal bile acid concentrations. The University of Arizona Human Subjects committee and local hospital committees approved the UDCA study, and written informed consent was obtained from each participant before study enrollment.

Measurement of fecal bile acid concentrations

Analysis of fecal bile acid levels for the UDCA trial was completed in conjunction with the parent clinical trial and has been described in detail elsewhere (6, 13). Briefly, at baseline, study participants provided 72-hour stool samples that were frozen and shipped overnight on dry ice to the University of Arizona Cancer Center (13). Samples were then homogenized with an equal weight of water for 15 minutes and centrifuged (13). Fecal water was comprised of the aqueous phase after centrifugation and was stored at −80°C. Measurement of bile acids was conducted using gas chromatography (6, 20). Results were obtained for lithocholic acid (LCA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), cholic acid (CA), UDCA, ursocholic acid (UC), and other bile acids, a category that included isodeoxycholic, isoursodeoxycholic, 7-ketolithocholic, and 12-ketolithocholic acids (6, 13). Concentrations of total primary bile acids were calculated by summing the values for CDCA and CA, whereas total secondary bile acid concentration was assessed by combining the remaining values.

Analysis of vitamin D metabolites

Analysis of 25(OH)D and $1,25(OH)_2D$ concentrations was performed at Heartland Assays (Ames, IA). A competitive chemiluminescence immunoassay was used for assessment of 25(OH)D concentrations (21). These analyses were conducted with quality assurance and quality control measures including a pooled serum sample analyzed with batches of study samples to monitor analytical precision and identify possible laboratory shifts over time, as well as testing of duplicates in different batches. The coefficient of variation was less than 7.0% for 25(OH)D analyses. For $1,25(OH)_2D$ concentrations, a ^{125}I-based radioimmunoassay was employed, as has been described in detail previously (22). The coefficient of variation was 11.5% for $1,25(OH)_2D$ analyses. All analyses were conducted in a blinded fashion. For 25(OH)D, participants were categorized by circulating concentrations into one of three categories: deficient (<20 ng/mL), inadequate ($\geq$20 and <30 ng/mL), and adequate ($\geq$30 ng/mL; refs. 23–26); whereas for $1,25(OH)_2D$, tertiles were created on the basis of the distribution of values among the participants.

Dietary and physical activity data

Data for dietary intake were obtained from the Arizona Food Frequency Questionnaire (AFFQ), a semiquantitative, 175-item food frequency questionnaire that asked respondents to report how often and how much they consumed of each food item over the past 12-month period for over 800 foods (27). The survey has been tested for reliability and validity in our prior chemoprevention trials of adenoma recurrence (27). Data for physical activity were obtained via the Arizona Activity Frequency Questionnaire (AAFQ), a 59-item, validated questionnaire that groups physical activity by leisure, recreational, household, and "other" activity categories (28). Output included metabolic equivalents (MET) units per day and per activity, number of hours per day per activity, and number of activities reported by respondents for each category.

Outcome variables

Adenoma characteristics, including number, size, location, and histology, were obtained from the medical record and the pathology report for each subject (29). Recurrent adenomas were defined as any colorectal adenoma detected at colonoscopy at least six months after randomization. Adenomas were classified as advanced if they had a diameter of 1 cm or more and/or at least 25% tubulovillous or villous histology. Colorectal adenocarcinomas were included in the advanced recurrence group, whereas all other adenomas were classified as non-advanced. In subjects with more than one adenoma, size and characterization of the histologic type was based on the largest and/or most advanced adenoma.

Statistical analyses

Baseline participant characteristics were compared by tertile of total fecal bile acid concentrations with means and standard deviations for continuous variables, and frequencies and percentages for categorical variables. Means and SDs for total, primary and secondary, and individual bile acids were calculated for each category of 25(OH)D concentration and each tertile of $1,25(OH)_2D$. Unconditional logistic regression modeling was used to evaluate the adjusted ORs (95% confidence intervals) for the associations between vitamin D metabolites and the odds of having had high versus low fecal bile acid concentrations based on the median concentration of each bile acid. Potential confounding was assessed for age, body mass index (BMI), sex, race, season of blood draw, family

Table 1. Baseline characteristics of participants ($n = 651$) by tertile of baseline fecal bile acid concentrations

	Total fecal bile acids (μg/mL)			
	***n* =220**	***n* = 217**	***n* = 214**	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Baseline characteristic	**94.2 ± 34.9**	**220.1 ± 45.8**	**656.4 ± 458.8**	***P*[a]**
Male (*n*, %)	169 (76.8)	136 (62.7)	123 (57.5)	<0.001
Age (y, mean ± SD)	67.7 ± 8.0	65.8 ± 8.1	65.3 ± 8.5	<0.01
Race (white, *n*, %)	205 (93.2)	205 (94.5)	196 (91.6)	0.30
BMI (kg/m2, mean ± SD)	27.3 ± 3.8	28.4 ± 5.1	28.9 ± 5.4	<0.001
Previous polyps[b] (yes, *n*, %)	101 (45.9)	94 (43.3)	97 (45.3)	0.91
Family history CRC[c] (yes, *n*, %)	69 (31.4)	55 (25.3)	58 (27.1)	0.35
Physical activity (kJ/d, mean ± SD)	10,524.3 ± 2,644.4	9,924.9 ± 2,549.2	10,004.1 ± 2,712.0	<0.05
Dietary intake (mean ± SD)				
Energy (kcal/d)	2,064.2 ± 860.3	1,880.7 ± 772.7	1,933.5 ± 824.8	0.09
Protein (g/d)	76.6 ± 32.7	71.3 ± 28.3	70.8 ± 30.3	<0.05
Total fat (g/d)	62.3 ± 32.8	56.5 ± 26.8	60.2 ± 31.6	0.46
Saturated fat (g/d)	19.8 ± 11.5	18.1 ± 9.2	19.5 ± 11.5	0.75
Carbohydrate (g/d)	299.6 ± 132.2	267.3 ± 117.3	274.8 ± 122.4	<0.05
Total fiber (g/d)	24.2 ± 11.9	21.4 ± 10.2	20.5 ± 9.4	<0.001
Calcium (mg/d)	1,075.0 ± 562.7	990.9 ± 461.5	949.6 ± 462.5	<0.01

[a]*P* values calculated using ANOVA for categorical baseline characteristics and simple linear regression for continuous variables.
[b]History of polyps before qualifying exam; data missing for 29 participants.
[c]Family history of colorectal cancer in at least one first-degree relative.

history of colorectal cancer, current smoking, history of previous polyps, physical activity, and aspirin use, and mean daily dietary intake of energy, protein, total fat, saturated fat, carbohydrate, total fiber, and calcium. A confounding variable was included in the final analysis if it changed the point estimate by 10% or greater (30); thus, the final adjusted models for the association between vitamin D metabolites and baseline bile acids included age, sex, BMI, physical activity, and calcium intake. In addition, linear regression models were used to evaluate the association between continuous values for vitamin D metabolite concentrations and continuous variables for each bile acid. We then conducted stratified analyses of the effect of UDCA treatment on adenoma recurrence by category of 25 (OH)D and tertile of $1,25(OH)_2D$ concentration to ascertain whether there was effect modification by vitamin D metabolites. The Stata statistical software package (version 10.0, Stata Corporation) was used for data management and statistical analysis.

Results

Table 1 presents the baseline characteristics of study participants by tertile of total fecal bile acid concentrations at baseline. Males, older individuals, and those with lower BMI and greater

Table 2. Association between baseline 25(OH)D and $1,25(OH)_2D$ concentrations and baseline fecal bile acid concentrations

	Vitamin D status [*n*, mean 25(OH)D ± SD]			
	Deficient	**Inadequate**	**Adequate**	
Baseline bile acid concentrations	***n* = 146**	***n* = 281**	***n* = 224**	
(mean μg/mL, ± SD)	**<20 ng/mL**	**≥20 and <30 ng/mL**	**≥30 ng/mL**	***P*[a]**
Total bile acids	392.1 ± 359.1	338.9 ± 405.2	267.2 ± 303.3	<0.01
Primary	86.9 ± 186.6	66.0 ± 176.8	41.0 ± 115.2	<0.01
Secondary	305.2 ± 234.4	273.2 ± 297.0	226.2 ± 217.0	<0.001
Chenodeoxycholic	14.3 ± 30.7	10.9 ± 34.2	5.5 ± 14.3	<0.01
Cholic	72.6 ± 167.0	55.0 ± 160.9	35.5 ± 104.3	<0.05
Lithocholic	26.3 ± 28.6	19.3 ± 18.0	17.8 ± 23.5	<0.001
Deoxycholic	155.6 ± 116.2	142.7 ± 110.5	120.5 ± 90.4	<0.001
Ursodeoxycholic	17.0 ± 32.2	18.8 ± 100.6	11.1 ± 31.3	0.36
Ursocholic	38.6 ± 88.4	43.1 ± 196.5	32.5 ± 148.8	0.66
Other[b]	67.6 ± 59.3	49.1 ± 42.5	44.3 ± 59.9	<0.001
	Tertile of $1,25(OH)_2D$ concentrations (*n*, mean ± SD)			
	Tertile 1	**Tertile 2**	**Tertile 3**	
Baseline bile acid concentrations	***n* = 230**	***n* = 220**	***n* = 201**	
(mean μg/mL, ± SD)	**23.0 ± 4.4 pg/ml**	**33.6 ± 2.8 pg/ml**	**47.1 ± 8.4 pg/ml**	***P*[a]**
Total bile acids	339.5 ± 357.5	321.8 ± 386.6	315.8 ± 350.6	0.50
Primary	66.6 ± 153.3	67.0 ± 188.8	51.2 ± 136.6	0.33
Secondary	272.8 ± 267.9	254.8 ± 257.8	264.6 ± 252.2	0.72
Chenodeoxycholic	10.4 ± 31.2	10.5 ± 23.8	8.4 ± 29.1	0.47
Cholic	56.2 ± 141.1	56.4 ± 169.8	42.8 ± 121.2	0.35
Lithocholic	19.5 ± 23.0	20.3 ± 20.0	21.4 ± 25.5	0.38
Deoxycholic	141.4 ± 109.6	132.8 ± 105.6	140.0 ± 102.9	0.87
Ursodeoxycholic	21.7 ± 112.3	12.9 ± 26.5	12.1 ± 28.0	0.15
Ursocholic	34.1 ± 101.4	38.5 ± 184.6	43.3 ± 188.0	0.55
Other[b]	56.1 ± 68.7	50.4 ± 45.0	47.7 ± 40.9	0.10

[a]*P* values calculated using simple linear regression modeling.
[b]Other includes isodeoxycholic, isoursodeoxycholic, 7-ketolithocholic, and 12-ketolithocholic acids.

Table 3. Adjusted[a] ORs (95% CIs) for the associations between baseline concentrations of vitamin D metabolites and high versus low baseline fecal bile acid concentrations

	Vitamin D status [*n*, mean 25(OH)D ± SD]			
Low vs. high[b] baseline bile acid concentrations	**Deficient *n* = 146 <20 ng/mL**	**Inadequate *n* = 280 ≥20 and <30 ng/mL**	**Adequate *n* = 224 ≥30 ng/mL**	**P_{trend}[c]**
Total low				
High	1.00	0.87 (0.57–1.35)	0.61 (0.38–0.97)	<0.05
Primary low				
High	1.00	0.71 (0.46–1.09)	0.61 (0.38–0.96)	<0.05
Secondary low				
High	1.00	0.97 (0.62–1.50)	0.66 (0.41–1.06)	<0.05
Chenodeoxycholic low				
High	1.00	0.54 (0.35–0.83)	0.39 (0.24–0.63)	<0.001
Cholic low				
High	1.00	0.66 (0.43–1.02)	0.56 (0.36–0.90)	<0.05
Lithocholic low				
High	1.00	0.89 (0.58–1.36)	0.68 (0.43–1.08)	0.08
Deoxycholic low				
High	1.00	1.09 (0.70–1.68)	0.83 (0.52–1.33)	0.33
Ursodeoxycholic low				
High	1.00	0.88 (0.58–1.36)	0.67 (0.42–1.07)	0.09
Ursocholic low				
High	1.00	0.70 (0.46–1.07)	0.68 (0.43–1.08)	0.13
Other[d] low				
High	1.00	0.63 (0.41–0.97)	0.60 (0.38–0.96)	<0.05
	Tertile of $1,25(OH)_2D$ concentrations *n* (mean ± SD)			
Low vs. high[b] baseline bile acid concentrations	**Tertile 1 *n* = 230 22.6 ± 4.6 pg/ml**	**Tertile 2 *n* = 220 33.5 ± 2.8 pg/ml**	**Tertile 3 *n* = 201 46.8 ± 7.9 pg/ml**	**P_{trend}[c]**
Total (Low)				
High	1.00	0.89 (0.60–1.31)	1.18 (0.79–1.76)	0.44
Primary (Low)				
High	1.00	0.86 (0.59–1.28)	0.88 (0.60–1.31)	0.52
Secondary (Low)				
High	1.00	1.03 (0.70–1.53)	1.22 (0.82–1.83)	0.34
Lithocholic (Low)				
High	1.00	1.31 (0.89–1.92)	1.21 (0.82–1.80)	0.33
Deoxycholic (Low)				
High	1.00	1.04 (0.71–1.55)	1.26 (0.84–1.89)	0.23
Chenodeoxycholic (Low)				
High	1.00	0.90 (0.60–1.34)	0.75 (0.49–1.14)	0.18
Cholic (Low)				
High	1.00	0.82 (0.56–1.20)	0.82 (0.55–1.22)	0.32
Ursocholic (Low)				
High	1.00	0.78 (0.53–1.15)	0.81 (0.54–1.20)	0.28
Ursodeoxycholic (Low)				
High	1.00	1.05 (0.71–1.56)	1.04 (0.69–1.56)	0.85
Other[d](Low)				
High	1.00	1.07 (0.73–1.58)	1.04 (0.70–1.55)	0.84

[a]Models adjusted for age, sex, BMI, physical activity, and calcium intake.
[b]Cut-off point for low versus high was median value for each bile acid (mean μg/mL): primary, 7.4; secondary, 197.6; chenodeoxycholic acid, 0.0; cholic acid, 0.0; lithocholic acid, 15.1; deoxycholic acid, 111.4; ursodeoxycholic acid, 0.0; ursocholic acid, 0.0; other, 39.2; total, 214.0.
[c]*P* values calculated using logistic regression modeling with vitamin D status as a categorical exposure variable.
[d]Other includes isodeoxycholic, isoursodeoxycholic, 7-ketolithocholic, and 12-ketolithocholic acids.

physical activity were significantly less likely to have high total bile acid concentrations. Regarding dietary intake, protein, carbohydrate, total fiber, and calcium were each significantly inversely associated with total fecal bile acid concentrations.

As shown in Table 2, in unadjusted analyses baseline concentrations of total ($P < 0.01$), primary ($P < 0.01$), and secondary ($P < 0.001$) bile acids were significantly inversely associated with baseline 25(OH)D levels. Individual bile acids, including CDCA ($P < 0.01$), CA ($P < 0.05$), LCA ($P < 0.001$), DCA ($P < 0.001$), and other bile acids ($P < 0.001$) also exhibited significant inverse relationships with 25(OH)D concentrations. Conversely, neither UDCA ($P = 0.36$) nor UC ($P = 0.66$) were significantly associated with 25(OH)D. Furthermore, circulating concentrations of 1,25 $(OH)_2D$ were not statistically significantly associated with any bile acid.

Table 3 presents the results of adjusted logistic regression models of the association between vitamin D metabolite concentrations and the odds of fecal bile acid concentrations above (high) versus equal to or below (low) the median. After adjustment for age, sex, BMI, physical activity, and calcium intake, adequate concentrations of 25(OH)D were statistically significantly associated with reduced odds for high levels of total (OR,

Table 4. Unadjusted and adjusted linear regression models for 25(OH)D, 1,25 $(OH)_2D$, and baseline fecal bile acid concentrations

Baseline bile acid concentrations (μg/mL)	25(OH)D (ng/mL) β-coefficient (SE)				$1,25(OH)_sD$ (pg/mL) β-coefficient (SE)			
	Unadjusted	P[a]	Adjusted[b]	P[a]	Unadjusted	P[a]	Adjusted[b]	P[a]
Total bile acids	−4.49 (1.40)	<0.001	−2.56 (1.50)	0.09	−1.45 (0.25)	0.25	−0.80 (1.28)	0.54
Primary	−1.64 (0.62)	<0.01	−1.21 (0.67)	0.07	−0.64 (0.56)	0.26	−0.41 (0.57)	0.48
Secondary	−2.86 (1.00)	<0.01	−1.25 (1.07)	0.21	−0.81 (0.90)	0.37	−0.39 (0.91)	0.67
Chenodeoxycholic	−0.28 (0.11)	<0.01	−0.32 (0.12)	<0.01	−0.01 (0.02)	0.55	−0.08 (0.10)	0.46
Cholic	−1.36 (0.56)	<0.05	−0.88 (0.60)	0.14	−0.003 (0.003)	0.26	−0.33 (0.52)	0.52
Lithocholic	−0.26 (0.09)	<0.01	−0.20 (0.09)	<0.05	0.02 (0.02)	0.36	0.10 (0.08)	0.21
Deoxycholic	−1.74 (0.49)	<0.001	−0.61 (0.43)	0.16	0.002 (0.004)	0.72	0.35 (0.37)	0.35
Ursodeoxycholic	−0.16 (0.27)	0.55	−0.16 (0.27)	0.55	−0.01 (0.006)	0.08	−0.46 (0.25)	0.06
Ursocholic	−0.28 (0.62)	0.66	−0.28 (0.62)	0.66	−0.001 (0.003)	0.84	0.07 (0.58)	0.90
Other[c]	−0.77 (0.20)	<0.001	−0.61 (0.22)	<0.01	−0.02 (0.008)	<0.01	−0.45 (0.19)	<0.05

[a]*P* value calculated using linear regression modeling.

[b]Models adjusted for age, sex, and BMI.

[c]Other includes isodeoxycholic, isoursodeoxycholic, 7-ketolithocholic, and 12-ketolithocholic acids.

0.61; 95% CI, 0.38–0.97), and primary (OR, 0.61; 95% CI, 0.38–0.96) fecal bile acids, as well as CDCA (OR, 0.39; 95% CI, 0.24–0.63), CA (OR, 0.56; 95% CI, 0.36–0.90), and other bile acids (OR, 0.60; 95% CI, 0.38–0.96). No statistically significant associations were observed for $1,25(OH)_2D$ and high versus low fecal bile acid concentrations.

We next examined the association between continuous variables for 25(OH)D, $1,25(OH)_2D$, and bile acid concentrations (Table 4). Similar to the findings presented in Table 2 by category of vitamin D metabolites, in unadjusted analyses, statistically significant inverse associations were observed between 25(OH)D concentrations and all bile acids except for UDCA and UC. After adjustment for potential confounders, associations were attenuated, with CDCA ($P < 0.01$), LCA ($P < 0.05$), and other bile acids ($P < 0.01$) remaining significant. For $1,25(OH)_2D$ and bile acids, no significant associations were observed with the exception of an inverse relationship with other bile acids in both adjusted and unadjusted models.

Finally, we sought to determine whether baseline concentrations of vitamin D metabolites modified the effect of UDCA treatment versus placebo on overall or advanced colorectal adenoma recurrence. A prior publication by our group demonstrated that sex modified the effect of UDCA treatment on adenoma recurrence; therefore, we stratified these results by sex. As shown in Table 5, in models adjusted for age, BMI, and season of blood draw, there were no statistically significant differences for UDCA treatment effect by category of vitamin D metabolites for either overall adenoma recurrence or advanced recurrence among men or women.

Discussion

To our knowledge, this is the first epidemiologic study of the association between circulating vitamin D metabolite concentrations and fecal bile acid levels. The results demonstrated that higher circulating 25(OH)D concentrations were significantly associated with lower baseline fecal bile acid concentrations for all but UDCA and UC. After adjustment for potentially confounding variables, the relationships remained statistically significant for total and primary bile acid concentrations, for CDCA and CA individually, and for the category of other bile acids. In contrast, blood levels of $1,25(OH)_2D$ were not related to baseline fecal bile acid concentrations, and no modification of the effect of UDCA on colorectal adenoma recurrence was observed by levels of either vitamin D metabolite.

BAs are required for lipid digestion and metabolism (31), and the primary BAs, CDCA and CA, are produced in the liver from the precursor, cholesterol (3). The poor water solubility of lipids presents problems for digestion because the substrates are not easily accessible to the digestive enzymes in the aqueous phase, and the lipolytic products tend to aggregate to larger complexes that make poor contact with the cell surface and therefore are not easily absorbed. This latter problem is overcome by "solubilization" of the lipid products with amphipathic bile acids. Bile acids are cholesterol clearance metabolites that emulsify the products of dietary fat digestion for intestinal absorption. They undergo active transport in the ileum, and enterohepatic (re)circulation via the portal system. As shown in Fig. 1, bile acid synthesis occurs exclusively in liver, and is catalyzed by 7α- and 12α-hydroxylation of cholesterol as well as side chain oxidation to a carboxylate moiety, yielding CA and CDCA as primary bile acids, the latter of which are subsequently 7-dehydroxylated by microbial enzymes in the intestinal lumen to deoxycholic and lithocholic secondary bile acids, respectively. Figure 1 illustrates the integrative crosstalk between bile acid and vitamin D metabolism, which may form the molecular basis for the observations reported in the current study. These pathways constitute an elaborate autoregulatory cascade that is affected by vitamin D, and mediated by nuclear receptors/transcription factors, for the maintenance of hepatic cholesterol catabolism to bile acids, as well as lowering the risk of colon cancer by the detoxification of secondary bile acids. Thus, although BAs clearly have a key role in fat metabolism, carcinogenic effects in the colorectum are likely present after prolonged exposure, and these may be mitigated through the vitamin D pathway.

More than 75 years ago, DCA was identified as a potential carcinogen (32); since that time, the role of bile acids in carcinogenesis has been refined and several mechanisms of action have been proposed. First, it has been hypothesized that chronic exposure to bile acids in response to a high-fat diet may result in the selection of apoptosis-resistant cells in the colon, leading to tumorigenesis (5). Next, several lines of evidence suggest that hydrophobic bile acids may increase oxidative stress via the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS; ref. 5). In addition, some epidemiologic studies have shown that higher bile acid concentrations in the blood or serum, particularly CDCA, are observed in patients with colorectal cancer compared with controls (5, 33). Finally, the emerging bile acid–gut microbiome axis suggests that there is considerable

Table 5. Adjusted[a] ORs (95% CI) for treatment effect of UDCA by concentrations of 25(OH)D, of 1,25$(OH)_2$D, and sex

	Recurrence				Advanced recurrence			
	Women		Men		Women		Men	
Vitamin D metabolite	*n* (%) recur UDCA/placebo	OR (95% CI)	*n* (%) recur UDCA/placebo	OR (95% CI)	Percentage of recur UDCA/placebo	OR (95% CI)	Percentage of recur UDCA/placebo	OR (95% CI)
25(OH)D status								
Deficient								
<20 ng/mL	30 (42.3)/23 (32.9)	1.61 (0.80–3.24)	28 (45.2)/24 (48.0)	0.88 (0.39–1.96)	13 (18.6)/7 (10.0)	2.21 (0.79–6.15)	8 (12.9)/7 (14.0)	0.99 (0.29–3.40)
Inadequate								
≥20 and <30 ng/mL	26 (32.9)/33 (44.6)	0.65 (0.33–1.29)	76 (43.2)/72 (43.9)	0.83 (0.53–1.29)	8 (10.1)/7 (9.5)	0.99 (0.31–3.11)	18 (10.3)/22 (13.4)	0.77 (0.39–1.53)
Adequate								
≥30 ng/mL	11 (31.4)/16 (33.3)	0.81 (0.31–2.17)	71 (42.0)/75 (48.7)	0.99 (0.64–1.53)	6 (17.1)/5 (10.6)	1.15 (0.28–4.64)	25 (14.8)/31 (20.1)	0.70 (0.38–1.31)
$P_{interaction}$[b]		0.15		0.81		0.59		0.93
1,25(OH)2D tertile								
Tertile 1								
23.0 ± 4.4 pg/mL	33 (42.3)/31 (42.5)	1.09 (0.56–2.11)	61 (48.0)/56 (49.6)	1.01 (0.60–1.70)	12 (15.6)/8 (11.0)	1.55 (0.57–4.22)	19 (15.1)/20 (17.7)	1.01 (0.47–2.17)
Tertile 2								
33.6 ± 2.8 pg/mL	16 (31.4)/18 (32.7)	0.85 (0.36–1.99)	60 (42.3)/60 (45.8)	0.97 (0.59–1.59)	6 (11.8)/5 (9.1)	1.39 (0.38–5.12)	18 (12.7)/25 (19.1)	0.68 (0.34–1.35)
Tertile 3								
47.1 ± 8.4 pg/mL	18 (32.1)/23 (35.9)	0.81 (0.36–1.86)	54 (39.1)/55 (44.4)	0.83 (0.51–1.38)	9 (16.1)/6 (9.5)	1.37 (0.42–4.46)	14 (10.1)/15 (12.1)	0.82 (0.36–1.85)
$P_{interaction}$[b]		0.82		0.93		0.98		0.44

[a]Models adjusted for age, BMI, and season of blood draw.

[b]$P_{interaction}$ calculated with a likelihood-ratio test.

genetic and environmental variation in not only microbiota-mediated metabolism of bile acids, but also the potential for impact of bile acid composition in the colon on gut microbiome–host physiology interactions (34).

In contrast, UDCA is a hydrophilic bile acid that been examined in detail as a potential chemopreventive agent for colorectal cancer (6–11). In our randomized, placebo-controlled UDCA trial, no overall effect of the intervention was observed, although there was a statistically significant reduction in the formation of lesions with high-grade dysplasia (6). Secondary analyses of this trial revealed that treatment with UDCA reduced the risk of advanced recurrent adenomas among men, but not women (12). Furthermore, an investigation of the genetic variation in cholesterol 7α-hydroxylase (*CYP7A1*) demonstrated that there were significant differences in treatment effect among three SNPs in this gene (13). Because in-depth investigations of the UDCA trial have identified several patient characteristics that may allow for targeted approaches to the use of UDCA in chemoprevention, as well as the growing literature of crosstalk between the bile acid and vitamin D pathways, we sought to determine whether vitamin D metabolite concentrations might also be related to bile acid levels and/or may modify the effect of UDCA treatment on adenoma recurrence.

In cross-sectional analyses and after adjustment for potentially confounding variables such as age, sex, BMI, physical activity, and calcium intake, we found that circulating concentrations of 25(OH)D were inversely associated with total and primary fecal bile acid levels. There are several potential mechanisms of actions that aid in the understanding of this relationship, as outlined in Fig. 1 and discussed further here. The VDR is now recognized as a bile acid sensor, and binding of either LCA or the potent vitamin D metabolite, 1,25$(OH)_2$D, activates the VDR. In fact, the crystal structure of the VDR ligand–binding pocket in a complex with LCA and an SRC-2 coactivator peptide dramatically reveals the binding of two LCA molecules to VDR. One LCA molecule binds in the ligand-binding pocket, and a second LCA is bound to a site located on the VDR surface (35). When associated with either the 1,25$(OH)_2$D and/or LCA ligand(s), activated VDR increases the hepatic expression of the cytochrome P450 enzyme CYP3A4 (14, 17, 18; Fig. 1). CYP3A4-catalyzed hydroxylation of bile acids is a key step in reducing the hydrophobicity, and thus the toxicity, of bile acids (36). Therefore, activation of CYP3A4 by either LCA- or 1,25$(OH)_2$D-binding to the VDR commences catabolism of LCA and other bile acids, ultimately reducing bile acid concentrations (37), despite the fact that CDCA, CA and other bile acids do not themselves bind VDR (14). The key role for CYP3A4 in mediating detoxification of bile acids, via a mechanism that is dependent on VDR, was recently demonstrated in a study with mice that possess an intestine-specific disruption of VDR ($Vdr^{\Delta IEpC}$) and exhibit a significant accumulation in bile acid levels. When $Vdr^{\Delta IEpC}$ mice are crossed with a CYP3A4-humanized mouse line that expresses high levels of CYP3A4 in the gut, the resultant $Vdr^{\Delta IEpC}$/CYP3A4 hybrid strain does not exhibit accumulation of bile acids, along with a reversal of LCA-induced hepatotoxicity (38). Thus, the interplay between VDR and CYP3A4 is critical to the likely *in vivo* role of VDR as a bona fide bile acid sensor that helps to effect BA homeostasis (Fig. 1).

In the current work, although a significant link was observed between 25(OH)D and bile acids levels, no association between circulating concentrations of the primary VDR ligand, 1,25$(OH)_2$D, and any bile acid was observed. Potential reasons for this include the homeostatic control of 1,25$(OH)_2$D in

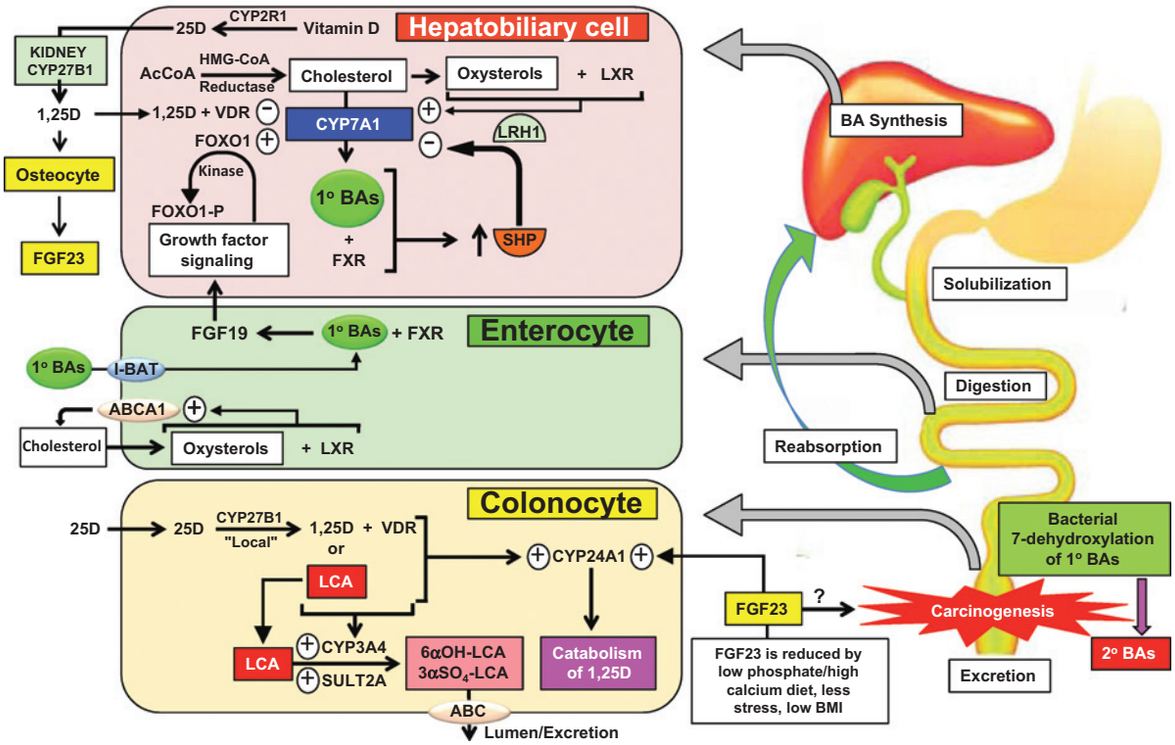

Figure 1.
Regulation of cholesterol, bile acid, and vitamin D metabolism. An integrative model illustrating the interplay between vitamin D and bile acid metabolism, as mediated by nuclear receptors/transcription factors, and the potential mechanisms that may drive colon carcinogenesis. The first committed/rate-limiting step in the synthesis of bile acids from cholesterol is the introduction of a hydroxyl group at carbon #7 of the sterol ring by the enzyme cholesterol 7α-hydroxylase (encoded by *CYP7A1*). Catabolism of cholesterol to bile acids is regulated by: (i) oxysterols that induce the expression of *CYP7A1* via the liganding of LXRα in cooperation with LRH-1 to feed-forward enhance bile acid synthesis, and (ii) bile acids that repress the expression of *CYP7A1* via binding to FXR to induce SHP, which in turn neutralizes LRH-1, ultimately inhibiting bile acid synthesis. Other transcription factors/nuclear receptors, besides LXRα and FXR that regulate the expression of *CYP7A1* are: LRH-1, SHP, VDR, RXR, and FOXO1. FOXO1 is phosphorylated in response to FGF19 and insulin signaling, and removed from the nucleus to abolish its induction of *CYP7A1*. Thus, feedback repression of *CYP7A1* is regulated by a trio of nuclear receptors: FXR, SHP, LRH-1, plus phosphorylation/dephosphorylation of FOXO1. The sequence of molecular events is as follows: Bile acids bind to FXR, the primary bile acid sensor, inducing the transcription of *SHP* in liver, and *FGF19* in small intestine. Elevated SHP protein inactivates LRH-1 by forming heteromeric complex to elicit promoter-specific repression of *CYP7A1* and *SHP*. In the intestine, FGF19 signals that the gut is processing large amounts of bile acids, and FGF19 circulates to the liver to signal the activation of a kinase that phosphorylates FOXO1 (thereby excluding it from the nucleus) to suppress hepatobiliary *CYP7A1* expression. Insulin similarly signals the activation of phosphorylation of FOXO1 in liver to repress *CYP7A1*; thus, insulin increases cholesterol levels. Vitamin D is converted to 25(OH)D by CYP2R1 in the liver, and circulating 25(OH)D is metabolized to the $1,25(OH)_2D$ hormone by CYP27B1 in kidney and extrarenal tissues such as colon. VDR is also a bile acid sensor that, when liganded, represses *CYP7A1* in liver to reduce cholesterol catabolism, as well as triggers LCA detoxification in colon by inducing *CYP3A4* and *SULT2A* when VDR is liganded with either lithocholic acid or $1,25(OH)_2D$. In bone (osteocytes), $1,25(OH)_2D$ induces FGF23, a phosphaturic hormone that induces *CYP24A1* in colon to initiate catabolism of $1,25(OH)_2D$ to inactive metabolites. This latter action of FGF23 tempers the ability of $1,25(OH)_2D$ to reduce colon carcinogenesis through induction of *CYP3A4/SULT2A*, and FGF23 may have additional independent tumor-promoting actions in the colon (41). LXR, liver X receptor; FXR, farnesoid X (bile acid) receptor; LCA, lithocholic acid; BAs, bile acids; ABC, ATP-binding cassette transporters; 1,25D, 1,25-dihydroxyvitamin D; 25D, 25-hydroxyvitamin D.

circulation, which may have limited the variation of this metabolite between study subjects. Second, because many tissues express 1α-hydroxylase, and as such can convert 25(OH)D to $1,25(OH)_2D$ locally rather than systemically, it is possible that the key activity for the $1,25(OH)_2D$-bile acids feedback loop may occur in bursts at the cellular level and cannot be detected via measurement of circulating concentrations. Thus, the relevant biomarker appears to be the 25(OH)D precursor metabolite that serves as a substrate for locally-expressed CYP27B1 to produce intestinal $1,25(OH)_2D$ (Fig. 1). Interestingly, as described above with genetic variation in cholesterol 7α-hydroxylase (*CYP7A1*) and the significant differences in UDCA treatment effect among SNPs in this gene (13), the *CYP27B1* gene also possesses several SNPs that modulate its activity in terms of $1,25(OH)_2D$ synthesis (39), suggesting that genetic variation and identification of relevant multigene SNPs will be vital in personalized medicine approaches to UDCA and/or vitamin D chemoprevention.

In addition to the absence of a relationship between vitamin D metabolites and UDCA and UC concentrations at baseline, in prospective analyses, there was no evidence of effect modification of UDCA by 25(OH)D or $1,25(OH)_2D$ on colorectal adenoma recurrence. These findings are in contrast with a recent publication demonstrating that 25(OH)D concentrations were significantly associated with response to UDCA among patients with primary biliary cirrhosis, with lower concentrations of 25(OH)D observed in non-responders (40). The different study population and endpoints used in that study may account for the contrasting results observed herein, as the primary findings of the UDCA trial

revealed no overall effect of the intervention on recurrent adenomas. Because subsequent analyses revealed that UDCA treatment significantly reduced the risk of an advanced colorectal lesion in men, but not women (12), the prospective assessment of effect modification by vitamin D metabolite concentrations was stratified by sex. Although for men we observed a similar relationship as that previously reported between UDCA and a reduction in risk for advanced adenoma recurrence, no heterogeneity of treatment effect was observed by categories of either 25(OH)D or 1,25 $(OH)_2D$.

In summary, the current work demonstrated a significant inverse association between the vitamin D metabolite 25(OH)D and fecal bile acid concentrations; after adjustment for confounding, these results were largely confined to primary bile acids. These findings support prior work showing that vitamin D has a role in bile acid regulation and metabolism (14, 36, 37), and indicate that vitamin D should be considered as a potential preventive or therapeutic strategy in bile-acid related pathology of the colon (Fig. 1). Conversely, the absence of an association with baseline concentrations of UDCA and the lack of effect modification of UDCA treatment on adenoma recurrence does not support a role for circulating vitamin D metabolites in UDCA-mediated effects on colorectal neoplasia. However, further work regarding the potential role of genetic variation in the vitamin D pathway on UDCA on colorectal cancer prevention or targeted treatment is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: E.T. Jacobs, P. Lance
Development of methodology: E.T. Jacobs
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.S. Alberts, P. Lance
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.T. Jacobs, M.E. Martínez, D.J. Roe, P.W. Jurutka
Writing, review, and/or revision of the manuscript: E.T. Jacobs, M.R. Haussler, L.N. Kohler, P. Lance, M.E. Martínez, D.J. Roe, P.W. Jurutka
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.T. Jacobs, D.S. Alberts, P.W. Jurutka
Study supervision: D.S. Alberts

Grant Support

This work was supported by Public Health Service grants R01CA140285 (to E.T. Jacobs and P.W. Jurutka), and PO1CA041108 (to D.S. Alberts). This work was also supported by P30CA023074 (University of Arizona Cancer Center Support grant).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 3, 2016; revised March 24, 2016; accepted April 13, 2016; published OnlineFirst May 2, 2016.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.
2. Steele CB, Rim SH, Joseph DA, King JB, Seeff LC. Colorectal cancer incidence and screening—United States, 2008 and 2010. MMWR Surveill Summ 2013;62Suppl 3:53–60.
3. Ajouz H, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. World J Surg Oncol 2014;12:164.
4. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mut Res 2005;589:47–65.
5. Barrasa JI, Olmo N, Lizarbe MA, Turnay J. Bile acids in the colon, from healthy to cytotoxic molecules. Toxicol *In Vitro* 2013;27:964–77.
6. Alberts DS, Martinez ME, Hess LM, Einspahr JG, Green SB, Bhattacharyya AK, et al. Phase III trial of ursodeoxycholic acid to prevent colorectal adenoma recurrence. J Natl Cancer Inst 2005;97:846–53.
7. Tung BY, Emond MJ, Haggitt RC, Bronner MP, Kimmey MB, Kowdley KV, et al. Ursodiol use is associated with lower prevalence of colonic neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Ann Intern Med 2001;134:89–95.
8. Serfaty L, De Leusse A, Rosmorduc O, Desaint B, Flejou JF, Chazouilleres O, et al. Ursodeoxycholic acid therapy and the risk of colorectal adenoma in patients with primary biliary cirrhosis: an observational study. Hepatology 2003;38:203–9.
9. Singh S, Khanna S, Pardi DS, Loftus EV Jr, Talwalkar JA. Effect of ursodeoxycholic acid use on the risk of colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease: a systematic review and meta-analysis. Inflam Bowel Dis 2013;19:1631–8.
10. Serfaty L. Chemoprevention of colorectal cancer with ursodeoxycholic acid: pro. Clin Res Hep Gastro 2012;36Suppl 1:S53–60.
11. Carey EJ, Lindor KD. Chemoprevention of colorectal cancer with ursodeoxycholic acid: cons. Clin Res Hep Gastro 2012;36Suppl 1:S61–4.
12. Thompson PA, Wertheim BC, Roe DJ, Ashbeck EL, Jacobs ET, Lance P, et al. Gender modifies the effect of ursodeoxycholic acid in a randomized controlled trial in colorectal adenoma patients. Cancer Prev Res 2009;2:1023–30.
13. Wertheim BC, Smith JW, Fang C, Alberts DS, Lance P, Thompson PA. Risk modification of colorectal adenoma by CYP7A1 polymorphisms and the role of bile acid metabolism in carcinogenesis. Cancer Prev Res 2012;5:197–204.
14. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, et al. Vitamin D receptor as an intestinal bile acid sensor. Science 2002;296:1313–6.
15. Schmidt DR, Holmstrom SR, Fon Tacer K, Bookout AL, Kliewer SA, Mangelsdorf DJ. Regulation of bile acid synthesis by fat-soluble vitamins A and D. J Biol Chem 2010;285:14486–94.
16. Wang Z, Schuetz EG, Xu Y, Thummel KE. Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. J Steroid Biochem Mol Bio 2013;136:54–8.
17. Thompson PD, Jurutka PW, Whitfield GK, Myskowski SM, Eichhorst KR, Dominguez CE, et al. Liganded VDR induces CYP3A4 in small intestinal and colon cancer cells via DR3 and ER6 vitamin D responsive elements. Biochem Biophys Res Comm 2002;299:730–8.
18. Jurutka PW, Thompson PD, Whitfield GK, Eichhorst KR, Hall N, Dominguez CE, et al. Molecular and functional comparison of 1,25-dihydroxyvitamin D(3) and the novel vitamin D receptor ligand, lithocholic acid, in activating transcription of cytochrome P450 3A4. J Cell Biochem 2005;94:917–43.
19. Chen J, Zhao KN, Chen C. The role of CYP3A4 in the biotransformation of bile acids and therapeutic implication for cholestasis. Ann Translational Med 2014;2:7.
20. Hess LM, Krutzsch MF, Guillen J, Chow HH, Einspahr J, Batta AK, et al. Results of a phase I multiple-dose clinical study of ursodeoxycholic acid. Cancer Epidemiol Biomarkers Prev 2004;13:861–7.
21. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. Methods Enzymol 1997;282:174–86.
22. Hollis BW. Assessment of circulating 25(OH)D and 1,25(OH)2D: emergence as clinically important diagnostic tools. Nutr Rev 2007;65:S87–90.
23. Jacobs ET, Alberts DS, Foote JA, Green SB, Hollis BW, Yu Z, et al. Vitamin D insufficiency in southern Arizona. Am J Clin Nutr 2008;87:608–13.
24. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006;81:353–73.
25. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998;351:805–6.

26. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 2005;135:317–22.
27. Martinez ME, Marshall JR, Graver E, Whitacre RC, Woolf K, Ritenbaugh C, et al. Reliability and validity of a self-administered food frequency questionnaire in a chemoprevention trial of adenoma recurrence. Cancer Epidemiol Biomarkers Prev 1999;8:941–6.
28. Staten LK, Taren DL, Howell WH, Tobar M, Poehlman ET, Hill A, et al. Validation of the Arizona Activity Frequency Questionnaire using doubly labeled water. Med Sci Sports Exercise 2001;33: 1959–67.
29. Martinez ME, Sampliner R, Marshall JR, Bhattacharyya AB, Reid ME, Alberts DS. Adenoma characteristics as risk factors for recurrence of advanced adenomas. Gastroenterology 2001;120:1077–83.
30. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. Am J Epidemiol 1989;129:125–37.
31. Staels B, Fonseca VA. Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. Diabetes Care 2009;32 Suppl 2:S237–45.
32. Cook JW, Kennaway EL, Kennaway NM. Production of tumours in mice by deoxycholic acid. Nature 1940;145:627.
33. Tong JL, Ran ZH, Shen J, Fan GQ, Xiao SD. Association between fecal bile acids and colorectal cancer: a meta-analysis of observational studies. Yonsei Med J 2008;49:792–803.
34. Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. Curr Opinion Gastroenterol 2014;30:332–8.
35. Belorusova AY, Eberhardt J, Potier N, Stote RH, Dejaegere A, Rochel N. Structural insights into the molecular mechanism of vitamin D receptor activation by lithocholic acid involving a new mode of ligand recognition. J Med Chem 2014;57:4710–9.
36. Stedman C, Robertson G, Coulter S, Liddle C. Feed-forward regulation of bile acid detoxification by CYP3A4: studies in humanized transgenic mice. J Biol Chem 2004;279:11336–43.
37. Nishida S, Ozeki J, Makishima M. Modulation of bile acid metabolism by 1alpha- hydroxyvitamin D3 administration in mice. Drug Metab Dispos 2009;37:2037–44.
38. Cheng J, Fang ZZ, Kim JH, Krausz KW, Tanaka N, Chiang JY, et al. Intestinal CYP3A4 protects against lithocholic acid-induced hepatotoxicity in intestine-specific VDR- deficient mice. J Lipid Res 2014;55:455–65.
39. Jacobs ET, Van Pelt C, Forster RE, Zaidi W, Hibler EA, Galligan MA, et al. CYP24A1 and CYP27B1 polymorphisms modulate vitamin D metabolism in colon cancer cells. Cancer Res 2013;73:2563–73.
40. Guo GY, Shi YQ, Wang L, Ren X, Han ZY, Guo CC, et al. Serum vitamin D level is associated with disease severity and response to ursodeoxycholic acid in primary biliary cirrhosis. Aliment Pharmacol Ther 2015;42:221–30.
41. Jacobs ET, Martinez ME, Buckmeier J, Lance P, May M, Jurutka P. Circulating fibroblast growth factor-23 is associated with increased risk for metachronous colorectal adenoma. J Carcinogenesis 2011;10:3.