RESEARCH COMMUNICATION

Significance of Fecal Deoxycholic Acid Concentration for Colorectal Tumor Enlargement

Atsuko Kawano[1], Hideki Ishikawa[2], Toshiki Kamano[3], Motonari Kanoh[4], Kazuhiro Sakamoto[5], Tomiyo Nakamura[6], Toru Otani[7], Toshiyuki Sakai[2], Koichi Kono[1]

Abstract

Deoxycholic acid (DCA) has been shown to promote proliferation of colonic carcinoma cells in many fundamental studies. However, no large-scale prospective clinical study providing direct evidence for an association of DCA with progress of colorectal tumor development in humans has been reported to date. To address this question, we conducted a two-step epidemiological study applying enzyme-linked immunosorbent assays to measure fecal cholic acid (CA) and DCA concentrations. Firstly, we compared bile acid concentrations of fecal samples from 366 patients who had multiple colorectal tumors removed endoscopically (tumor group) with those from 24 controls without abnormality in their large intestine (control group). Secondly, the tumor group was followed-up to evaluate the association between fecal bile acid concentrations and recurrence of colorectal tumors four years later. Fecal DCA level in the tumor group were significantly higher than that in the controls, whereas there was no difference in CA levels between the two groups. In the tumor group, a subgroup with high DCA level had higher recurrence risk of large adenomas (> 3 mm) four years later than the low DCA subgroup (odds ratio:1.85, 95% confidence interval: 1.12-3.05). This trend was observed more strongly in the left side colon. In conclusion, a high fecal DCA concentration may be a promoter of colorectal tumor enlargement.

Keywords: Colorectal cancer - bile acids - prospective cohort study - deoxycholic acid

Asian Pacific J Cancer Prev, **11**, 1541-1546

Introduction

In recent years, the incidence of colorectal cancer (CRC) is rapidly increasing in Japan. One of the causes is suggested to be a rapid increase in animal fat intake between 1960 and 1970 (Yang et al., 2002). As the possible mechanism by which an increase in animal fat intake might promote CRC occurrence, involvement of intestinal secondary bile acids has been suggested. An increase in the intake of animal fat leads to the increase in primary bile acids in the large intestine, which results in an elevated level of secondary bile acids through the metabolic action of the intestinal flora. Many reported studies have shown that secondary bile acids promote proliferation of colonic cells in vitro or colon tumor progression in experimental animals (Reddy et al., 1977; Rafter et al., 1986; Lapré and Van der Meer, 1992; Peiffer et al., 1997; Hori et al., 1998; Ochsenkühn et al., 1999; Kozoni et al., 2000; Song et al., 2005).

On the other hand, the results of epidemiological studies in humans are equivocal. The main reasons for this might be failure to completely eliminate the influence of existing CRC on bile acid levels, and the small number of cases studied. In the former case, intestinal bleeding and/or changes in peristalsis and dietary contents may affect the CRC patients' intestinal flora as well as their fecal bile acids concentration. In the latter case, fecal bile acid levels are known to be inconsistent among individuals and to fluctuate largely even in the same individual. Reliable results could, therefore, not be obtained by small sample size.

Furthermore, it remains unclear at which time point bile acids would affect CRC pathogenesis because many of previous studies were performed in patients with advanced cancer. Patients who had colorectal adenoma, precancerous lesion of CRC, removed by colonoscopy are considered to belong to a high-risk group. A prospective cohort study in this group of patients in order to investigate the association between fecal bile acids and adenoma recurrence would provide the answer to this question, i.e., at which time point bile acids may play a role in CRC pathogenesis.

*[1]Division of Preventive and Social Medicine, Department of Hygiene and Public Health, Osaka Medical College, Osaka, [2]Department of Molecular-Targeting Cancer Prevention, Kyoto Prefectural University of Medicine, Kyoto, [3]Medical Health Center, Tokyo Rinkai Hospital, Tokyo, [4]Division of Sports and Health Sciences, Daito Bunka University, Saitama, [5]Department of Coloproctological Surgery, Juntendo University Hospital, Tokyo, [6]Division of Health Sciences, Osaka University Graduate School of Medicine, Osaka, [7]Otani Clinic, Osaka. *For correspondence : cancer@gol.com*

We have already reported a large-scale randomized clinical trial to determine whether dietary fiber and Lactobacillus casei strain Shirota prevent the recurrence of colorectal tumors. At entry of the trial, fecal samples from all participants were obtained. In the present study, we measured fecal bile acids in patients with and without colorectal tumors observed under total colonoscopy, and evaluated the association between fecal bile acids and the recurrence of colorectal tumors.

Materials and Methods

Study design

This study consists of two different designs. One is a cross-sectional study in patients who underwent total colonoscopy at a single institution and the other is a prospective cohort study in patients who had colorectal tumors removed by this procedure. In the former study, a cut-off point was determined in order to divide subjects into high- and low deoxycholic acid (DCA) groups in the latter study, which was performed with data from our randomized clinical trial for cancer prevention.

Subjects

Tumor group: The tumor group refers to the participants of a randomized clinical trial performed at the Osaka Medical Center for Cancer and Cardiovascular Diseases. We have previously described the study in detail.[10] In brief, the subjects were men and women aged 40 to 65 years who had had two or more colorectal tumors (adenomas and/or early cancers) removed by total colonoscopy within three months of recruitment. The patients were randomly allocated into four regimens receiving Lactobacillus casei preparation, wheat bran biscuit, both or neither. The intervention lasted for four years. The enrollment period of this trial was between June 1993 and September 1997. Total colonoscopy for observation was performed two and four years after the start of the regimen. All polyps discovered by this procedure were resected and examined histologically. Dietary assessments were conducted by means of a 3-day diet record at entry by trained nutritionists.

A total of 380 patients completed the trial. However, the following number of patients failed to collect fecal sample at entry: 4 in the L. casei group, 4 in the wheat bran group, 3 in the both-administered group and 3 in the neither-administered group. After excluding these 14 cases, 366 patients (302 men and 64 women) remained for analysis.

Control group: The subjects of the control group were recruited among the patients who presented to the above-mentioned institution within the period of the clinical trial. From a total of 2,026 patients who underwent total colonoscopy between January 1996 and March 1997, 106 men en women aged 40 to 80 years with no abnormality (colorectal tumor, diverticulum or inflammatory bowel disease) observed under this procedure as well as no history of colectomy were selected and invited by mail to participate in the trial. Twenty-four patients consented to give fecal samples.

The study protocol was reviewed and approved by the Ethics Committee of the Osaka Medical Center for Cancer and Cardiovascular Diseases. Written consent was obtained from all patients.

Measurement of fecal bile acids

Fecal sample was collected from each subject as follows. The entire feces of one defecation in the previous evening or in the morning of the first consultation with a nutritionist at entry were directly collected in a black plastic bag covering a bowl. The bag was closed and placed into another transparent plastic bag containing oxygen absorber (AGELESS®) and sealed. Placed in a cooling box with ice, the sample was transported to the hospital. Immediately after arrival, each sample was weighed, to it was added the same amount of water and stirred thoroughly. The entire sample was then frozen at -30°C, freeze-dried under negative pressure, and powdered in a mill. All samples were stored at -30°C until required for analysis performed at Juntendo University. The concentrations of DCA and cholic acid (CA) in each sample were measured by an enzyme-linked immunosorbent assay (ELISA) developed by our collaborator Kamano et al., (1999) using rabbit antisera against DCA and CA. The concentration of each bile acid was adjusted according to the content of water of each sample.

Statistical Analysis

An unpaired Student's t-test and a χ^2 test were performed to compare the tumor group with the control group. For evaluating association of fecal DCA with tumor recurrence, logistic regression models were applied to adjust for age, sex, smoking status, alcohol consumption, and body mass index (BMI). Besides, intakes of calcium, carotene and soluble fiber were added to these factors to adjust data for evaluating association of fecal DCA with colorectal tumor recurrence after four years. For all the analyses, statistical significance was established at $p < 0.05$ for the 2-tailed test. Computation was performed using SPSS® software.

Results

Comparison between the tumor and control groups

Table 1 shows the baseline characteristics of the subjects. The mean age of the tumor group was lower than that of the control group and the former included a greater proportion of men than the latter. The subjects in the tumor group had higher intakes of alcohol and animal protein and less intake of insoluble dietary fiber compared with those in the control group.

Next, we compared the fecal concentrations of CA, DCA, and DCA/CA between the tumor group and control group (Table 2). The concentration of DCA in the tumor group was significantly higher than that in the control group, and the significant difference was also observed in both men and women in a separate analysis by sex. As for the levels of CA and DCA/CA, there was no significant difference between the two groups.

Then, by evaluating the distribution of the fecal DCA concentration in the tumor group and control group,

Table 1. Baseline Characteristics of Subjects and the Amount of Dietary Daily Intake

	Tumor group (n = 366)		Control group (n = 24)		
	Means±SD	(Range)	Means±SD	(Range)	P value
Age, years	55.0±6.1	(40-65)	59.8±10.6	(41-78)	0.04
Male sex, number (%)	302 (82.5%)		13 (54.1%)		0.001
Height, cm	164.1±7.6	(144.0-185.0)	161.2±11.0	(143.0-183.0)	0.22
Body weight, kg	64.1±9.8	(37.0-99.0)	60.4±10.8	(44.0-86.0)	0.11
BMI, kg/ m^2x100	23.8±2.6	(16.8-32.2)	23.3±2.4	(18.7-27.1)	0.25
Alcohol intake, mg/day	31.2±26.4	(0.0-138.0)	17.2±19.0	(0.0-57.5)	0.02
Current smoker, number (%)	165 (45.0%)		6 (25.5%)		0.05
Dietary intake					
Total energy, kcal/day	2109.2±395.7	(851.0-3854.7)	1963.9±400.7	(1174.3-2698.3)	0.08
Fat, g/day	54.5±15.3	(19.2-122.1)	53.8±15.0	(26.5-88.2)	0.82
Calcium, mg/day	650.4±240.3	(186.6-1851.0)	717.4±274.0	(295.0-1325.3)	0.19
Carotenoid, mg/day	2927.9±1687.0	(145.1-11139.4)	3199.1±2053.3	(263.3-7800.3)	0.45
Total dietary fiber, g/day	15.2±4.2	(4.9-32.2)	17.1±7.0	(4.4-33.1)	0.20
Soluble dietary fiber, g/day	3.3±1.2	(0.6-9.4)	3.6±1.4	(0.9-6.4)	0.25
Insoluble dietary fiber, g/day	11.8±3.1	(3.6-22.7)	13.4±5.6	(3.5-26.7)	0.02
Protein, g /day	83.3±16.9	(36.9-136.5)	77.7±20.6	(40.6-108.7)	0.20
Animal protein, g/day	46.8±13.5	(14.2-82.4)	39.8±15.2	(19.2-69.8)	0.04
Vegetable protein, g/day	36.5±7.7	(14.0-69.7)	37.8±10.3	(16.1-57.3)	0.44

Table 2. Bile Acid Concentration (mg/g wet feces) of the Tumor Group Compared to which of the Control Group by Enzyme-Linked Immunosorbent Assay.[a]

	Total (n = 390)			Male (n = 315)			Female (n = 75)		
	Tumor group	Control group	P value	Tumor group	Control group	P value	Tumor group	Control group	P value
Bile acid	(n = 366)	(n = 24)		(n = 302)	(n = 13)		(n = 64)	(n = 11)	
CA[b]	4.82±4.81	3.60±4.27		5.09±5.02	3.41±3.64		3.58±3.43	3.81±5.10	
	(0.33-51.94)	(0.23-14.99)	0.18	(0.33-51.94)	(0.68-11.42)	0.13	(0.38-14.99)	(0.23-14.99)	0.88
DCA[c]	17.28±12.93	9.97±6.07		17.88±13.08	11.78±5.99		14.49±11.91	7.84±5.70	
	(1.40-84.65)	(1.49-22.22)	< 0.001	(1.79-84.65)	(3.29-22.22)	0.004	(1.40-61.66)	(1.49-19.27)	0.007
DCA[c]/CA[b]	7.89±7.35	6.51±5.04		7.70±7.30	5.96±3.96		8.78±7.57	7.18±6.22	
	(0.17-35.93)	(0.26-19.00)	0.21	(0.17-35.93)	(0.94-12.34)	0.15	(0.26-26.33)	(0.26-19.00)	0.45

[a]Values are means±SD. Ranges are given between parenthesis; [b]CA; cholic acid; [c]DCA; deoxycholic acid

Table 3. Incidence of Colorectal Tumor Before Recruitment According to the Fecal DCA[a] Concentration

		DCA[a] (mg / g wet feces)		OR[b]	95% CI
		≤13.8	>13.8		
Control group, number of subjects		18	6	1	
Tumor group, number of subjecs		181	185	2.44	(0.92-6.48)
	Tumor more than 4 lesions	87 (48.0%)	101 (54.5%)	2.64	(0.95-7.33)
	Largest size of tumor > 9mm	81 (44.7%)	89 (48.1%)	3.02	(1.09-8.35)
	High grade tumor[3]	108 (59.6%)	110 (59.4%)	2.31	(0.84-6.38)

[a]DCA: deoxycholic acid; [b] Adjusted for age, gender, body mass index, alcohol and smoking; [c]High grade tumor: adenoma with severe atypia and cancer in situ

we determined a cut-off point between these groups as 13.8 mg/g wet feces at which the sum of sensitivity and specificity was its maximum. According to this cut-off point, the subjects of the tumor group were divided into two subgroups; the "above 13.8" group refers to the patients whose fecal DCA concentration was higher than 13.8 mg/g wet feces, while the "under 13.8" group refers to those with fecal DCA concentration of 13.8 mg/g wet feces or below.

Characteristics of tumors, i.e., the number, size and histology of adenomas, detected by the initial colonoscopy before recruitment were evaluated according to the fecal DCA concentration (Table 3). Among the subjects who had tumor(s) larger than 9 mm, those with DCA "above 13.8" had significantly higher odds ratio (OR) of 3.02 (95% confidence interval (CI) 1.09-8.35) than that in the controls after adjustment for age, sex, BMI, smoking status, and alcohol intake. The OR of the size of tumor was higher than that of the number or the grade of atypia.

Evaluation of the tumor group according to the fecal DCA concentration

Table 4 shows the clinical characteristics in the tumor group according to the fecal DCA level. The "above 13.8" group included a significantly greater proportion of men than the "under 13.8" group. Furthermore, the mean body weight was higher and the intakes of calcium, carotene and dietary fiber were lower in the "above 13.8" group compared with the "under 13.8" group. There was no difference in alcohol intake, smoking status, and intakes of other nutrients between the two groups.

Prospective cohort study in the tumor group

Recurrence of colorectal tumor observed four years

Table 4. Characteristics and Dietary Daily Intake in the Tumor Group According to Fecal DCA[a] Concentration

	DCA[a] (mg/g wet feces)				
	≤13.8 (n=181)		>13.8 (n=185)		
	Means±SD	(Range)	Means±SD	(Range)	P value
Age, years	55.5±6.0	(40-65)	54.5±6.1	(42-65)	0.13
Male sex, number (%)	144 (79.5%)		158 (85.4%)		0.009
Height, cm	163.6±7.9	(144.0-185.0)	164.8±7.4	(144.0-182.0)	0.16
Weight, kg	63.3±9.8	(37.0-98.0)	65.5±9.9	(43.0-99.0)	0.04
BMI, kg/ m^2x100	23.5±2.5	(16.8-32.2)	24.0±2.7	(18.0-32.0)	0.07
Alcohol intake, mg/day	29.4±25.9	(0.0-138.0)	33.5±26.6	(0.0-115.0)	0.15
Current smorker, number (%)	77 (42.5%)		83 (44.8%)		0.83
Dietary intake					
Total energy, kcal/day	2097.0±412.4	(851.0-3273.1)	2121.1±379.6	(1157.3-3854.7)	0.56
Fat, g/day	54.5±16.3	(25.3-122.1)	54.5±14.4	(19.2-99.5)	0.96
Fat/total energy, %	23.4±5.3	(12.5-49.5)	23.2±5.1	(10.1±35.5)	0.69
Calcium, mg/day	690.7±254.1	(186.6-1851.0)	616.3±219.8	(191.0-1403.7)	0.002
Carotenoid, mg/day	3200.6±1815.3	(323.3-11139.4)	2664.1±1511.4	(145.1-8011.8)	0.002
Total dietary fiber, g/day	15.8±4.3	(4.9-32.2)	14.6±4.0	(4.9-27.3)	0.01
Soluble dietary fiber, g/day	3.5±1.2	(1.1-9.4)	3.2±1.1	(0.6-6.4)	0.005
Insoluble dietary fiber, g/day	12.2±3.2	(3.6-22.7)	11.4±3.1	(4.0-20.9)	0.02
Protein, g/day	84.4±17.1	(36.9-125.0)	82.9±17.0	(43.7-136.5)	0.41
Animal protein, g/day	45.5±13.8	(14.9-81.3)	46.4±13.4	(14.2-82.4)	0.41
Vegetable protein, g/day	36.8±7.6	(14.0-58.5)	36.5±7.7	(18.0-69.7)	0.70

[a]DCA; deoxycholic acid

Table 5. Recurrence of Colorectal Tumor on the 4th Year After the Intervention

	DCA[a] (mg/g wet feces)		Relative risk[b]	95% CI	OR[b,c]	95% CI	OR[b,d]	95% CI
	≤13.8 (n=181)	>13.8 (n=185)						
Number of tumors At least one	89 (49.1%)	102 (55.1%)	1.12	(0.92-1.36)	1.34	(0.87-2.06)	1.32	(0.85-2.05)
Number of tumors > 3	11 (6.1%)	12 (6.4%)	1.06	(0.48-2.36)	1.02	(0.42-2.49)	1.02	(0.41-2.52)
Tumor with moderate to sever atypia	67 (37.15%)	78 (42.2%)	1.13	(0.88-1.47)	1.32	(0.85-2.06)	1.3	(0.83-2.03)
Size of tumor > 3 mm	38 (20.9%)	60 (32.4%)	1.54	(1.08-2.19)	1.82	(1.11-2.96)	1.85	(1.12-3.05)
Right side colon[e]	25 (13.8%)	37 (20.0%)	1.44	(0.91-2.30)	1.45	(0.82-2.57)	1.55	(0.86-2.78)
Left side colon[f]	15 (8.2%)	28 (15.1%)	1.82	(1.00-3.30)	1.98	(1.00-3.92)	2.07	(1.02-4.21)
Rectum	6 (3.3%)	7 (3.7%)	1.14	(0.39-3.33)	1.35	(0.42-4.36)	1.22	(0.35-4.21)
Size of tumor > 9 mm	1 (0.5%)	6 (3.2%)	5.87	(0.71-48.27)	9.92	(0.97-100.75)	11.54	(1.16-114.97)

[a] DCA: deoxycholic acid; [b] The relative risk and OR as compared with the group with DCA ≤ 13.8; [c] Adjusted for age, gender, BMI, alcohol, smoking and intervention group; [d] Adjusted for age, gender, BMI, alcohol, smoking, intervention group, calcium intake, carotene intake and soluble dietary fiber intake.; [e] Right side colon: cecum, ascending colon and transverse colon; [f] Left side colon: descending colon and sigmoid colon

after the collection of fecal samples in the tumor group is shown in Table 5. The recurrence risk of a large-sized adenoma (> 3 mm) in the "above 13.8" group was significantly higher than that in the "under 13.8" group. This trend was not changed after adjustment for age, sex, BMI, alcohol intake, smoking status, intakes of calcium, carotene, soluble dietary fiber, and intervention group. Especially, the relative risk of developing tumor larger than 9 mm was markedly higher in the "above 13.8" group; the adjusted OR was 11.54 (95% CI: 1.16-114.97). With regard to the site, we observed stronger association between fecal DCA level and tumor larger than 3 mm in the left side colon, i.e., descending colon and sigmoid colon. There was no significant difference between the two subgroups in the number of tumors or the grade of atypia.

Discussion

This epidemiological study demonstrated that a high fecal DCA concentration was strongly associated with the progress of colorectal adenoma, known precancerous lesion of CRC.

Animal experiments using rodents administered chemical carcinogens suggested that secondary bile acids, mainly deoxycholic acid and lithocholic acid (LCA), could have the ability to promote colorectal tumors (Reddy et al., 1977; Rafter et al., 1986; Hori et al., 1998; Kozoni et al., 2000). Some experimental studies showed that bile acids, especially secondary bile acids, could disrupt the integrity of the colonic mucosal cell membrane and the increased cell loss would stimulate compensatory cell renewal by increased mucosal proliferation (Lapré and Van der Meer, 1992; Peiffer et al., 1997; Song et al., 2005).

On the other hand, the results of clinical studies investigating a relationship between fecal bile acids and CRC are inconsistent. Three case-control studies reported that total fecal bile acids promoted the development of CRC and/or colorectal adenoma (Hill et al., 1975; Mudd et al., 1980; Murray et al; 1980), and two studies reported similar results with secondary bile acids (Reddy et al., 1975; Reddy and Wynder, 1977). Owen et al. reported the association of LCA/DCA with the risk of CRC and/or

adenoma, suggesting LCA/DCA as a beneficial biomarker (Owen et al., 1986; 1987; 1992; Little et al., 2002). However, there are six reports finding no association of the concentration of total fecal bile acids or each bile acid with CRC (Kaibara et al., 1983; Tanida et al., 1984; Breuer et al., 1985; Imray et al., 1992; Roy et al., 1999; Meance et al., 2003). One nested case-control study was reported as a prospective study, showing no significant association of total fecal bile acids concentration, DCA concentration or LCA/DCA with CRC occurrence (Haines et al., 2000). The conceivable reasons for this inconsistency are small sample size of cases, possible influence of existing cancer or adenoma on the patient's fecal bile acid composition, and extreme difficulty in performing a highly accurate measurement of fecal bile acids.

In contrast, our study has the following strong points. The number of cases was sufficiently large. Also the influence of existing tumor was completely eliminated by excluding patients with advanced cancer or those with a history of colectomy, and by endoscopic removal of all adenomas several weeks before fecal samples were collected. Moreover, the entire feces obtained from one defecation were processed into homogeneous freeze-dried powder as a whole, which enabled us to perform an accurate measurement. Lastly, the patients with resected colorectal tumors were prospectively followed-up to observe tumor recurrence after the collection of fecal samples. Therefore, our findings obtained from this study are considered to be highly reliable.

Haines et al. reported no association of fecal DCA concentration or LCA/DCA with the risk of CRC in their prospective study (Haines et al., 2000). However, they also observed slightly elevated fecal DCA levels in patients with CRC and their endpoint was CRC alone unlike our study including all tumors (mainly adenomas.) Therefore, the results of these two studies would not be considered to conflict with each other.

Dietary fiber, calcium and carotene, which showed a significant negative correlation with fecal DCA concentration in this study, are suggested to be protective against CRC occurrence in many epidemiological studies. Thus, there is a strong possibility of DCA playing an important role in the risk reduction mechanism by intakes of dietary fiber, calcium and carotene. Dietary fiber may alter the intestinal flora leading to the changes in fecal DCA concentration, whereas detailed mechanisms for calcium and carotene to reduce CRC risk are still unknown.

On the other hand, there are some points to note in this study. Firstly, there is a marked imbalance between the number of men and women in our subjects. This is because the test institution often accepts referrals of company employees through periodic health-check offered by their employer, which is a common practice in Japan. Since the proportion of women in regular employment is still small, this was reflected in our study population. Although the similar trend was observed in separate analysis by sex, the results of this study should be interpreted in women with caution. Secondly, there were some differences between fecal bile acid concentrations previously reported by Kamano (Kamano et al., 1999), who is also a collaborator of this study, and the measurements in the present study. This may be explained by the different treatment of fecal samples. Kamano et al. collected a part of feces and analyzed them directly, whereas we added water to the entire feces, freeze-dried and powdered before analysis. Since all samples in this study were treated in exactly the same way, measurements within this study are considered to be highly comparable.

In conclusion, our study demonstrated in a large human population the association of DCA with the progress of colorectal tumor, particularly tumor enlargement, which was previously implicated by fundamental studies. These findings clarify a part of CRC pathogenesis and also suggest possibility of fecal DCA concentration being high-risk marker for CRC.

Acknowledgments

The authors thank Tomoko Aoyama, Tomoko Saeki and Ayako Nasu for their administrative support, and Kazuko Kimura and Ikuko Takeyama for their dietary instruction. We are also grateful to Kyoko Leuven-Uchiyama for her translating this manuscript.

References

Breuer NF, Dommes P, Jaekel S, et al (1985). Faecal bile acid excretion pattern in colonic cancer patients. *Dig Dis Sci*, **30**, 852-9.

Haines A, Hill MJ, Thompson MH, et al (2000). A prospective study of faecal bile acids and colorectal cancer. *Eur J Cancer Prev*, **9**, 317-23.

Hill MJ, Drasar BS, Williams REO, et al (1975). Fecal bile acids and clostridia in patients with cancer of the large bowel. *Lancet*, **1**, 535-9.

Hori T, Matsumoto K, Sakaitani Y, et al (1998). Effect of dietary deoxycholic acid and cholesterol on fecal steroid concentration and its impact on the colonic crypt cell proliferation in azoxymethane-treated rats. *Cancer Lett*, **124**, 79-84.

Imray CH, Radley S, Davis A, et al (1992). Faecal unconjugated bile acids in patients with colorectal cancer or polyps. *Gut*, **33**, 1239-45.

Ishikawa H, Akedo I, Otani T, et al (2005). Randomized trial of dietary fiber and Lactobacillus casei administration for prevention of colorectal tumors. *Int J Cancer*, **116**, 762-7.

Kaibara N, Sasaki T, Ikeguchi M, et al (1983). Fecal bile acid and neutral sterols in Japanese with large bowel carcinoma. *Oncology*, **40**, 255-8.

Kamano T, Mikami Y, Kurasawa T, et al (1999). Ratio of primary and secondary bile acids in feces: possible marker for colorectal cancer? *Dis Colon Rectum*, **42**, 668-72.

Kozoni V, Tsioulias G, Shiff S, et al (2000). The effect of lithocholic acid on proliferation and apoptosis during the early stages of colon carcinogenesis: differential effect on apoptosis in the presence of a colon carcinogen. *Carcinogenesis*, **21**, 999-1005.

Lapré JA, Van der Meer R (1992). Diet-induced increase of colonic bile acids stimulates lytic activity of fecal water and proliferation of colonic cells. *Carcinogenesis*, **13**, 41-4.

Little J, Owen RW, Femandez F (2002). Asymptomatic colorectal neoplasia and fecal characteristics: a case-control study of subjects participating in the Nottingham fecal occult blood screening trial. *Dis Colon Rectum*, **45**, 1233-41.

Meance S, Boutron-Ruault MC, Myara A, et al (2003). Fecal primary bile acids and serum cholesterol are associated with colorectal adenomas. *Dig Dis Sci*, **48**, 1751-7.

Mudd DG, McKelvey STD, Norwood W, et al (1980). Faecal bile acid concentrations of patients with carcinoma or increased risk of carcinoma in the large bowel. *Gut*, **21**, 587-90.

Murray WR, Blackwood A, Trotter JM, et al (1980). Faecal bile acids and Clostridia in the etiology of colorectal cancer. *Br J Cancer*, **41**, 923-8.

Ochsenkühn T, Bayerdörffer E, Meining A, et al (1999). Colonic mucosal proliferation is related to serum deoxycholic acid levels. *Cancer*, **85**, 1664-9.

Owen RW, Day DW, Thompson MH (1992). Faecal steroids and colorectal cancer: steroid profiles in subjects with adenomatous polyps of the large bowel. *Eur J Cancer Prev*, **1**, 105-12.

Owen RW, Dodo M, Thompson MH, et al (1987). Fecal steroids and colorectal cancer. *Nutr Cancer*, **9**, 73-80.

Owen RW, Henly PJ, Thompson MH, et al (1986). Steroids and cancer: faecal bile acid screening for early detection of cancer risk. *J Steroid Biochem*, **24**, 391-4.

Peiffer LP, Peters DJ, McGarrity TJ (1997). Differential effects of deoxycholic acid on proliferation of neoplastic and differentiated colonocytes in vitro. *Dig Dis Sci*, **42**, 2234-40.

Rafter JJ, Eng VW, Furrer R, et al (1986). Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon. *Gut*, **27**, 1320-9.

Reddy BS, Mastromarino A, Wynder EL (1975). Further leads on metabolic epidemiology of large cancer. *Cancer Res*, **35**, 3403-6.

Reddy BS, Watanabe K, Weisburger JH, et al (1977). Promoting effect of bile acids in colon carcinogenesis in germ-free and conventional F344 rats. *Cancer Res*, **37**, 3238-42.

Reddy BS, Wynder EL (1977). Metabolic epidemiology of colon cancer: fecal bile acids and neutral sterols in colon cancer patients and patients with adenomas polyps. *Cancer*, **39**, 1533-9.

Roy P, Owen RW, Faivre J, et al (1999). Faecal neutral sterols and bile acids in patients with adenomas and large bowel cancer: an ECP case-control study. *Eur J Cancer Prev*, **8**, 409-15.

Song S, Byrd JC, Koo JS, et al (2005). Bile acids induce MUC2 overexpression in human colon carcinoma cells. *Cancer*, **103**, 1606-14.

Tanida N, Hasaka Y, Shimoyama T, et al (1984). Comparison of faecal bile acid profiles between patients with adenomatous polyps of the large bowel and healthy subjects in Japan. *Gut*, **25**, 824-32.

Yang CX, Kuroishi T, Huang XE, et al (2002). Correlation between food consumption and colorectal cancer: an ecological analysis in Japan. *Asian Pac J Cancer Prev*, **3**, 77-83.