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Fecal Bile Acids in Two Japanese Populations with Different Colon Cancer Risks[1]

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ABSTRACT

Some workers have associated fecal bile acids with colon cancer frequency. They suggest that the risk for colon cancer increases with a rise in the level of total and degraded fecal bile acids. The Japanese in Hawaii, who are at high risk for this cancer, had higher concentrations of deoxycholic acid (a degraded bile acid) in their fecal specimens than did the people in Akita, Japan, who are at low risk. However, the findings for the other bile acids were unremarkable or inconsistent. These data were suggestive, but not strongly supportive, of a relationship between fecal bile acid patterns and colon cancer risk.

INTRODUCTION

Several studies have suggested that persons who are at high risk for colon cancer have more total and degraded fecal bile acids, such as deoxycholic and lithocholic acids, than do persons who are at low risk for colonic cancer (8, 17). These studies involved 11 to 26 members of ethnic or diet groups from different countries which included Britain, United States, Uganda, Japan, and India. A recent study, however, gave contrasting results (10). There were no differences in the bile acid patterns between the Danes in Copenhagen, who are at high risk for colon cancer, and the Finns in a rural providence of Finland, who are at low risk.

In the present study, we compared the fecal bile acid patterns in 2 ethnically similar but geographically separate groups with a marked difference in their colon cancer risk pattern.

MATERIALS AND METHODS

Participants of the Japan-Hawaii Cancer Study in Honolulu, Hawaii, and Akita Prefecture, Japan, formed the population base of this investigation (15). Japanese men and women, ages 30 to 74, were randomly selected to submit a stool specimen for bile acid analysis. If a person had a previous resection for a gastrointestinal lesion or was currently receiving antibiotic medication, he or she was disqualified from the study. Over 95% of the qualified selectees agreed to participate. In all, there were 247 participants from Hawaii and 122 participants from Akita.

The method of stool collection has been described elsewhere (2). The donor took home a dry ice container, a polyethylene bag, and a metal toilet-bowl frame. After the fecal specimen was expelled into the bag, it was frozen at −20° until the time of analysis.

Much of the methodology for bile acid analysis in the present paper was derived from the work of Grundy *et al.* (3), with minor modification of the gas chromatography technique to achieve better resolution of the bile acid peaks. Gas chromatographic analysis was carried out on a Tracor Model MT-222 gas chromatograph equipped with flame ionization detectors and a 1.85-m 1% Hi-Eff-8BP, Gas Chrom Q, 100/120 mesh column (Applied Science Laboratories, Inc.). Nitrogen was used as the carrier gas with an 80-ml/min flow rate, and injection temperature was 230° whereas the detector temperature was 240°. Temperature programming was used in the column oven with an initial temperature of 215° and a final oven temperature of 230° programmed at a rate of 1°/min. Resolution was improved by this linear temperature programming, especially the separation of the methyl deoxycholate TMS[3] derivative and the methyl chenodeoxycholate TMS derivative. Individual bile acid peaks were recorded on a Hewlett-Packard Model 338A integrator, and the area under each curve was determined in relation to the response factor of the internal standard.

A calibration curve was determined by using known standards prior to the runs for each day. Seven standards were available from Applied Science. In the order of elution, they were cholic, cholanic, deoxycholic, chenodeoxycholic, hyodeoxycholic, lithocholic, and ursodeoxycholic acids. This mixture of bile acids was converted to the TMS ethers and used each day to check the performance of the column and integrator. Analysis of unknown bile acid mixtures was carried out only when the analysis of this standard bile acid mixture agreed within 10% of previous values. The relative retention times (ratio of peak retention time of specific bile acid to peak retention time of 5α-cholestane) of the unknown bile acids were as follows: bile acid 1, 1.36; bile acid 2, 1.60; bile acid 3, 1.96; bile acid 5, 1.22; bile acid 7, 3.81; bile acid 8, 5.16; and bile acid 9, 2.58.

The primary bile acids, cholic and chenodeoxycholic acids, are degraded to deoxycholic and lithocholic acids, respectively. These degraded or secondary bile acids are then converted further into other degraded bile acids.

A diet recall questionnaire was used to estimate each subject's weekly consumption of different food items found in a Western or Japanese diet. The details of the dietary method were published previously (4). Briefly, it was de-

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[3] The abbreviation used is: TMS, trimethylsilyl.

signed to quantitate the recent intakes of specific food items with the assistance of photographs of weighed small, medium, and large portions of each item.

The blood pressure was recorded on the left arm with the person in a sitting position. Diastolic blood pressure was based on the disappearance of the sound. The hematocrit was based on the micro technique.

The statistical tests used in this paper were the 2-tailed *t* test (Ref. 19, pp. 91–119) the Mantel-Haenszel test (12), and analysis of covariance (Ref. 19, pp. 419–446). Adjustment for age and sex was done simultaneously by the direct method (1).

RESULTS

The comparison of Japanese men and women in Honolulu and Akita by weight, height, blood pressure, and other parameters is given in Table 1. The men in Hawaii were taller, heavier, and had greater hematocrit values than did their counterparts in Japan, who had higher levels of systolic blood pressure. The Japanese women in Hawaii also had slightly greater hematocrit values than did the women in Japan.

The food items in the questionnaire were separated into Western or Japanese foods on an *a priori* basis. The age- and sex-adjusted mean quantitative weekly intake of the different items were then computed and are shown in Table 2. Of the Western foods, the Japanese in Hawaii consumed much more beef, bacon, lettuce, celery, and coffee than did their Japan counterparts. With respect to the Japanese foods, the people in Akita ate much more dried fish, tofu (soy bean curd), rice, hakusaizuke (pickled Chinese cabbage), takuwan (pickled turnip), ume (pickled plum), and genmai cha tea than did Japanese residents in Hawaii. The same pattern of dietary differences was present for both men and women.

Table 1

Age-adjusted comparisons between study subjects in Hawaii and Akita

	Hawaii	Akita	p^a
Men			
No.	165	70	
Av. age	58.1	54.6	
Colon cancer incidence[b]	22.4	5.6	
Ht (cm)	164.7	160.0	<0.001
Wt (kg)	65.6	56.7	<0.001
Systolic blood pressure (mm Hg)	133.9	141.2	0.025
Diastolic blood pressure (mm Hg)	85.3	84.2	0.562
Hematocrit (%)	44.4	43.4	0.042
Women			
No.	82	52	
Av. age	51.7	50.0	
Colon cancer incidence[b]	18.8	5.4	
Ht (cm)	152.0	150.1	0.086
Wt (kg)	54.1	52.0	0.265
Systolic blood pressure (mm Hg)	128.6	132.2	0.300
Diastolic blood pressure (mm Hg)	81.0	80.5	0.795
Hematocrit (%)	40.0	38.6	0.031

[a] Two-tailed *t* test.
[b] Annual age-adjusted incidence per 100,000. From Ref. 20. The rates per Miyagi Prefecture, which adjoins Akita, were used to represent the Akita rates.

Table 2

Age- and sex-adjusted mean weekly consumption (g/week) of Western and Japanese food items by study subjects in Hawaii and Akita

Food item	Hawaii	Akita	p^a
Western			
Beef	151.3	10.1	<0.001
Wieners	30.1	30.6	0.944
Bacon	10.2	1.1	<0.001
Sliced tomatoes	135.6	177.8	0.140
Lettuce	275.2	89.2	<0.001
Celery	21.6	5.8	<0.001
Coffee	2706.3	12.4	<0.001
Milk	622.0	510.7	0.304
Japanese			
Sashimi (raw fish)	48.0	35.3	0.065
Dried fish	16.5	776.0	<0.001
Dried cuttlefish	0.9	1.1	0.764
Kamaboko (fish cake)	20.8	15.6	0.120
Tofu (soy bean curd)	89.1	239.3	<0.001
Rice	1367.1	1970.0	<0.001
Hakusaizuke (pickled Chinese cabbage)	46.8	151.1	<0.001
Takuwan (pickled turnip)	13.5	85.0	<0.001
Ume (pickled plum)	4.6	16.0	<0.001
Tsukudani (seaweed paste)	1.0	1.9	0.098
Green tea	632.3	1047.3	0.046
Genmai cha tea	410.3	1305.7	<0.001

[a] Two-tailed *t* test.

We then compared the bile acid pattern between the 2 groups in Table 3, which gives the age- and sex-adjusted percentage of those with measurable levels of the respective bile acids. More persons in Japan than in Hawaii had measurable levels of lithocholic acid (a degraded bile acid product), chenodeoxycholic acid (a primary bile acid), and ursodeoxycholic acid. With respect to the unknown degraded bile acids, Peak 1 was more commonly found among the people in Hawaii, whereas Peaks 2, 5, and 7 were more common in Japan. For those with measurable levels, we then compared the geometric mean values of the fecal bile acids of the subjects in Hawaii and Japan in Table 4. We used geometric means instead of arithmetic means because of the skewed distribution of most of the bile acid values. The Hawaii men and women had higher mean values for deoxycholic and cholic acids and for unknown Peaks 1, 2, and 7, whereas the Akita subjects had higher values for unknown Peak 8.

DISCUSSION

Because of the work by Hill (5, 6, 8, 9), Reddy (17, 18), and their respective coworkers, much interest has focused on the possible relationship of bile acids to colon cancer development. It has been hypothesized that a Western diet, rich in fatty foods, affects the intestinal bacterial flora and increases bile acids in the colon where degradation of these substances can take place, especially by nuclear dehydrogenating bacteria. Many of the bile acids act as cocarcinogens in animal model systems (16), and it has been postulated that their further bacterial degradation may form carcinogens (5).

To test this hypothesis, we have studied the diet and bile acid patterns of the Japanese in Hawaii and Akita Prefecture, Japan. Because of the wide variation in fecal flora, fecal bile acid composition, and in daily diet, large numbers (247 from Hawaii and 122 from Akita) were included in the study. Marked dietary differences were noted between the 2 population groups. The Hawaii Japanese consumed much more American foods (beef, bacon, lettuce, celery, coffee) but much less Japanese foods (dried fish, tofu, rice, etc.) than did their counterparts in Akita, Japan.

Differences of a lesser magnitude were also present between the Japanese men in Hawaii and Akita with respect to weight, height, and hematocrit values. Similar findings among the men have also been observed by others, using larger population samples (11).

When comparisons were made in fecal bile acid concentrations between the 2 Japanese groups, we noted several important findings. (a) The Hawaii subjects had a much higher concentration of deoxycholic acid than did subjects in Akita Prefecture, although a similar percentage from both groups had measurable amounts of this major degraded bile acid. This was the only finding which was consistent with the work of Hill et al. (8) and Reddy and Wynder (17). (b) Unknown bile acid 1 was found in more specimens in Hawaii and at higher concentrations than in Akita specimens. Further work must be done to isolate and identify this bile acid. (c) More people in Akita than in Hawaii had measurable amounts of the following degraded bile acids: lithocholic acid; ursodeoxycholic acid; and unknown bile acids 2, 5, and 7. On the basis of past studies, this pattern was contrary to expected findings.

Table 3

Age- and sex-adjusted percentages with measurable amounts of specific bile acids among study subjects in Hawaii and Akita

Bile acids	Hawaii	Akita	p^a
Lithocholic acid	75.2	85.8	0.033
Deoxycholic acid	85.3	87.3	0.492
Chenodeoxycholic acid	34.9	51.5	<0.001
Cholic acid	81.3	86.7	0.169
Cholanic acid	79.4	77.7	0.301
Hyodeoxycholic acid	21.0	23.7	0.349
Ursodeoxycholic acid	15.2	28.2	0.008
Unknown bile acid 1	31.6	16.7	0.004
Unknown bile acid 2	16.1	23.6	0.048
Unknown bile acid 3	9.3	8.9	0.418
Unknown bile acid 5	2.8	15.0	0.002
Unknown bile acid 7	2.9	13.3	0.004
Unknown bile acid 8	4.4	5.9	0.225
Unknown bile acid 9	26.1	23.6	0.341

[a] Mantel-Haenszel test.

The bile acid analyses of Hill and Aries (7) and Reddy and Wynder (17) are based upon the gas chromatographic method of Grundy et al. (3). Our fecal bile acid analysis also followed Grundy's procedure. We converted the bile acids to the same TMS ethers and methyl esters used by Grundy, Hill, and Reddy to form stable bile acid derivatives suitable for gas chromatography. Our only departure from the gas chromatographic procedure of these investigators, as described in detail in an earlier paper (14), was in the gas chromatographic separation of the bile acids using temperature programming which increased the separation of the bile acid derivatives.

Past studies (13) have shown that fecal bile acid levels can vary by sex and age. On this basis, we adjusted for these 2 factors in our data analysis. However, sex and age adjustment did not noticeably alter the results in Table 3, except for cholanic acid and unknown Peak 5, or in Table 4, except for unknown Peaks 7 and 8. The unadjusted results for the people in Hawaii and Akita were 80.6 and 74.6%, respectively, for cholanic acid; 3.6 and 13.9% for unknown Peak 5; 0.41 and 0.23 for unknown Peak 7; and 0.26 and 1.04 for unknown Peak 8. Because of the small numbers of subjects (11 to 26) in each tested group by Hill et al. (8) and Reddy and Wynder (17), it is unlikely that they adjusted for differences in age and sex distributions in each studied group.

The present study does not disprove or support a major role of bile acids in the etiology of large-bowel cancer. The possibility exists that colon cancer-prone individuals with

Table 4

Age- and sex-adjusted geometric mean values (mg/g, dry-weight, feces) of bile acids among study subjects with measurable values in Hawaii and Akita

Bile acids	Hawaii	Akita	p^a
Total bile acids	8.61 (0.48, 61.18)[b]	7.45 (0.10, 95.30)	0.187
Lithocholic acid	1.25 (0.20, 17.31)	1.12 (0.01, 11.03)	0.491
Deoxycholic acid	3.64 (0.11, 41.88)	2.18 (0.02, 30.23)	0.001
Chenodeoxycholic acid	2.16 (0.09, 23.19)	1.74 (0.11, 14.57)	0.275
Cholic acid	0.82 (0.01, 35.36)	0.41 (0.01, 24.23)	<0.001
Cholanic acid	0.64 (0.02, 8.10)	0.49 (0.01, 7.35)	0.079
Hyodeoxycholic acid	0.52 (0.01, 3.62)	0.64 (0.02, 18.45)	0.595
Ursodeoxycholic acid	0.62 (0.01, 24.39)	0.70 (0.01, 21.24)	0.710
Unknown bile acid 1	0.20 (0.01, 7.86)	0.09 (0.01, 3.93)	0.046
Unknown bile acid 2	0.15 (0.01, 5.06)	0.07 (0.01, 0.84)	0.031
Unknown bile acid 3	0.25 (0.01, 8.10)	0.10 (0.01, 1.87)	0.128
Unknown bile acid 5	0.38 (0.07, 1.24)	0.29 (0.01, 2.14)	0.638
Unknown bile acid 7	0.59 (0.09, 3.63)	0.19 (0.03, 2.05)	0.076
Unknown bile acid 8	0.18 (0.03, 1.13)	1.98 (0.01, 6.47)	0.025
Unknown bile acid 9	1.45 (0.11, 6.23)	1.75 (0.22, 13.86)	0.434

[a] Analysis of covariance with 2-tailed t test.
[b] Numbers in parentheses, minimum and maximum values.

unusually high bile acid production and bacterially degraded bile acids might be few in number and may have been missed in the random selection of subjects used in this study. However, a more likely explanation is that the increased frequency of colon cancer in the Hawaii Japanese population can be due to an as yet unidentified carcinogen in human fecal specimens.

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