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Effects of Konjac Glucomannan on Putative Risk Factors for Colon Carcinogenesis in Rats Fed a High-Fat Diet

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The aim of this study was to determine effects of konjac glucomannan (KGM) in a high fat corn oil diet on risk factors of colon carcinogenesis, that is, fecal β-glucuronidase, mucinase, and bile acids, and on preventive factors, that is, fecal microflora and cecal short-chain fatty acids (SCFAs). Sprague-Dawley rats (n = 8 animals per group) were fed a normal-fat fiber-free (5% corn oil, w/w) or high-fat (25% corn oil, w/w) diet containing no fiber, KGM (5%, w/w), or inulin (5%, w/w, as a prebiotic control) for 4 weeks. Results indicated that the high-fat fiber-free diet significantly elevated the fecal β-glucuronidase and mucinase activities and total bile acid concentration and decreased cecal SCFA contents, as compared with its normal-fat counterpart. The incorporation of KGM, as well as inulin, into the high-fat fiber-free diet beneficially reduced the fecal β-glucuronidase and mucinase activities and lithocholic acid (secondary bile acid) concentration. Although KGM elevated the daily fecal total bile acid excretion, the change was due to the primary, instead of the secondary, bile acids. In addition, KGM beneficially promoted the daily fecal excretion of bifidobacteria and lactobacilli and cecal SCFA contents, as compared with the high-fat fiber-free diet. Therefore, the present study suggests that KGM potentially attenuated the high fat-induced risk in colon carcinogenesis.

KEYWORDS: Konjac glucomman; β-glucuronidase; mucinase; short-chain fatty acid; microflora; bile acid

INTRODUCTION

Colorectal cancer is among the leading causes of cancer mortality in developed countries (*1*). Epidemiological studies summarize a protective role of dietary fiber-containing foods (*1*). However, mechanisms whereby dietary fiber modulates the colorectal cancer remain to be illustrated. It is generally accepted that dietary fiber may decrease incidence of colorectal cancer by increasing colonic bulk, reducing the transit time (*1*,*2*), and promoting the production of short-chain fatty acids (SCFAs) that have been shown to induce apoptosis, cell cycle arrest, and differentiation of colon cancer cells (*3*, *4*). Recent studies also suggest that probiotics, such as bifidobacteria and lactobacilli, may reduce the colon carcinogenesis (*5*,*6*). The anticarcinogenic effect of soluble dietary fibers may be partially mediated by their prebiotic effects. However, effects of dietary fibers on gut microflora-associated risk factors of colorectal cancer (*7*) such as β-glucuronidase that hydrolyzes procarcinogen into carcinogen (*8*) and mucinase that hydrolyzes the protective mucin coat in the intestinal wall and thus exposes the underlying colonocytes to the luminal carcinogens (*9*) are not fully investigated.

Konjac glucomannan (KGM), consisting of D-glucose and D-mannose units joined together with β-1,4 glycosidic bond linkages, is a fermentable soluble fiber derived from the tubers of *Amorphophallus konjac* C. koch (*10*). Although KGM is traditionally consumed as jelly and noodles in Japan and Taiwan for centuries, it recently is consumed as a functional food since it is shown to improve serum cholesterol and blood glucose levels (*10*, *11*), bowel movement, and colonic microflora (*12*,*13*). Besides these beneficial functions, KGM may prevent the risk of colon carcinogenesis with a normal level of dietary fat by reducing fecal concentration of secondary bile acids in mice (*14*) and the toxicity of fecal water obtained from mice toward a model of colonocytes (*14*, *15*). In addition, addition KGM into a normal-fat diet has been shown to reduce tumorigenesis in rat model of experimentally induced colon cancer (*16*).

Epidemiological studies (*1*) and animal treated with carcinogens (*17*, *18*) demonstrated a positive association between fat intake and colon tumor incidence. A high-fat intake has been shown to increase fecal β-glucuronidase activity and secondary bile acids (*17*, *19*). However, the role of fat on fecal mucinase, microflora, and short-chain fatty acids remains to be investigated. In addition, mechanisms underlying the anticarcinogenic potential of dietary fibers in the matrix of high-fat diet have not been clearly demonstrated. Therefore, the effects of dietary fat (25% vs 5% corn oil, w/w) on potential precancerous risk factors of colon carcinogenesis, that is, fecal β-glucuronidase, mucinase, and secondary bile acids, and their effects on preventive factors, that is, fecal bifidobacteria and lactobacilli and cecal SCFAs, in rats fed a fiber-free diet, were determined in this study. We further determined effects of KGM or inulin (as a prebiotic control) in the high-fat fiber-free diet on these modulated factors of colon carcinogenesis.

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MATERIALS AND METHODS

Animals. Five-week-old Sprague-Dawley rats were obtained from BioLASCO Taiwan Co., Ltd. (Yi-Lan, Taiwan). Animals were housed four per plastic cage in an animal holding room maintained on a 12 h light−dark cycle at 24 ± 1 °C and 50% humidity. All animals were allowed free access to water and food throughout the study. Animal care followed the guidelines of the National Research Council (*20*) and was approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University.

Experimental Design. Rats were fed with a standard rodent diet (Rodent Laboratory chow diet 5001, Purina Co., USA) during the acclimatizing period (1 week) and then randomly divided into four diets (n = 8 animals per group) modified from the AIN-76 diet (*21*): (1) normal-fat fiber-free diet, (2) high-fat (25%, w/w) fiber-free diet, (3) high-fat diet with 5% (w/w) konjac glucomannan (Fukar Co., Taipei, Taiwan), and (4) high-fat diet with 5% (w/w) inulin (Sentosa Co., Taipei, Taiwan). The purity of KGM was 80%, and that of inulin powder was 85.5% as indicated by the manufacturer. The compositions of diets were shown in **Table 1**. In order to reduce the spillage, the powder diet was mixed with an equal weight of distilled water and made into dough. Food intake was determined daily, and body weight was measured twice weekly. Rats were individually housed in plastic cage and then placed into metabolic cages during days 21−28. Feces voided by each rat during days 24−27 were collected in ice-bathed tubes. The lyophilized feces of each animal were individually pooled and pulverized together for preparation of multiday composites and then were stored at −20 °C until analyses for β-glucuronidase and mucinase activities, bile acids, and microflora. Rats were anesthetized with carbon dioxide on day 28 after an overnight fasting. The cecums were dissected, and their contents were scraped with glass slides into a tube. The cecal contents were weighed, immediately frozen in liquid nitrogen, and stored at −20 °C until analysis for SCFAs. Triplicate determinations were carried out for all analyses.

Fecal β-Glucuronidase and Mucinase Activities. Fecal β-glucuronidase activity was measured from the release of 2-nitrophenol from synthetic substrates using the method described by Marteau et al. (*22*). An aliquot (0.5 g) of dry fecal sample was homogenized in 25 mL of phosphate-buffered saline (0.1 mol/L, pH 7.4) for 1 min in an ice bath, followed by centrifugation at 10000*g*, 4 °C, for 10 min. An aliquot (0.5 mL) of supernatant was mixed with 0.25 mL of substrate (52 mmol/L) at 37 °C for 2, 5, and 10 min, respectively. Another set using distilled water to substitute the substrate served as sample blank. The readings of products at 405 nm of samples were subtracted with those of their corresponding sample blanks. The concentrations of products were quantified by comparing to a standard curve of 4-nitrophenol (Sigma). To analyze the fecal mucinase activity, an aliquot (0.5 mL) of supernatant was mixed with 0.5 mL of 0.5% (w/w) porcine gastric mucin (Sigma Chemical Co., St. Louis, MO, USA) at 37 °C for 10 and 15 min, and the product, reducing sugars, was determined by the Smogyi method (*23*). Sample blanks were also used to eliminate the false reading of products. Enzyme activities were calculated from the linear reaction rates. The protein content in the fecal sample was determined by a protein assay reagent (Bio-Rad, Hercules, CA, USA). The enzyme activity was expressed as IU/mg of protein.

Fecal Microflora. Changes in fecal bacteria population were assessed using fluorescence *in situ* hybridization method (FISH) as described previously (*12*, *13*). The genotypic probes targeting 16S rRNA of bacteria were Bif164, Laa1, and Ncib10748, specific for bifidobacteria (*24*), lactobacilli (*25*), and clostridia (*26*), respectively. The nucleic acid stain 4′,6-diamidino-2-phenylindole solution was used for total bacterial counts (*12*, *13*). Probe fluorescence was detected with a Zeiss Axioskop2 microscope (Carl Zeiss, Jena, Germany) fitted for epifluorescence microscope with a 100 W mercury bulb (HBO 103), a 20 × Plan-neofluar objective, a filter set 01, 09, and 20, and a cooled charge-coupled device video camera (MacroFire, Model S99831, Optronics, Goleta, CA). The microbial concentrations are expressed as log counts/g of feces while the proportion of each bacterial genus to total bacteria was calculated using their original counts.

Bile Acids in Fecal Water. Fecal water was prepared with the method described by Rieger et al. (*27*) with slight modification. The lyophilized fecal composites were rehydrated to 3-fold their original fecal wet weight, followed by centrifugation at 36000*g*, 4 °C, for 2 h. The supernatant fluid, fecal water, was used for bile acid analysis. Bile acids were extracted and derivatized according to the method described by Czubayko et al. (*28*) using hyodeoxycholic acid (Sigma) as internal standard. The extraction efficiency of bile acid (determined with hyodeoxycholic acid) was 52.8 ± 0.02%, 55.1 ± 0.04%, 55.3 ± 0.02%, and 50.2 ± 0.04% in the normal-fat fiber-free, high-fat fiber-free, KGM, and inulin group, respectively. Samples were dissolved in cyclohexane before they were injected onto a gas chromatograph (GC-14B; Shimadzu Corp., Kyoto, Japan) fitted with a fused silica column (HP-5, 0.25 mm × 0.25 μm × 60 m; Agilent Technology, Santa Clara, CA), an automatic on-column injection system (AOC-20, Shimadzu Corp.), and a flame ionization detector. The injector and detector temperature was 300 and 320 °C, respectively, and the initial oven temperature was 150 °C for 3 min, increasing to 270 at 30 °C/min increment and then maintained at 270 °C for 64 min, with the carrier, N_2, at 2 mL/min. Peaks area were analyzed with a C-R6A Chromatopac (Shimadzu Corp.). The total bile acid denotes for the sum of cholic, chenodeoxycholic, deoxycholic, and lithocholic acids determined in this study. The proportion of secondary bile acids (percent of total bile acids) denotes for the ratio of the sum of deoxycholic and lithocholic acid concentration to the total bile acid concentration × 100.

Table 1. Composition of Experimental Diets[a]

	normal fat	high fat		
ingredients	fiber-free (g/kg of diet)	fiber-free (g/kg of diet)	KGM (g/kg of diet)	inulin (g/kg of diet)
corn starch	700	500	437.5	441.5
casein	200	200	200	200
corn oil	50	250	250	250
inulin[b]				58.5
KGM[c]			62.5	
methioine	3	3	3	3
choline	2	2	2	2
AIN mineral mix 76-A	35	35	35	35
AIN vitamin mix 76-A	10	10	10	10
total energy (MJ/kg of diet)	17.0	21.1	20.1	20.1

[a] The diets were modified from AIN-76 (*21*). [b] The purity of inulin was 85.5%. [c] The purity of konjac glucomannan was 94.8%.

Cecal Short-Chain Fatty Acids. Cecal contents were analyzed for acetate, propionate, *i*-butyrate, and *n*-butyrate with 4-methyl-*n*-valeric acid (Sigma) as an internal standard as described previously (*29*). After evaporating the ether extract, the SCFAs were dissolved in 10% phosphate solution before being injected into a gas chromatograph (GC-14B; Shimadzu Corp.) fitted with a glass capillary column (0.25 mm × 30 m, Stabilwax-DA; Restek Corp., Bellefonte, PA, USA) and a flame ionization detector. The temperature of injector port and detector was 250 °C, and the initial oven temperature was 100 °C, increasing to 200 at 6 °C/min. The flow rate of carrier, N_2, was 1 mL/min. Peak areas were analyzed with a C-R6A Chromatopac (Shimadzu Corp.).

Statistical Analysis. Data were presented as means ± SE and analyzed using SPSS version 10.0 (SPSS, Inc., Chicago, IL). Fecal bacterial counts were log transformed before statistical analysis. The diet effects were determined using one-way ANOVA followed by the least significant differences (LSD) test. A p value < 0.05 was considered to be statistically significant.

RESULTS

The energy intake, weight gain, and feed efficiency (g of daily weight gain/kJ of daily feed intake) were similar between rats fed the normal-fat fiber-free diet and its high-fat counterpart (**Table 2**). The incorporation of KGM and inulin into the high-fat fiber-free diet significantly suppressed the energy intake by 10.3% (p = 0.002) and 11.6% ($p < 0.001$), respectively, and the body weight gain by more than 30%. In agreement with that, the feed efficiencies of high-fat KGM and inulin diets were significantly lower than that of their fiber-free counterpart.

The high-fat fiber-free diet significantly increased fecal β-glucuronidase and mucinase activity by 145% and 52% as compared with its normal-fat counterpart (**Figure 1**). The incorporation of KGM

Table 2. Effects of Normal-Fat Fiber-Free Diet and High-Fat Diets on Energy Intake, Weight Gain, and Feed Efficiency of Sprague-Dawley Rats[a]

	normal fat	high fat		
	fiber-free	fiber-free	KGM	inulin
feed intake (g/day)	20.0 ± 0.1b	15.1 ± 0.6a	14.7 ± 0.4a	14.2 ± 0.4a
energy intake (kJ/day)	335.9 ± 0.9b	328.4 ± 9.0b	294.7 ± 7.3a	290.4 ± 7.0a
weight gain (g/day)	6.5 ± 0.3b	6.2 ± 0.3b	4.6 ± 0.1a	4.5 ± 0.2a
feed efficiency[b] (%)	1.9 ± 0.1b	1.8 ± 0.1b	1.6 ± 0.1a	1.6 ± 0.1a

[a] Data are expressed as mean ± SE (n = 8 animals per group). Different letters across a row denote significant differences between treatments according to one-way ANOVA followed by LSD test ($p < 0.05$). [b] Feed efficiency (%) = [daily body weight gain (g)/daily feed intake (kJ)] × 100.

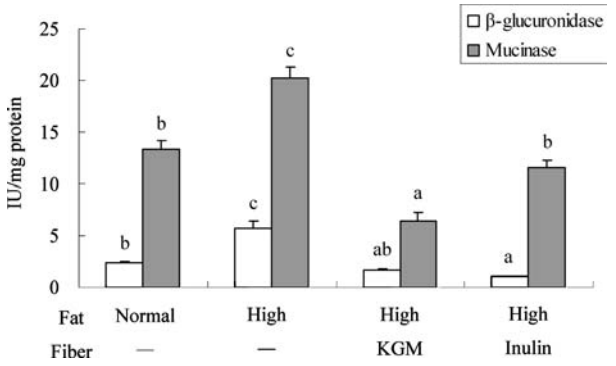

Figure 1. Fecal β-glucuronidase and mucinase activities in rats fed various diets. Different letters denote significant differences across groups for each individual enzyme as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$).

and inulin into the high-fat fiber-free diet significantly reduced β-glucuronidase activity by 71% ($p < 0.001$) and 82% ($p < 0.001$), respectively, to levels similar to that shown in the normal-fat fiber-free diet. The incorporation of KGM and inulin into the high-fat fiber-free diet significantly reduced mucinase activity by 68% ($p < 0.001$) and 43% ($p < 0.001$), respectively, to levels even lower than that shown in the normal-fat fiber-free diet.

The high-fat fiber-free diet increased the concentration of primary bile acids, cholic acid ($p = 0.004$) and chenodeoxycholic acid ($p < 0.001$), and the total bile acids ($p < 0.001$), but not the secondary bile acids (deoxycholic acid and lithocholic acid), as compared with its normal-fat counterpart (**Table 3**). KGM and inulin did not reduce the fecal concentration of total bile acids. However, the fecal cholic acid concentration was greater in the KGM ($p < 0.001$) and inulin ($p = 0.006$) group, respectively, than that in the high-fat fiber-free group, while the lithocholic acid concentration was lower in the KGM ($p = 0.01$) and inulin ($p = 0.048$) group, respectively. Therefore, addition of KGM and inulin significantly reduced the proportion of secondary bile acids to 31.5 ± 3.5% and 31.0 ± 1.6%, respectively, as compared to that (42.6 ± 3.7%) in the high-fat fiber-free group.

The daily fecal mass was similar between rats fed the normal-fat fiber-free diet (1.6 ± 0.2 g/day) and its high-fat counterpart (1.3 ± 0.1 g/day). The incorporation of KGM and inulin into the high-fat diet significantly elevated the fecal output to 1.9 ± 0.1 g/day ($p = 0.005$) and 1.9 ± 0.2 g/day ($p = 0.01$), respectively. Therefore, the effects of various diets on daily fecal output of bile acids were determined (**Table 3**). The high-fat fiber-free diet increased the daily fecal output of cholic acid ($p = 0.04$) and chenodeoxycholic acid ($p = 0.001$) and total bile acids ($p = 0.027$), but not the secondary bile acids, as compared with its normal-fat counterpart. The incorporation of KGM ($p < 0.001$) and inulin ($p < 0.001$) further elevated the fecal output of cholic acid to 3.2- and 2.7-fold that in the high-fat fiber-free group, respectively, and both ($p < 0.05$) elevated fecal output of total bile acids to ~1.5-fold. However, the daily fecal output of lithocholic acid, a secondary bile acid, tended to decrease by the addition of KGM ($p = 0.088$) as compared with its high-fat counterpart.

Table 3. Concentration and Daily Fecal Excretion of Bile Acids in Sprague-Dawley Rats Fed Various Diets[a,b]

	normal fat	high fat		
	fiber-free	fiber-free	KGM	inulin
Concentration (nmol/g of Wet Feces)				
CA	46.4 ± 7.1a	194.2 ± 22.6b	431.5 ± 59.3d	333.8 ± 15.5c
CDCA	38.5 ± 3.5a	332.4 ± 46.2b	250.9 ± 24.3b	333.6 ± 51.0b
DCA (a)	275.1 ± 21.3	290.0 ± 55.9	270.6 ± 28.3	239.2 ± 21.1
LCA (b)	79.2 ± 14.4ab	112.5 ± 27.6b	36.7 ± 10.1a	55.6 ± 21.2a
total[c]	439.2 ± 36.3a	929.2 ± 110.5b	989.7 ± 77.2b	962.1 ± 70.1b
a + b	354.4 ± 30.1	402.5 ± 66.0	307.3 ± 35.8	294.7 ± 20.5
Daily Fecal Excretion (nmol/Day)				
CA	74.1 ± 10.2a	253.9 ± 28.3b	821.4 ± 91.8c	695.7 ± 68.3c
CDCA	61.9 ± 8.1a	437.7 ± 51.4b	504.1 ± 54.1bc	704.6 ± 127.8c
DCA (a)	455.1 ± 55.3	384.9 ± 71.3	495.1 ± 67.5	452.3 ± 81.2
LCA (b)	126.6 ± 19.4	149.5 ± 37.7	74.4 ± 22.2	105.7 ± 36.3
total	717.7 ± 69.8a	1226.1 ± 125.9b	1895.1 ± 144.7c	1958.2 ± 231.9c
a + b	581.6 ± 65.9	534.5 ± 83.4	569.6 ± 84.4	558.0 ± 85.7

[a] Data are presented as mean ± SE (n = 8 animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$). [b] CA, cholic acid, CDCA, chenodeoxycholic acid, DCA, deoxycholic acid, LCA, lithocholic acid. [c] The sum of bile acids determined.

The concentration (log counts/g of feces), the daily fecal output (log counts/daily fecal mass), and proportion of individual genus of bacteria to total bacteria were not different between the normal-fat and the high-fat fiber-free groups (**Table 4**). The incorporation of KGM decreased only the fecal clostridia concentration ($p = 0.007$) and significantly increased the daily fecal output of bifidobacteria ($p = 0.04$) and lactobacilli ($p = 0.006$), as compared with the high-fat fiber-free diet, respectively. The incorporation of inulin into the high-fat fiber-free diet significantly increased the concentration of lactobacilli ($p = 0.041$) and decreased the concentration of clostridia ($p = 0.001$). The inulin group also elevated the daily output of bifidobacteria ($p = 0.004$) and lactobacilli ($p = 0.001$), respectively. Furthermore, KGM ($p = 0.004$) and inulin ($p = 0.002$) significantly decreased the relative ratio of clostridia, respectively, as compared with the high-fat fiber-free diet.

The cecal concentration (μmol/g of cecal content) of propionate and cecal contents (μmol/cecum) of each SCFA (acetate, propionate, *i*-butyrate, and *n*-butyrate) were significantly lower in the high-fat fiber-free group as compared to those in the normal-fat counterpart (**Table 5**). The incorporation of KGM promoted only the cecal *i*-butyrate concentration ($p = 0.037$), as compared with the high-fat fiber-free diet, and elevated the cecal content of each SCFA to the level similar to that shown in the normal-fat fiber-free group. Inulin diet did not significantly elevate the cecal concentration of any SCFA but significantly elevated cecal contents of acetate ($p = 0.014$) and propionate ($p = 0.026$), as compared with the high-fat fiber-free diet.

DISCUSSION

This was the first study to indicate that a high corn oil diet could modulate fecal bacteria mucinase activity and reduced the production of short-chain fatty acids, which may jeopardize the

Table 4. Concentration and Daily Output of Fecal Microflora in Sprague-Dawley Rats Fed Various Diets[a]

	normal fat	high fat		
	fiber-free	fiber-free	KGM	inulin
		log Counts/G of Feces		
bifidobacteria	9.67 ± 0.09a	9.72 ± 0.14ab	9.89 ± 0.08ab	10.03 ± 0.08b
lactobacilli	9.65 ± 0.06a	9.68 ± 0.10a	9.85 ± 0.11ab	9.97 ± 0.08b
clostridia	9.86 ± 0.05bc	10.05 ± 0.08c	9.63 ± 0.13ab	9.46 ± 0.10a
total[b]	10.71 ± 0.03a	11.09 ± 0.09b	11.00 ± 0.09b	10.92 ± 0.08ab
		log Counts/Day of Feces		
bifidobacteria	9.88 ± 0.07ab	9.84 ± 0.15a	10.15 ± 0.08bc	10.32 ± 0.05c
lactobacilli	9.86 ± 0.05a	9.77 ± 0.09a	10.15 ± 0.09b	10.27 ± 0.07b
clostridia	10.07 ± 0.06b	10.16 ± 0.09b	9.88 ± 0.14ab	9.72 ± 0.13a
total	10.92 ± 0.01a	10.87 ± 0.22a	11.30 ± 0.06b	11.22 ± 0.03ab
		% Total Bacteria		
bifidobacteria	9.5 ± 1.6ab	6.5 ± 2.0a	10.0 ± 2.0ab	13.7 ± 0.5b
lactobacilli	8.8 ± 1.1ab	5.2 ± 0.8a	9.1 ± 1.3ab	11.7 ± 2.0b
clostridia	14.6 ± 1.9b	12.6 ± 1.7b	5.2 ± 1.1a	4.6 ± 1.0a

[a] Data are presented as mean ± SE (n = 8 animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$). [b] Total bacteria were quantified by DAPI.

Table 5. Concentration and Total Output of Cecal Short-Chain Fatty Acids in Sprague-Dawley Rats Fed with Various Diets[a]

	normal fat	high fat		
	fiber-free	fiber-free	KGM	inulin
		μmol/g of Cecal Content		
acetate	43.8 ± 3.7	39.2 ± 3.2	49.9 ± 6.7	45.3 ± 4.2
propionate	15.9 ± 0.6b	11.2 ± 1.5a	13.3 ± 1.2ab	13.0 ± 1.3ab
i-butyrate	1.3 ± 0.2ab	0.9 ± 0.1a	1.7 ± 0.5b	0.8 ± 0.1a
n-butyrate	6.0 ± 0.3	5.2 ± 0.8	4.7 ± 0.4	4.7 ± 0.7
total SCFA[b]	67.1 ± 4.4	56.4 ± 5.0	69.6 ± 7.5	69.3 ± 5.7
		μmol/Cecum		
acetate	97.7 ± 19.5b	51.3 ± 9.1a	106.3 ± 8.1b	102.1 ± 15.1b
propionate	35.1 ± 5.6b	13.8 ± 2.6a	31.8 ± 4.9b	30.3 ± 6.1b
i-butyrate	3.2 ± 0.5b	1.1 ± 0.2a	3.2 ± 0.6b	2.1 ± 0.5ab
n-butyrate	12.9 ± 1.5b	6.0 ± 1.0a	11.9 ± 2.2b	11.5 ± 2.8ab
total SCFA	148.9 ± 26.7b	72.1 ± 12.3a	153.2 ± 15.1b	146.1 ± 24.2b

[a] Data are presented as mean ± SE (n = 8 animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by LSD test ($p < 0.05$). [b] Sum of acetate, propionate, *i*-butyrate, and *n*-butyrate.

integrity of colonocytes and subsequently increase the risk of colonic tumorigenesis. In addition, this study agreed with previous studies (*17*, *19*) that high corn oil intake enhanced the fecal bacteria β-glucuronidase activity and concentrations of lithocholic and total bile acids. Therefore, this study found a new underlying mechanism for the carcinogenic effect of high corn oil intake. Although how dietary corn oil increase the mutagenic load of feces remains unclear, authors suggest that low amount of dietary carbohydrate in the high-fat diet may shift colonic bacteria to synthesize β-glucuronidase and mucinase instead of carbohydrate-metabolizing enzymes.

The roles of KGM and inulin in a high corn oil diet on putative risk factors of colon carcinogenesis were first investigated in this study. The current study showed that both KGM and inulin ameliorated the high fat-induced mutagenic load to colonocytes by reducing the colonic bacteria β-glucuronidase and mucinase activities and concentrations of lithocholic acid. In addition, KGM and inulin promoted the growth of colonic bifidobacteria and lactobacilli, in agreement with previous studies (*13*, *29*), and production of short-chain fatty acids. Therefore, the current study suggested that addition of KGM or inulin into a high corn oil diet beneficially decreased the risk of colonic carcinogenesis partially by modulating the colonic bacteria enzyme activities.

The increased fecal bile acid excretion due to a high-fat diet is considered a major risk for colon carcinogenesis (*17*, *18*). The current study examined the fecal bile acid profile and indicated that dietary corn oil mainly increased fecal excretion of primary bile acids, instead of the secondary bile acids. It is known that bacteria 7α-dehydroxylase which converts the primary into the secondary bile acids exists in clostridia (*30*) and eubacteria (*31*) but is not detected in lactobacilli and bifidobacteria (*32*). Since the fecal concentration of clostridia was similar between the normal-fat and the high-fat fiber-free groups, we suppose that the bacteria 7α-dehydroxylase activity may remain similar between these two groups. Therefore, the concentration of secondary bile acid did not increase in the high-fat fiber-free group in the present study.

The use of viscous dietary fibers on colon carcinogenesis prevention is of concern because they may increase the delivery of bile acids to the colon and increase the contact of carcinogenic bile acid to the colonocytes. Results of our study demonstrated that KGM increased the fecal output of total bile acids, in agreement with a previous study (*11*). However, this increased output was due to the primary, instead of the secondary, bile acids. In addition, the conversion of primary to secondary bile acids was reduced by KGM as shown in the reduced proportion of the secondary bile acids. Although mechanisms remained unclear, we proposed that KGM may hinder the contact between primary bile acids and their metabolizing enzymes. Moreover, KGM has been shown to speed up the transit time, which may prevent the primary bile acids from subsequent transformation into toxic metabolites by the intestinal microflora. In addition, the metabolism of primary bile acids was not stimulated by KGM, which was consistent with the unchanged fecal clostridia population. Furthermore, the increased cecal SCFA contents in the KGM group may acidify colon contents which would depress the 7α-dehydroxylase activity (*33*).

Previous studies showed that the incorporation of KGM and inulin into a normal-fat fiber-free diet significantly reduced the fecal water toxicity toward a model of colonocytes and reduced the proportion of secondary to total bile acid in feces (*14*). The current study observed that incorporation of KGM and inulin into the high-fat fiber-free diet significantly decreased the fecal lithocholic acid concentration and slightly modulated the deoxycholic acid concentration. Previous studies have shown that the secondary bile acids, such as lithocholic acid and deoxycholic acid, were cytotoxic for normal colonic mucosa cells and caused a compensatory mucosal proliferation, which is related to higher risk of colon carcinogenesis (*34*). Therefore, the incorporation of KGM and inulin in the high-fat fiber-free diet has the potential to reduce the fecal toxicity toward colonocytes.

The principal metabolites of fermentable fibers are SCFAs, namely, acetate, propionate, and butyrate. The SCFAs, in particular *n*-butyrate, have been shown to be utilized as an energy source in normal intestinal epithelia (*35*), induce apoptosis, and inhibit proliferation of transformed cells (*36*). This study was the first to demonstrate that a high-fat diet resulted in a lower cecal SCFA production than did its normal-fat counterpart, which may lead to a greater risk of colonic carcinogenesis. However, the incorporation of KGM and inulin into this high-fat fiber-free diet almost diminished the fat-induced reduction in the cecal SCFA

contents. Therefore, this result suggests that KGM and inulin may reduce the high fat diet-induced risk of colon carcinogenesis partially by offering the fermentation substrate for colonic microflora, supporting normal intestinal epithelia (*35*) and suppressing the neoplasia.

Rats fed with the high-fat KGM and inulin diets have lower food and caloric intake as compared with their high-fat fiber-free counterpart. It has been reported that energy restriction in animals inhibits the growth of spontaneous or experimentally induced tumors (*37*). The underlying mechanisms for the anticarcinogenic effects of energy restriction include modulating oxidative DNA damage, enhancing DNA repair, and modulation in adrenal metabolism, insulin metabolism, and various aspects of gene expression (*38*). However, the link between energy restriction and the fecal enzyme activities and bile acid levels has never been reported and remains to be investigated.

In conclusion, KGM, as well as its prebiotic control inulin, diminished the high corn oil-induced alterations in the fecal β-glucuronidase and mucinase activities and cecal short-chain fatty acid contents. These soluble fibers also reduced the fecal lithocholic acid concentration and enhanced the fecal output of bifidobacteria and lactobacilli. Therefore, KGM and inulin potentially prevent the risk of colon carcinogenesis associated with a high-fat intake.

ABBREVIATIONS USED

KGM, konjac glucomannan; SCFA, short-chain fatty acid; FISH, fluorescence *in situ* hybridization; DAPI, 4′,6-diamidino-2-phenylindole.

LITERATURE CITED

(1) *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*; American Institute for Cancer Research: Washington, DC, 2007.

(2) Burkitt, D. P. Epidemiology of cancer of the colon and rectum. *Cancer* **1971**, *28*, 3−13.

(3) Coradini, D.; Pellizzaro, C.; Marimpietri, D.; Abolafio, G.; Daidone, M. G. Sodium butyrate modulates cell cycle-related proteins in HT29 human colonic adenocarcinoma cells. *Cell Prolif.* **2000**, *33*, 139−146.

(4) Emenaker, N. J.; Calaf, G. M.; Cox, D.; Basson, M. D.; Qureshi, N. Short-chain fatty acids inhibit invasive human colon cancer by modulating uPA, TIMP-1, TIMP-2, mutant p53, Bcl-2, Bax, p21 and PCNA protein expression in an in vitro cell culture model. *J. Nutr.* **2001**, *131*, 3041s−3046s.

(5) Reddy, B. S.; Rivenson, A. Inhibitory effect of *Bifidobacterium longum* on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline, a food mutagen. *Cancer Res.* **1993**, *53*, 3914−3918.

(6) McIntosh, G. H.; Royle, P. J.; Playne, M. J. A probiotic strain of *L. acidophilus* reduces DMH-induced large intestinal tumors in male Sprague-Dawley rats. *Nutr. Cancer* **1999**, *35*, 153−159.

(7) Hambly, R. J.; Rumney, C. J.; Fletcher, J. M.; Rijken, P. J.; Rowland, I. R. Effects of high- and low-risk diets on gut microflora-associated biomarkers of colon cancer in human flora-associated rats. *Nutr. Cancer* **1997**, *27*, 250−255.

(8) Klinder, A.; Glei, M.; Pool-Zobel, B. Prebiotics and reduction of risk of carcinogenesis: review of experimental and human data. In *Handbook of Prebiotics*; Gibson, G., Roberfroid, M., Eds.; CRC Press: Boca Raton, FL, 2008; pp 295−328.

(9) Miller, R. S.; Hoskins, L. C. Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a "most probable number" method. *Gastroenterology* **1981**, *81*, 759−765.

(10) Doi, K. Effect of konjac fibre (glucomannan) on glucose and lipids. *Eur. J. Clin. Nutr.* **1995**, *49*, s190−s197.

(11) Chen, H.-L.; Sheu, W.-H.; Tai, T.-S.; Liaw, Y.-P.; Chen, Y.-C. Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects—a randomized double-blind trial. *J. Am. Coll. Nutr.* **2003**, *22*, 36−42.

(12) Chen, H.-L.; Cheng, H.-C.; Liu, Y.-J.; Liu, S.-Y.; Wu, W.-T. Konjac acts as a natural laxative by increasing stool bulk and improving colonic ecology in healthy adults. *Nutrition* **2006**, *22*, 1112−1119.

(13) Chen, H.-L.; Cheng, H.-C.; Wu, W.-T.; Liu, Y.-J.; Liu, S.-Y. Supplementation of konjac glucomannan into a low-fiber Chinese diet promoted bowel movement and improved colonic ecology in constipated adults: a placebo-controlled, diet-controlled trial. *J. Am. Coll. Nutr.* **2008**, *27*, 102−108.

(14) Chen, H.-L.; Lin, Y.-M.; Wang, Y.-C. Comparative effects of cellulose and soluble fibers (pectin, konjac glucomannan, inulin) on fecal water toxicity toward Caco-2 cells, fecal bacteria enzymes, bile acid, and short-chain fatty acids. *J. Agric. Food Chem.* **2010** (DOI: 10.1021/jf102127k).

(15) Yeh, S.-L.; Lin, M.-S.; Chen, H.-L. Inhibitory effects of a soluble dietary fiber from *Amorphophallus konjac* on cytotoxicity and DNA damage induced by fecal water in Caco-2 cells. *Planta Med.* **2007**, *73*, 1384−1388.

(16) Mizutani, T.; Mitsuoka, T. Effect of Konjac mannan on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in Fischer 344 rats. *Cancer Lett.* **1983**, *19*, 1−6.

(17) Reddy, B. S.; Mangat, S.; Sheinfil, A.; Weisburger, J. H.; Wynder, E. L. Effect of type and amount of dietary fat and 1,2-dimethylhydrazine on biliary bile acids, fecal bile acids, and neutral sterols in rats. *Cancer Res.* **1977**, *37*, 2132−2137.

(18) Reddy, B. S. Dietary fat and its relationship to large bowel cancer. *Cancer Res.* **1981**, *41*, 3700−3705.

(19) Reddy, B. S.; Mangat, S.; Weisburger, J. H.; Wynder, E. L. Effect of high-risk diets for colon carcinogenesis on intestinal mucosal and bacterial beta-glucuronidase activity in F344 rats. *Cancer Res.* **1977**, *37*, 3533−3536.

(20) *Guide for the Care and Use of Laboratory Animals*; Publication 85-23, rev.; National Research Council, National Institute of Health: Bethesda, MD, 1985.

(21) Report of the American Institute of Nurtition ad hoc committee on standards for nutritional studies. *J. Nutr.* **1977**, *107*, 1340−1348.

(22) Marteau, P.; Pochart, P.; Flourie, B.; Pellier, P.; Santos, L.; Desjeux, J. F.; Rambaud, J. C. Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am. J. Clin. Nutr.* **1990**, *52*, 685−688.

(23) Smogyi, M. Notes on sugar determination. *J. Biol. Chem.* **1952**, *195*, 19−23.

(24) Jansen, G. J.; Wildeboer-Veloo, A. C.; Tonk, R. H.; Franks, A. H.; Welling, G. W. Development and validation of an automated, microscopy-based method for enumeration of groups of intestinal bacteria. *J. Microbiol. Method* **1999**, *37*, 215−221.

(25) Wang, R. F.; Cao, W. W.; Cerniglia, C. E. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Appl. Environ. Microbiol.* **1996**, *62*, 1242−1247.

(26) Nagahama, M.; Nagayasu, K.; Kobayashi, K.; Sakurai, J. Binding component of *Clostridium perfringens* iota-toxin induces endocytosis in Vero cells. *Infect. Immun.* **2002**, *70*, 1909−1914.

(27) Rieger, M. A.; Parlesak, A.; Pool-Zobel, B. L.; Rechkemmer, G.; Bode, C. A diet high in fat and meat but low in dietary fibre increases the genotoxic potential of "faecal water". *Carcinogenesis* **1999**, *20*, 2311−2316.

(28) Czubayko, F.; Beumers, B.; Lammsfuss, S.; Lutjohann, D.; von Bergmann, K. A simplified micro-method for quantification of fecal excretion of neutral and acidic sterols for outpatient studies in humans. *J. Lipid Res.* **1991**, *32*, 1861−1867.

(29) Chen, H.-L.; Fan, Y.-H.; Chen, M.-E.; Chan, Y. Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice. *Nutrition* **2005**, *21*, 1059−1064.

(30) Ridlon, J. M.; Kang, D. J.; Hylemon, P. B. Bile salt biotransformations by human intestinal bacteria. *J. Lipid Res.* **2006**, *47*, 241−259.

(31) Hirano, S.; Nakama, R.; Tamaki, M.; Masuda, N.; Oda, H. Isolation and characterization of thirteen intestinal microorganisms capable of 7 alpha-dehydroxylating bile acids. *Appl. Environ. Microbiol.* **1981**, *41*, 737−745.

(32) Takahashi, T.; Morotomi, M. Absence of cholic acid 7 alpha-dehydroxylase activity in the strains of *Lactobacillus* and *Bifidobacterium*. *J. Dairy Sci.* **1994**, *77*, 3275−3286.
(33) Christl, S. U.; Bartram, H. P.; Paul, A.; Kelber, E.; Scheppach, W.; Kasper, H. Bile acid metabolism by colonic bacteria in continuous culture: effects of starch and pH. *Ann. Nutr. Metab.* **1997**, *41*, 45−51.
(34) Bernstein, H.; Bernstein, C.; Payne, C. M.; Dvorakova, K.; Garewal, H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat. Res.* **2005**, *589*, 47−65.
(35) Roediger, W. E. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **1982**, *83*, 424−429.
(36) Scharlau, D.; Borowicki, A.; Habermann, N.; Hofmann, T.; Klenow, S.; Miene, C.; Munjal, U.; Stein, K.; Glei, M. Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat. Res.* **2009**, *682*, 39−53.
(37) Kritchevsky, D. Colorectal cancer: the role of dietary fat and caloric restriction. *Mutat. Res.* **1993**, *290*, 63−70.
(38) Kritchevsky, D. Caloric restriction and experimental carcinogenesis. *Hybridoma Hybridomics* **2002**, *21*, 147−151.

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