

## Ameliorative effects of konjac glucomannan on human faecal $\beta$ -glucuronidase activity, secondary bile acid levels and faecal water toxicity towards Caco-2 cells

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### Abstract

Konjac glucomannan (KGM) has been shown to increase human colon microbial ecology and reduce faecal toxicity in mice. The main goal of the present study was to assess the effects of a KGM supplement into a low-fibre diet on precancerous markers of colon cancer in a double-blind, placebo- and diet-controlled study. Adult volunteers consumed defined diets supplemented with konjac (4.5 g/d) or placebo (maize starch) for 4 weeks. Stools collected before and at the end of the supplementation were analysed for  $\beta$ -glucosidase,  $\beta$ -galactosidase and  $\beta$ -glucuronidase activities, microflora and bile acids. Faecal water was co-incubated with Caco-2 cells, a model of human colonocytes, to determine the cytotoxicity and DNA-damaging effect as assessed by the comet assay. The results indicated that the KGM supplement significantly decreased faecal  $\beta$ -glucuronidase activity by 25.6 (SE 7.8)% and faecal secondary bile acid level by 42.4 (SE 11.8)%. In contrast, consuming the defined diet supplemented with placebo for 4 weeks did not improve these determinants. The KGM-supplemented diet, but not the placebo diet, significantly increased the survival rate (%) of Caco-2 cells co-incubated with faecal water for 1 and 3 h, respectively. In addition, KGM significantly reduced the DNA damage induced by the faecal water alone or in combination with H<sub>2</sub>O<sub>2</sub>. The faecal bifidobacteria and lactobacilli levels increased only with the KGM-supplemented diet. Therefore, we conclude that supplementation of KGM into a low-fibre diet improved the faecal microbial ecology and metabolites, which may contribute to the reduced toxicity of faecal water and precancerous risk factors of human colon cancer.

**Key words:** Konjac glucomannan:  $\beta$ -Glucuronidase: Bile acids: DNA damage: Cytotoxicity

Colorectal cancer is the third leading cause of cancer mortality in the USA<sup>(1)</sup>. Dietary fibre, the food component that is metabolised mainly in the caecum and colon, has been considered a modulator for incidence of colorectal cancer. However, the results from epidemiological studies have been inconsistent<sup>(2)</sup>. It has been concluded that an increased intake of food containing dietary fibre is associated with a decreased risk of colorectal cancer<sup>(2)</sup>. However, the precise mechanisms for the probable protective role of dietary fibre remain unclear. It has been proposed that dietary fibres dilute faecal toxin content, decrease transit time and increase stool weights<sup>(2)</sup>, all of which decrease the exposure of colonocytes to mutagens. However, it is generally thought that viscous soluble dietary fibres adsorb bile acids and therefore enhance their faecal excretion<sup>(3)</sup>. It is important to clarify the modulatory role of soluble fibres on the faecal secondary bile acid level

since it exerts adverse effects on epithelial barrier function and is thought to be related to tumour promotion<sup>(4,5)</sup>.

Another modulatory role of soluble dietary fibre in faecal mutagen load that needs further study is the bacterial enzymes that hydrolyse pro-carcinogenic glycosides into carcinogenic aglycosides<sup>(6)</sup>. Therefore, soluble dietary fibres may reduce the risk of colorectal cancer partially by reducing faecal carcinogen formation that is modulated by the colonic bacterial profile. Secondly, SCFA, especially butyrate, derived from fermentation of dietary fibres, are shown to increase apoptosis and suppress the growth of colon cancer cells<sup>(7,8)</sup>. Another means for dietary fibres to promote colon health is by increasing the population of probiotics such as bifidobacteria<sup>(9)</sup>.

Konjac glucomannan (KGM), derived from the tuber of *Amorphophallus konjac* C. Koch from the botanical family Araceae, is a well-known soluble dietary fibre

**Abbreviations:** HBSS, Hanks balanced salt solution; KGM, konjac glucomannan.

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consumed in Taiwan, Japan and China. KGM is a  $\beta$ -(1  $\rightarrow$  4)-linked polysaccharide composed of a D-glucosyl and D-mannosyl backbone that is lightly branched, possibly through  $\beta$ -(1  $\rightarrow$  6) glucosyl units<sup>(10)</sup>. The addition of KGM to a low-fibre diet has been shown to normalise bowel movement in constipated children<sup>(11)</sup> and adults<sup>(12)</sup> and to increase faecal weights and SCFA production<sup>(12,13)</sup>, which suggest a protective role of KGM in the colon. In addition, several studies have demonstrated that supplementation of KGM into a fibre-free feed in mice reduced faecal water-induced cytotoxicity and DNA damage effects in Caco-2 cell lines, a model for colonic epithelium cells<sup>(14,15)</sup>. These findings suggest that KGM supplementation can reduce the toxicity of colonic content in humans, and subsequently may exert chemopreventive function for colorectal cancer. However, the roles of KGM in humans on colonic bacterial activity, faecal secondary bile acid levels and faecal water toxicity have never been clarified.

A 10% KGM feed has been shown to reduce the incidence of dimethylhydrazine-induced colon tumours in rats<sup>(16)</sup>. However, this result may not be applicable to humans since the amount of fibre administered to animals is greater than the amount that human subjects could routinely consume. In order to determine the protective effects of KGM on colon cancer risk, the present study determined the effects of a KGM supplement in adults on faecal toxicity towards Caco-2 cells and potential pre-neoplastic risk factors of colorectal cancer, such as faecal toxin-producing enzyme activities and bile acids, in a double-blind, placebo-controlled and diet-controlled trial.

## Methods

### Subjects

Adult volunteers were recruited via advertisements in communities near to Chung Shan Medical University (Taichung City, Taiwan). We recruited healthy adults who passed bowel movement more than three times a week, with normal serum cholesterol concentration (<2400 mg/l), and were willing to comply with the experimental protocol and defined meals. The criteria for exclusion were self-reported lactose intolerance, non-omnivorous diet, habitual consumption of probiotic food and any perception of chronic bowel irregularity. The recruited volunteers were stratified by sex and then were randomly divided into a placebo or KGM group. Each group consisted of one male and fourteen female adults. The mean age was 41.2 (SE 3.6) and 40.0 (SE 3.4) years, respectively, while the BMI was 23.5 (SE 1.0) and 23.9 (SE 1.0) kg/m<sup>2</sup>, respectively, for the volunteers in the placebo and KGM group.

### Experimental design

Before the experimental period, the volunteers were instructed to record their 3 d food intake in order to

assess their energy intake from which the investigators designed their experimental diet. Nutrient analysis was done using local nutrient composition tables<sup>(17)</sup>. The double-blind, placebo-controlled, diet-controlled study consisted of a 4-week placebo or glucomannan (KGM) period. The volunteers consumed 7 d cycle menus of a typical low-fibre Taiwanese diet as described in a previous study<sup>(13)</sup> that met their energy requirement with a typical nutrient pattern of the adult population in Taiwan<sup>(18)</sup>. The volunteers consumed the meals in the experimental unit and were not allowed to consume additional food. The exact energy and nutrient intake during the experiment were determined by subtracting the leftovers from the offered meal. KGM or placebo was taken as capsules (0.5 g/capsule) during each meal with 0.5–1.0 g (one to two capsules) during days 1–7 to prevent potential bowel complication. During the rest of the study, the dose was maintained at 1.5 g (three capsules) per meal, 4.5 g/d, which has been shown effective to promote faecal bifidobacteria growth in human subjects<sup>(12,13)</sup>. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethics Committee of the Chung Shan Medical University Hospital. A written informed consent was obtained from all the subjects.

### Faecal collection

The volunteers each collected voided faeces during the week before and during days 21–28 of the study. The samples were stored in ice buckets and immediately sent to our laboratory. Aliquots (approximately 10%, w/w) of each faeces were homogenised with twofold of deionised water in a blender and were stored at  $-20^{\circ}\text{C}$  until they were lyophilised. The lyophilised faeces from an individual were pooled together to be a faecal composite and stored at  $-20^{\circ}\text{C}$  for further analysis.

### Faecal bacteria enzyme activity

The  $\beta$ -glucosidase,  $\beta$ -galactosidase and  $\beta$ -glucuronidase activities were measured from the release of 2-nitrophenol from synthetic substrate, *p*-nitrophenyl- $\beta$ -D-glucopyranoside, *p*-nitrophenyl- $\beta$ -D-galactopyranoside and 4-nitrophenyl- $\beta$ -D-glucuronide, respectively, as described by Marteau *et al.*<sup>(19)</sup>. An aliquot (0.5 g) of dry faecal composite was sonicated in 15 ml buffer (0.1 M-Na<sub>2</sub>HPO<sub>4</sub>, 0.15 M-NaCl, pH 7.4) in an ice bath for 1 min, followed by centrifugation at 10 000 g, 4°C for 10 min. An aliquot (0.5 ml) of supernatant was mixed with 0.25 ml of substrate (52 mM) at 37°C for 2, 5 and 10 min, respectively, from which the initial reaction rate was determined. The reaction product was measured at 405 nm. The protein contents in the samples were analysed using a protein assay reagent

(Life Science Research, Hercules, CA, USA). The enzyme activity was expressed as  $\mu\text{mol}/\text{min}$  per mg protein.

### Faecal water preparation

Faecal water represents the portion of colonic content that directly contacts the colonic epithelium cells and is a good tool to assess the role of dietary intervention in colon carcinogenesis<sup>(20)</sup>. Faecal water was prepared with the method described by Rieger *et al.*<sup>(20)</sup>, with slight modification. The lyophilised faecal composites were rehydrated to threefold their original faecal wet weight, followed by centrifugation at 36 000 g, 4°C for 2 h. The supernatant fluid, i.e. faecal water, was used immediately for incubation with the Caco-2 cells. The remaining faecal water was stored in  $-20^{\circ}\text{C}$  for bile acids analysis.

### Analysis of bile acids in faecal water

A 50  $\mu\text{g}$  hyodeoxycholic acid (Sigma Chemical Company, St Louis, MO, USA) was added to 0.5 ml faecal water as an internal standard. After a 1 h mild alkaline hydrolysis with 1 ml 5 M-NaOH at 90°C, bile acids were extracted and derivatised as the method described by Czubayko *et al.*<sup>(21)</sup>. The samples were dissolved in 0.5 ml of cyclohexane before they were injected onto a gas chromatograph (GC-14B; Shimadzu Corporation, Tokyo, Japan) fitted with a fused silica column (HP-5, 0.25 mm  $\times$  0.25  $\mu\text{m}$   $\times$  60 m; Agilent Technologies, Inc., Santa Clara, CA, USA), an automatic on-column injection system (AOC-20; Shimadzu Corporation) and a flame ionisation 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°C at 30°C/min increment and then maintained at 270°C for 64 min. The flow rate of the carrier,  $\text{N}_2$ , was 2 ml/min. Peak areas were analysed with a C-R6A Chromatopac (Shimadzu Corporation). Data are expressed as faecal bile acid content:

$$\begin{aligned} \text{Wet faeces } (\mu\text{mol}/\text{g}) \\ &= \text{concentration in faecal water } (\mu\text{mol}/\text{ml}) \\ &\quad \times \text{faecal water ratio } (\text{ml}/\text{g wet faeces}). \end{aligned}$$

### Quantification of faecal microflora

The changes in faecal bacteria population were assessed using the fluorescence *in situ* hybridisation method as described previously<sup>(12,13)</sup>. The genotypic probes targeting 16S rRNA of bacteria were Bif164, Laa1, Bac303 and Ncib10748, specific for bifidobacteria<sup>(22)</sup>, lactobacilli<sup>(23)</sup>, bacteroides<sup>(24)</sup> and clostridia<sup>(25)</sup>, respectively. The nucleic acid stain 4'-6-diamidino-2-phenylindole solution was used for total bacterial counts<sup>(12,13)</sup>. 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  $\times$  Plan-neofluar objective, a filter set 01, 09 and 20, and a cooled charge-coupled device video camera (Macro-Fire, Model S99831; Optronics, Goleta, CA, USA). The bacteria concentrations are expressed as  $\log_{10}$  counts/g wet faeces. The proportion of specific bacteria (% total bacteria) is calculated as the faecal concentration (counts/g faeces) of this genus of bacteria quantified by specific probe divided by that of all bacteria quantified by 4'-6-diamidino-2-phenylindole.

### Cell culture

Caco-2 cells were used as a model of colonocytes to assess the role of the supplement on cytotoxicity and DNA-damaging effect of faecal water as described previously<sup>(14,15)</sup>. The Caco-2 cells were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan, ROC) and were cultured as described previously<sup>(14,15)</sup>. The cells were harvested at approximately 90% confluence and re-suspended in Hank's balanced salt solution (HBSS) at a concentration of  $2 \times 10^6$  cells/ml for further analysis of cytotoxicity and for the comet assay.

### Viability of Caco-2 cells with faecal water

Cytotoxicity of faecal water towards Caco-2 cells was determined for each volunteer in triplicate. The cell suspension (450  $\mu\text{l}$ ,  $2 \times 10^6$  cells/ml HBSS) was incubated with 50  $\mu\text{l}$  HBSS as control or faecal water at 37°C for 1 or 3 h in a gently shaking water-bath<sup>(14)</sup>. The cell survival rate (% to initial cell number) was determined by trypan blue exclusion staining.

### Comet assay

The DNA-damaging effect of faecal water was determined individually by the comet assay, as described previously<sup>(14,15,20)</sup>. Briefly, the Caco-2 cell suspension (450  $\mu\text{l}$ ,  $2 \times 10^6$  cells/ml HBSS) was incubated with 50  $\mu\text{l}$  of HBSS as control or faecal water for 3 h, followed by centrifugation at 850 g. The harvested cells were re-suspended in low-melting-point agarose (in PBS) and were then placed between two layers of normal melting-point agarose on frosted glass microscope slides. Another set of slides was further treated with 75  $\mu\text{M}$ - $\text{H}_2\text{O}_2$  for 5 min on ice. The slides were subjected to electrophoresis (300 mA at 25 V for 25 min) after the cells were lysed and DNA was unwinded. The slides were stained with ethidium bromide for analysis of the comet images using a fluorescence microscope (Olympus/BX51; Center Valley, PA, USA) equipped with the Interactive Image Analysis Comet Assay III (Perceptive Instrument; Haverhill, Suffolk, UK). The DNA strand breaks were expressed as tail moment. Each value presented was calculated from three batches

of experiments, each of which included two slides with at least 200 random comets.

### Statistical analyses

The data were analysed using Statistical Package for Social Sciences version 10.0 (SPSS, Inc., Chicago, IL, USA) and presented as mean values with their standard errors. The concentrations of faecal bacteria were log transformed before analysis. Differences that occurred after 4 weeks of dietary interventions were analysed using paired Student's *t* tests. The relative change (% difference before and at the end of intervention) was compared by the unpaired Student's *t* test. The differences were considered significant at  $P < 0.05$ .

## Results

### Faecal $\beta$ -glucosidase, $\beta$ -galactosidase and $\beta$ -glucuronidase activities

Faecal  $\beta$ -glucosidase,  $\beta$ -galactosidase and  $\beta$ -glucuronidase activities did not significantly change with the placebo-supplemented diet (placebo diet) (Table 1). The KGM-supplemented diet did not modulate faecal  $\beta$ -galactosidase activity. However, the KGM diet significantly increased faecal  $\beta$ -glucosidase activity by 10.5 (SE 5.9)% ( $P = 0.012$ ) and decreased faecal  $\beta$ -glucuronidase activity by 31.5 (SE 7.2)% ( $P = 0.008$ ). The relative change in  $\beta$ -glucuronidase activity (%) caused by the placebo and KGM was significantly different ( $P = 0.001$ ).

### Faecal bile acids

Consuming the placebo diet did not significantly change the level of any bile acid in the faecal water (Table 2). However, on the one hand, the KGM diet significantly decreased the concentration of total secondary bile acids, sum of deoxycholic acid, lithocholic acid and ursodeoxycholic acid, from 1.56 (SE 0.41) to 0.88 (SE 0.20)  $\mu\text{mol/g}$  wet faeces ( $P < 0.001$ ). The lithocholic acid level alone was significantly decreased by 13.18 (SE 0.3)% ( $P = 0.041$ )

with the KGM diet. On the other hand, the KGM diet significantly increased the concentration of total primary bile acids, sum of cholic acid and chenodeoxycholic acid from 0.67 (SE 0.14) to 0.97 (SE 0.18)  $\mu\text{mol/g}$  wet faeces ( $P < 0.001$ ) and the chenodeoxycholic acid alone was significantly increased by 53.9 (SE 14.9)%. The proportion of secondary bile acids to total bile acids was 66.7 (SE 3.3)% in the beginning of the KGM period, which was significantly decreased to 47.4 (SE 4.7)% with the KGM diet. In contrast, the relative proportion of secondary bile acid did not change after 4 weeks of the placebo diet.

### Human faecal water-induced cell death of Caco-2 cells

The cell survival rate (% to original number) with 1 and 3 h treatment of control medium was 95.2 (SE 0.7) and 91.2 (SE 0.8)%, respectively, which were reduced as treated with faecal water from either group of volunteers. Consuming the placebo diet for 4 weeks did not alter the survival rate of the Caco-2 cells co-incubated with 1 h, 90.2 (SE 0.8)% at week 0 *v.* 89.3 (SE 1.1)% at week 4, or 3 h faecal water, 83.4 (SE 0.7)% at week 0 *v.* 82.7 (SE 0.6)% at week 4. In contrast, consuming the KGM diet significantly increased the survival rate of Caco-2 cells co-incubated with either 1 h, 87.5 (SE 0.9)% at week 0 *v.* 90.9 (SE 0.7)% at week 4 ( $P = 0.001$ ), or 3 h faecal water, 81.1 (SE 0.8)% at week 0 *v.* 84.3 (SE 0.9)% at week 4 ( $P = 0.001$ ).

### Human faecal water-induced DNA damage in Caco-2 cells

Consuming the placebo diet for 4 weeks significantly increased the faecal water-induced DNA damage (denoted as tail moment) of Caco-2 cells (week 0 *v.* week 4,  $P < 0.05$ ), implying that the placebo diet increased the DNA-damaging effect of faecal water (Table 3). In contrast, the KGM diet significantly decreased the DNA damage induced by the faecal water (week 0 *v.* week 4,  $P < 0.001$ ). The KGM diet further significantly reduced the tail moment of the Caco-2 cells treated with faecal water in combination with  $\text{H}_2\text{O}_2$  from 54.0 (SE 0.7) to 44.9 (SE 0.8) (week 0 *v.* week 4,  $P < 0.001$ ).

**Table 1.** Faecal bacteria enzyme activities in adult volunteers supplemented with 4 weeks of placebo or konjac glucomannan (Mean values with their standard errors,  $n = 15$ )

	Placebo						Konjac glucomannan					
	Week 0		Week 4		Change $\dagger\dagger$ (%)		Week 0		Week 4		Change (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Faecal enzymes ( $\mu\text{mol/min}$ per mg protein)												
$\beta$ -Glucosidase	7.8	1.1	8.8	1.0	11.5	8.4	9.7	0.9	11.8*	1.2	10.5	5.9
$\beta$ -Galactosidase	10.1	1.6	11.6	1.7	13.8	7.0	13.7	1.3	13.7	1.2	3.7	5.0
$\beta$ -Glucuronidase	5.5	0.8	6.3	1.0	19.9	10.8	7.5	0.9	5.3**	0.8	-31.5**	7.2

Mean values were significantly different between week 4 and week 0 within a group (as analysed by paired Student's *t* test): \* $P < 0.05$ , \*\* $P < 0.01$ .

$\dagger$  Change (%) = (enzyme activity ( $\mu\text{mol/min}$  per mg protein) at week 4 - enzyme activity at week 0)/enzyme activity at week 0.

$\ddagger$  Mean values were significantly different between placebo and KGM groups ( $P < 0.01$ ; Student's *t* test).

**Table 2.** Faecal bile acid profile in adult volunteers supplemented with 4 weeks of placebo or konjac glucomannan(Mean values with their standard errors, *n* 15)

	Wet faeces ( $\mu\text{mol/g}$ )							
	Placebo				Konjac glucomannan			
	Week 0		Week 4		Week 0		Week 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Primary bile acids								
Cholic acid	0.27	0.07	0.23	0.05	0.25	0.05	0.27	0.07
Chenodeoxycholic acid	0.52	0.11	0.40	0.13	0.42	0.11	0.70**	0.13
Secondary bile acids								
Deoxycholic acid	0.92	0.20	0.82	0.13	1.07	0.16	0.61	0.16
Lithocholic acid	0.47	0.15	0.56	0.17	0.28	0.08	0.15*	0.04
Ursodeoxycholic acid	0.12	0.02	0.16	0.03	0.21	0.05	0.12	0.07
Total bile acids	2.30	0.36	2.17	0.34	2.23	0.52	1.84	0.31

Mean values were significantly different between week 4 and week 0 within a group (as analysed by paired Student's *t* test):\**P*<0.05, \*\**P*<0.01.

### Faecal microflora

The placebo diet did not modulate the faecal concentration of any genus of bacteria determined in the present study, while the KGM diet elevated the faecal concentrations of bifidobacteria (week 0 *v.* week 4, *P*=0.038), lactobacilli (week 0 *v.* week 4, *P*=0.005) and total bacteria (week 0 *v.* week 4, *P*=0.036) (Table 4). The KGM diet also increased the relative proportion (% of total bacteria) of faecal bifidobacteria and lactobacilli.

### Characteristics and dietary intake before and during the study

The energy and macronutrients of volunteers between the placebo and the KGM groups were not significantly different (Table 5). The percentages of energy from proteins, carbohydrates and fat were approximately 15, 50 and 35%, with low fibre intake, <12 g/d (excluding the KGM).

### Discussion

This was the first study to show the role of KGM in reducing the risk of human colorectal cancer and suggest

its possible mechanisms. The daily KGM supplement (4.5 g/d) into a low-fibre Taiwanese diet beneficially reduced the faecal  $\beta$ -glucuronidase activity and secondary bile acid level. This soluble dietary fibre further ameliorated human faecal water-induced cytotoxicity and DNA-damaging effects towards Caco-2 cells, a model for colonic epithelium cells.

Instead of a high dose (100 g/kg diet) of KGM used in previous animal studies<sup>(16,26)</sup>, results from the present study showed that daily supplementation of a relatively small amount of KGM was sufficient to reduce some toxin formation by reducing bacterial  $\beta$ -glucuronidase in the human colon. The reduced  $\beta$ -glucuronidase activity is likely to be due to the decreased clostridia population that produce a higher amount of  $\beta$ -glucuronidase than do lactobacteria or bifidobacteria<sup>(27)</sup>. In contrast, the present study did not observe effects of KGM on  $\beta$ -galactosidase activity. The modulatory role of non-digestible carbohydrates in faecal  $\beta$ -glucosidase activity has not been consistent among studies<sup>(28–32)</sup>. Similarly to effects of galacto-oligosaccharide, inulin and resistant starch<sup>(29–32)</sup>, the present study also observed an increase in faecal  $\beta$ -glucosidase activity with supplementation of KGM,

**Table 3.** DNA damage (denoted as tail moment) of Caco-2 cells treated with faecal water only or in combination with H<sub>2</sub>O<sub>2</sub>(Mean values with their standard errors, *n* 15)

	Tail moment (DNA damage score)							
	Placebo				Konjac glucomannan			
	Week 0		Week 4		Week 0		Week 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Faecal water	12.4	0.6	13.1*	0.6	13.8	0.5	11.0***	0.5
Faecal water + 75 $\mu\text{M}$ -H <sub>2</sub> O <sub>2</sub>	49.8	1.7	50.7	1.6	54.0	0.7	44.9***	0.8

Mean values were significantly different between week 4 and week 0 within a group (as analysed by paired Student's *t* test):\**P*<0.05, \*\*\**P*<0.001.



**Table 4.** Changes in faecal bacterial population of adult volunteers supplemented with placebo or konjac glucomannan(Mean values with their standard errors, *n* 15)

	Placebo				Konjac glucomannan			
	Week 0		Week 4		Week 0		Week 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Log <sub>10</sub> counts/g wet faeces								
Bifidobacteria	9.39	0.04	9.42	0.04	9.24	0.09	9.48*	0.09
Lactobacilli	8.86	0.08	8.94	0.10	8.63	0.07	8.90**	0.08
Bacteroides	8.90	0.12	8.94	0.10	8.68	0.09	8.76	0.09
Clostridia	9.32	0.06	9.40	0.05	9.33	0.09	9.24	0.08
Total	10.17	0.03	10.21	0.03	10.17	0.05	10.24*	0.05
Total bacteria (%)								
Bifidobacteria	17.3	1.4	16.6	1.1	10.3	1.3	16.7*	1.8
Lactobacilli	5.8	0.8	6.4	0.9	3.1	0.5	5.3*	0.2
Bacteroides	6.9	0.9	6.1	0.7	3.7	0.6	4.1	0.6
Clostridia	16.1	2.2	16.5	1.5	16.5	2.0	11.2*	1.3

Mean values were significantly different between week 4 and week 0 within a group (as analysed by paired Student's *t* test): \**P*<0.05, \*\**P*<0.01.

while fructo-oligosaccharide and guar gum hydrolysate (galactomannan polymer) do not exert this effect in humans<sup>(28,31)</sup>. In the present study, the KGM diet increased faecal  $\beta$ -glucosidase activity probably because faecal bacteria liberated  $\beta$ -glucosidase to decompose the glucomannan unit of KGM. Therefore, the present study suggests that KGM could reduce the formation of toxins that was mediated by the  $\beta$ -glucuronidase enzyme in the colon.

The present study investigated whether the KGM supplement would influence the level of carcinogenic bile acids by assessing the bile acid profile in the faecal water. The faecal water represents the portion of colonic content that directly contacts the colonic epithelium cells and is a good tool to assess the role of dietary intervention in colon carcinogenesis<sup>(20)</sup>. Previous studies have shown that hydrophilic bile acids are better tools for predicting colonic carcinogenesis than do total bile acids<sup>(33,34)</sup>.

**Table 5.** Daily dietary intake of adult volunteers in placebo or konjac glucomannan group during the present study\*(Mean values with their standard errors, *n* 15)

	Placebo		Konjac glucomannan	
	Mean	SE	Mean	SE
Energy (MJ/d)	7.7	0.1	7.9	0.1
Carbohydrates				
g/d	233.2	3.2	240.5	5.6
%	50.4	0.5	50.4	0.7
Protein				
g/d	68.8	1.1	70.4	2.3
%	14.9	0.2	14.8	0.3
Fat				
g/d	71.7	1.8	73.9	1.7
%	34.8	0.5	34.9	0.7
Dietary fibre† (g/d)	11.1	0.1	11.3	0.2

\* Mean values were not significant different between placebo and konjac glucomannan group.

† Data exclude the dietary fibre from the supplements.

The present study therefore demonstrated the effect of KGM on the concentration and profile of bile acids in the faecal water. In contrast to the promoted effect of KGM on total bile acids in faeces<sup>(35)</sup>, KGM tended to decrease the total bile acids in the faecal water although without a statistical difference from the placebo. Particularly, KGM significantly decreased the secondary bile acid concentrations in the aqueous phase of faeces in the present study. The acidic faecal environment resulting from SCFA, the KGM fermentation products<sup>(13)</sup>, may facilitate the precipitation of hydrophilic deconjugated bile acids and inhibit their enzymatic formation from primary bile acids<sup>(36,37)</sup>. The reduced formation of secondary bile acids in faeces from the volunteers that consumed the KGM supplement suggests a protective role of KGM in human colonic carcinogenesis.

The present study agreed with previous studies that faecal water caused cell death and DNA damage in the human adenocarcinoma-derived cell lines<sup>(14,15,38,39)</sup>. KGM supplementation reduced the cytotoxicity of human faecal water towards Caco-2 cells compared with the placebo in the present study, which agreed with a previous observation in mice<sup>(14,15)</sup>. The present study assessed the viability of the Caco-2 human adenocarcinoma cell by its ability to exclude trypan blue into the cells that indicates the integrity of the cell membrane. The greater cytoprotective effect of KGM may be related to its fermentation products, such as butyrate<sup>(40,41)</sup>. In addition, the KGM supplement reduced the production of secondary bile acids that was shown to cause damage of tight junctions<sup>(40)</sup>, which further contributed to the reduced cytotoxicity of faecal water towards Caco-2 cells. Therefore, we suggest that fermentation of KGM and the modulatory role of KGM on microbial metabolites contribute to the decreased faecal water cytotoxicity.

In the present study, we assessed the acute effects of faecal water on DNA damage of Caco-2 cells. The KGM supplement in adult volunteers demonstrated a protective effect on faecal water-induced DNA damage, which was in agreement with our previous observation in mice<sup>(14,15)</sup>. The result was also supported by previous studies that demonstrated probiotics alone or fermentation of non-digestible oligosaccharides by probiotics ameliorated the genotoxicity of faecal water in human colonic adenocarcinoma cells<sup>(38,39)</sup>. The Caco-2 cells were further co-incubated with faecal water in combination with H<sub>2</sub>O<sub>2</sub> in the present study to determine the protective effect of KGM against oxidative stress. The result indicated that the KGM supplement increased the defensive capacity of faecal water against H<sub>2</sub>O<sub>2</sub>, which may be mediated by free-radical-scavenging ability derived from fermentation of KGM by lactic acid bacteria<sup>(42)</sup>. In addition, butyrate produced from KGM fermentation<sup>(13)</sup> may protect human colon cells from DNA damage as well<sup>(43,44)</sup>.

The present study confirmed the prebiotic role of KGM in healthy adults, i.e. increasing bifidobacteria and lactobacilli, which agreed with our previous observation in mice and in adult human subjects<sup>(12,45)</sup>. Although there is no direct experimental evidence for a cancer-suppressive role of probiotics in human subjects, there is a wealth of evidence emerging from laboratory studies<sup>(9)</sup>. We suggest that KGM supplementation may inhibit faecal toxicity partially by improving the human colonic microflora profile.

In conclusion, the present study suggests that a KGM supplement in healthy adults may beneficially exert chemopreventive effects for human colorectal cancer by a reduction of faecal water toxicity. These effects were likely to be associated with a reduction in  $\beta$ -glucuronidase activity and hydrophilic secondary bile acids and promotion of the colonic probiotic population.

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