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Konjac Glucomannan and Inulin Systematically Modulate Antioxidant Defense in Rats Fed a High-Fat Fiber-free Diet

Wen-Tzu Wu[†] and Hsiao-Ling Chen[*,†,‡]

[†]School of Nutrition, Chung Shan Medical University, Taichung, Taiwan

[‡]Department of Nutrition, Chung Shan Medical University Hospital, Taichung, Taiwan

ABSTRACT: The aim of this study was to investigate the effects of konjac glucomannan (KGM) and inulin on the balance between pro-oxidative status and antioxidative defense systems in the colon, liver, and plasma of rats fed a high-fat fiber-free diet. Male Sprague−Dawley rats (n = 8 animals per group) were fed a high-fat (25% corn oil, w/w) fiber-free diet or that supplemented with KGM or inulin fiber (5%, w/w) for 4 weeks. The index of pro-oxidative status, malondialdehyde (MDA), and blood lymphocyte DNA damage; the antioxidative defense, that is, antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase) in the colonic mucosa and liver; and the plasma antioxidant levels were determined. The fermentation of fiber was shown in fecal short-chain fatty acids. Incorporation of KGM and inulin into the high-fat fiber-free diet beneficially reduced the MDA levels of the colon and liver and DNA damage in blood lymphocytes. On the other hand, both fibers enhanced the antioxidative defense systems by up-regulating the gene expressions of glutathione peroxidase and catalase in the colonic mucosa and of superoxide dismutase and catalase in the liver. Furthermore, KGM and inulin promoted antioxidative status in the blood by elevating the α-tocopherol level. KGM and inulin were well-fermented in rats and increased the concentration and daily excretion of fecal short-chain fatty acids, especially acetate and butyrate. These results suggest that in vivo utilization of KGM and inulin stimulated both local and systemic antioxidative defense systems in rats.

KEYWORDS: konjac glucomannan, inulin, malondialdehyde, DNA damage, antioxidant enzymes, short-chain fatty acid

INTRODUCTION

Free radical damage is believed to cause cell and tissue damage that ultimately results in morbidities such as cancer and cardiovascular disease.[1,2] Studies have been conducted to explore the antioxidative capacities of phytochemicals such as flavanols, flavonols, and anthocyanidins.[3−5] However, the role of a major component of plants, dietary fiber, in eliminating the free radicals and ultimately defending against oxidative stress has been rarely studied.

Dietary fibers are metabolized in the colon, where they beneficially modulate the microflora profile and bowel habit.[6] In addition, some dietary fibers are considered to exert beneficial physiological functions beyond the digestive system, such as improving lipid metabolism in the liver[7] and reducing blood inflammation indices in diabetic patients.[8] The colon, the interface between the large population of microflora and the colonic content, is constantly challenged by either the diet-derived or endogenous reactive oxidative substances (ROS). An imbalance in the cellular redox system leading to elevated ROS level in the colon causes gastrointestinal tract dysfunction and even colorectal cancer.[9,10] Recent studies have shown that polysaccharides derived from a tropical fruit, litchi (*Litchi chinensis* Sonn.), exerted in vitro free radical scavenging effects.[11] Xanthan gum, a soluble dietary fiber, has been shown to prevent the autoxidation of soybean oil and to exert iron-chelating ability.[12] These studies suggest an antioxidative capacity for dietary fibers. However, the antioxidative capacity derived from fermentation of dietary fiber is not well established.

Konjac glucomannan (KGM), consisting of D-glucose and D-mannose units joined together with β-1,4 glycosidic bond linkages, is a highly polymerized viscous fiber derived from the tubers of *Amorphophallus konjac* C. Koch.[13] KGM has been made into jelly and noodles that are commonly consumed in Japan and Taiwan. Inulin, a mixture of fructo-oligosaccharides, derived from the tuber of chicory (*Cichorium intybus*), is another purified dietary fiber that is widely used as a supplement in foods. We previously reported that fermentation of KGM, as well as oligofructose, by several lactic acid bacteria exerted in vitro antioxidative capacities[14] and that supplementation of KGM and inulin into a fiber-free diet reduced the DNA damaging effect of fecal water toward Caco-2 cells, a model of human colonocytes.[15] These studies suggest that KGM and inulin may reduce oxidative stress in the colon. Furthermore, because the fermentation products of soluble dietary fibers may be carried to the liver and the circulation system, KGM and inulin may modulate the redox status beyond the digestive system, which has never been investigated.

The aim of the present study was to determine the effects of KGM and inulin on the balance between pro-oxidative status and antioxidative defense system in Sprague−Dawley rats fed a high-fat fiber-free diet. Lymphocyte DNA damage and the lipid oxidation product, malondialdehyde (MDA), in the colonic tissues, liver, and plasma reflect pro-oxidative status. Antioxidative defense systems were assessed by measuring plasma antioxidant levels and through expression of antioxidant enzymes in the proximal and distal colonic mucosa cells and in the liver.

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Table 1. Composition (Grams per Kilogram of Diet) of Experimental Diets[a]

ingredient	fiber-free	KGM	inulin
corn starch	500	437.5	441.5
casein	200	200	200
corn oil	250	250	250
inulin[b]			58.5
KGM[c]		62.5	
methionine	3	3	3
choline	2	2	2
AIN Mineral Mix 76-A	35	35	35
AIN Vitamin Mix 76-A	10	10	10
total energy (MJ/kg diet)	21.1	20.1	20.1

[a] The diets were modified from AIN-76.[17] [b] The purity of inulin was 85.5%. [c] The purity of KGM was 94.8%.

MATERIALS AND METHODS

Animals. Five-week-old male Sprague−Dawley rats (BioLASCO Taiwan Co., Ltd., Yi-Lan, Taiwan) were housed in plastic cages in an animal room maintained on a 12 h light−dark cycle at 24 ± 1 °C and 50% humidity. Animals were allowed free access to water and food throughout the study. Animal care followed the guidelines of the National Research Council,[16] and the experiment was approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University.

Experimental Design. Initially, rats were fed a standard rodent diet (Rodent Laboratory chow diet 5001, Purina Co., St. Louis, MO) during 1 week of acclimatization. Afterward, animals were randomly assigned to the high-fat (25% corn oil, w/w) modified AIN-76 diets[17] (n = 8 animals per group) containing no dietary fiber or 5% (w/w) konjac glucomannan fiber (Fukar Co., Taipei, Taiwan) or inulin fiber (Sentosa Co., Taipei, Taiwan). The compositions and caloric densities of experimental diets are shown in Table 1. Fresh feces were collected during the last week of the experiment as described previously.[18] After 4 weeks, rats were anesthetized by carbon dioxide after an overnight fast, and blood samples collected from the abdominal aorta were placed into heparinized tubes, from which an aliquot (~0.3 mL) was used to isolate lymphocytes for analysis of DNA damage; the remaining blood samples were centrifuged at 550g for 10 min to obtain the plasma for analysis of MDA and antioxidants. For determination of plasma ascorbic acid, an aliquot of plasma was mixed with an equal volume of 5% (w/v) metaphosphoric acid (Sigma Chemical Co., St. Louis, MO) to remove the protein, and the samples were analyzed immediately.[19] The remaining plasma was stored at −80 °C for analyses of α-tocopherol and MDA within a month. The liver was dissected into five pieces and frozen immediately. Colons were removed, cut longitudinally, flushed with saline to remove the contents, and blotted dry with a paper towel. A 0.5 cm^2 tissue from the proximal and distal colon was dissected for determination of MDA. The mucosa of the remaining colon was scraped with a glass slide immediately and placed into the RNAase-free phosphate-buffered saline. The tissue and mucosa samples were stored at −20 °C for subsequent analysis.

MDA Levels in the Colon, Liver, and Plasma. MDA levels were determined using 1,1,3,3-tetraethoxypropane (Sigma) as the standard using the method described by Lee and Csallany.[20] The proximal and distal colon tissue samples and hepatic tissues (0.2 g) were homogenized in 9-fold volumes of 50 mM potassium phosphate buffer containing 1.15% (w/v) potassium chloride (pH 7.0) and centrifuged at 12000g for 10 min to obtain the supernatant. An aliquot (0.1 mL) of the supernatant or plasma was mixed with 25 μL of 0.2% (w/v) butylated hydroxytoluene and 12.5 μL of 10 N NaOH at 60 °C for 30 min. After incubation with a trichloroacetic acid solution (7.2% w/v, containing 1% w/v potassium iodide) in ice for 10 min, samples were centrifuged at 12000g for 10 min. An aliquot of this supernatant (0.5 mL) was mixed with 1 mL of 0.6% (w/v) thiobarbituric acid (TBA) at 95 °C for 1 h, and the MDA−TBA adduct was extracted with n-butanol. The MDA−TBA adduct was eluted with a mixture of phosphate buffer (50 mM) and methanol (65:35, v/v) at 0.8 mL/min in an HPLC system (Jasco, Tokyo, Japan) equipped with a C18 reverse phase column (LiChroCART 250-4, Merck, Darmstadt, Germany) capped with a guard column (LiChrospher 100 RP-18e, Merck) at 532 nm and quantified with a standard curve. The tissue MDA content was further calibrated with the protein measured by the method of Bradford using a commercial reagent (Life Science Research, Hercules, CA).

Plasma α-Tocopherol and Ascorbic Acid levels. Plasma α-tocopherol was extracted and analyzed according to the method described by Catignani and Bieri[21] with the HPLC system described above. In brief, an aliquot (0.2 mL) of plasma was mixed with an internal standard (0.1 mL of α-tocopheryl acetate, 105.7 μM, Sigma) and extracted twice with n-hexane. After removal of the hexane under a stream of nitrogen, samples were dissolved in 99% (v/v) methanol immediately before being eluted with 98% (v/v) methanol (1.2 mL/min) from a C18 reverse phase column (LiChroCART 250-4, Merck). The absorbance of α-tocopherol was determined at 290 nm and quantified with a standard curve. The plasma α-tocopherol levels were calibrated with the total lipid level determined by using the method of Frings and Dunn.[22] Plasma ascorbic acid was determined according to the method described previously[19] with a HPLC system described above. The sample was eluted with potassium phosphate buffer (0.1 M, pH 3.5) with a flow rate of 0.8 mL/min. The ascorbic acid was detected at 245 nm and quantified with a standard curve.

Blood Lymphocyte DNA Damage. An aliquot (3 mL) of whole blood was gently layered to the top of an equal volume of a density medium (Histopaque-1077, Sigma) and centrifuged at 1000g for 10 min according to the method described previously.[23] The lymphocyte layer was carefully transferred to a tube and gently washed twice with 5 mL of RPMI1640 medium (Gibco Life Technologies, Grand Island, NY).[24,25] The viability of lymphocytes was determined using the trypan blue assay.[26] With ≥98% cell viability, lymphocytes (5×10^5/mL) were suspended in 1% (w/v) low-melting-point agarose and then layered onto a layer of normal-melting-point agarose (1%, w/v) on a frosted glass microscopy slide. After application of a top layer of normal-melting-point agarose, the slides were immersed in a cold lysing solution (10 mM Tris, 1% w/v sodium N-laurylsarcosine, 0.1 mM Na_2EDTA, 2.5 M NaCl, 1% v/v Triton X-100, 10% v/v DMSO, pH 10) for 1 h at 4 °C. The slides were further treated with either 50 μL of saline or formamidopyrimidine glycosylase (FPG, 1 μg/mL, Sigma) at 37 °C for 30 min for quantification of the oxidized nucleic acids. The FPG is a specific enzyme that nicks the DNA at sites of oxidized purine.[27] After being washed with a saline solution, the slides were allowed to unwind for 20 min in an alkaline solution (0.3 M NaOH, 1 mM Na_2EDTA), followed by electrophoresis at 25 V and 300 mA for 20 min. Duplicate slides were prepared from each animal, and the DNA breakages from at least 100 lymphocytes per slide were determined. The image was analyzed using the Interactive Image Analysis Comet Assay III (Perceptive Instrument, Haverhill, U.K.). DNA damage was denoted as tail moment (% of DNA in tail × tail length).

Relative Gene Expressions of Antioxidant Enzymes in Colonic Mucosa and Liver. The expression of glutathione peroxidase, superoxide dismutase, and catalase genes was determined using quantitative real-time polymerase chain reaction (PCR). The tissue RNA was extracted with REzol C&T reagent (PROtech Technology, Taipei, Taiwan) according to the method provided. Briefly, colonic mucosa or hepatic tissue (50 mg) was homogenized in 1 mL of REzol

Table 2. MDA Levels (Nanomoles per Milligram of Protein) in the Proximal Colon, Distal Colon, and Liver of Sprague−Dawley Rats Fed a High Corn Oil Diet Containing No Fiber, 5% (w/w) KGM, or Inulin[a]

	fiber-free	KGM	inulin
colon			
proximal	2.72 ± 0.19 b	2.31 ± 0.15 b	1.56 ± 0.16 a
distal	3.79 ± 0.33 b	2.65 ± 0.24 a	3.03 ± 0.28 ab
liver	1.03 ± 0.12 b	0.68 ± 0.04 a	0.59 ± 0.06 a

[a] Data are presented as the mean ± SE (n = 8 animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by Duncan's test ($p < 0.05$).

C&T. After the addition of 0.2 mL of chloroform, the samples were vigorously mixed for 15 s, followed by centrifugation 12000g for 15 min at 4 °C. The supernatant (aqueous phase) was mixed with an equal volume of isopropanol (J. T. Baker, Deventer, The Netherlands), and the RNA pellet was precipitated with centrifugation, 12000g, for 10 min at 4 °C. After washing with 75% ethanol, the RNA was dissolved in RNA-free ultrapure water and stored at −70 °C until analysis. The concentration and quality of RNA were determined by the 260/280 nm absorbance. cDNA was synthesized using random primers (Applied Biosystems Life Technologies, Foster City, CA) in a thermal cycler (TaKaRa Biomedical, Shuzo, Japan) at 25 °C for 10 min, at 37 °C for 2 h, and at 85 °C for 5 min.

The quantitative real-time PCR for mRNA levels was performed using TaqMan gene expression assays (Applied Biosystems) with the StepOne Real-Time PCR system (model 7700, Applied Biosystems). The assay identification (accession number of NCBI gene reference shown in parentheses) of primers for the target genes, glutathione peroxidase, superoxide dismutase, and catalase, and for the internal reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were Rn0057799_g1 (NM_030826.3), Rn01477289_m1 (NM_017050.1), Rn00560930_m1 (NM_012520.1), and Rn99999916_s1 (NM_017008.3), respectively. The exact primer and probe sequences used for real-time PCR in this study were not provided due to the proprietary issue and policy of the supplier. The PCR reaction was performed at 50 °C for 2 min, at 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The relative gene expression of each enzyme was normalized to that of GAPDH and was furthered calculated relative to their fiber-free counterpart using the comparative threshold cycle (C_t) method. The fold difference in gene expression was calculated as $2^{-\Delta\Delta Ct}$.

Fecal Short-Chain Fatty Acids. Fecal acetate, propionate, and butyrate were extracted with methyl ether with 4-methyl-n-valeric acid (Sigma) as an internal standard according to the method described previously.[18] The fecal short-chain fatty acids were dissolved in 10% phosphate solution before they were injected into a gas chromatograph (GC-14B; Shimadzu Corp., Kyoto, Japan) fitted with a glass capillary column (0.25 mm × 30 m, Stabilwax-DA, Restek Corp., Bellefonte, PA) and a flame ionization detector. The temperature of the injector port and detector was 250 °C, and the initial oven temperature was 100 °C, increasing to 200 °C at 6 °C/min. The flow rate of N_2 was 1 mL/min. Peak areas were analyzed with a C-R6A Chromatopac (Shimadzu Corp.).

Statistical Analysis. Data are presented as the mean ± SE and analyzed using SPSS (version 12.0, SPSS Inc., Chicago, IL). The diet effects were determined using one-way ANOVA followed by the post hoc analysis using Duncan's test. A p value of ≤0.05 was considered to be statistically significant.

RESULTS

The MDA content of the proximal colon was significantly lowered by the inulin diet 43% ($p < 0.001$), whereas that of the distal colon was reduced by the KGM diet ∼30% ($p = 0.011$), as compared with the high-fat fiber-free counterpart (Table 2). The reductions by the KGM diet of the MDA content in the proximal colon (∼15%, $p = 0.1$) and that of the inulin diet in the distal colon (∼20%, $p = 0.077$) were not statistically significant. The MDA content in the liver was significantly decreased by the KGM and inulin diets by 34% ($p = 0.008$) and 43% ($p = 0.001$), respectively, as compared with the fiber-free diet.

Plasma α-tocopherol levels were significantly increased by the KGM and inulin diets by 66% ($p = 0.002$) and 41% ($p = 0.044$), respectively, as compared with the high-fat fiber-free diet (Table 3). Plasma ascorbic acid and MDA levels were not affected by the incorporation of either fiber into the diet.

The KGM and inulin diets reduced DNA damage (denoted as tail moment) of blood lymphocytes to approximately half ($p < 0.001$, respectively) of that shown in the fiber-free counterpart (Figure 1). The FPG treatment increased the tail moment of blood lymphocytes in all three groups of rats. KGM and inulin diets significantly reduced the tail moment in combination with FPG treatment by 43% ($p < 0.001$) and 54% ($p < 0.001$), respectively, as compared to the fiber-free counterpart. The FPG-induced tail moment in the fiber-free group was 3.1 ± 0.1, which was decreased by KGM by 31% ($p < 0.001$) and by inulin by 56% ($p < 0.001$).

The transcriptions of glutathione peroxidase, superoxide dismutase, and catalase in the colonic mucosa and the liver were evaluated by quantitative real-time PCR normalized by GAPDH as an internal standard (Figure 2). In the proximal colonic mucosa, the incorporation of KGM and inulin into the high-fat fiber-free diet similarly and significantly increased the glutathione peroxidase gene expression ∼4-fold ($p = 0.033$ and 0.037, respectively), but did not modulate the superoxide dismutase and catalase transcript levels (Figure 2A). In the distal colonic mucosa, KGM and inulin significantly increased the glutathione peroxidase gene expression (2.6 ± 0.6)-fold ($p = 0.017$) and (2.4 ± 0.3)-fold ($p = 0.033$), respectively, and significantly increased the catalase gene expression (2.2 ± 0.2)-fold ($p = 0.016$) and (2.3 ± 0.4)-fold ($p = 0.011$), respectively (Figure 2B). However, gene expression of superoxide dismutase in the colonic mucosa was not modulated by either fiber (Figure 2A,B). In the liver, the KGM and inulin diets significantly increased superoxide dismutase gene expression (1.7 ± 0.2)-fold ($p = 0.05$) and (2.0 ± 0.2)-fold ($p = 0.005$), respectively (Figure 2C). In addition, KGM and inulin significantly increased the hepatic catalase gene expression (1.5 ± 0.1)-fold ($p = 0.012$) and (1.8 ± 0.2)-fold ($p < 0.001$), respectively. The gene expression of glutathione peroxidase in the liver was similar among groups.

The KGM diet significantly increased fecal acetate ($p = 0.01$), butyrate ($p = 0.004$), and total short-chain fatty acid ($p = 0.09$) concentrations, as compared with the high-fat fiber-free diet (Table 4). In contrast, the inulin diet slightly elevated the fecal acetate concentration ($p = 0.069$) and significantly caused a 1.6-fold increase ($p < 0.001$) in the butyrate concentration, as compared with the fiber-free counterpart. In addition, the KGM diet elevated the daily fecal output (μmol/day) of acetate to 2.5-fold ($p < 0.001$) and approximately tripled that of propionate ($p = 0.027$) and butyrate ($p < 0.001$), respectively, as compared with the fiber-free counterpart, whereas the inulin diet elevated the daily fecal acetate excretion by approximately 57% ($p = 0.022$) and almost tripled ($p = 0.001$) the daily butyrate excretion. The proportion of butyrate was significant higher in the inulin diet as compared with the fiber-free counterpart ($p = 0.005$).

Table 3. Plasma α-Tocopherol, Ascorbic Acid, and MDA Levels in Sprague−Dawley Rats Fed a High Corn Oil Diet Containing No Fiber, 5% (w/w) Konjac Glucomannan, or Inulin[a]

	fiber-free	KGM	inulin
α-tocopherol (μmol/g plasma lipid)	1.87 ± 0.23 a	3.10 ± 0.24 b	2.63 ± 0.28 b
ascorbic acid (mM)	0.20 ± 0.04	0.21 ± 0.03	0.19 ± 0.03
MDA (μM)	8.2 ± 0.6	7.9 ± 0.5	7.4 ± 0.5

[a] Data are expressed as the mean ± SE (n = 8 animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by Duncan's test ($p < 0.05$).

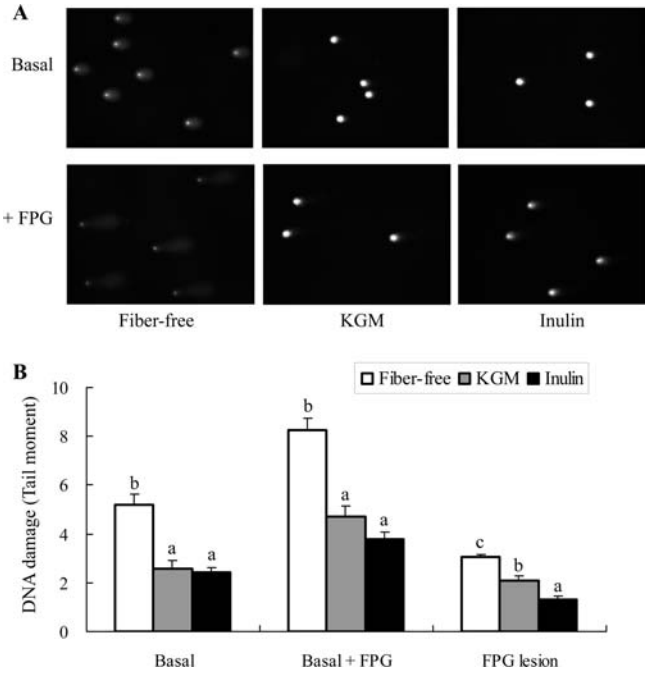

Figure 1. (A) Representative electrophoresis and (B) DNA damage of lymphocytes with basal condition or in combination with 1 μg/mL FPG (basal + FPG). Lymphocytes were isolated from Sprague−Dawley rats fed a high corn oil diets containing no fiber (white bars), 5% (w/w) KGM (gray bars), or inulin (black bars). The DNA damage was determined by comet assay and expressed as tail moment. Bars are presented as the mean ± SE (n = 8 animals per group). Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by Duncan's test ($p < 0.05$). KGM, konjac glucomannan; FPG, formamidopyrimidine DNA-glycosylase.

DISCUSSION

This was the first study to indicate that two purified soluble dietary fibers, KGM and inulin, effectively alleviated oxidative stress in rats fed a high-fat fiber-free diet. KGM and inulin effectively reduced the lipid peroxidative product, MDA, in either the distal or proximal colon. This reduced oxidative stress was likely to be mediated by the enhanced gene expression of glutathione peroxidase in the proximal and distal colonic mucosa and the catalase gene expression in the distal colonic mucosa. Furthermore, KGM and inulin diets reduced the MDA levels in the liver, the site of metabolism of many compounds absorbed from the gastrointestinal tract. These beneficial effects of fibers in the liver were related to the enhanced hepatic gene expression of superoxide dismutase and catalase. Besides, KGM and inulin improved the plasma antioxidative status and α-tocopherol/total lipid level, which may protect blood cells from oxidative stress and further reduce the oxidative damage of lymphocyte DNAs.

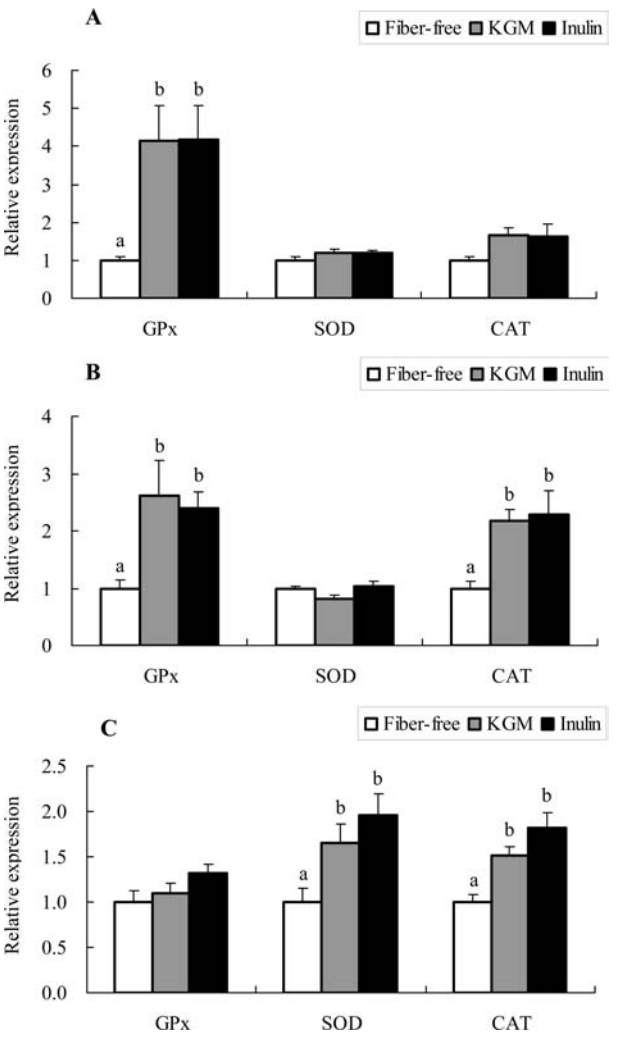

Figure 2. Gene expression of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in the (A) proximal, (B) distal colonic mucosa, and (C) liver of Sprague−Dawley rats fed a high corn oil diet containing no fiber, 5% (w/w) KGM, or inulin. Data (mean ± SE, colonic mucosa, n = 4 animals per group; liver, n = 8 animals per group) are reported as fold difference with respect to fiber-free diet after normalization for GAPDH. Different letters denote significant differences across groups as analyzed by one-way ANOVA followed by Duncan's test ($p \leq 0.05$). KGM, konjac glucomannan.

The effect of feeding a purified dietary fiber, in the absence of other antioxidant phytochemicals, on the intestinal redox system has not been well-documented. A recent study demonstrated that a mixture of grape fiber and polyphenol induced a glutathione redox system in the proximal colonic mucosa of rats.[28]

Table 4. Concentration, Daily Excretion, and Relative Proportion of Fecal Short-Chain Fatty Acids (SCFA) in Sprague−Dawley Rats Fed a High Corn Oil Diet Containing No Fiber, 5% (w/w) KGM, or Inulin[a]

	fiber-free	KGM	inulin
	Concentration (μmol/g of Wet Feces)		
acetate	64.3 ± 7.9 a	104.4 ± 6.8 b	90.9 ± 11.0 ab
propionate	10.9 ± 2.2	22.4 ± 4.9	12.2 ± 3.3
butyrate	5.1 ± 1.1 a	9.9 ± 0.8 b	13.2 ± 0.9 c
total SCFA[b]	80.3 ± 10.5 a	136.7 ± 11.7 b	116.2 ± 13.9 ab
	Daily Fecal Excretion (μmol/Day)		
acetate	155.2 ± 18.6 a	389.9 ± 16.8 c	244.7 ± 30.0 b
propionate	26.4 ± 6.0 a	84.4 ± 25.1 b	29.6 ± 5.6 a
butyrate	12.3 ± 2.9 a	38.8 ± 4.9 b	35.5 ± 2.0 b
total SCFA	193.8 ± 26.0 a	513.2 ± 40.7 c	309.8 ± 30.6 b
	Relative Ratio (% Total)		
acetate	80.9 ± 2.8	77.3 ± 2.0	78.0 ± 2.1
propionate	13.1 ± 1.9	15.5 ± 2.1	9.5 ± 1.7
butyrate	6.1 ± 0.9 a	7.3 ± 0.3 a	12.5 ± 2.0 b

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

Another study indicated that fermented wheat aleurone induced antioxidative defenses, such as the expression of glutathione *S*-transferase π in the human colon cells, and the expression of both catalase and glutathione *S*-transferase π in the HT-29 cells derived from a human adenocarcinoma.[29] However, the role of dietary fiber per se cannot be concluded from these studies. Results of the current study determined that KGM and inulin effectively enhanced the gene expression of glutathione peroxidase involved in glutathione recycling, as well as the expression of catalase involved in the elimination of hydroxyl radicals in the colon. We suggest that the modulatory roles of these dietary fibers in gene expression of colonocytes are independent of any other dietary components and are likely to be mediated by their fermentation products, the short-chain fatty acid, and the intestinal microflora.

Short-chain fatty acids play an important role in colon physiology.[30] Butyrate is considered to be particularly essential for colonocytes as it is a preferred energy source, a potent controller of cell growth and differentiation, and an inducer of apoptosis.[31] Apart from its role in cell growth, butyrate also has been shown to modulate antioxidant defense of colonocytes[29,32,33] and to protect the hydrogen peroxide-induced DNA damage in isolated human colonocytes and HT29 cells.[32] Rectal administration of butyrate in healthy humans also has been shown to increase the glutathione level in the colonic mucosa.[33] However, the sole effect of butyrate may not be as great as the whole mixture of fermentation products on antioxidative enzymes.[29] Therefore, it is likely that butyrate mediates part of the antioxidant effects from dietary fiber fermentation. Our previous study has shown that both KGM and inulin promoted the cecal short-chain fatty acid production in the same animal model as the current study.[18] Furthermore, the current study demonstrated that both KGM and inulin significantly promoted the concentration and daily excretion of fecal short-chain fatty acids, especially acetate and butyrate. Therefore, the results from these two studies support a role for fermentation of KGM and inulin in the elevation of antioxidant enzyme expression in the epithelium throughout the colon and the subsequent reduction in the formation of the lipid peroxidation product MDA.

The mechanisms by which KGM and inulin modulated the balance between pro- and antioxidation are not known. Several in vitro studies have demonstrated that many species of bifidobacteria and lactobacilli reduce the lipid peroxidation, increase the free radical scavenging ability, and chelate free ferrous ions.[34,35] Both KGM and inulin supplementations are known to increase the amounts of fecal bifidobacteria and lactobacilli in mice fed a normal-fat fiber-free diet[15] and in rats fed a high-fat fiber-free diet.[18] Therefore, it is suggested that the antioxidative capacity of KGM and inulin is partially mediated via its prebiotic role.

Soluble dietary fibers may also exert their antioxidative capacity through fermentation products.[14,36] An in vitro study has demonstrated that fermentation products of KGM and fructo-oligosaccharide by several colonic lactic acid bacteria exerted free radical scavenging effects.[14] In addition, fecal soluble material from rats supplemented with KGM had been shown to enhance the iron-chelating capacity,[36] thus reducing the initiation of free radical cascades. Furthermore, feeding KGM and inulin to mice reduced the DNA damaging effect of fecal soluble substances toward Caco-2 cells, a model of colonocyte.[36] All of these results suggest that KGM and inulin may reduce the oxidative challenge to the colonocytes due to the antioxidant capacity of their fermentation products.

The effects of dietary fibers on hepatic antioxidant enzymes have been rarely examined. Oxidative stress was induced by a cystine-rich fiber-free diet, and pectin effectively retained the total superoxide dismutase and Cu,Zn-superoxide dismutase activities in the liver.[37] A recent study indicated that fructo-oligosaccharide, similar to vitamin E, effectively reduced alterations in the hepatic superoxide dismutase and glutathione peroxidase activities in mice subcutaneously administered D-galactose.[38] Our study demonstrates that the hepatic MDA level (nmol/mg protein) was effectively reduced by >30% with either KGM or inulin, which was in agreement with the elevated gene expressions of the hepatic superoxide dismutase and catalase. Therefore, previous and current studies support the utilization of dietary fiber in the large intestine to exert antioxidative effects in the liver.

The effect of fiber consumption on the blood antioxidative status also has not been well studied. Oligofructoses have been shown to reduce the plasma thiobarbituric acid-reactive substances (TBARS) and promote the plasma vitamin E/triglycerol levels in rats fed a high-fructose diet.[39] A placebo-controlled and diet-controlled trial indicated that fructo-oligosaccharide supplement beneficially reduced the plasma TBARS in constipated nursing-home residents.[40] In agreement with these previous studies, the current study indicates that both KGM and inulin diets reduced the index of plasma oxidative stress and DNA breakage of lymphocytes and increased the plasma α-tocopherol/total lipid level, as compared with the fiber-free counterpart. Although the mechanism is unclear, the prebiotic effect of KGM and inulin may partially contribute to the reduced blood oxidative status, similar to a previous study showing decreased oxidative stress markers in human blood and urine with probiotic-fermented milk consumption.[41]

In conclusion, addition of 5% (w/w) KGM or inulin into a high-fat fiber-free diet effectively up-regulated the gene expressions of antioxidant enzymes throughout the colon and in the

liver. The in vivo antioxidative effects of dietary fibers may reach the whole body by reducing the oxidative damage toward the blood lymphocytes and increasing plasma antioxidant levels. The local and systemic antioxidative effects of KGM and inulin are likely to be related to their fermentation in the cecum and colon and their prebiotic effects.

AUTHOR INFORMATION

Corresponding Author

*Postal address: School of Nutrition, Chung Shan Medical University, No.110, Sec.1 Jianguo N. Road, Taichung City 402, Taiwan. Phone: +886-4-24730022, ext. 12207. Fax: +886-4-23248175. E-mail: hlchen908@gmail.com.

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ABBREVIATIONS USED

KGM, konjac glucomannan; MDA, malondialdehyde; ROS, reactive oxidative substances; FPG, formamidopyrimidine glycosylase; PCR, polymerase chain reaction.

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