科技部補助專題研究計畫成果報告
期末報告

以高脂低纖維飲食及致癌劑誘發大腸病變模式探討蒟蒻纖維、菊糖寡醣及纖維素調節急性基因損傷、腫瘤形成、抗腫瘤免疫及相關機制(第 2 年)

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處 理 方 式 ：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢
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中 華 民 國 103 年 07 月 10 日

中 文 摘 要 ： 膳食纖維是最主要的植物機能成份，我們先前研究發現水溶性蒟蒻纖維與菊糖寡醣同樣都有降低糞便水毒性的作用，也證實蒟蒻纖維及數種寡醣經乳酸菌體外代謝後具有抗氧化作用，活體實驗更證實可提高血液抗氧化分子、降低全身性氧化壓力及啟動腸道表皮細胞、肝臟抗氧化機制，凸顯蒟蒻纖維及菊糖寡醣的多重保健功效，因此本實驗室原擬於三年內有系統地探討蒟蒻纖維、菊糖寡醣、纖維素對自發性大腸腫瘤(sporadic colon cancer)發展各時期之抑制作用，並分析膳食纖維的預防作用與體內抗氧化性及腸道內容物成分之關連性，然而因為最終本計畫獲得 2 年補助，另外增加 2 年博士後研究員補助，因此專注進行原計畫之前 2 年部分。本報告第一年探討蒟蒻纖維及菊糖寡醣對於急性 AOM 誘發之毒性(已經發表於 Food Chem)。第二年則發展 AOM 合併高脂飲食誘發之大腸癌前期病變模式，分為 30 及 45 周兩個時期，各測量動物生長、異常腺窩病灶(ACF)數量、病理切片觀察、血液氧化壓力指標以及免疫指標、大腸菌相以及短鏈脂肪酸。再者，博士後研究員則針對研究過程發現之嚴重肝臟病變探討 AOM 合併高脂飲食誘發肝臟發炎性脂肪肝之病理以及介入纖維素的效應，以 PCR array 篩出可能被調控之發炎、脂質代謝等基因群，再輔以促發炎細胞激素等測量。

中文關鍵詞： 蒟蒻、菊醣、致癌物、高脂、基因損傷、異常腺窩病灶、發炎

英 文 摘 要 ： Dietary fiber is a major functional ingredient in plants. We have indicated that soluble fibers such as konjac glucomannan (KGM) and inulin oligosaccharides reduce the fecal water toxicity and exerted in vitro and in vivo anti-oxidative effects. We originally planned to explore effects of KGM and inulin on various stages of sporadic colon cancer development in three years. However, this study was funded for two years, in addition with postdoctoral fellowship. Therefore, we re-designed this study and focused on effects of these two fibers on the colonic carcinogenesis at the initiation stage and on early promotion stage of tumorgenesis, instead of carcinogenesis. In the first year, we determined effects of KGM and inulin on AOM-induced acute DNA damage on the colon, and the underlying cellular mechanisms. Results of the first year has been published in Food Chemistry. In the second year, we

tried to develop a colonic adenoma model with high fat (20% oil, w/w) low-fiber (1% cellulose) and several injection of AOM. Mice were sacrificed 30 and 45 week after the first initiation treatment. We determined the body weight, feed intake twice per week, aberrant crypt foci (ACF), histology of the colon, blood MDA, cytokines, fecal microbiota and short-chain fatty acids.

英文關鍵詞： konjac glucomannan, inulin, AOM, high-fat, gene damage, ACF, inflammation

目 錄

中文摘要

膳食纖維是最主要的植物機能成份，我們先前研究發現水溶性蒟蒻纖維與菊糖寡醣同樣都有降低糞便水毒性的作用，也證實蒟蒻纖維及數種寡醣經乳酸菌體外代謝後具有抗氧化作用，活體實驗更證實可提高血液抗氧化分子、降低全身性氧化壓力及啟動腸道表皮細胞、肝臟抗氧化機制，凸顯蒟蒻纖維及菊糖寡醣的多重保健功效，因此本實驗室原擬於三年內有系統地探討蒟蒻纖維、菊糖寡醣、纖維素對自發性大腸腫瘤(sporadic colon cancer)發展各時期之抑制作用，並分析膳食纖維的預防作用與體內抗氧化性及腸道內容物成分之關連性，然而因為最終本計畫獲得 2 年補助，另外增加 2 年博士後研究員補助，因此專注進行原計畫之前 2 年部分。本報告第一年探討蒟蒻纖維及菊糖寡醣對於急性 AOM 誘發之毒性(已經發表於 Food Chem)。第二年則發展 AOM 合併高脂飲食誘發之大腸癌前期病變模式，分為 30 及 45 周兩個時期，各測量動物生長、異常腺窩病灶(ACF)數量、病理切片觀察、血液氧化壓力指標以及免疫指標、大腸菌相以及短鏈脂肪酸。再者，博士後研究員則針對研究過程發現之嚴重肝臟病變探討 AOM 合併高脂飲食誘發肝臟發炎性脂肪肝之病理以及介入纖維素的效應，以 PCR array 篩出可能被調控之發炎、脂質代謝等基因群，再輔以促發炎細胞激素等測量。

ABSTRACT

Dietary fiber is a major functional ingredient in plants. We have indicated that soluble fibers such as konjac glucomannan (KGM) and inulin oligosaccharides reduce the fecal water toxicity and exerted *in vitro* and *in vivo* anti-oxidative effects. We originally planned to explore effects of KGM and inulin on various stages of sporadic colon cancer development in three years. However, this study was funded for two years, in addition with postdoctoral fellowship. Therefore, we re-designed this study and focused on effects of these two fibers on the colonic carcinogenesis at the initiation stage and on early promotion stage of tumorgenesis, instead of carcinogenesis. In the first year, we determined effects of KGM and inulin on AOM-induced acute DNA damage on the colon, and the underlying cellular mechanisms. Results of the first year has been published in Food Chemistry. In the second year, we tried to develop a colonic adenoma model with high fat (20% oil, w/w) low-fiber (1% cellulose) and several injection of AOM. Mice were sacrificed 30 and 45 week after the first initiation treatment. We determined the body weight, feed intake twice per week, aberrant crypt foci (ACF), histology of the colon, blood MDA, cytokines, fecal microbiota and short-chain fatty acids.

關鍵字

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Key words：konjac glucomannan, inulin, AOM, high-fat, gene damage, ACF, inflammation

第一年計劃 (第一作者為博士後研究員，本人為通訊作者)

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Effects of konjac glucomannan, inulin and cellulose on acute colonic responses to genotoxic azoxymethane

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ABSTRACT

Mice were fed low-fibre, or that supplemented with soluble fibre (konjac glucomannan, KGM; inulin), or insoluble fibre (cellulose) to determine how these three fibres modulated the acute colonic responses to an azoxymethane (AOM) treatment. Results indicated that KGM and inulin exerted greater anti-genotoxic effects compared to cellulose and up-regulated the gene expressions of glutathione *S*-transferase and antioxidant enzymes. The apoptotic index in the distal colon was the greatest and the expression of Bcl-2 was the lowest in the KGM group 24 h after the AOM treatment. On the other hand, the proliferative index and expression of Cyclin D1 were lower in all fibre groups. Furthermore, KGM increased cecal short-chain fatty acid contents, and both KGM and inulin increased fecal probiotic concentrations. This study suggested that soluble fibres were more effective than cellulose on ameliorating AOM-induced genotoxicity by up-regulating antioxidant enzyme genes, and enhancing epithelium apoptosis by down-regulating Bcl-2.

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1. Introduction

Colon cancer is the leading cause of death worldwide, and dietary factors are known to be capable of regulating the colon carcinogenesis (Ferlay et al., 2010). Epidemiological studies have suggested the reverse association between intake of dietary fibre, the indigested parts of plant materials, and the risk of colon cancer (Aune et al., 2011; Dahm et al., 2010). Underlying potential mechanisms, whereby dietary fibres may influence the development of colon carcinogenesis, include increased fecal bulk, reduced colonic transit time and diluted fecal toxin contents, which consequently reduce the exposure of colonic mucosa to the luminal carcinogens (American Institute for Cancer Research, 2007; Spiller, 2001). In addition, the interaction between dietary fibre and colonic microbiota and bile acids, and the production of short-chain fatty acids (SCFA) resulting from fermentation, are believed to protect against colon cancer development (Young, Hu, Le, & Nyskohus, 2005). Butyrate, in particular, is one of the SCFA that serves as the major energy source of colonocytes (Roediger, 1982) and has been shown to enhance apoptosis and inhibit proliferation in the colonic cells *in vitro* (Chai, Evdokiou, Young, & Zalewski, 2000; Zhang et al., 2010).

Konjac glucomannan (KGM), derived from the tubers of *Amorphophallus konjac* C. Koch, is composed of β-1,4-linked D-glucose and D-mannose units joined together with branches through β-1,6-glucosyl units (Doi, 1995). The viscous polymer can be processed into various vegetarian food products and commonly consumed in the Asian countries such as Japan and Taiwan. Inulin, a mixture of fructo-oligosaccharides derived from the tuber of chicory (*Cichorium intybus*), is a well-known prebiotic and widely used as a supplement in functional food. Both KGM and inulin have been shown to increase the production of SCFA and stimulate the growth of bifidobacteria and lactobacilli in animal and human studies (Chen, Cheng, Wu, Liu, & Liu, 2008; Chen, Lin, & Wang, 2010). In addition, these two soluble fibres have also been shown to up-regulate the antioxidant enzymes in the colon (Wu & Chen, 2011b). On the other hand, cellulose, a poorly-fermented insoluble fibre, increases fecal bulk and may therefore reduce the fecal toxic concentration, but does not increase the fecal butyrate level (Chen et al., 2010).

Azoxymethane (AOM) is commonly used to induce experimental animal model of colon carcinogenesis (Rosenberg, Giardina, &

Abbreviations: AI, apoptotic index; AOM, azoxymethane; Bcl-2, B cell leukemia; CAT, catalase; GPX2, glutathione peroxidase 2; GST-π, glutathione *S*-transferase π; KGM, konjac glucomannan; PI, proliferative index; qPCR, quantitative real-time polymerase chain reaction; SCFA, short-chain fatty acid; SOD1, superoxide dismutase 1.

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Tanaka, 2009). The AOM is metabolized into methylazoxymethanol that causes DNA adducts (Weisburger, 1971). The potential cellular defense mechanisms, such as antioxidant machinery and apoptosis, and the compensatory response to apoptosis, such as cell proliferation, may occur after the DNA damage (Bellamy, Malcomson, Harrison, & Wyllie, 1995; Fan & Bergmann, 2008). Therefore, it is generally considered that increased DNA damage or/and insufficient apoptosis response against the DNA damage leads to an increased risk of carcinogenesis. We have previously demonstrated that supplementation of KGM, inulin or cellulose into a low-fibre diet reduced acute DNA damages in Caco-2 cell, a colonocyte cell line model, caused by fecal water treatment (Chen et al., 2008), as inulin exerted greater suppressive effect compared to KGM and cellulose. However, effects of these three fibres, on colonic DNA damage, antioxidant enzymes, apoptotic and proliferative responses induced by AOM, have not been shown *in vivo*.

The main goal of this study was to examine effects of two soluble fibres (KGM, inulin) and one insoluble fibre (cellulose), over 24 h, after the AOM administration on colonic DNA damage, cell cycle homeostasis, and gene expression of related cellular mechanisms in mice. We also determined the SCFA in the cecum and fecal microbiota.

2. Methods and materials

2.1. Animals

Male C57BL/6J mice were obtained at 5 weeks of age from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Every three mice were housed in a solid-bottomed plastic cage, with stainless wire-bar lid and wood shavings for bedding, in a animal holding room maintained on a 12-h light-dark cycle at 24 ± 1 °C and 50% humidity. All animal were allowed free access to water and food in the study. Animal care followed the guidelines of the National Research Council (1985) and was approved by the Institutional Animal Care and Use Committee (IACUC) of Chung Shan Medical University (approved number 1077).

2.2. Experimental design

After 1 week of acclimatisation, mice (6-week-old) were randomly divided into four groups ($n = 12$ per group) and fed either modified AIN-76 (American Institute of Nutrition, 1977) high-fat (20% corn oil, w/w) low-fibre (1% cellulose) diet or that supplemented with another 5% (w/w) fibre derived from KGM (80%, Fukar Co., Taipei, Taiwan), inulin (85.5%, Sentosa Co., Taipei, Taiwan), or cellulose (99.9%, Sigma Chemical Co., St. Louis, MO) for 3 weeks. The composition of the low-fibre diet was as follows (g/kg): casein, 200; corn starch, 540; corn oil, 200; AIN-76A mineral mix, 35; AIN-76A vitamin mix, 10; methionine, 3; choline bitartrate, 2; cellulose, 10. The amount of corn starch was substituted by dietary fibre, with correction of the purities to formulate the fibre-supplemented diet. Daily food intake and body weight were recorded throughout the study. Mice were individually housed and fecal outputs were collected during days 17–21. Mice were anaesthetized with CO_2 before or 24 h after a single intraperitoneal injection of AOM (10 mg/kg body weight, Sigma) on day 22. A midline incision was made to dissect the cecum from where the contents were removed and weighed. The cecal contents were immediately stored at −20 °C for further analysis of SCFA. The entire colon was then removed and flushed clean with ice-cold sterile saline. Segments (0.5 cm) of the distal colon were fixed in 10% (v/v) buffered formalin overnight and embedded in paraffin for further immunohistological examination. The remaining colons were immediately processed for colonocyte isolation.

2.3. Isolation of colonocytes

The colonocytes were isolated according to the method described by Pool-Zobel et al. (1993) with slight modification. Briefly, colonic tissues were washed in a phosphate buffered saline containing penicillin (10 units/ml, Gibco Life Technologies, Foster City, CA) and streptomycin (10 mg/ml, Sigma) at 37 °C with shaking, for three times, each for 10 min. Tissues were then treated with collagenase (type XI, 125 units/ml, Sigma) for 30 min at 37 °C and was then centrifuged at 800g for 10 min to collect the colonocytes. Half of the isolated colonocytes were used to determine the DNA damage, while the other halves were processed for RNA isolation to determine the expression of target genes.

2.4. Comet assay

The DNA damages of colonocytes were determined using the Comet assay as described previously (Wu & Chen, 2011b). The viability of isolated colonocytes was determined using the trypan blue assay (Phillis, 1973). With ⩾90% cell viability, cells (5×10^5/ml) were suspended in 1% (w/v) low-melting-point agarose which was layered onto a layer of 1% (w/v) normal-melting-point agarose on a frosted glass slide. After application of a third layer of 1% normal-melting-point agarose, the slides were immersed in a cold lysing solution (10 mM Tris, 1% sodium *N*-laurylsarcosine, 0.1 mM Na_2EDTA, 2.5 M NaCl, 1% Triton X-100, 10% dimethylsulphoxide, pH 10) for 1 h at 4 °C. 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 mouse, and the DNA breakages from at least 100 cells per slide were determined. The image was analysed using the Interactive Image Analysis Comet Assay III (Perceptive Instrument, Haverhill, Suffolk, UK). DNA damage was denoted as tail moment (% of DNA in tail × tail length).

2.5. Relative gene expressions

The gene expressions of antioxidant enzymes, superoxide dismutase 1 (SOD1), catalase (CAT), glutathione peroxidase 2 (GPX2), detoxification enzyme, glutathione *S*-transferase π (GST), B cell leukemia (Bcl-2) oncogene that suppresses cell apoptosis (Willis, Day, Hinds, & Huang, 2003), and Cyclin D1 (Ccnd1), a cell cycle regulator that controls transition from the G1 to S phase (Fu, Wang, Li, Sakamaki, & Pestell, 2004), were determined by using quantitative real-time polymerase chain reaction (qPCR). The RNAs was isolated according to the method described previously Ferlay et al. (2010). Briefly, colonocytes were homogenised (5×10^5 cells/ml) in REzol™ C&T reagent (PROtech Technology, Taipei, Taiwan). After addition of 0.2 ml chloroform, the samples were vigorously mixed for 15 s, followed by centrifugation 12,000g for 15 min at 4 °C. The supernatant was mixed with an equal volume of isopropanol (J. T. Baker, Deventer, The Netherlands), and the RNA pellet was precipitated with centrifugation, 12,000g 10 min at 4 °C. After washing with 75% ethanol, the RNA was dissolved in RNA-free water for further complementary DNA (cDNA) synthesis. The quality of RNA were determined by the 260/280 nm absorbance. The cDNA was synthesized using random primers (Applied Biosystems Life Technologies) in a thermal cycler (TaKaRa Biomedical, Shuzo, Japan).

The qPCR 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 parenthesis) of primers for the target genes SOD1, CAT, GPX2, GST, Bcl-2, and Ccnd1 was Mm01344233_g1 (NM_011434.1), Mm00437992_m1

(NM_009804.2), Mm00850074_g1 (NM_030677.2), Mm04213618_gH (NM_013541.1), Mm00477631_m1 (NM_009741.3), Mm00432359_m1 (NM_007631.2), respectively, and that for the internal reference gene β-actin was Mm00607939_s1 (NM_007393.3). The exact primer and probe sequences were not provided due to the proprietary issue and policy of the supplier. The gene expression of each target gene was first normalised to that of its own internal reference gene β-actin. The relative gene expression of the experimental group was compared to that of the control group at 0 h according to the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

2.6. Immunohistochemical staining

The apoptosis and proliferation of colonic epithelium were determined by using the immunohistochemical staining. Paraffin sections (5 μm) were dewaxed, rehydrated through descending alcohol concentration and treated with 20 μg/ml proteinase K (Sigma) for 20 min. Endogenous peroxidase was removed by treatment with 3% hydrogen peroxide (H_2O_2) for 20 min. The epithelial cells undergoing apoptosis were determined by using the TUNEL method according to the manufacturer's instruction (ApopTag S7101, Millipore, Temecula, CA) and counterstained with methyl green. The 3′ hydroxyl ends of broken DNA strand were enzymatically labelled with digoxigenin nucleotides and were then treated with anti-digoxignin antibody bound to peroxidase. A negative control was prepared for each animal to monitor the non-specific reaction. The apoptotic index (AI), the ratio (%) of TUNEL-positive to total epithelium cells was determined from at least 40 crypts randomly selected from each animal.

The Ki-67 protein, a marker shown during cell proliferation, in the colonic epithelial cells was determined with a polyclonal antibody (Millipore). After inhibition of endogenous peroxidase activity by 3% H_2O_2, the Ki-67 antibody was then applied at 1:300 dilutions for 1 h at room temperature. The stains were shown by using a biotinylated secondary antibody and detection system (IHC Select Immunoperoxidase Secondary Detection System, Millipore) according to the manufacturer's instruction. The proliferative index (PI), i.e. the ratio of Ki-67 positive to total cells, was determined in the whole crypt column and upper-third crypt, respectively.

2.7. Cecal SCFA

Cecal SCFA was extracted with methyl ether, according to the method described previously (Wu & Chen, 2011a), using 4-methyl-*N*-valeric acid (Sigma) as an internal standard. The re-dissolved sample was analysed by gas chromatography (GC-14B; Shimadzu Corp., Kyoto, Japan) using a glass capillary column (0.25 mm × 30 m, Stabilwax-DA, Restek Corp., Bellefonte, PA) with a flame ionisation detector and peak areas were collected with a C-R6A Chromatopac (Shimadzu Corp.).

2.8. Fecal microbiota

Fecal bacteria population were determined by using fluorescence *in situ* hybridization method (FISH), as described previously (Chen, Cheng, Liu, Liu, & Wu, 2006). The genotypic probes were specifically designed to target 16S rRNA of bifidobacteria (Jansen, Wildeboer-Veloo, Tonk, Franks, & Welling, 1999), lactobacilli (Wang, Cao, & Cerniglia, 1996), and clostridia (Nagahama, Nagayasu, Kobayashi, & Sakurai, 2002). The nucleic acid stain 4′ 6-diamidino-2-phenylindole was used for total bacterial counts (Chen et al., 2006). Probe fluorescence was detected with a Zeiss Axioskop2 microscope (Carl Zeiss, Jena, German) 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 concentration is expressed as $\log_{10}$ counts/g feces.

2.9. Statistical analysis

Values were presented as means ± SEM and analysed using SPSS version 14.0 (SPSS Inc., Chicago, IL). The diet effects at a time point were determined using one-way ANOVA followed by Tukey's test. The time effect of AOM within each dietary group was determined using the Student's *t*-test. A P value <0.05 was considered statistically significant.

3. Results

The growth and physical activity were normal in all groups throughout the experimental period. The calorie intake was 56.4 ± 3.4, 51.8 ± 2.7, 52.2 ± 1.1 and 52.6 ± 1.6 kJ/d in the low-fibre, KGM, inulin and cellulose group, respectively. The daily weight gain of low-fibre, KGM, inulin and cellulose group was 0.20 ± 0.01, 0.16 ± 0.01, 0.15 ± 0.01, and 0.17 ± 0.02 g/d, respectively. Both caloric intake and weight gain were similar across groups.

The DNA damage (tail moment) of colonocytes at 0 h was the greatest in the low-fibre group (Fig. 1), which was significantly decreased with dietary supplementation of KGM (P = 0.015) and inulin (P = 0.003), respectively.

The single AOM injection significantly increased the DNA damage at 24 h as compared with the respective counterpart at 0 h (P < 0.05, respectively). The tail moment at 24 h was still the greatest in the low-fibre group, 4.5 ± 0.2, which was significantly reduced by all fibres. KGM, inulin and cellulose decreased the DNA damage by 27% (P < 0.001), 40% (P < 0.001) and 16% (P = 0.006), respectively, as compared with that in the low-fibre group.

Addition of dietary fibre into the low-fibre diet did not affect the expression of SOD1 in the isolated colonocytes at 0 h (Fig. 2A). The AOM treatment increased the SOD1 expression in all groups except the cellulose group. The SOD1 gene expression was enhanced to the greatest with KGM, from 1.54 ± 0.17 at 0 h to 2.88 ± 0.16 at 24 h (P < 0.001 vs. low-fibre at 24 h). However, cellulose group

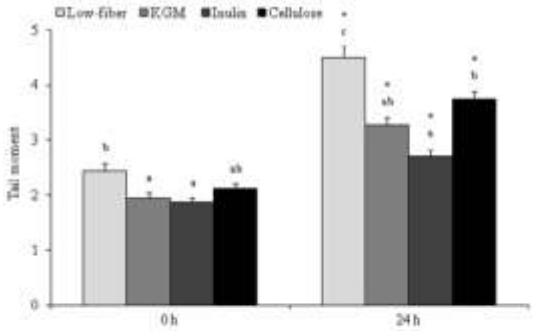

Fig. 1. DNA damage (denoted as tail moment) of colonocytes. C57BL/6J mice were fed a high-fat (20%, w/w) low-fibre (1% cellulose, w/w) diet or that supplemented with 5% (w/w) KGM, inulin or cellulose for 3 weeks and then sacrificed 0 or 24 h after an AOM injection (10 mg/kg BW, i.p.). DNA damages of colonocytes isolated from mice were determined by using comet assay. Values are means ± SEM (n = 6 per group). Different letters denoted significant differences across dietary groups at the same time point as analysed by one-way ANOVA followed by Tukey's test (P < 0.05). * at 24 h denoted the significant differences compared to that at 0 h as analysed by Student's *t*-test. KGM, konjac glucomannan.

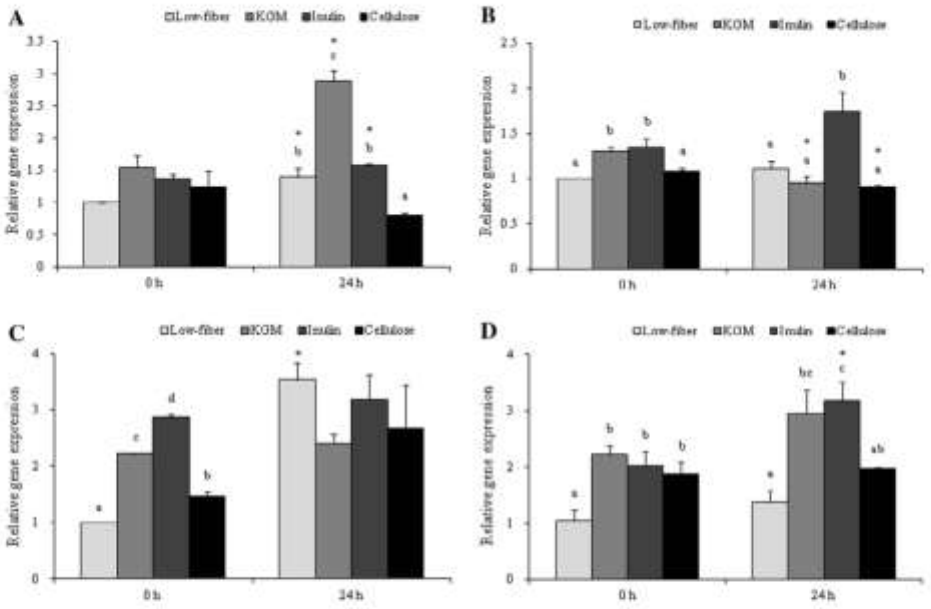

Fig. 2. Relative gene expressions of (A) SOD1, (B) CAT, (C) GPX2 and (D) GST in the colonocytes. Relative gene expression was normalised using internal control gene β-actin, and compared to that of the control group at 0 h according to the $2^{-\Delta\Delta Ct}$ method. Values are means ± SEM ($n = 6$ per group). Different letters denoted significant differences across dietary groups at the same time point as analysed by one-way ANOVA followed by Tukey's test ($P < 0.05$). * at 24 h denoted the significant differences compared to that at 0 h as analysed by Student's *t*-test. KGM, konjac glucomannan.

slightly decreased SOD1 gene expression from 1.24 ± 0.23 at 0 h to 0.79 ± 0.03 at 24 h ($P = 0.081$). The relative gene expressions of CAT were greater in the KGM and inulin groups than that in the low-fibre and cellulose groups at 0 h (Fig. 2B). The AOM treatment decreased the CAT expressions in the KGM ($P = 0.002$) and cellulose ($P = 0.004$) groups, but not in the inulin group, and the inulin group had the greatest CAT expression among groups at 24 h. The relative gene expression of GPX2 was increased with either soluble or insoluble fibre supplementation at 0 h (Fig. 2C). The AOM treatment induced the relative GPX2 expression only in the low-fibre group, to a level similar to that shown in the fibre-supplemented groups at 24 h. The relative gene expression of GST was also enhanced with either type of fibre supplementation at 0 h ($P < 0.05$) (Fig. 2D). However, the relative GST expressions at 24 h were greater only in the KGM ($P = 0.002$) and inulin ($P = 0.001$) groups, but not in the cellulose group, as compared with that in the low-fibre counterpart.

The original (0 h) AI in the distal colon was similar among groups (Fig. 3). The AOM treatment significantly increased the AI in the low-fibre, KGM and inulin groups by one-fold ($P < 0.001$), ~120% ($P < 0.001$) and ~80% ($P < 0.001$), respectively, and slightly increased that in the cellulose group ($P = 0.06$). In addition, the AI at 24 h was greater only in the KGM group ($P = 0.001$), but not in the inulin and cellulose groups, as compared with that in the low-fibre counterpart. The representative images of AOM-induced apoptosis are shown in the Supplementary data (A).

The PI of the whole crypt at 0 h was greater in mice fed either fibre-supplemented diet than that in the low-fibre group (Table 1). The AOM treatment significantly increased the PI of the whole crypt only in the low-fibre group ($P < 0.001$), not in any fibre-supplemented groups. The PIs of the whole crypt at 24 h were significant lower in the KGM, inulin and cellulose groups for 30% ($P < 0.001$), 19% ($P = 0.004$) and 31% ($P < 0.001$), respectively, than

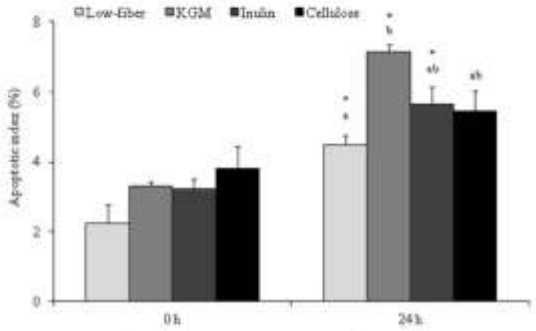

Fig. 3. Apoptotic index in the distal colon at 0 or 24 h of AOM treatment. The TUNEL assay was used to determine apoptosis as described in Section 2. Apoptotic index (%) = the ratio of TUNEL-positive to total epithelium cells. Values are means ± SEM ($n = 6$ per group). Different letters denoted significant differences across dietary groups at the same time point as analysed by one-way ANOVA followed by Tukey's test ($P < 0.05$). * at 24 h denoted the significant differences compared to that at 0 h as analysed by Student's *t*-test. KGM, konjac glucomannan.

that in the low-fibre counterpart. We further examined the PI of the upper-third crypt. The PI in the low-fibre group was the greatest among groups at either time point. Addition of KGM, inulin and cellulose into the low-fibre diet significantly decreased that by 26% ($P = 0.001$), 35% ($P = 0.007$) and 37% ($P = 0.006$), respectively, at 0 h, and 42% ($P < 0.001$), 34% ($P = 0.004$) and 40% ($P < 0.001$), respectively, 24 h after the AOM treatment. The representative images of Ki-67 positive stains are shown in the Supplementary data (B).

The relative expression of Bcl-2, an anti-apoptotic gene, at 0 h was similar among groups (Fig. 4A). However, the Bcl-2 gene expression was significant lower in the fibre-supplemented group

Table 1
Effect of different diets on proliferative index of the whole or upper-third crypt in the distal colon at 0 or 24 h after an AOM treatment.[1]

	Low-fibre	KGM	Inulin	Cellulose
Proliferative index (%)				
0 h				
Whole crypt	30.3 ± 2.0^{a}	40.0 ± 2.3^{b}	37.7 ± 0.5^{b}	38.9 ± 2.3^{b}
Upper-third crypt	55.2 ± 1.6^{b}	40.6 ± 1.9^{a}	36.0 ± 3.0^{a}	34.6 ± 2.5^{a}
24 h				
Whole crypt	$44.8 \pm 1.6^{b,*}$	$31.5 \pm 1.1^{a,*}$	$36.3 \pm 1.4^{a,*}$	$30.8 \pm 2.5^{a,*}$
Upper-third crypt	59.0 ± 2.8^{b}	$34.5 \pm 1.4^{a,*}$	39.0 ± 2.4^{a}	35.3 ± 2.1^{a}

[1] Data are expressed as means ± SEM ($n = 6$ per group). Different superscript letters denote significant differences across groups as analysed by one way ANOVA followed by Tukey's test ($P < 0.05$). * at 24 h denote significant difference compared to that at 0 h as analysed by Student's *t*-test.

compared to the low-fibre counterpart 24 h after the AOM treatment. KGM exerted the greatest suppressive effect on Bcl-2 gene expression, followed by the inulin and then cellulose groups. Furthermore, the relative expression of Cyclin D1, a proliferation-related gene, at 0 h was increased with all types of dietary fibre examined in this study, and was the greatest in the cellulose group (Fig. 4B). However, the relative gene expression of Cyclin D1 at 24 h, in the low-fibre group, was significantly up-regulated as compared to that at 0 h ($P < 0.001$), and was greater ($P < 0.05$) than that in either fibre-supplemented group.

The cecal acetate and propionate contents were not affected by any dietary fibre examined in this study at 0 h, but the butyrate contents were greater in the KGM ($P = 0.047$) and inulin ($P < 0.001$) groups as compared to that in the low-fibre counterpart, respectively (Table 2). Most individual cecal SCFA content was not affected with the AOM treatment, except that the butyrate content was significantly increased with the AOM treatment in the KGM group by more than one-fold. In addition, the KGM group had the greatest acetate, propionate, butyrate and the total SCFA contents among groups at 24 h.

Addition of cellulose into the low-fibre diet significantly increased wet and dry fecal mass by 38% (1.26 ± 0.08 g per day, $P = 0.021$) and 64% (0.92 ± 0.06 g per day, $P < 0.001$), respectively. KGM and inulin tended to increase the wet fecal mass, but the effect was not statistically significant.

After 3 weeks of dietary treatment, the fecal bifidobacteria concentration ($\log_{10}$ counts per g feces) in KGM and inulin was 10.99 ± 0.02 and 10.98 ± 0.02, respectively, which was significant greater ($P < 0.001$, respectively) than that in the low-fibre counterpart (10.27 ± 0.01). Addition of KGM and inulin into the low-fibre

Table 2
Effect of different diets on cecal short-chain fatty acids analysed prior to or 24 h after an AOM treatment.[1]

	Low-fibre	KGM	Inulin	Cellulose
μmole/cecum				
0 h				
Acetate	10.3 ± 0.7	12.4 ± 3.8	13.0 ± 2.9	7.7 ± 1.1
Propionate	1.2 ± 0.3	1.4 ± 0.5	1.6 ± 0.4	0.8 ± 0.2
Butyrate	0.6 ± 0.1^{a}	0.9 ± 0.1^{b}	1.3 ± 0.1^{c}	0.8 ± 0.1^{ab}
Total SCFA	12.1 ± 0.7	14.7 ± 4.2	15.9 ± 3.3	9.3 ± 1.3
24 h				
Acetate	9.0 ± 0.9^{ab}	13.4 ± 1.2^{b}	12.4 ± 1.0^{ab}	8.9 ± 0.7^{a}
Propionate	0.7 ± 0.2^{a}	1.7 ± 0.4^{b}	1.5 ± 0.2^{ab}	0.8 ± 0.1^{ab}
Butyrate	0.6 ± 0.1^{a}	$1.9 \pm 0.3^{b,*}$	1.1 ± 0.2^{ab}	0.9 ± 0.1^{a}
Total SCFA	10.2 ± 1.1^{a}	17.0 ± 1.8^{b}	15.0 ± 1.3^{ab}	10.5 ± 0.7^{a}

[1] Data are expressed as means ± SEM ($n = 6$ per group). Different superscript letters denote significant differences across groups as analysed by one way ANOVA followed by Tukey's test ($P < 0.05$). * at 24 h denote significant difference compared to that at 0 h as analysed by Student's *t*-test. Total SCFA = acetate + propionate + butyrate.

diet also increased the fecal lactobacilli ($\log_{10}$ counts per g feces) from 10.36 ± 0.03 to 10.98 ± 0.05 ($P < 0.001$) and 10.99 ± 0.04 ($P < 0.001$), respectively. Furthermore, addition of KGM and inulin into the low-fibre diet increased total bacteria ($\log_{10}$ counts per g feces) from 10.50 ± 0.05 to 11.74 ± 0.03 ($P = 0.015$) and 11.78 ± 0.05 ($P = 0.006$), respectively. However, none of the dietary fibre examined in the present study significantly changed the fecal clostridia concentration.

4. Discussion

To our knowledge, this was the first *in vivo* study which compared the effects of soluble and insoluble fibres on the DNA integrity of colonocytes and examined the underlying mechanisms in mice fed a Western-like diet. Colonocytes were constantly challenged with the toxicity of colonic contents, which could lead to carcinogenesis. The present results indicated that dietary fibres, especially soluble fibre, effectively ameliorated the genotoxicity of the high-fat low-fibre diet at 0 h. These results were in agreement with our previous *in vitro* results showing that dietary fibres reduced the DNA damage of Caco-2 cells induced by fecal water of mice fed high-fat diet (Chen et al., 2010; Yeh, Lin, & Chen, 2007). Besides, our previous study has indicated that dietary fibres, such as KGM and inulin, up-regulated the GPX2 expression in the distal colon (Wu and Chen, 2011b). In agreement with that, the present result indicated that both soluble and insoluble fibres up-regulated

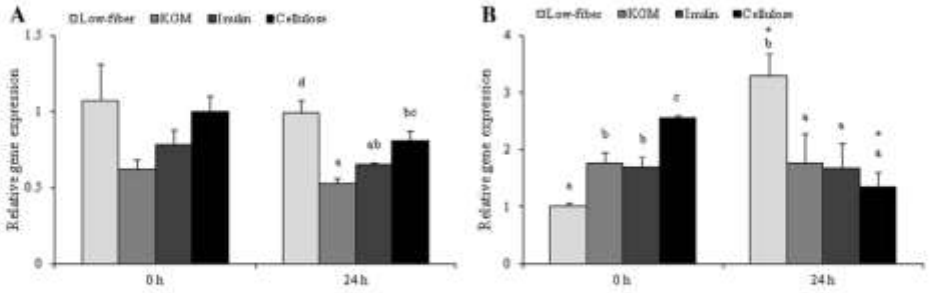

Fig. 4. Relative gene expressions of (A) Bcl-2, (B) Cyclin D1 in the colonocytes. Relative gene expression was normalised using internal control gene β-actin, and compared to that of the control group at 0 h according to the $2^{-\Delta\Delta Ct}$ method. Values are means ± SEM ($n = 6$ per group). Different letters denoted significant differences across dietary groups at the same time point as analysed by one-way ANOVA followed by Tukey's test ($P < 0.05$). * at 24 h denoted the significant differences compared to that at 0 h as analysed by Student's *t*-test. KGM, konjac glucomannan.

the gene expressions of GPX2, while soluble fibre exerted greater effect than cellulose. In addition, this study also examined the gene expression of GST, and confirmed addition of either fibre up-regulated the gene. Therefore, the present study suggested that both soluble and insoluble fibres positively protected the colonic epithelium, while KGM and inulin were more effective than cellulose. These protective effects of fibres were likely to be mediated by up-regulation of antioxidant and detoxified enzymes, GPX2 and GST, in the colonocytes.

Earlier studies have indicated that soluble fibres promoted colonic epithelium proliferation (Comalada et al., 2006; Hino et al., 2010). However, the role of fibre on colonic apoptosis has not been examined. The imbalance between cell proliferation and apoptosis could lead to risk in carcinogenesis. Therefore, we also compared effects of soluble fibres and cellulose on the apoptosis and proliferation in the distal colon in the basal state (without the AOM challenge). We found that in the matrix of a high-fat diet, all dietary fibres examined in the present study similarly increased the PI of the whole crypt at 0 h. Furthermore, in order to differentiate the normal cell proliferation in the basal crypt and the "risky" proliferation in the upper crypt (Morini et al., 2005), we further specifically measured the PI in the upper-third crypt. We then found that all dietary fibre examined presently significantly decreased, instead of increasing, PI of the upper third crypt. Therefore, we suggested that addition of dietary fibre, regardless of solubility, into a high-fat low-fibre diet may maintain the normal proliferation and differentiation of colonic epithelium cells. On the other hand, the present study also found that all dietary fibres tended to increase the apoptosis of colonic epithelium cells. Therefore, results regarding the cell apoptosis and proliferation in the distal colon suggested that all dietary fibres examined in this study promoted epithelium turnover without increasing the uncontrolled cell proliferation. The increased cecal butyrate contents, especially in the soluble fibre groups, could supply energy for normal turnover of normal colon epithelium (Roediger, 1982).

The present study further examined effects of dietary fibres on the colonic responses during the initiation stage of carcinogenesis caused by AOM. Results confirmed the genotoxic effect of AOM and indicated that soluble fibres were more effective than cellulose on reducing AOM-induced DNA damages with concordant up-regulation of the colonic antioxidant enzymes, including SOD1, CAT and GST. The antioxidant enzymes in the colonocytes were likely to ameliorate the genotoxicity derived from AOM. Therefore, this study suggested that KGM and inulin effectively ameliorated the AOM-induced DNA damage partially by promoting the antioxidant machinery in the colonocytes.

We further determined the epithelium apoptosis after the AOM treatment since this cell death response appears to be an innate biological mechanism for protection against tumorigenesis. We found that AOM induced the AI at 24 h in all dietary groups, which was in agreement with a previous observation (Hu, Martin, Le, & Young, 2002). Among fibres examined in this study, KGM had the greatest effects on both promoting AI as well as reducing the transcription of Bcl-2, which suggests that KGM could exert the greatest effect on up-regulating apoptotic mechanisms against the AOM challenge. The current study further found that all dietary fibres significantly reduced the AOM-induced cell proliferation in the upper-third and whole crypt, suggesting protective effect of either soluble or insoluble fibre on carcinogen-induced hyper-proliferation of the distal colon. Therefore, KGM effectively induced colonic epithelium apoptosis and all fibres examined presently reduced proliferation after the AOM challenge, which suggest their protective effects on the initiation of carcinogenesis.

Butyrate could be involved in the anti-genotoxic effects of soluble fibres observed in this study before and after the AOM treatment. A previous study showed that butyrate protected against H_2O_2-induced genetic damage in primary colon cells (Abrahamse, Pool-Zobel, & Rechkemmer, 1999). This effect of butyrate may contribute to the significant lower cellular DNA damage in the KGM and inulin groups before the AOM treatment, and slightly lower damage in the cellulose group. Furthermore, soluble fibre-supplemented groups had an increased cecal butyrate content and decreased DNA damage of colonocytes after the AOM treatment, which supported the potential role of butyrate in the DNA repair process (Kerr et al., 2013). In addition, *in vitro* cell line studies have shown that butyrate activated the intrinsic pathway of apoptosis and sensitised cancer cells to apoptosis mediated by the extrinsic pathway (Pajak, Gajkowska, & Orzechowski, 2009; Wang, Luo, & Xia, 2009). Previous studies also suggested that the butyrate-induced apoptosis was primarily associated with regulation of gene expressions of pro- and anti-apoptotic proteins such as Bcl-2 protein family, by inhibiting the activity of histone deacetylase (Fung, Cosgrove, Lockett, Head, & Topping, 2012). The role of KGM in epithelium apoptotic responses was in agreement with the increased cecal butyrate content and decreased Bcl-2 gene expression. Therefore, the butyrate derived from fermentation of soluble fibre that occurred after the AOM treatment, could primarily modulate the cellular pathways to apoptosis instead of proliferation.

Another mechanism that could mediate the anti-genotoxic effect of KGM and inulin is the colonic microbiota. The present study, in agreement with previous studies, demonstrated the prebiotic effects of KGM and inulin (Wu & Chen, 2011a; Yeh et al., 2007).

Probiotic supplement is shown to reduce genotoxic potential of fecal water in patients with atopic dermatitis (Roessler, Forssten, Glei, Ouwehand, & Jahreis, 2012). Therefore, the increased fecal bifidobacteria concentration in the soluble fibre-supplemented groups may lead to a lower fecal toxic load and ameliorate colonic DNA damages. In addition, recent studies have shown that bifidobacteria and lactobacilli have anticancer properties (Clark, Robien, & Slavin, 2012; Verma & Shukla, 2013). Although mechanisms have not been fully understood, studies suggest that probiotics or their metabolite may ameliorate the transformation of AOM to toxic methylazoxymethanol by reducing colonic β-glucuronidase activity (Matsumoto, Takata, & Komeiji, 1979; Wu and Chen, 2011a), inhibiting proliferation and inducing apoptosis of colonocytes (Kumar et al., 2013).

Addition of cellulose into the low-fibre diet also ameliorated the AOM-induced DNA damage. However, the efficacy of cellulose was not as great as soluble fibres. When compared with the low-fibre groups at 24 h, cellulose did not significantly enhance gene expressions of any antioxidant enzyme examined, increase cecal SCFA and fecal bifidobacteria or lactobacilli concentrations. However, cellulose significantly reduced the gene expressions of Bcl-2 and Cyclin D1, and PI. Therefore, we suggested that the cellulose could still contribute to the cellular signals to modulate the cellular response to AOM. However, these effects may not be mediated by SCFA and underlying mechanism remains to be investigated.

In summary, the current study indicated that both soluble fibres and cellulose maintained normal cell turnover of crypts at the distal colon in mice fed a high-fat low-fibre diet. As mice were attacked by a carcinogen (AOM), dietary fibres ameliorated colonic DNA damage, with efficacy in the order of inulin > KGM > cellulose. In addition, dietary fibres increased cellular apoptosis response to AOM, with efficacy in the order of KGM > inulin > cellulose. The greater effects of soluble fibres may be mediated by butyrate and probiotics.

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hybridization, in this study, was done by using the upright fluorescence microscope, provided by the Instrument Center of Chung Shan Medical University, which is supported by National Science Council, Ministry of Education and Chung Shan Medical University, Taichung, Taiwan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.01.065.

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第二年計劃

中文摘要

目的: 本實驗的研究目的是建立高脂低纖維合併化學致癌劑 azoxymethane (AOM)之小鼠模式，並探討在起始期就補充兩種劑量(2.5, 5%, w/w)蒟蒻纖維或菊糖對大腸腫瘤發生的影響。

材料方法: 將六週齡大之 C57BL/6J 雄性小鼠隨機分為下列幾組: vehicle 控制組(腹腔注射生理食鹽水，1 次/週×7，20%玉米油及 1%纖維素飼料)及 AOM 控制組(10 mg/kg BW)，以及 AOM 注射並於飼料中添加 2.5%、5%蒟蒻纖維及菊糖組，共餵飼 30 及 45 週後進行犧牲，分析結腸異常腺窩病灶(ACF)及石蠟病理切片，以進行組織病變觀察。糞便分析則包括菌相定量及短鏈脂肪酸濃度。血液分析包括脂質過氧化物濃度，以及免疫相關細胞激素 TNF-α 及 IL-10 濃度測定。

結果: 本研究顯示高劑量蒟蒻或菊糖寡糖能在 30 周起降低後端大腸 ACF，不但如此，45 周時高劑量蒟蒻或菊糖寡糖可有效降低前端大腸病變嚴重度，使得高異常病灶 (ACF/focus)的數目降低。蒟蒻在抑制末端結腸異常腺窩病灶 (ACF/focus)的效果優於菊糖，但是對總 ACF 數目的抑制效果則蒟蒻及菊糖類似，可能與改善菌相、產生有抑制細胞病變的短鏈脂肪酸有關。

結論: 在低纖維(1% w/w)飼料中添加蒟蒻或菊苣纖維能防止高脂合併化學致癌劑 azoxymethane (AOM)造成之大腸前後端病變，並且呈現劑量效應。

關鍵字： 蒟蒻纖維、菊糖、結腸異常腺窩病灶、菌相、短鏈脂肪酸

ABSTRACT

Objective: The main purpose of this study was to investigate the effects of two doses of konjac glucomannan (KGM) or inulin supplementation on colonic carcinogenesis induced by a high-fat low fiber diet in combination with azoxymethane (AOM).

Materials and Methods: Male C56BL/6J mice (6-week-old) were randomly divided into the following groups: vehicle (saline i.p., once per week for 7 weeks) control (20% corn oil, 1% cellulose diet), and AOM (10 mg/kg BW) groups that were fed control diet and fiber supplemented groups fed with 2.5% or 5% (w/w) of KGM and inulin. Mice were sacrificed after 30 and 45 weeks of diet and carcinogenic initiation, respectively.

Results: High doses of KGM or inulin could reduce the ACF density in the distal colon since week 30, and these fiber supplementation could reduce the ACF density in the proximal colon at week 45. The effect of KGM on inhibiting the highly developed (3 crypt/focus) was greater than that of inulin. However, the inhibitory effects of KGM and inulin on total ACF numbers were similar. The underlying mechanism of the anti-carcinogenic effects of fiber could be associated with colonic microbiota and short-chain fatty acids.

Conclusion: Supplementation of KGM or inulin into a low-fiber high fat diet could effectively reduced the colonic carcinogenesis in a dose-dependent manner.

Key words: konjac glucomannan, inulin, aberrant crypt foci, microbiota, short-chain fatty acid

前言

惡性腫瘤位居國人十大死亡原因之首位，根據衛福部最新公布之資料顯示，2011 年大腸癌癌症發生人數再創新高，六度蟬聯癌症發生人數第一名，死亡率則排名癌症死亡第三位(衛生福利部統計處, 2014)，因此值得我們針對大腸直腸癌之預防做深入之研究。大腸直腸癌發生由複雜的基因因素與環境因素交互作用產生，基因因素分為先天及後天基因突變，環境因素則包括飲食內容及生活型態等。World Cancer Research Fund/American Institute for Cancer Research 歸納大量流行病學研究後指出，有助於降低直結腸癌危險性之飲食因子為葉酸、鈣及含膳食纖維的蔬菜水果(*Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*, 2007)。膳食纖維是蔬菜水果之主要成分，但是與葉酸、複雜的天然抗氧化成分以及植化素同時存在，不易釐清膳食纖維本身作用以及機制。因此，本研究以高脂飼料合併化學藥劑的方式誘發小鼠大腸病變以探討膳食纖維調節急性基因損傷、腫瘤形成過程及相關機制。

實驗目的

本研究目的是探討在 AOM 誘發大腸癌之誘發期，於高脂低纖維飼料(20%油脂，1%纖維素)中添加 2.5%及 5%之蒟蒻纖維或菊糖寡醣，對於 30 週及 45 週後小鼠大腸癌前期病變指標-結腸異常腺窩病灶(ACF)、大腸癌腫瘤、體內抗氧化作用及發炎相關指標的影響，並探討相關腸道內影響因子如菌相及短鏈脂肪酸濃度。

研究方法

1. 動物品種來源及飼養

向國家實驗研究院實驗動物中心購入 5 週齡大的 C57BL/6J 雄鼠。實驗期間將雄鼠每 3 隻分置於含木屑墊料的飼養籠中，並放置於自動照光控制(12 小時日夜循環)與室溫控制 25℃的專業動物房內，給予自由飲水和攝食。

2. 動物飼料

飼料配方如表一，Vehicle 及 AOM 組為控制飼料、纖維補充組分為低劑量蒟蒻纖維組

(L-KGM, 2.5%)、高劑量蒟蒻纖維組(H-KGM, 5%)、低劑量菊糖寡糖組(L-Inulin, 2.5%)及高劑量菊糖寡糖組(H-Inulin, 5%)。

3. 實驗設計

本實驗以高脂(20% W/W corn oil)合併化學致癌劑 Azoxymehtane (AOM) 誘發 C57BL/6J 雄鼠為大腸前期病變實驗模式。於 6 週齡大時進行隨機分組，Vehicle 組接受 0.9% NaCl 注射及控制飼料，其它 5 組為實驗組，分別為 AOM 控制組，低劑量蒟蒻纖維組(L-konjac glucomannan, L-KGM)，高劑量蒟蒻纖維組(H- konjac glucomannan, H-KGM)，低劑量菊糖寡醣組(L-Inulin)及高劑量菊糖寡醣組(H-Inulin)。介入 30 週及 45 週，期滿前收取 2 天新鮮糞便，之後禁食 24 小時，隔天秤重並犧牲。犧牲當天收取血液，大腸分為前、後兩大段分析結腸異常腺窩病灶(ACF)及石蠟病理切片，以進行組織病變觀察。糞便分析則包括菌相定量及短鏈脂肪酸濃度。血液分析包括脂質過氧化物濃度，以及免疫相關細胞激素 TNF-α 及 IL-10 濃度測定。

4. **統計**

本實驗所得到的數據皆以平均數(mean) ± 標準誤 (standard error of mean, SEM)表示，研究結果皆以社會科學軟體(SPSS Version 12.0 for Windows, SPSS, Chicago, IL, USA)進行統計分析。再以 LSD test 進行同時間點的組間比較，當 $p < 0.05$ 為組間具有顯著性差異。

結果

在體重增加方面，如 Fig 1 顯示，於誘發期第 30 周及 45 周時，各組體重皆無顯著差異。

在大腸長度方面如 Fig 2 顯示，於誘發期第 30 周時，AOM 組大腸長度最短，vehicle 組介於 AOM 以及纖維組之間，H-KGM、L-inulin 及 H-inulin 大腸長度顯著高於 AOM 組，顯示補充水溶性纖維有降低大腸病變引起的大腸縮短現象。但是到了 45 周時 vehicle、AOM、各纖維介入組之大腸長度皆無組間差異。

新鮮糞便重量及盲腸內容物重量結果如表 2 顯示。在 30 週組中，糞便重量方面各組皆無顯著差異。而在盲腸內物重方面，Vehicle 組及 AOM 組顯著低於 L-KGM 組、H-KGM 組及 H-Inulin 組 ($p<0.05$)。在 45 組週中，在糞便重量方面，L-Inulin 組顯著低於 Vehicl 組及 L-KGM 組 ($p<0.05$)。而在盲腸內物重方面，則是 H-KGM 組顯著高於 AOM 組及 L-Inulin 組 ($p<0.05$)。

前端大腸 ACFs 數目結果如表 3 顯示。Vehicle 組因未注射 AOM，其 ACF 數目皆為 0。30 週時 H-KGM 組及 H-Inulin 之 ACF 總數皆顯著低於其餘各組 ($p<0.05$)。45 週時 3 crypt/focus 的數目 H-KGM 組顯著低於 AOM 組 ($p<0.05$)，且 H-KGM 組及 H-Inulin 之 ACF 總數量皆顯著低於其餘各組 ($p<0.05$)。因此本研究顯示高劑量蒟蒻或菊糖寡糖能在 30 周起降低 ACF，不但如此，45 周時高劑量蒟蒻或菊糖寡糖可有效降低前端大腸病變嚴重度，使得高異常病灶(ACF/focus)的數目降低。

末端大腸 ACFs 數目結果如表 3 顯示。30 週時 AOM 組之 3 crypt/focus 及 ACF 總數量顯著高於其餘各組，且 L-KGM 組顯著低於 L-Inulin 組及 H-Inulin 組($p<0.05$)，而 AOM 組之 ACF 總數皆顯著高於其餘各組($p<0.05$)。45 週時 3 crypt/focus 的數目也是 AOM 組最高，兩種 KGM 組以及高菊糖組顯著低於 AOM 組 ($p<0.05$)，在 ACF 總數量方面則 H-KGM 組及 H-Inulin 皆顯著低於其餘各組 ($p<0.05$)。因此本研究顯示蒟蒻在抑制末端大腸高異常病灶(ACF/focus)的效果優於菊糖，但是對總 ACF 數目的抑制效果則蒟蒻及菊糖類似。

表一、動物飼料配方[1]

	Control	L-KGM	H-KGM	L-Inulin	H-Inulin
		g/kg			
Corn Starch	440	408.75	377.5	410.76	381.52
Sucrose	100	100	100	100	100
Casein	200	200	200	200	200
Corn Oil	200	200	200	200	200
α-Cellulose	10	10	10	10	10
KGM[1]	-	31.25	62.5	-	-
Inulin[1]	-	-	-	29.24	58.48
Modified AIN-Mineral Mix-76A[2]	35	35	35	35	35
AIN-Vitamin Mix-76A	10	10	10	10	10
Methionine	3	3	3	3	3
Choline	2	2	2	2	2
Energy Density (kcal/g)	4.76	4.63	4.51	4.64	4.52

[1]The purity of KGM and inulin was 80% and 85.5%, respectively.

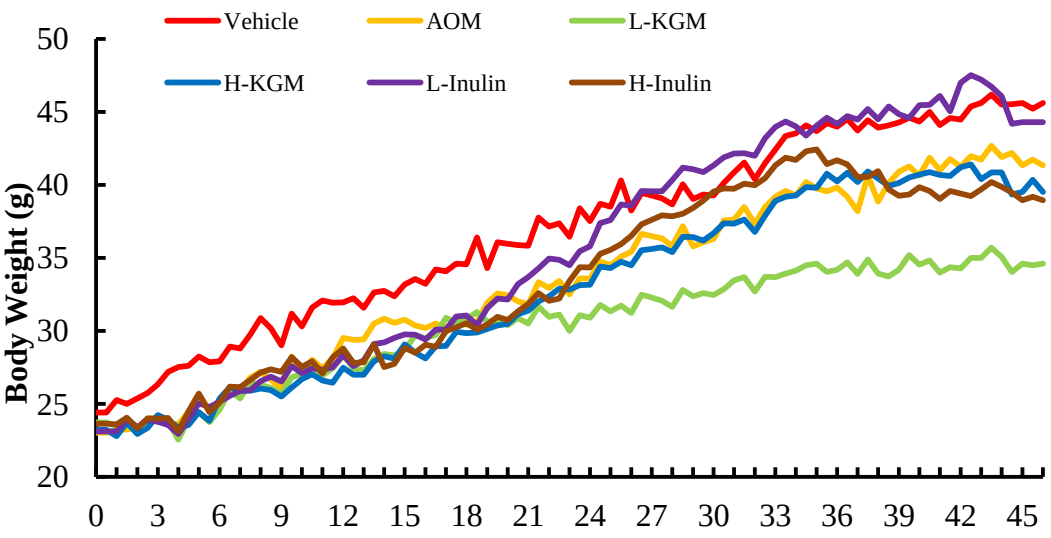


Fig. 1 體重變化情形

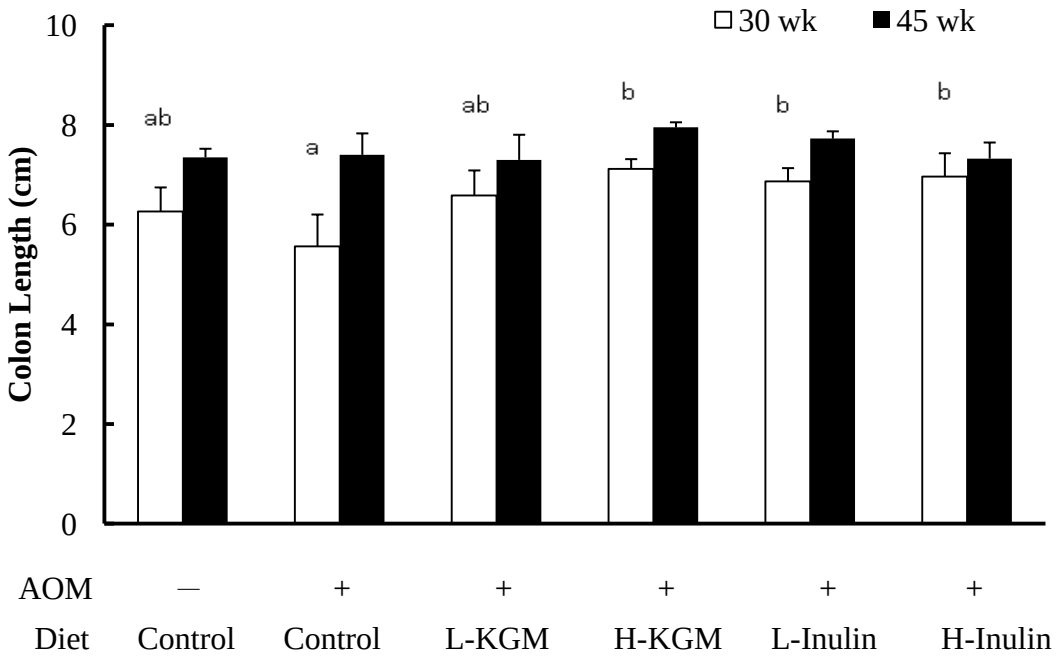


Fig. 2 誘發期第 30 及 45 周各組大腸長度

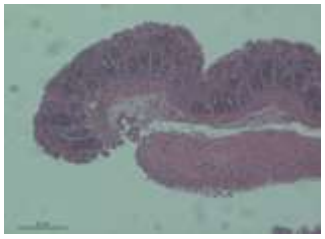
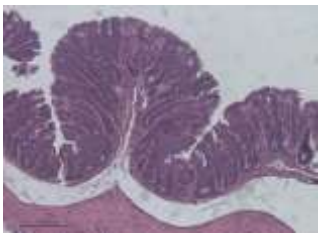
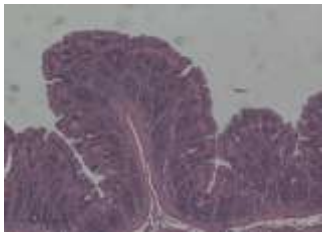
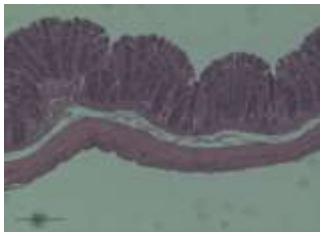
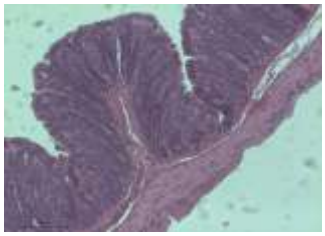
 Vehicle	 AOM	 L-KGM
 H-KGM	 L-Inulin	 H-Inulin

Fig. 3 誘發第 30 周各組小鼠之結腸組織病理觀察

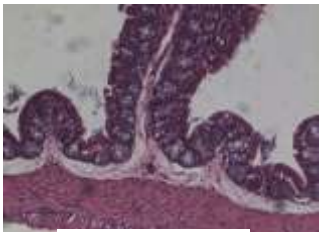
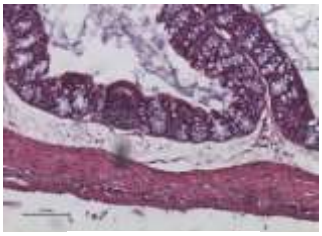
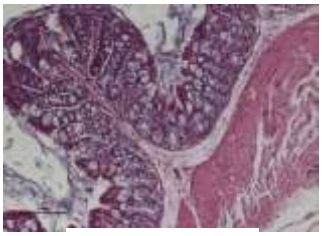
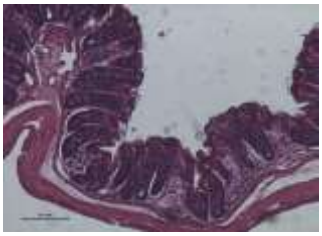
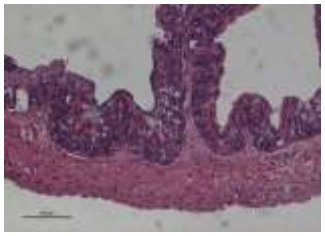
 Vehicle	 AOM	 L-KGM
 H-KGM	 L-Inulin	 H-Inulin

Fig. 4 誘發第 45 周各組小鼠之結腸組織病理觀察

表 2、補充蒟蒻及菊糖纖維對注射 AOM 雄鼠新鮮糞便重量及盲腸內容物重量之影響 [1,2]

	Vehicle	AOM	L-KGM	H-KGM	L-Inulin	H-Inulin
Fresh Feces (g/d)						
30 wk	0.37 ± 0.04	0.32 ± 0.04	0.36 ± 0.06	0.34 ± 0.04	0.31 ± 0.02	0.38 ± 0.04
45 wk	0.70 ± 0.19^{B}	0.53 ± 0.09^{AB}	0.76 ± 0.14^{B}	0.54 ± 0.09^{AB}	0.39 ± 0.09^{A}	0.54 ± 0.02^{AB}
Cecal Content (g/cecum)						
30 wk	0.11 ± 0.01^{a}	0.10 ± 0.02^{a}	0.20 ±0.03^{b}	0.20 ± 0.03^{b}	0.14 ± 0.01^{ab}	0.20 ± 0.06^{b}
45 wk	0.20 ± 0.03^{AB}	0.14 ± 0.01^{A}	0.19 ± 0.03^{AB}	0.26 ± 0.02^{B}	0.17 ± 0.02 ^{A}	0.18 ± 0.03^{AB}

[1]Data are expressed as mean ± SEM

[2]Different lower case and capitalized letters denote significant differences across groups at 30 wk and 45 wk, respectively, as analyzed by one-way ANOVA followed by LSD ($p < 0.05$).

表 3、補充蒟蒻及菊糖纖維對注射 AOM 雄鼠前端結腸 ACF 數目之影響 [1,2]

Group	≧3 crypts/ focus		Total ACF	
	30 wk	45 wk	30 wk	45 wk
AOM	1.0 ± 0.6	1.7 ± 0.8^{B}	3.6 ± 0.4^{b}	5.0 ± 0.0^{B}
L-KGM	0.4 ± 0.3	0.7 ± 0.7^{A}	2.8 ± 0.7^{b}	4.3 ± 1.1^{B}
H-KGM	0.3 ± 0.3	0.3 ± 0.6^{A}	1.8 ± 0.6^{a}	2.3 ± 0.3^{A}
L-Inulin	0.8 ± 0.4	1.0 ± 0.4^{AB}	2.8 ± 0.4^{b}	3.5 ± 0.3^{B}
H-Inulin	0.6 ± 0.3	0.5 ± 0.4^{A}	2.0 ± 0.4^{a}	2.3 ± 0.4^{A}

[1]Data are expressed as mean ± SEM

[2] Different lower case and capitalized letters denote significant differences across groups at 30 wk and 45 wk, respectively, as analyzed by one-way ANOVA followed by LSD ($p < 0.05$).

血液脂質過氧化物 MDA 濃度方面， 30 週 AOM 組之脂質過氧化物濃度顯著高於其他組 ($p<0.05$)。在補充高劑量 KGM 及 Inulin 皆能有效降低脂質過氧化物濃度 ($p<0.05$)。45 週組之組間差異類似 30 週。

血漿促發炎細胞激素方面，30 週 AOM 組之 TNF-α 濃度顯著高於 Vehicle 組 ($p<0.05$)。在補充 KGM 及 Inulin 後，無論是低或高劑量皆能有效降低 TNF-α 分泌 ($p<0.05$)。反之在 IL-10 方面，Vehicle 組之濃度較 AOM 組高 ($p<0.05$)，高劑量 KGM 及 Inulin 組濃度較 AOM 組高 ($p<0.05$)，且 H-KGM 組及 H-Inulin 組之 IL-10 濃度與 Vehicle 組相似 ($p > 0.05$)。

30 週組之每日糞便菌相在 *Bifidobacterium spp.* 方面，AOM 組菌相數量顯著低於 Vehicle 組 ($p<0.05$)。在補充 KGM 及 Inulin 後，無論是低或高劑量皆能有效促進 *Bifidobacterium spp.* 生長 ($p<0.05$)，且除了 L-KGM 組以外，H-KGM 組及 H-Inulin 組之數量與 Vehicle 組相似。在 *Lactobacillus spp.* 方面，AOM 組菌相數量顯著低於 Vehicle 組 ($p<0.05$)。在補充 KGM 及 Inulin 後，無論是低或高劑量皆能有效促進 *Lactobacillus spp.* 生長 ($p<0.05$)，且除了 L-KGM 組以外，H-KGM 組及 H-Inulin 組之數量與 Vehicle 組相似。在 *Clostridium spp.* 方面，AOM 組菌相數量顯著低於 Vehicle 組 ($p<0.05$)。在補充 KGM 及 Inulin 後，低劑量組能有效促進 *Clostridium spp.* 生長 ($p<0.05$)，且與 Vehicle 組相似。在總菌方面，AOM 組菌相數量顯著低於 Vehicle 組 ($p<0.05$)。在補充 KGM 及 Inulin 後，無論是低或高劑量皆能有效促進總體菌相生長 ($p<0.05$)，且除了 L-KGM 組以外，H-KGM 組及 H-Inulin 組之數量與 Vehicle 組相似。45 周之菌項改變趨勢與 30 周類似。

30 週組之每日糞便乙酸排出量方面，Vehicle 組約為 AOM 組的 2 倍 ($p<0.05$)。補充 KGM 及 Inulin 組之乙酸排出量顯著高於 AOM 組。在丙酸方面，Vehicle 組之排出量顯著高於 AOM 組 ($p<0.05$)。在補充 KGM 或 Inulin 後，無論是低或高劑量皆能有效促進丙酸排出量 ($p<0.05$)。在丁酸方面，H-KGM、Inulin 組皆顯著高於 AOM 組。在總短鏈脂肪酸方面，Vehicle 組之排出量為 AOM 組的 1.2 倍($p<0.05$)。在補充高劑量 KGM 及 Inulin 後，可降低 AOM 的效應，使總短鏈脂肪酸排出量與 Vehicle 組相似。45 周之短鏈脂肪酸趨勢與 30 周類似。

討論

本研究採用 AOM 化學致癌小鼠模式，在實驗設計及飼料設計有一些特別的考量。第一，大部分研究探討測試纖維於 promotion stage 的作用，但是本研究希望探討纖維從 initiation stage 到 promotion stage 的作用。第二，前人利用 AOM 誘發大腸癌模式之基礎飼料大部分是已含足

夠的纖維素(5% w/w 相當於人類每天 25 g 纖維)而再添加測試纖維於基礎飼料，但是高纖維飼料可能造成營養密度過低，使動物不容易存活，因此本研究之基礎飲食原本採取無纖維高脂肪飼料，但是於前置實驗發現接受無纖維高脂肪基礎飼料的小鼠於接受 AOM 注射後難以存活，因此正式實驗將纖維量調整至 1% w/w (相當於人類每天 5 g 纖維)。第三，本研究之基礎飼料模擬目前國人飲食傾向高脂低纖維飲食型態，前人研究發現富含 n-6 PUFA 的玉米油可順利誘發動物模式的大腸癌變(Reddy, Burill, & Rigotty, 1991)，因此本研究之基礎飲食採取高玉米油(20% w/w)低纖維(1% w/w)飼料，如此作法不但模擬了國人飲食型態，實驗飼料添加膳食纖維之後亦能保障營養素密度且正確反應出纖維的保健功效。第四，基於本實驗設計與前人模式皆不同，動物對 AOM 耐受不佳，因此採用較低劑量 AOM 多次注射的誘發方式，並且觀察不同階段(30 及 45 週)的變化。

人類及 DMH/AOM 動物模式癌變過程中與發炎有關，在異常黏膜突間叢聚、adenoma 及 adenocarcinoma 皆發現 COX-2 過度表現以及動物體內腸道、肝臟、心臟等氧化壓力上升(Chen & Huang, 2009) 。因此 DMH/AOM 造成氧化傷害、發炎、影響 cell cycle、proliferation、apoptosis、基因突變等皆是造成細胞癌化的機制。因此本研究觀察 AOM 動物模式癌變過程中，體內氧化壓力指標、發炎相關指標、大腸異常黏膜突間叢聚數目，並且測量腸道內具有降低大腸癌變的短鏈脂肪酸以及菌相。

根據 Ghirardi 等人(Ghirardi, Nascimbeni, Villanacci, Fontana, Di Betta, & Salerni, 1999)的研究顯示，於 F344 大鼠體內注射致癌藥劑 AOM，經過 30 週後，可於結腸後端發現顯而易見的異常黏膜突間叢聚、adenoma 及 adenocarcinoma。然而本實驗採用高玉米油但低劑量 AOM 注射，經過 30 周後觀察到 ACF 但是沒有 adenoma，在結腸後端誘發的 ACFs 數目會隨著膳食纖維添加的種類及比例不同而有顯著差異。在前人的研究中亦顯示，於嚙齒動物注射致癌藥劑 AOM 會增加體內發炎現象的上升，包括促進 PGE_2、IL-6 及 TNF-α 等發炎相關激素分泌(Minoura, Takata, Sakaguchi, Takada, Yamamura, Hioki, et al., 1988,(Popivanova, Kitamura, Wu, Kondo, Kagaya, Kaneko, et al., 2008)。在本實驗中同樣可以發現促發炎的細胞激素 TNF-α 因注射 AOM 後明顯上升。另外，在本實驗中還可以發現注射 AOM 後可明顯造成體內氧化壓力使血液 MDA 濃度上升。在 Hendrickse 等人的研究亦證實，脂質過氧化物 MDA 之濃度為治療大腸癌的重要目標之一 (Hendrickse, Kelly, Radley, Donovan, Keighley, & Neoptolemos, 1994)。

膳食纖維可促進腸道蠕動、有益腸道益生菌生長並並增加短鏈脂肪酸生成，因此，可能具有預防結腸癌發生的功效。已有學者發現在 promotion stage 餵食嚙齒動物含菊糖飼料後可降低大腸癌前指標 ACF 的誘發，並抑制生成大腸腫瘤 (Verghese, Rao, Chawan, & Shackelford, 2002)。在本實驗亦可發現相同情形，餵食菊糖組別之 ACF 誘發數目可較未額外添加纖維的 AOM 組少，但未有顯著性差異。此現象可能是由於本實驗是建立在一般成人可接受的纖維範圍內(相當於人類每天 5~30 g 纖維)，而非如同過往研究多半給予高纖維飲食，因此降低了組別間的差距。

結論與建議

本研究顯示高劑量蒟蒻或菊糖寡糖能在 30 周起降低 ACF，不但如此，45 周時高劑量蒟蒻或菊糖寡糖可有效降低前端大腸病變嚴重度，使得高異常病灶 (ACF/focus)的數目降低。蒟蒻在抑制末端大腸高異常病灶 (ACF/focus)的效果優於菊糖，但是對總 ACF 數目的抑制效果則蒟蒻及菊糖類似。

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博士後研究員成果

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中文摘要

目的: 本實驗的研究目的是建立高脂低纖維合併化學致癌劑 azoxymethane (AOM)之小鼠模式，並探討蒟蒻纖維、菊糖及纖維素對肝臟脂肪的影響。

材料方法: 將六週齡大之 C57BL/6J 雄性小鼠隨機分為下列幾組: vehicle 控制組(腹腔注射生理食鹽水，1 次/週×7，20%玉米油及 1%纖維素飼料)及 AOM 控制組(10 mg/kg BW)，以及 AOM 注射並於飼料中添加 5%蒟蒻纖維、菊糖及纖維素組，共餵飼 30 週後進行犧牲，觀察肝臟組織切片及分析肝臟細胞激素濃度，並以 PCR 微陣列分析調節脂肪代謝相關基因。

結果: 根據 H&E 染色結果顯示，補充蒟蒻纖維明顯減少脂肪堆積於肝臟中。相較於 AOM 組，蒟蒻纖維組顯著降低肝臟 interlukin (IL)-6 濃度；而菊糖組則顯著增加 IL-10 濃度。PCR 微陣列結果則顯示 AOM 及纖維素組共同 up-regulation 的基因有 2 個(Alox12 與 Fads3)，共同 down-regulation 的基因有 4 個(Hmgcr、IL-1β、IL-6 及 Ptgs2)。

結論: 本研究推測膳食纖維減少因高脂肪造成的肝臟中脂肪堆積，可能透過調節肝臟中脂肪代謝相關基因，因此而改變肝臟組織免疫反應。

關鍵字： 蒟蒻纖維、菊糖、纖維素、PCR 微陣列

Abstract

Objective: The main purpose of this study was to investigate the effects of konjac glucomannan (KGM), inulin and cellulose supplementation into a high-fat low fiber diet on liver lipid in an azoxymethane (AOM) injection rodent model.

Materials and Methods: Male C56BL/6J mice (6-week-old) were randomly divided into the following groups: vehicle (saline i.p., once per week for 7 weeks) control (20% corn oil, 1% cellulose diet), and AOM (10 mg/kg BW) groups that were fed control diet and addition of 5% KGM, inulin and cellulose groups. Mice were sacrificed after 30 weeks. The histopathological observation,

cytokine concentrations (TNFα, IL-6 and IL-10) and PCR array of lipid regulation in the liver were determined.

Results: The fatty liver was ameliorated in the presence of KGM group. KGM supplementation significantly reduced the liver pro-inflammatory cytokine, IL-6, concentration. However, inulin supplementation promoted the anti-inflammatory cytokine, IL-10, concentration. Lipid regulation of PCR array was showed that two genes (Alox12 and Fads3) were up-regulated and 4 genes (Hmgcr、IL-1β、IL-6 and Ptgs2) were down- regulated in the AOM and cellulose groups simultaneously.

Conclusion: These results suggested that KGM, inulin and cellulose may reduce the lipid accumulation in the liver through the regulation of lipid-related gene expression in the AOM-injection mice model.

關鍵字： 蒟蒻纖維、菊糖、纖維素、PCR 微陣列

Key words: konjac glucomannan, inulin, cellulose, PCR array

(一)前言

本實驗室已發表多篇研究蒟蒻纖維、菊糖寡醣與纖維素於動物與人體實驗，包括降低糞便水毒性、促進腸道蠕動、膽酸代謝及促進益生菌生長的功效。本研究計畫原先預同時比較不同種類的膳食纖維在化學致癌(azoxymethane, AOM)模式動物之大腸腫瘤生成過程中的作用機制(30 週以及 45 週)，由於 45 週後仍尚未於大腸部位產生腫瘤，卻發現 30 週犧牲的動物肝臟外觀產生許多小瘤(圖一)，經 H&E 染色發現是嚴重脂肪肝(圖二)，因此後續實驗分析則改為分析飼養 30 週的小鼠肝臟組織。

(二)文獻

(1)蒟蒻纖維、菊糖寡糖、纖維素簡介

蒟蒻(*Amorphophallus konjac*)為天南星科蛇芋屬多年生宿根性塊莖草本植物，蒟蒻塊莖富含葡甘聚醣是一種黏稠水溶性纖維，製成之蒟蒻果凍、素料及低卡食品頗受國人歡迎(1)。菊糖 inulin 的食物來源為菊苣塊莖(*Cichorium intybus*)、洋蔥、大蒜、蘆筍等，菊糖由果糖以β2→1 方式鍵結之低黏稠性水溶性纖維，聚合度介於 3-60 之間，可區分為菊糖寡醣、高分子菊糖纖維等商品，其中菊糖寡醣常添加於具有調節腸道功能的保健食品(2)。纖維素為葡萄糖以β1 → 4 方式鍵結之聚合物，普遍存在植物細胞壁，屬於不可溶性膳食纖維。本實驗室已發現補充蒟蒻

纖維及菊糖寡醣此兩種膳食纖維，皆具有調降血脂之功效(3)。

(2)高脂飲食

非酒精性脂肪肝(non-alcoholic fatty liver, NAFLD)與營養過剩有關,肝臟可儲存多餘的脂肪，許多研究皆指出高脂飲食與脂肪肝的發生高相關。倘若長期脂肪累積在肝細胞中，則會發展成慢性發炎的情況，造成肝細胞損傷然後修復結痂，如此反覆過程即稱為NAFLD或nonalcoholic steatohepatitis (NASH)，並且，最終肝臟硬化且衰竭(4)。前人研究指出n-6不飽和脂肪酸可抑制肝臟脂肪生成作用(lipogenesis)，透過抑制相關基因表現(5)。

(3) 膳食纖維降低血脂之機制

一、抑制膽固醇吸收及排出: 水溶性纖維在腸道內形成凝膠，可以阻隔膽固醇，影響膽固醇與消化酶、膽酸與腸黏膜的接觸；增加其從腸道排出，抑制膽固醇的腸肝循環，進一步降低肝臟中膽酸濃度，為維持二者的體內平衡，肝臟會帶償性利用膽固醇代謝為膽酸，同時膽固醇的生物合成速率也加快，另外，膳食纖維可加速腸蠕動，縮短食物在腸道的停留期。已知關華膠可藉由減慢小腸黏膜液體層之流動性，干擾小腸對膽固醇吸收(6)。

二、減少脂質與膽固醇合成

膳食纖維於大腸中經腸道菌發酵後產生之短鏈脂肪酸，由肝門靜脈吸收至肝臟中，可能扮演調節膽固醇的角色，研究指出丙酸(propionate)可抑制肝臟中膽固醇生合成反應速率限制酵素 HMG-CoA reductase 活性，因而降低膽固醇合成，減少血液膽固醇濃度(7)。

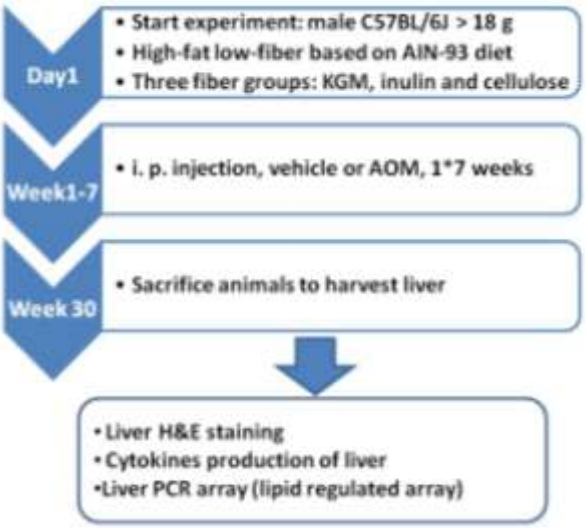
(4) AOM 代謝

AOM 是目前最常用來誘發嚙齒動物自發性大腸癌的化學致癌劑(8)。AOM 後經肝臟 P450 之 CYP2E1 轉換為 methylazoxymethanol (MAM)，再形成具有高度活性的 methyldiazonium ion，隨著膽汁進入腸道或藉由血液接觸腸道細胞而導致病變。AOM 亦造成動物體內腸道、肝臟、心臟等氧化壓力上升(8)。因此 AOM 造成氧化傷害、發炎、影響 cell cycle、 proliferation、apoptosis、基因突變等皆是造成細胞癌化的機制。有研究指出 AOM 造成 C57BL/6J 小鼠小囊泡性脂肪肝(microvesicular steatosis)、肝血竇擴張(sinusoidal dilatation)、肝小葉壞死(centrilobular necrosis)等等肝臟損傷(9)。

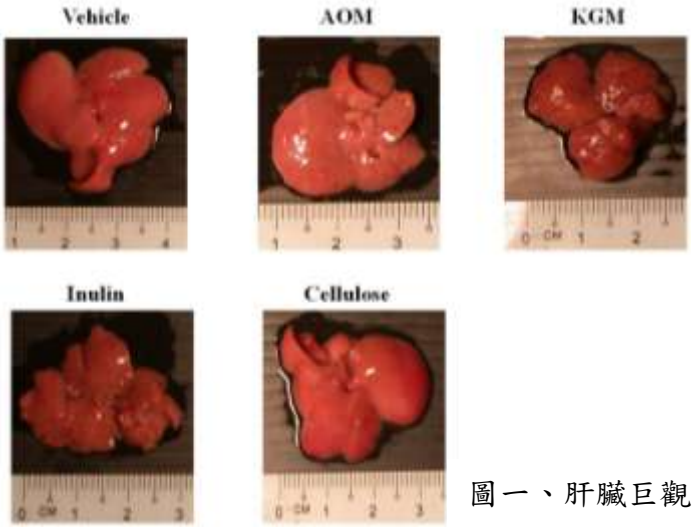
(三) 研究目的

以高脂(20%玉米油, w/w)低纖維(1% cellulose，相當於人類每天 5 g 纖維)飲食之 male C57Bl/6J 小鼠作為癌症控制組，探討添加 5%蒟蒻纖維、菊糖寡醣及纖維素於 AOM 注射(10 mg/kg BW，每週一次，共 7 次)對肝臟的影響。

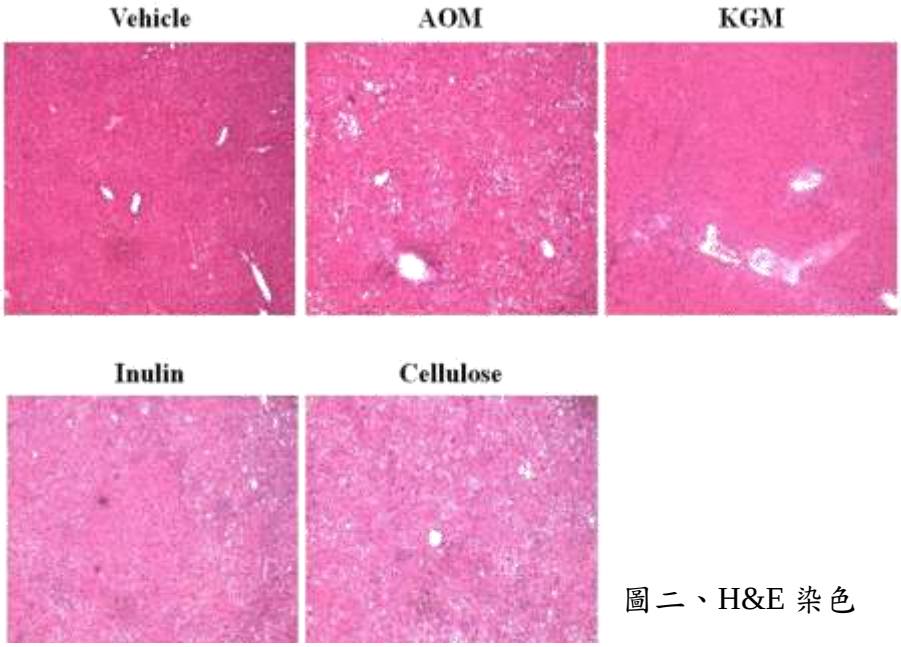
(四)研究方法



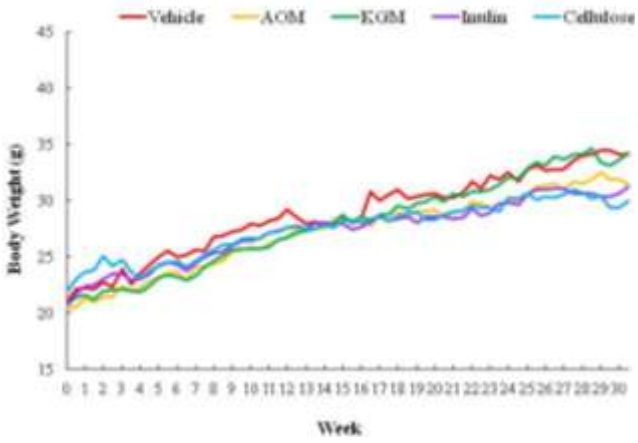
(五)結果



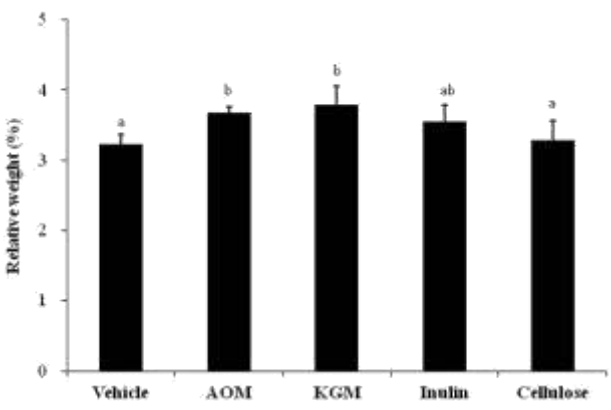
圖一、肝臟巨觀



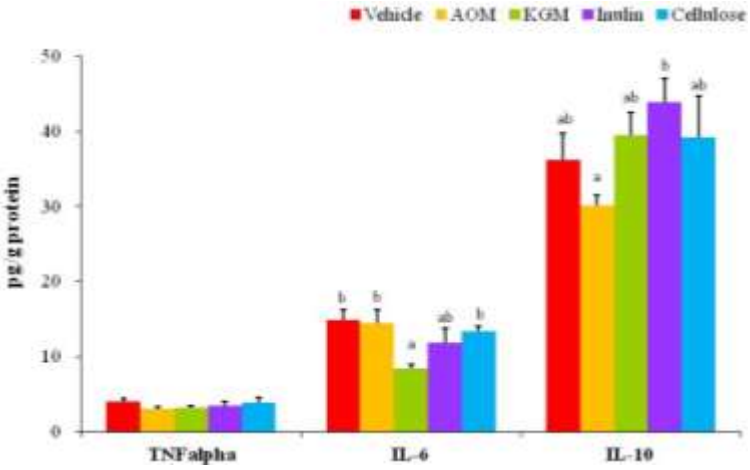
圖二、H&E 染色



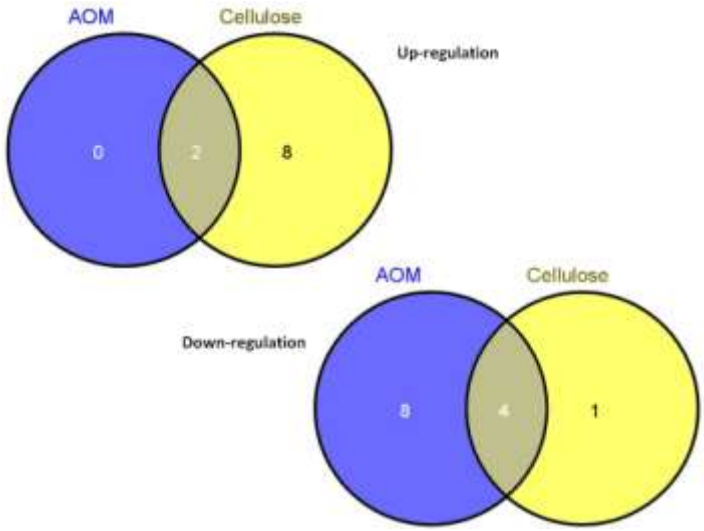
圖三、體重變化



圖四、肝臟相對重量



圖五、肝臟中細胞激素濃度



圖六、PCR array

表一顯示膳食纖維補充可顯著降低體重增加，然而蒟蒻纖維與菊糖組能量攝取顯著低於纖維素組。

圖二肝臟 H&E 染色結果顯示，補充蒟蒻纖維組與 Vehicle 組相似，AOM 組別肝臟切片則呈現很多小囊泡性脂肪空洞；另外肝臟相對重量，AOM 與蒟蒻纖維素組顯著高於纖維素組(圖四)。

肝臟中 TNFα各組之間沒有差異(圖五)，然而相較於 AOM 組，蒟蒻纖維組肝臟 IL-6 濃度顯著降低，菊糖組則顯著增加肝中 IL-10 濃度。

因 H&E 染色結果發現，腹腔注射 AOM 連續 7 週，並同時餵食高脂肪低纖維飼料 30 週後，形成嚴重脂肪肝，因此 PCR 微陣列分析則針對脂質調控做分析，先以 sterol regulatory element-binding protein 1 c、Peroxisome proliferator-activated receptor a、fatty acid synthase 及 Acetyl-CoA carboxylase 測試五組之間差異，結果纖維素組表現最大，因此選定 vehicle、AOM 及纖維素三組做 PCR 微陣列分析，結果以 vehicle 當成 1 的結果顯示(圖六)，AOM 及纖維素共同 up-regulation 的基因有 2 個(Alox12 與 Fads3)，共同 down-regulation 的基因有 4 個(Hmgcr、IL-1β、IL-6 及 Ptgs2)。

表一、補充膳食纖維對體重增加與每日能量攝取之影響

	Vehicle	AOM	KGM	Inulin	Cellulose
Body weight gain (mg/d)	74.9 ± 13.8^{b}	66.7 ± 21.6^{b}	38.5 ± 9.2^{a}	41.1 ± 3.1^{a}	35.1 ± 10.2^{a}
Energy intake (kcal/day)	13.1 ± 2.4^{ab}	12.7 ± 1.7^{ab}	11.9 ± 0.2^{a}	11.9 ± 0.5^{a}	14.9 ± 0.5^{b}

(六)參考文獻

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國科會補助專題研究計畫出席國際學術會議心得報告-1

陳曉鈴部分　　日期：103 年 5 月 8 日

計畫編號	NSC101－2320－B－040－018－MY2		
計畫名稱	以高脂低纖維飲食及致癌劑誘發大腸病變模式探討蒟蒻纖維、菊糖寡醣及纖維素調節急性基因損傷、腫瘤形成、抗腫瘤免疫及相關機制		
出國人員姓名	陳曉鈴	服務機構及職稱	中山醫學大學營養學系教授
會議時間	2014 年 4 月 26 日至 2014 年 4 月 30 日	會議地點	San diego, CA, USA
會議名稱	(中文) (英文) Experimental Biology 2014		
發表題目	(中文) (英文) Konjac glucomannan and inulin modulated the immune function in a murine model of dextran sodium sulfate-induced colitis		

一、參加會議經過

Experimental Biology 是生物醫學界最大的聯合會議，是由 American Association of Anatomists (AAA)、The American Physiological Society (APS)、American Society for Biochemistry and Molecular Biology (ASBMB)、American Society for Investigative Pathology (ASIP)、American Society for Nutrition (ASN)以及 American Society for Pharmacology & Experimental Therapeutics (ASPET)等 6 個學會所組成，因此有機會與近 14,000 名跨領域的學者面對面討論。以下是議程表。

	SATURDAY, APRIL 26, 2014				SUNDAY, APRIL 27, 2014		
	8:00 – 10:00 AM	10:30 AM – 12:30 PM	12:45 – 2:45 PM	3:00 – 5:00 PM	8:00 – 10:00 AM	10:30 AM – 12:30 PM	3:00 – 5:00 PM
Ballroom 20 D	**Fortification and Health: Opportunities and Challenges** K. Wiemer and J. Dwyer			**Energy Drinks: Current Knowledge and Critical Research Gaps** B.C. Sorkin and P.M. Coates	**Nutri-Metabolomics** N. Moustaid-Moussa and F. Assadi-Porter	**Presidential Symposium: Malnutrition and Inflammation: Intimate Partners** G. Jensen, *ASN President*	**Unscientific Beliefs About Scientific Topics in Nutrition** D.B. Allison and A.W. Brown
31 ABC	**Circulating Vitamin D and Risk of Breast and Colorectal Cancer** S. Smith-Warner and R. Ziegler	**Dietary Patterns Methods Project** J. Reedy		**Insights and Perspectives on Dietary Modifications to Reduce the Risk of CVD** B. Bradley and D. Baer	**How Should we Collect Dietary Data for Research?** R. Bailey and C. Zizza	***12:45 – 2:45 PM* Food and Nutrition Board Update** A. Yaktine S. Murphy	**Are Biofortified Staple Food Crops Improving Vitamin A and Iron Status in Women and Children?** J.P. Peña-Rosas and F. de Moura
Education Track Rm. 29AB	**8:00 AM – 9:30AM Clinical Emerging Leaders Award Competition**	**10:00 AM – 12:00 PM The Postdoctoral Research Award Competition**	**12:30 – 2:00 PM ASN Young Minority Investigator Oral Competition**	**2:30 – 5:00 PM Graduate Student Research Award Competition**	**Best Practices for Your Research Toolkit** R.A. Creasy	***12:45 – 2:45 PM* USDA-NIFA Funding Opportunities** D. Chester and J. Williams	**Nutrition Competencies in Health Professionals' Education and Training: A New Paradigm** P.M. Kris-Etherton and E. Saltzman
32B					Medical Nutrition: Interventions for the Treatment and Prevention of Nutrition-Related Diseases		Nutrition Epi and MAC: Epidemiologic Methods in Examining Health Outcomes in Diverse Populations
32A			PhenHRIG: Phenolic Compounds and Human Cognitive Function: Food for Thought		Nutrition Education and Knowledge of Medical Students and Practicing Clinicians		Determinants of Lactogenesis, Duration and Other Indicators of Lactation Success
30D		Nutritional Epi: Innovation and Validation of Dietary Assessment Tools and Their Applications			Global Nutrition: Infant & Young Child Feeding		Health and Food Systems Approaches in Community and Public Health
30C		Obesity: Physical Activity and Chronic Disease			EMM: : Diet and/or Exercise Regulation of Food Intake		EMM: Obesity and the Metabolic Syndrome
30B		Lactation: Bioactive Compounds and Other Milk Constituents			Effects of Dietary Bioactive Components on Experimental Models of Chronic Disease Risk		Antioxidant and Anti-inflammatory Effects of Dietary Bioactive Components
30A		Carotenoids. Retinoids and Health			Vitamins and Minerals: Zinc and Iron		~~Vitamins and Minerals: B~~ Vitamins and One-Carbon Metabolism
29D				Obesity: Diet, Behavior, Devices and Surgery	Diet and Cancer: Animal Studies		Diet and Cancer: Clinical and Human Studies
29C		Medical Nutrition: Nutrition and Inflammation			Aging: Nutrition and Cognition Across the Lifespan		Animal Research Models of Fetal Programming and Neonatal Development

This overview includes sessions programmed by ASN's Scientific Program Committee.
View ASN's *Society Highlights* and *Guest Society Highlights* in the onsite program for Council, RIS and other activities.

American Society for Nutrition at Experimental Biology at Experimental Biology 2014 – San Diego, CA

All sessions listed are in the San Diego Convention Center unless otherwise noted.

	MONDAY, APRIL 28, 2014			TUESDAY, APRIL 29, 2014		
	8:00 – 10:00 AM	10:30 AM – 12:30 PM	3:00 – 5:00 PM	8:00 – 10:00 AM	10:30 AM – 12:30 PM	3:00 – 5:00 PM
Ballroom 20 D	**Neurocognition: The Food-Brain Connection** M. Kelley and N.A. Khan		**Optimizing Protein Quantity and Distribution to Improve Health Outcomes** H. J. Leidy and W. W. Campbell	**It's Alive Microbes and Cells in Human Milk and their Potential Benefits to Mother and Infant** L.Bode and M. McGuire	**Human Milk Oligosaccharides** L. Bode and S. Donovan	**The Science of Cocoa Flavanols Bioavailability, Emerging Evidence and Proposed Mechanisms** J. Blumberg
		E.V McCollum Lecture 1:45 – 2:45 PM K. Dewey			***W.O. Atwater Lecture 12:45 – 1:45 PM*** D. Allison	**Ballroom: 20 BC** ***DANONE Award Lecture 5:00-6:15 PM*** G. Hotamisligil
31 ABC	**Dietary Whole Grain-Microbiota Interactions** N.L. Keim and R.J. Martin	**International Breast Cancer and Nutrition** C. M. Weaver and D. Teegarden	**Novel Mathematical Models for Investigating Topics in Obesity** S. B. Heymsfield and D. B. Allison	**Beyond Blood Pressure. New Paradigms in Sodium Intake Reduction and Health Outcomes** J.King and K. Reimers	**Modifying Eating Behavior Novel Approaches for Reducing Body Weight** M. A McCrory and N. Gletsu-Miller	**Research Advances and Considerations for Investigating the Human Diet, Nutrient Utilization and Microbiota Interface Across the Life Course** C. Davis and J. McDermid
Education Track Rm. 29AB	**International Forum - Brazil**	**International Forum - China**	**The Future of Nutrition Research at NIH** C. Davis and S. Ohlhorst	**Successful Scientist - What's the Winning Formula?** A.J. Stull and E. Ciappio	**Historical Impact of Nutritional Epidemiology** D. H. Alpers and D.s Bier	**International Forum - Japan**
32B	Nutrition Epi: Dietary Supplements and Bioactives	Nutrition Epi: Exploring Geographic Based Methods in Nutrition Epi Research	**International Forum-South America**		Global Nutrition: Household Food Insecurity & Social Determinants	
32A	Experimental Animal Research Models of Nutrient Metabolism	Global Nutrition: Prenatal Micronutrient Interventions	Nutrition Epi: Epi Research Addressing Diet and Health Outcomes	Nutrition Epi: Advancing Nutritional Epidemiology with Public Use and Commercial Data Sets	Nutrition Education: Health Eating Behaviors Across the Lifespan	Global Nutrition: Bio-behavioral Outcomes of Micronutrient Interventions
30D	Effects of Lactation/Breastfeeding on the Recipient Infant and/or Lactating Mother	Aging: Nutrition, Physical Performance and Bone Health	Food Security and its Connections to Nutrition and Health	Nutrition Epi: Nutrition and Chronic Disease Epi	Health Disparities and Promoting Health in Diverse Populations	Community and Public Health Nutrition: Food Environment
30C	EMM: Metabolic Phenotyping, Metabolomics and Biomarkers	EMM: Protein and Amino Acid Metabolism	EMM: Dietary Factors Affecting Lipid Metabolism	EMM: Energy Balance, Macronutrients and Weight Management	EMM: Protein Intake and Health Implications	Obesity: Body Composition
30B	Cardiovascular Effects of Dietary Bioactive Components	Dietary Bioactive Components of Medicinal, Functional and Whole Foods	Bioavailability, Metabolism and Biomarkers of Dietary Bioactive Components	Mechanisms of Action and Molecular Targets of Dietary Bioactive Components	Nutrition Immunology	Nutrition Immunology: Nutrition, Infection and Immunity
30A	Vitamins and Minerals: Micronutrient Interventions	Vitamins and Minerals: Fat Soluble Vitamins and Chronic Disease	Nutrient-Gene Interactions: Nutritional Regulation of Epigenetics	Nutrient-Gene Interactions: Nutrition and the Genome	Nutrient-Gene Interactions in Obesity and Inflammation	
29D	Community and Public Health Nutrition Interventions	Diet and Cancer: Molecular Targets	Aging: Nutrition Interventions for Risk Factor Modification in Chronic Disease	Vitamins and Minerals: Selenium	Public Policy Nutrition: Nutrition Research and Surveillance to Improve the Health of the US Population	Nutrition Translation: Food Related Behaviors and Implications for Food Policy
29C	Nutrition Education: Childhood Obesity Prevention (I)	Nutrition Education: Childhood Obesity Prevention (II)	Nutrition Education: Nutrition Education and Behavior Change			

YOGURT IN NUTRITION
INITIATIVE FOR A BALANCED DIET

2nd GLOBAL SUMMIT ON THE HEALTH EFFECTS OF YOGURT

8:00 am	**Welcome & Introduction**, *Sharon Donovan and Raanan Shamir*
8:05 – 8:30 am	**History of yogurt and current patterns of consumption** *Speaker: Mauro Fisberg / Moderator: Andrew Prentice*
8:30 – 10:00 am	**Yogurt and health in the life cycle** Yogurt consumption associated with adequate nutrient intake and decreased metabolic diseases in children and adolescents (including latest results of HELENA study) *Speaker: Luis Moreno* Nutr Rev 2014;72:68-81 Impact of Yogurt on appetite control and energy balance and body composition *Speaker: Angelo Tremblay / Moderator: Barbara Rolls* Importance of milk protein on the health status of the elderly (> 50 years health status) *Speaker: Robert R. Wolfe / Moderator: René Rizzoli* Dairy protein and musculoskeletal health: Report of the EU working group *Speaker: René Rizzoli / Moderator: Robert R. Wolfe*
10:00 – 10:15 am	*Break*
10:15 – 11:45 am	**Future hot topics** Dietary dairy product intake and incident type 2 diabetes *Speaker: Nita Forouhi / Moderator: Sharon Donovan* Gut microbiota & health: What's new? *Speaker: Olivier Goulet / Moderator: Raanan Shamir* Microbiota and the Gut-Brain axis *Speaker: John Bienenstock / Moderator: Raanan Shamir* Yogurt and sustainability: Energy and protein conversion by dairy cows *Speaker: Toon Van Hooijdonk / Moderator: Chris Cifelli*
11:45 am – 12:15 pm	**Posters session** Short presentations from selected posters dedicated on yogurt *Moderator: Sharon Donovan*
12:15-12:30 pm	**Wrap up: Key role of yogurt for the future** *Speaker: Frans Kok*
12:30 pm	**Conclusion**, *Sharon Donovan and Raanan Shamir*
12:30 – 2:00 pm	**Tasting session with Ellie Krieger** After a short presentation, we will have a tasting session featuring yogurt in all different ways. Recipes come from Ellie's award-winning cookbooks. A signing session of her last book ***Weeknight Wonders*** will follow.

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April 26-30, 2014

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YI-CHUN HAN

TAICHUNG TAIWAN

STUDENT

二、與會心得

今年除了發表海報之外，也參與台灣營養學會及中華民國免疫學會與美國營養學會 (American Society of Nutrition, ASN)合作會議，針對促成台灣營養學會在 ASN 主辦會議中發表營養相關議題專欄、協助拓展台灣學生國際觀及相關學會、贊助商合作等議題進行商討。

以下為合作會議照片記錄:


台灣營養學會與美國營養學會 (American Society of Nutrition, ASN)合作會議


中華民國免疫學會與美國營養學會 (American Society of Nutrition, ASN)合作會議

此次活動與腸道免疫、腸道代謝物相關的資訊很多，可見目前研究重點著重於腸道相關功效，我已針對腸道相關研究多年，目前也著手進行 Nutritional immunology 相關研究，因此感覺受益良多，增進更多相關研究方向資訊。

三、發表論文全文或摘要

Konjac glucomannan and inulin modulated the immune function in a murine model of dextran sodium sulfate-induced colitis.

Yi-Chun Han, Hsiao-Ling Chen: School of Nutrition, Chung Shan Medical University, Taichung, Taiwan, ROC

This study was to investigate the protective effects of konjac glucomannan (KGM) and inulin on colonic colitis in a dextran sodium sulfate (DSS)-induced murine model.

During the 21-d diet period, six-week-old C57BL/6J mice were randomly assigned and fed AIN-93 diet (vehicle, DSS group) or fiber-supplemented (KGM 2%, Inulin 2%, or KGM 1% + Inulin 1% w/w) diet. In the following 5-d DSS period, all mice were fed AIN-93 fiber-free diet while drinking water with DSS (3% w/v) was offered only to DSS and fiber groups. MICE were sacrificed on the 3rd of DSS-withdrawn period. The colonic tissues were collected to determine the pathohistology and gene expression of tight junction proteins by RT-PCR. The plasma TNF-α and IL-10 were also analyzed.

DSS group had the greatest weight loss and DAI throughout the DSS period and its index of colitis continued to increase in the DSS-withdrawn period. At the end of the study, the crypt depth was reduced at the proximal colon and severe colitis occurred at the distal colon in the DSS group, which was ameliorated with fiber supplementation. In addition to that, soluble fiber normalized the DSS-induced alteration in the plasma TNF-α and IL-10 levels and the gene expression of occludin.

Pre-supplementation of KGM and inulin prevented the colitis-related symptoms in the DSS-induced colitis.

This study was supported by the National Science Council Grant NSC-101-2320-B-040-018-MY2, Taiwan.

Abstract Number: 1853

Poster Session Title Dietary Bioactive Components: Antioxidant and Anti-inflammatory Effects of Dietary Bioactive Components

Day of Presentation: Monday, April 28, 2014

Program Number: 830.2

Poster Board Number: C364

陳曉鈴教授與蔡嘉哲教授於發表之海報前合影

陳曉鈴教授與與會日本教授於發表之海報前合影

四、建議

感謝國科會贊助。

此次有幸參與台灣營養學會及中華民國免疫學會與美國營養學會 (American Society of Nutrition, ASN)合作會議，針對推廣營養相關議題討論，建議相關政府機構可藉由推廣台灣各類學會之相關會議進行國際合作，進而提升台灣觀光率及專業領域之交流。

五、攜回資料名稱及內容

於營養學會發表之時段表及各發表之名稱、學者名片、廠商資料、會議資料等。

國科會補助專題研究計畫出席國際學術會議心得報告-2

韓怡君部分　　日期：103 年 5 月 8 日

計畫編號	NSC101－2320－B－040－018－MY2		
計畫名稱	以高脂低纖維飲食及致癌劑誘發大腸病變模式探討蒟蒻纖維、菊糖寡醣及纖維素調節急性基因損傷、腫瘤形成、抗腫瘤免疫及相關機制		
出國人員姓名	陳曉鈴	服務機構及職稱	中山醫學大學營養學系教授
會議時間	2014 年 4 月 26 日至 2014 年 4 月 30 日	會議地點	San diego, CA, USA
會議名稱	(中文) (英文) Experimental Biology 2014		
發表題目	(中文) (英文) Konjac glucomannan and inulin modulated the immune function in a murine model of dextran sodium sulfate-induced colitis		

一、參加會議經過

今年 Experimental Biology 2014 於 4 月 26 - 30 日在 San diego convention center 舉行，此次會議中，在 Dietary Bioactive Components: Antioxidant and Anti-inflammatory Effects of Dietary Bioactive Components 的類別中，以海報張貼的方式發表研究題目『Konjac glucomannan and inulin modulated the immune function in a murine model of dextran sodium sulfate-induced colitis』，張貼的時間是 4 月 28 日，在 presentation time (1:45pm - 2:45pm)時間內與會學者互相交流，從中培養聽力以及口語表達能力，同時也是接受先進指教的好機會。

今年除了發表海報之外，也有幸參與台灣營養學會及中華民國免疫學會與美國營養學會 (American Society of Nutrition, ASN)合作會議，更加增進了國際觀以及對於台灣營養學會拓展推廣台灣營養的了解。

陳曉鈴教授與學生韓怡君於會議中心合影

二、與會心得

1. Experimental Biology 2013 學術研討會由六個協會共同舉辦，除了 American Society of Nutrition 之外，亦能聆聽其他領域有興趣的演講。感謝國科會此次補助出席此國際會議。

2. 透過國際會議中海報論文發表研究成果與來自各地區學者交流，相信能促進國內與國際交流的發展。並能有效培養聽力以及口語表達能力。

3. 透過合作會議，了解國際合作以協助推廣台灣營養的重要性，更能增加國際觀。

4. 會場中參展的廠商，展示許多生物醫學相關的最新技術服務、藥品以及儀器，透過新儀器與技術的認識，日後應用獲益良多。

三、發表論文摘要

Konjac glucomannan and inulin modulated the immune function in a murine model of dextran sodium sulfate-induced colitis.

Yi-Chun Han, Hsiao-Ling Chen: School of Nutrition, Chung Shan Medical University, Taichung, Taiwan, ROC

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DSS group had the greatest weight loss and DAI throughout the DSS period and its index of colitis continued to increase in the DSS-withdrawn period. At the end of the study, the crypt depth was reduced at the proximal colon and severe colitis occurred at the distal colon in the DSS group, which was ameliorated with fiber supplementation. In addition to that, soluble fiber normalized the DSS-induced alteration in the plasma TNF-α and IL-10 levels and the gene expression of occludin.

Pre-supplementation of KGM and inulin prevented the colitis-related symptoms in the DSS-induced colitis.

This study was supported by the National Science Council Grant NSC-101-2320-B-040-018-MY2, Taiwan.

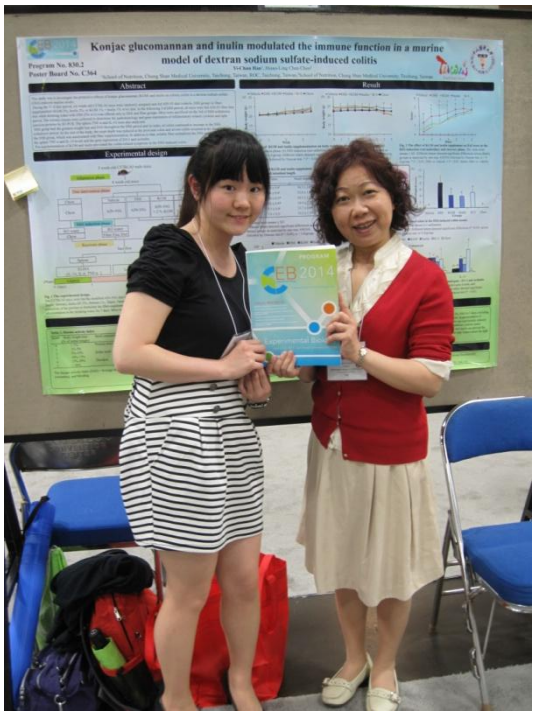
Abstract Number: 1853

Poster Session Title Dietary Bioactive Components: Antioxidant and Anti-inflammatory Effects of Dietary Bioactive Components

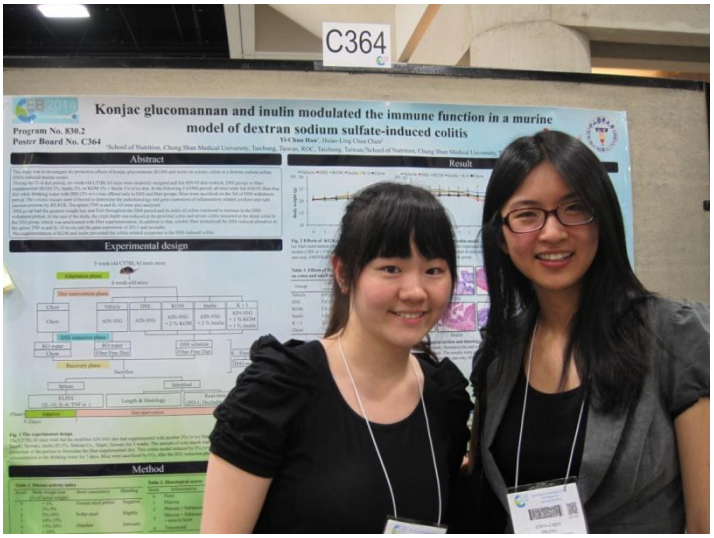
Day of Presentation: Monday, April 28, 2014

Program Number: 830.2

Poster Board Number: C364



陳曉鈴教授與學生韓怡君於發表之海報前合影



學生韓怡君與與會交流學生於發表之海報前合影

四、建議

參與國際會議除了可以增加國際觀，增加英語聽力、口語能力外，過程中可與世界各地學者交流，不僅可以當場請益，更能幫助拓展人際網絡，對個人能力提升或是提升國際聲譽都有幫助。感謝國科會給予補助出席國際會議，期望政府單位可以繼續於科學研究上的投資及獎勵。

五、攜回資料名稱及內容

會議手冊及論文發表張貼日程表手冊各一本、紀念背包一個。

科技部補助計畫衍生研發成果推廣資料表

日期:2014/06/26

科技部補助計畫	計畫名稱: 以高脂低纖維飲食及致癌劑誘發大腸病變模式探討蒟蒻纖維、菊糖寡醣及纖維素調節急性基因損傷、腫瘤形成、抗腫瘤免疫及相關機制
	計畫主持人: 陳曉鈴
	計畫編號: 101-2320-B-040-018-MY2　　學門領域: 保健營養

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：陳曉鈴	計畫編號：101-2320-B-040-018-MY2
計畫名稱：以高脂低纖維飲食及致癌劑誘發大腸病變模式探討蒟蒻纖維、菊糖寡醣及纖維素調節急性基因損傷、腫瘤形成、抗腫瘤免疫及相關機制	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	1	1	100%		
		博士後研究員	1	1	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	2	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	1. 參與 American Society of Nutrition 年會(Experimental Biology Conference), 並與會長親自會面討論兩國學會合作 2. 獲得科技部特殊傑出人才獎 3. 協助食品安全產業 4. 建立黃豆製品標準安全製造流程

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

第一年成果已經發表, 第 2 年成果整理撰寫中。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

本報告第一年探討蒟蒻纖維及菊糖寡醣對於急性 AOM 誘發之毒性(已經發表於 Food Chem)。第二年則發展 AOM 合併高脂飲食誘發之大腸癌前期病變模式，分為 30 及 45 周兩個時期，各測量動物生長、異常腺窩病灶(ACF)數量、病理切片觀察、血液氧化壓力指標以及免疫指標、大腸菌相以及短鏈脂肪酸。再者，博士後研究員則針對研究過程發現之嚴重肝臟病變探討 AOM 合併高脂飲食誘發肝臟發炎性脂肪肝之病理以及介入纖維素的效應，以 PCR array 篩出可能被調控之發炎、脂質代謝等基因群，再輔以促發炎細胞激素等測量。