行政院國家科學委員會專題研究計畫 成果報告

探討果寡醣、異麥芽寡醣對慣性便秘安養中心居民之大腸菌相、血液抗氧化狀態及大腸癌前危險指標之影響(第3年)
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中 華 民 國 99 年 09 月 21 日

行政院國家科學委員會補助專題研究計畫 ☑成果報告 □期中進報告

探討果寡醣、異麥芽寡醣對慣性便秘安養中心居民之大腸菌相、血液抗氧化狀態及大腸癌前危險指標之影響

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成果報告類型(依經費核定清單規定繳交)：□精簡報告　☑完整報告

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中　華　民　國　99 年 9 月 19 日

目錄

中文摘要

  本研究探討果寡醣生技寡醣對安養中心低活動力老人腸道生理、菌相的調節作用，以及血液過氧化狀態的影響。本研究於台中市安養中心進行，以固定飲食型態下分為安慰劑期、補充期及恢復期。研究成果已被接受刊登(Nutrition)並且已先行於網路上刊登，敬附論文於下。

關鍵詞: 果寡醣、老人、糞便細菌酵素、氧化壓力、短鏈脂肪酸、腸道功能

The Beneficial Effects of Fructo-oligosaccharides Supplementation on Fecal Bifidobacteria and Index of Peroxidation Status in Constipated Nursing-Home Residents—A Placebo-controlled, Diet-controlled Trial
將刊登於 Nutrition **2010 (in press)**

Abstract

Objective: This study assesses effects of fructo-oligosaccharides (FOS) supplementation on fecal bifidobacteria, lipid peroxidation index and indices of nutritional status, and whether effects of FOS sustained after its withdrawal, in constipated nursing-home residents. The associations of fecal bifidobacteria and blood measurements were also examined.

Methods: Six men and four women participated in a double-blind, diet-controlled study that consisted of a 4-wk placebo (3 mL fructose syrup) period, a 4-wk FOS (10 g/d) period and a 4-wk post period. Stools were collected during the last week of each period to determine the microflora and fecal weight. Fasting blood was collected at the end of each period and analyzed for thiobarbituric acid-reactive substances (TBARS) and biochemical indices.

  Results: Fecal counts (Log counts/g dry feces) and daily fecal output of bifidobacteria significantly increased with FOS as compared to the placebo. The effect on bifidobacteria output lasted through the post period. Plasma TBARS concentration was reduced by 16% and 21% in the FOS and the post period, respectively, as compared to that in the placebo period. The plasma cholesterol level was significantly reduced by approximately 7% in both the FOS and post period, respectively, as compared to that in the placebo period. The increases in fecal bifidobacteria output during the FOS period (Log CFU/d) was associated with decreases in plasma TBARS and plasma cholesterol, respectively.

Conclusion: Supplementation of FOS increases the daily output of bifidobacteria, and decreases plasma TBARS and cholesterol concentrations in constipated nursing-home elderly residents and these effects remained at the end the post period.

Keywords: fructo-oligosaccharides, thiobarbituric acid-reactive substances (TBARS), constipation, elderly, cholesterol

Introduction

The elderly population has high incidences of impaired oral function and bowel function, and chronic diseases.[1,2] It has been reported that the elderly people with poor oral function tend to select fewer dietary fiber sources, fruits and vegetables, which aggravate their gastrointestinal conditions.[3] A soluble dietary fiber supplement that could be easily incorporated into their ordinary diet may improve the bowel function or age-related syndromes.

Fructo-oligosaccharides (FOS) has been commonly applied to functional food as it could modulate the bowel function. Two studies have indicated that daily administration of 10-15 g of FOS to healthy young adults significantly improves defecation frequency and feeling of incomplete defecation.[4,5] FOS is poorly digested in the human small intestine, but is fermented by the resident microflora in the human colon.[4] Its bifidogenic effects in healthy adults have been extensively demonstrated in well-controlled human trials.[4,6,7] Aging has been associated with decreased fecal bifidobacteria concentrations, which may partially cause dysfunction of the intestine, decreased immunity, and greater susceptibility to disease.[8] Unfortunately, there is a lack of study that demonstrates the role for FOS in elderly subjects, especially in constipated, long-term care residents.

Free radical attack has been associated with neurodegenerative and cardiovascular diseases and cancer, all of which become more frequent with age.[9] Recent studies have found bifidogenic dietary fiber to have antioxidative effects [10-11]. An *in vitro* study demonstrates that fermentation of FOS by bifidobacteria exerts excellent eliminating effects on free radicals.[10] A clinical study also have suggested that consumption of prebiotics beneficially reduces blood oxidative status and elevates plasma antioxidant, such as α-tocopherol and uric acid, levels in men.[11] The possibility that FOS supplement may reduce the plasma pro-oxidative status in the elderly, as does a possible antioxidant mechanism for FOS, remains unexplored.

The main goal of this study was to assess effects of FOS supplementation on colonic microflora and lipid peroxidation status, and the association of these two measurements, in constipated nursing-home residents. A secondary objective was to determine if effects of FOS sustained after its withdrawal. The fecal characteristics, bowel functions, and selective plasma indices of nutritional status also were examined.

Materials and methods
Subjects

We recruited volunteers from a local nursing home. The volunteers who were on medication for treating constipation or otherwise had spontaneous bowel movements less than three times per week or had straining with passage of bowel movement under no medication were defined as constipated. The criteria for recruitment were stable physiological condition, long-term residence (> 6-months), chronic constipation (> 6-months), ability to chew soft or blended diet, not bed-ridden, and no tobacco or antibiotic use. In addition, subjects agreed to comply with the cycled menus, and specimen (blood and fecal) collections. All subjects were free of clinically wasting or terminal illness. Ten subjects (six males) aged 74.0 ± 3.3 participated in the study. Seven subjects were treated for constipation with MgO, Dulcolax, Sennapur, or combination of either two. All medical regimens and fluid intake were kept constant throughout the study. The activity of daily life (ADL) was moderate as graded 73.8 ± 3.3 point using Barthel index. [12] Most subjects were on regular and soft

diet; three were on blended diet.

Experimental design

This double-blind, placebo-controlled, diet-controlled study consisted of a 4-wk placebo (3 mL fructose syrup) period, a 4-wk FOS period, and a 4-wk post period. The study did not employ a crossover or randomized design because subjects could not tolerate long-term diet control. FOS was consumed as a drink and its dose was gradually increased from 5 g/d (first 7 d) to 10 g/d (d 8-28) to avoid potential intestinal distension. Neither sweetener was consumed during the post period. Spontaneous defecation and enema usage were recorded by the nurse. All stools voided during the last week of each period were individually collected in plastic bags to determine the fecal weight and microflora counts. Body weight was determined and fasting blood samples were collected on the last day of each period. The experimental protocol was approved by the ethics committee of Chung Shan Medical University Hospital; all volunteers provided written informed consent.

Diet and dietary intake assessment

Subjects consumed a 7-d cycled menu (Table 1) from one month before the study until the end of post period in order to exclude the possibility that dietary variation might confound the effects of FOS. The amount of food provided to individual subject in the experimental periods was based on the 3-d dietary assessment in the pre-experimental period. In order to assess the average intakes of energy, nutrients and dietary fiber, meals served to and left over by the subjects were weighed and recorded for three non-consecutive days during each period. Fluid intake was calculated from the fluid intake record and the water content of food. The nutrient contents of all food ingested by the subjects were determined using local nutrient composition tables. [13]

Supplements of antioxidants, oligosaccharides- or lactic acid bacteria-containing foods, were not allowed during the study. The composition (%) of FOS (Institute of Microbial Resources, Taichung, Taiwan) was fructosylnystose, 0.65; nystose (β-D-Fruf-(2$\rightarrow$1)- β-D-Fruf-(2$\rightarrow$1)- β-D-Fruf-(2$\rightarrow$1)-α-D-Glup), 10.51; 1-kestose (β-D-Fruf-(2$\rightarrow$1)-β-D-Fruf-(2$\rightarrow$1)-α-D-Glup), 28.41; sucrose, 14.26; glucose, 20.34; fructose, 2.83 and moisture, 23.0. The composition of fructose syrup (Pay Inc., Taipei, Taiwan) was fructose, 55; glucose, 20; and moisture, 25. The FOS supplement and fructose therefore provided similar amount of carbohydrates to the volunteers. FOS and fructose were administered to subjects in water as an afternoon snack.

Fecal collection

Nine of ten subjects completed the 7-d fecal collection for all three periods. The one who did not complete fecal collection in the post period was not included for statistical analysis. All feces voided were collected in plastic bag, vacuumed and sealed, and then stored in ice buckets and immediately sent to our laboratory. Feces were then homogenized in a stomacher. All lyophilized feces excreted by an individual were weighed and pooled together as a fecal composite for further analysis of fecal microflora.

Quantitation of fecal bifidobacteria, clostridia and total bacteria

Fecal bacteria were assessed using fluorescence *in situ* hybridization method (FISH).[14] The probes used were Bif164 [14] and Ib1 [15] specific for bifidobacteria and clostridia,

respectively, while the nucleic acid stain 4', 6-diamidino-2-phenylindole (DAPI) was used for total bacterial counts.[14] Fecal microbial counts are expressed as $\log_{10}$ counts/g dry feces. The daily microbial output was calculated by fecal count (count/g dry feces) × daily dry fecal weight (g/d) and expressed as $\log_{10}$ counts/d.

Blood preparation, and measurements of plasma biochemical indices and thiobarbituric acid-reactive substances (TBARS)

Venous blood samples (4 mL) were collected on the last day after a 12-h fast into a 10 mL vacutainer tube containing heparin, and centrifuged at $1000 \times g$ to obtain the plasma for analysis. Blood was assured to be non-hemolytic. Plasma total cholesterol, triglyceride, urea nitrogen, creatinine, albumin, uric acid, AST and ALT levels were determined using an automatic analyzer (CX5 Synchron, Beckman, USA).

Aliquots of plasma were stored at -80 °C for analysis of TBARS, according to the method described in Draper *et al*. with slight modifications.[16] Tenth mL of plasma or malonaldehyde dimethyl acetal (MDA) as the standard, and 30 μL 0.091 mol/L of butylated hydroxytoluene, were mixed with 0.2 mL 0.02 mol /L of thiobarbituric acid. The mixture was boiled for 30 min in the dark. After cooling to ambient temperature, TBARS was extracted with 0.5 mL of *n*-butanol. The absorbance at 532 nm was measured. TBARS concentration was calculated in reference to MDA standards.

Statistics

All data were expressed as means ± SEM and analyzed with SPSS version 10.0 for Windows (SPSS, Inc., Chicago, IL). The fecal bacterial counts were log-transformed. All data were with normal distribution and treated as parametric data. Data were analyzed with repeated measure ANOVA followed by LSD analysis to compare the difference between two periods. The association between fecal bifidobacteria excretion and plasma measurement was determined using Pearson's correlation. Effect was considered significant as $P < 0.05$.

Results

Subjects consumed the controlled defined diet all through the experiment; the dietary intake of nutrients and fluids are shown in Table 2. These subjects consumed similar amount of fluid, energy and nutrient among placebo, FOS and post period. The dietary fiber intake was approximately 1.55 g/mJ, 6.46 g/kcal.

Fecal wet weights are reported because use of enema may cause extra fluid excretion and overestimate the weight. Supplement of FOS increased fecal dry weight per week by 42% ($P = 0.024$), but not dry weight per stool ($P = 0.18$), as compared with fecal weights during the placebo period (Table 3). The dry weight and dry weight per stool were not significantly greater in the post period as compared to the placebo period. Supplementation of FOS did not increase spontaneous defecation frequency, but significantly reduced the use of enema ($P < 0.05$) as compared to those in the placebo period. The decreased use of enema with FOS supplement did not sustain through the post period.

Fecal counts (Log counts/g dry feces) of bifidobacteria significantly increased ($P =$

0.002); clostridia significantly decreased ($P = 0.02$); total bacteria counts did not significantly change in the FOS period as compared with the placebo period d (Table 4). FOS supplement also effectively increased the daily fecal outputs of bifidobacteria ($P = 0.004$) and total bacteria ($P = 0.03$). The increased bifidobacteria output with FOS supplementation lasted through the post period ($P = 0.01$). Bifidobacteria contributed to $11.9 \pm 3.8\%$ of total fecal bacteria in the placebo, which was not significantly different from that in the FOS ($12.5 \pm 4.5\%$) or post ($12.8 \pm 5.3\%$) period, respectively.

The plasma TBARS concentration in the placebo period was 12.5 ± 0.6 μmol/L, which was significantly reduced to 10.9 ± 0.5 ($P = 0.09$) in the FOS period, and 10.3 ± 0.4 ($P = 0.02$) in the post period. Plasma concentrations of cholesterol, triglyceride, urea nitrogen, creatinine, albumin, and uric acid during different periods are shown in Table 5. The plasma cholesterol level was significantly reduced by approximately 7% in the FOS period ($P = 0.043$) and post period ($P = 0.039$), respectively, as compared to that in the placebo period. Plasma urea nitrogen level was significantly reduced by ~15% in the FOS period ($P = 0.049$) as compared to that in the placebo period. Plasma concentrations of triglyceride, creatinine, albumin, uric acid and activities of AST and ALT remained unchanged during the study.

The increase in fecal bifidobacteria output during the FOS period (Log_{10} CFU/d) was significantly associated with decrease in plasma TBARS ($P = 0.02$) or cholesterol concentrations ($P = 0.04$) (Fig. 1), but not correlated with the decrease in plasma urea nitrogen concentration ($\gamma = 0.41$, $P = 0.31$).

The body weight (kg) was 51.4 ± 4.0, 52.8 ± 4.0 and 54.0 ± 4.0, with body mass index (kg/m^2) 20.8 ± 1.3, 21.4 ± 1.2 and 21.9 ± 1.2, in the placebo, FOS and placebo period, respectively. The body weights exerted time effect ($P = 0.022$), and the weights in the post period was significantly greater that in the placebo period.

Discussion

Our study, in agreement with a previous observation from a small group of constipated elderly men, observed that FOS supplementation significantly increased dry fecal mass (~ 0.41 g increase /g FOS ingested).[17] The bulky effect partially diminished at the end of post period, which suggested the metabolism of FOS was associated with its bulky effect. A previous study indicated that the bulky effect of fermentable fiber mainly was due to increased bacterial mass [18]. An increased bacterial population (CFU/g dry feces) has been observed in this study, which may partially mediates the bulky effect of FOS.

Although the defecation frequency did not increase with FOS, the decreased enema usage and slight change in fecal dry weight per stool suggest the passage of stool was more feasible and complete with FOS supplementation in our subjects. Since reduced swallowing and chewing ability limit the amount of fiber consumed by the Taiwanese elderly [1], supplementation of soluble oligosaccharides could be an important regimen to improve their bowel functions. The increased fecal dry mass and decreased enema usage did not sustain to

the post period, which suggest continuous supplementation is required to maintain significant improvement of bowel function in the chronically constipated older persons.

The bifidogenic effect of FOS in healthy adults has been extensively demonstrated in well-controlled human trials [6,7,19,20], but is rarely demonstrated in the elderly humans. These previous studies concluded that around 10 g of FOS per day effectively stimulated the proliferation of colonic bifidobacteria. Similar to previous studies, our study confirms that this dose (10 g, 4 wk) of FOS significantly increases fecal concentration and daily excretion of bifidobacteria in the constipated elderly. This bifidogenic effect did not fully sustain at the end of post period (28 d), which also was similar to a previous study that bifidogenic effect of FOS and partially-hydrolyzed guar gum mixture diminished at 14 day after its withdrawal [21]. However, we observed an increased daily output of fecal bifidobacteria in the post period as compared to that in the placebo period, which was likely to be due to slight, but not statistically significant, increases in both the fecal mass and fecal bifidobacteria concentration.

The antioxidative properties of prebiotics in rats [22] and humans [11] have been previously reported. We further demonstrated that FOS supplementation reduced plasma lipid peroxidative level in the elderly. In addition, our study demonstrated that the antioxidative effect of FOS supplementation was closely associated with its bifidogenic effect. This result is supported by previous *in vitro* studies that the intact cells [23,24], cell-free intracellular extract [23] or cell walls of lactic acid bacteria [25], and the fermentation of FOS by lactic acid bacteria [10] exert antioxidative effects. Although the mechanism is unclear, a recent study indicates that fermentation of FOS by lactic acid bacteria increases the radical scavenging ability and reduces the lipid peroxide.[10]   Therefore, colonic bifidobacteria are likely to be a vital component involved in the antioxidative effects of FOS in humans.

Although dietary supplementation of inulin (10%) has been reported to decrease total serum cholesterol level in normolipidemic rats [26] and reduce hepatic cholesterol level and atherosclerotic lesion area in aortic sinus in apolipoprotein E-deficient mice [27], the mechanism by which FOS exerted hypocholesterolemic effect remains to be investigated. The enhanced fecal bile acid output with FOS supplementation in mice [28] and inhibition of hepatic cholesterogenesis by propionate that is generated from fermentation [29] could partially mediate the hypocholesterol effects of FOS.   However, other mechanism was involved in this effect since the hypocholesterolemic effect remained after FOS was withdrawn. An *in vitro* study have suggested that lactic acid bacteria assimilate cholesterol from the culture medium.[30]   Therefore, it is hypothesized that FOS may increase fecal cholesterol excretion by stimulating bifidobacteria growth, which in turn reduces the blood cholesterol level. Our study found a close association between the FOS-induced increase in fecal bifidobacteria excretion and the FOS-induced decrease in plasma cholesterol level, which support that bifidobacteria may mediate the hypocholesterolemic effect of FOS in the elderly subjects.

The role of FOS on nitrogen metabolism has been extensively studied in normal rats and in renal-failure animal models.[31-33] These studies conclude that FOS reduces blood urea concentration and this effect is in a dose-dependent pattern.[32]   Our study extended this

finding that FOS reduced BUN in nursing-home residents whose renal function was normal. Although the increased daily excretion of bifidobacteria was not closely associated with the decreased BUN, FOS is likely to enhance the blood urea utilization by colonic microflora which therefore enhances the fecal disposal of nitrogen waste.[34]

Conclusion

Results from this study conclude that addition of 10 g of FOS per day to a low fiber diet can stimulate the dry fecal mass, proliferation and fecal excretion of bifidobacteria, and exert beneficial effects on blood antioxidative status, lipid, and urea status in constipated nursing-home residents. The beneficial effect of FOS on daily excretion of fecal bifidobacteria, and plasma TBARS and cholesterol levels sustain for 28 d after its withdrawal. bifidogenic function of FOS may mediate its antioxidative effects.

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Change in bifidobacteria
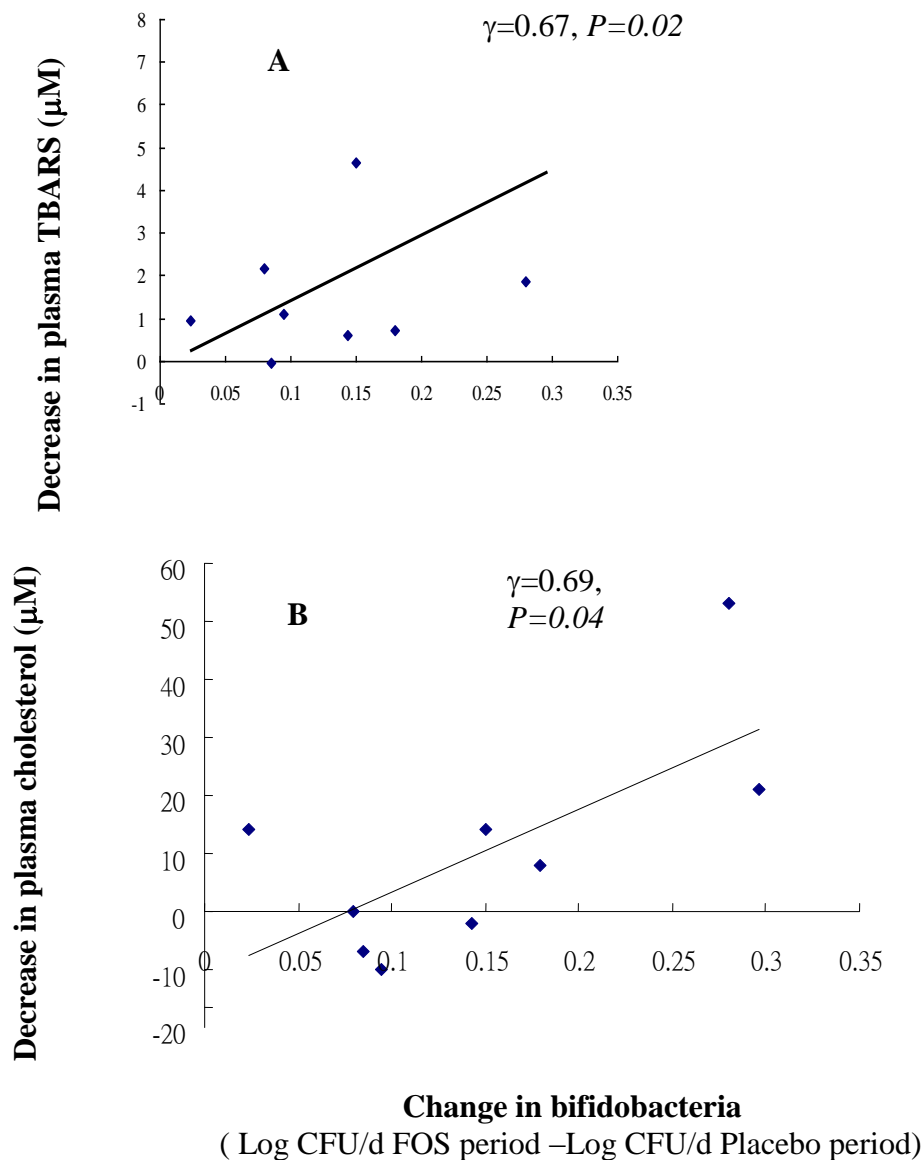(Log CFU/d FOS period –Log CFU/d Placebo period)

Fig. 1. Correlation between the changes in daily excretion of fecal bifidobacteria (Log CFU/d in FOS – Log CFU/d in placebo period) and the decrease in plasma (A) TBARS or (B) cholesterol concentration.   The association between fecal bifidobacteria excretion and plasma measurement was determined using Pearson's correlation.   Effect was considered significant as $P < 0.05$.

Table 1. Seven-day cycle menu[*]

Day	Menu
Day 1	
Breakfast	Rice porridge, pork shred, Chinese cabbage
Lunch	Rice/ porridge , fried tofu, Swordfish, Chinese spinach
Snack	Canned fruit cocktail
Dinner	Fried chicken, chicken wing, corn, ham, cabbage, Yuba,
Snack	Milk/soy milk, brown rice powder
Day 2	
Breakfast	Rice porridge, pork shred, bean sprout
Lunch	Rice/porridge, sweet potato leaves, pickle, Tofu, scrambled pork and carrot
Snack	Guava
Dinner	Rice/ porridge, bok choy, Chinese cabbage, kiss larval
Snack	Milk/soy milk, brown rice powder
Day 3	
Breakfast	Rice porridge, pork shred, canned peanut gluten, squash
Lunch	Rice /porridge, stewed pork, bamboo shoot, Chinese spinach, fish fillet, mushroom
Snack	Banana
Dinner	Rice/ porridge, egg, chicken nugget, bok choy, Chinese mushroom
Snack	Milk/soy milk, brown rice powder
Day 4	
Breakfast	Rice porridge, pork shred, pickle
Lunch	Rice/ porridge, roasted drumstick, bok choy, mushroom
Snack	Apple
Dinner	Rice/ porridge, pan-fried milk fish, carrot, pork slice, bamboo shoot
Snack	Milk/soy milk, brown rice powder
Day 5	
Breakfast	Rice porridge, pork shred, seaweed sauce
Lunch	Stir-fried noodles with carrot, onion, pork, cabbage
Snack	Watermelon
Dinner	Meatball, imitated chicken (soy product), sunset egg, bok choy
Snack	Milk/soy milk, brown rice powder
Day 6	
Breakfast	Rice porridge, pork shred, pickle, stewed peanut gluten
Lunch	Dumpling, vegetable soup
Snack	Grape

Dinner	Rice/porridge, pan-fried meat fish, bean sprout, carrot, sunset egg	
Snack	Milk/soy milk, brown rice powder	
Day 7		
Breakfast	Rice porridge, pork shred, stewed tofu	
Lunch	Scrambled noodle with carrot, onion, pork, shrimp, cabbage	
Snack	Banana	
Dinner	Rice/porridge, stewed fish, ground pork, bean noodle, cucumber	
Snack	Milk/soy milk, brown rice powder	

*The weight of each food provided to volunteers were individually designed to meet their dietary habit and energy requirement.

Table 2. The daily fluid, energy and nutrient intakes of subjects in the placebo, fructooligosaccharide-supplemented (FOS) and the post periods *

	Placebo	FOS	Post
Fluid (L)	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
Calories (MJ)	5.4 ± 0.6	5.4 ± 0.8	5.4 ± 0.8
Carbohydrate (g)	157.9 ± 4.5	157.2 ± 4.2	158.0 ± 4.4
Protein (g)	56.2 ± 3.0	56.4 ± 2.4	56.2 ± 2.7
Fat (g)	49.4 ± 1.3	49.2 ± 2.0	49.3 ± 1.8
Dietary fiber (g)	8.4 ± 0.3	8.4 ± 0.4	8.4 ± 0.3
Calcium (mg)	613.4 ± 24.6	613.0 ± 24.5	613.0 ± 24.5
Phosphorous (mg)	853.1 ± 45.5	853.1 ± 45.5	853.1 ± 45.5
Vitamin A (R.E.)	926.9 ± 84.2	927.9 ± 60.4	928.0 ± 59.2
Vitamin B_1 (mg)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Vitamin B_2 (mg)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Niacin (mg)	10.0 ± 0.7	10.0 ± 0.7	10.0 ± 0.7
Vitamin C (mg)	93.6 ± 2.9	93.6 ± 2.9	93.6 ± 2.9
Percentage of total calories (%)			
Carbohydrate	48.7 ± 0.9	48.4 ± 0.8	48.7 ± 0.7
Protein	17.3 ± 0.7	17.4 ± 0.7	17.2 ± 0.7
Fat	34.3 ± 0.6	34.2 ± 0.6	34.1 ± 0.6

*Data shown were the average of 3 nonconsecutive days during each period and expressed as means ± standard error of mean (n=10).

Table 3. Fecal output and bowel movement of subjects in the placebo, fructooligosaccharide-supplemented (FOS) and the post periods

	Placebo	FOS	Post
Fecal output			
Dry weight (g/wk)	53.2 ± 8.4 [a]	82.0 ± 11.1 [b]	71.8 ± 10.5 [ab]
Dry weight (g/stool)	13.3 ± 3.0	20.1 ± 3.3	16.7 ± 3.5
Bowel movement (no./wk)			
Spontaneous defecation	4.5 ± 0.8	4.2 ± 0.5	4.3 ± 0.8
Enema	0.4 ± 0.2 [b]	0.2 ± 0.1 [a]	0.5 ± 0.2 [b]

*Values in a row not sharing the same superscript denotes for significant difference ($P <$ 0.05) as analyzed by repeated ANOVA followed by LSD test. Values are means ± SEMs (n=9).

Table 4. Fecal bifidobacteria, clostridia and total bacteria counts (Log counts/g dry feces) of subjects in the placebo, fructooligosaccharide-supplemented (FOS) and the post periods

Fecal bacteria	Placebo	FOS	Post
Bifidobacterium	9.6 ± 0.1[a]	9.8 ± 0.1[b]	9.7 ± 0.1[a]
Clostridium	9.8 ± 0.1[b]	9.6 ± 0.1[a]	9.8 ± 0.1[b]
Total	10.8 ± 0.1	11.0 ± 0.1	10.9 ± 0.1

*Values in a row not sharing the same superscript denotes for significant difference ($P <$ 0.05) as analyzed by repeated ANOVA followed by LSD test. Values are means ± SEMs (n=9).

Table 5. Plasma biochemical parameters at the end of the placebo, fructooligosaccharide-supplemented (FOS) and the post periods

	Placebo	FOS	Post
Biochemical parameters			
Cholesterol (mmol/L)	4.8 ± 0.2[b]	4.5 ± 0.2[a]	4.5 ± 0.2[a]
Triglyceride (mmol/L)	1.9 ± 0.8	1.4 ± 0.2	1.8 ± 0.8
Urea nitrogen (mmol/L)	7.0 ± 0.8[b]	6.0 ± 0.6[a]	6.9 ± 1.0[b]
Creatinine (μmol/L)	106.1 ± 13.8	102.7 ± 34.5	106.1 ± 13.8
Albumin (g/L)	36.4 ± 0.5	35.7 ± 0.5	34.5 ± 0.8
Uric Acid (μmol /L)	331.3 ± 43.5	336.7 ± 44.4	306.3 ± 32.5
AST (U/L)	19.6 ± 1.0	19.9 ± 1.8	18.2 ± 1.3
ALT (U/L)	16.1 ± 2.6	17.1 ± 4.3	13.6 ± 1.4

AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*Values in a row not sharing the same superscript denotes for significant difference ($P <$ 0.05) as analyzed by repeated ANOVA followed by LSD test. Values are means ± SEMs (n=9).

中文摘要

　　本研究探討生技寡醣異麥芽寡醣對安養中心低活動力老人腸道生理、菌相的調節作用，以及血液過氧化狀態的影響。本研究於台中市安養中心進行，以固定飲食型態下分為安慰劑期、補充１期、補充２期及恢復期，各為期四周。研究成果已被接受刊登(Nutrition)並且已先行於網路上刊登，敬附論文於下。

關鍵詞:異麥芽寡醣、老人、糞便細菌酵素、氧化壓力、短鏈脂肪酸、腸道功能

Long-term supplementation of isomalto-oligosaccharides improved colonic microflora profile, bowel function and blood cholesterol levels in constipated elderly people—A placebo-controlled, diet-controlled trial
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ABSTRACT

Objectives: The main purpose of this study is to determine the long-term (8 wk) effects of isomalto-oligosaccharide (IO) supplementation on fecal microflora, bowel function, and biochemical indicators of nutritional status in constipated elderly subjects. We also assessed whether the effect of IO was sustained after its withdrawal.

Methods: Thirteen (5 male) constipated subjects (age 82.5 ± 1.9 years) participated in this diet-controlled study that consisted of a 4-wk placebo period, two 4-wk IO (10 g/d)-supplementation periods (IO1 and IO2), and a 4-wk post period. Fasting blood was collected on the last day of each period. Stools were collected during the last week of each period. The bowel function was monitored throughout the study.

Results: The fecal bifidobacteria, lactobacilli, and bacteroides counts (log counts/g wet feces) significantly increased and clostridia count decreased at the end of the IO1 period. The effects were more pronounced in the IO2 period, and then returned to the levels of the IO1 period at the end of the post period. Daily fecal excretion of acetate and propionate increased along with IO supplementation. The frequency of spontaneous defecation increased in the IO2 period, and wet fecal mass increased by 24% in both the IO1 and IO2 periods. The effects of IO on bowel function diminished in the post period. Plasma total and LDL-cholesterol levels were lower with 4- or 8-wk IO supplementation as compared with the placebo and post period, respectively.

Conclusions: IO supplementation into a low-fiber diet improved colonic microflora profile and bowel movement in a time-dependent fashion in constipated elderly subjects. These beneficial effects decreased after discontinuation of the supplements.

Key words: isomalto-oligosaccharide; bifidobacteria; *Clostridium perfringens*, cholesterol, bowel function, elderly

INTRODUCTION

Impaired bowel function, particularly constipation, is a common complaint of ill or inactive elderly people (1, 2). Populations with poor chewing ability need suitable dietary fiber supplements that can easily be incorporated into their ordinary diet to maintain regular bowel movements. Isomalto-oligosaccharides (IO), such as isomaltose, panose,

isomaltotriose and isomaltotetraose, naturally exist in Japanese fermented foods such as miso, soy sauce and sako and are commercially produced from corn starch to be a functional food ingredient (3). These oligomers contain α $1\rightarrow$ 6 glucosidic linkages (3) that resist endogenous digestion (4) and may exert a biological function similar to dietary fiber. Administration of 10 g of active components of IO for four week has been shown to improve the defecation frequency in a placebo-controlled and diet-controlled study that included only a small group of severely constipated elderly men (5). Therefore, IO is potentially a functional food to relieve constipation in the elderly. The elderly are likely to require long-term use of laxatives. However, the long-term effects of IO supplementation have not been determined.

Aging has been associated with decreased fecal bifidobacteria concentrations (6), which may partially be associated with dysfunction of the intestine, decreased immunity, and greater susceptibility to disease. Colonic microflora are responsible for a wide variety of metabolic activities that modulate human health. For instance, fermentation of dietary fibers by colonic microflora beneficially modulates colonic health through its metabolites, including the organic acids and short chain fatty acids that protect and support the normal turnover of the colonocytes (7, 8). Several studies have suggested that supplementation of IO in healthy adults increases the fecal bifidobacteria concentration (3, 9). However, there are a lack of studies demonstrating the modulatory effects of IO on colonic bacteria profile and the prolonged effects of the supplementation after its withdrawal in older populations.

The aim of this study was to evaluate the effects of eight weeks of ingestion of IO on fecal microflora, bowel function, fecal short chain fatty acids (SCFAs), and plasma biochemical indices in constipated elderly subjects, and to observe whether these effects were sustained after withdrawal for four weeks, in a diet-controlled, placebo-controlled trial.

SUBJECTS AND METHODS
Subjects

Residents living in a local nursing home were recruited into this study. The criteria for recruitment were; stable physiological condition, long-term residence (> 6 months), chronic constipation (> 6 months), ability to chew a soft or blended diet, not bed-ridden, and no tobacco or antibiotic use. In addition, subjects agreed to comply with the cycled menus and fecal specimen collection. The exclusion criteria were; subjects who were with plasma AST > 80 U/L, Cr > 132.6 μmol/L (1.5 mg/dL), or with a clinically terminal or wasting illness. Thirteen elderly subjects (5 males) with age (means ± SEM) 82.5 ± 1.9 years completed the study with good compliance. The mean weight and body mass index were 52.5 ± 2.9 kg and 20.9 ± 1.1 kg/m^2, respectively. Seven subjects had history of cerebrovascular accident. Ten of the subjects were treated for constipation with MgO, bisacodyl, sennoside, or a combination of any two. All medical treatments were kept constant throughout the study.

Experimental design

This double-blind, placebo-controlled, diet-controlled study consisted of a run-in period, 4-wk placebo (3 mL fructose syrup) period, two 4-wk IO periods (first and second 4 wk as IO1, IO2 period), and a 4-wk post period. Diets were designed for individual subjects based on an assessment before the run-in period. Subjects consumed their refined diet from the run-in period through the end of the post period. Isomalto-oligosaccharide was consumed as an afternoon drink, and the dose was gradually increased from 11 g/d (equivalent to 5.0 g of active component) to 22 g/d (equivalent to 10.0 g of active component) in the first seven days

of the IO1 period, and was kept at 22 g/d throughout the remaining IO period to avoid potential intestinal distension. IO was withdrawn during the post period. Body weight and height were determined on the last day of each period.

Three-days of food intake were assessed in each period to assure the constant nutrient and dietary fiber intake. Subjects were treated with enemas if spontaneous defecation did not occur in three days or as requested by the subjects. The incidence of spontaneous defecation and enema use was recorded every day and is expressed as incidence (no./wk) in this study. Stools voided during the last week of each period were individually collected in plastic bags and sent to the laboratory immediately to determine the fecal characteristics and microflora. The experimental protocol was approved by the Committee on Human Study at the Chung Shan Medical University Teaching Hospital. All subjects gave written informed consent.

Diet

Subjects were given a 7-day cycle menu (Table 1) designed by a registered dietitian in the ward, from one month before the experiment to the end of the post period in order to prevent the confounding effect of dietary variation. Four subjects consumed a blended diet while the remaining a soft or regular diet. Any other supplements, including antioxidants, other oligosaccharides- or lactic acid bacteria-containing foods, were not allowed during the study. The IO powder (Tamaru Enterprise Co., Taichung, Taiwan) contained 45% (w/w) IO active component, isomaltose 11.7%, panose 26.2%, isomaltotriose 1.95% and isomaltotetraose 5.15%. Fructose syrup (Pay Inc., Taipei, Taiwan), composed (w/w) of fructose (55%), glucose (20%), and moisture (25%), was used as the placebo. Both IO and fructose were administered to subjects in 100 mL drinking water as an afternoon snack.

Dietary intake

In order to assess the average intake of fluid, energy, nutrients and dietary fiber, meals and fluids served to and left over by the subjects were weighed and recorded for three non-consecutive days during each period. The nutrient content of all food ingested by the subjects was determined using local nutrient composition tables (10).

Measurement of fecal pH and moisture

Feces voided were immediately collected in plastic bags, vacuumed, sealed, and immediately sent to our laboratory in ice buckets within 4 hr of defecation. The pH was determined by inserting the probe to the center of the fecal homogenate. The fecal pH of each subject was taken from the mean values of 7-d fecal samples collected during the final week of each period. Aliquots of fresh feces were pooled together for analysis of short chain fatty acids. The remaining feces were homogenized with an equal weight of deionized water in a stomacher, lyophilized and stored at −20 °C. All lyophilized feces excreted by an individual were pooled together as a fecal composite for further analysis of fecal microflora. The fecal moisture was determined by comparing the wet and dry weights of fecal samples.

Quantitation of fecal bacteria

Fecal bacteria populations were assessed using the fluorescence *in situ* hybridization method (FISH), in which genotypic probes targeting 16S rRNA are designed for specific bacteria (11). The probes used in this study were Bif164 (5'-CAT CCG GCA TTA CCA CCC-3'), Laa1 (5'-CAT CCA GTG CAA ACC TAA GAG-3'), Bac303 (5'-CCA ATG TGG GGG ACC TT-3'), and Ib1 (5'-GAT GGA ACT GTA ACA AAA CT-3'), specific for

bifidobacteria (11), lactobacilli (12), bacteroides (13) and clostridia (14), respectively. The nucleic acid stain 4', 6-diamidino-2-phenylindole (DAPI) was used for total bacterial counts. The bacterial solution obtained during the fractionation of feces was fixed in a 3-fold volume of 4% paraformaldehyde solution overnight at 4 °C. The lyophilized fecal composite was individually fractionated using the method described previously (5) to separate fecal plant material from the bacteria. Aliquots (5 μL) of the fecal bacteria fraction were fixed on wells of microscopic slides following the method described previously (11, 15). To quantify the total fecal bacteria, slides were incubated with a nucleic acid stain, 4', 6-diamidino-2-phenylindole, as described previously (11, 15). Probe fluorescence was detected with a Zeiss Axioskop2 microscope (Carl Zeiss, Jena, German) fitted for epifluorescence microscopy 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 (CCD) video camera (MacroFire, Model S99831, Optronics, Goleta, CA). The microbial counts were expressed as $\log_{10}$ counts/g feces.

Measurement of fecal short chain fatty acids

Aliquots (0.5 g) of fecal composite were analyzed for acetate, propionate, *i*-butyrate and *n*-butyrate with 4-methyl-*n*-valeric acid as an internal standard, as described previously (16). The SCFAs extracted from the fecal samples were dissolved in 10% orthophosphoric acid solution immediately before they were injected onto a gas chromatograph (GC-14B, Shimadzu, Tokyo, Japan) fitted with a glass capillary column (0.25 mm, 30 m Stabilwax-DA, Restek Corp., Bellefonte, PA) and a flame ionization detector. The initial temperature of the oven was 100 °C and was raised to 200 °C at 6 °C /min. The temperature of the injection port and detector were both at 250°C. The flow rate of the carrier gas, N_2, was adjusted to be 1 mL/min. Peak areas were analyzed with a C-R6A Chromatopac (Shimadzu Corp., Tokyo, Japan).

Blood preparation and measurements of plasma biochemical indices

Venous blood samples (4 mL) were collected on the last day after a 12-h fast into a 10 mL vacutainer tube containing heparin, and centrifuged at $1000 \times g$ to obtain the plasma for analysis. Blood was assured to be non-hemolytic. Plasma sugar, total cholesterol, HDL-cholesterol, triglyceride, urea nitrogen, creatinine, albumin, and ALT levels were determined using an automatic analyzer (CX5 Synchron, Beckman, USA). LDL cholesterol levels were calculated based on the equation described by Friédewald *et al.* (17).

Statistical analysis

All data were expressed as means ± SEM and analyzed with SPSS version 14.0 for Windows (SPSS, Inc., Chicago, IL). The log-transformed bacteria counts and all other data were analyzed using General Linear Model repeated measures ANOVA followed by pair-wise LSD tests (18). A *P* value less than 0.05 was considered statistically significant.

Results

Fecal bacteria

Fecal counts (log counts/g wet feces) of bifidobacteria, lactobacilli, bacteroides, and total bacteria significantly increased at the end of the IO1 period, further increased at the end of the IO2 period, and remained greater in the post period than the level in the placebo period

(Table 2). In contrast, the fecal clostridia count significantly decreased at the end of the IO1 period as compared with the level in the placebo period, but this reduction was the most pronounced at the end of the IO2 period. At the end of the post period, the fecal clostridia counts remained lower than the level of the placebo period.

Bifidobacteria composed approximately 10% of total fecal bacteria at the end of the placebo period, which gradually increased to ~15% at the end of the IO2 period, and then decreased to a level slightly greater than the placebo level at the end of the post period. The relative counts (% total bacteria) of lactobacilli were double that of the placebo level at the end of the IO2 period. The relative counts of bacteroides remained constant throughout the study. Clostridia composed ~35% of total bacteria in the placebo period, to ~16% and ~9% in the IO1 and IO2 periods, respectively. The relative counts of clostridia remained at ~14% in the post period, similar to the level observed in the IO1 period.

Bowel function

The average frequency of spontaneous defecation and enema use throughout each period is shown in Table 3. The spontaneous defecation was low, only 1.2 per wk in the placebo period, which did not differ in the IO1 period, but significantly increased to 2.0 per wk in the IO2 period. However, the laxative effect of IO diminished in the post period. The frequency of enema usage tended to decrease with IO supplementation, but did not significantly differ among periods. Stool output was ~24% greater with either 4- or 8-wk IO supplementation as compared with the placebo. The increase in wet fecal mass was not sustained in the post period. Supplementation of IO for 4 and 8 weeks significantly increased the dry fecal mass by 16% and 12%, respectively, but this effect was not sustained to the post period. The fecal moisture and pH did not differ among groups.

Fecal short chain fatty acids

Acetate was the most dominant short chain fatty acid in feces collected in each period, with concentrations 42.6%, 89.2%, and 32% greater in the IO1, IO2, and post periods as compared with the level in the placebo period, respectively (Fig. 1A). Propionate was the second most dominant short chain fatty acid with a concentration 3.5-fold that of the level in the placebo period with 8-wk IO supplementation. The total short chain fatty acid concentrations were 42.6%, 89.2%, and 32.6% greater in the IO1, IO2, and post periods than that of the level of the placebo period, respectively. However, the fecal *i*-butyrate and *n*-butyrate concentrations did not differ significantly between periods. Furthermore, the daily fecal acetate excretion was 70.8%, 103.8%, and 45.2% greater in the IO1, IO2, and post periods than the level of the placebo period, respectively (Fig. 1B). The daily propionate output was significantly increased by 81.3% in the IO1 period, which further increased by 2-folds in the IO2 period, as compared with the output in the placebo period. The fecal *n*-butyrate outputs increased to 2- and 3-folds of the placebo level in the IO1 and IO2 periods, respectively. The daily outputs of total short chain fatty acids significantly increased by 75.9%, 134.0%, and 36.1% in the IO1, IO2, and post periods, respectively, as compared with the level in the placebo period. The fecal *i*-butyrate outputs did not significantly differ among periods.

Biochemical indices

The mean plasma biochemical measures of the subjects observed in this study were in the normal range for all periods (Table 4). The total and LDL cholesterol levels were lower at

the end of the IO1 and IO2 periods as compared with those in the placebo period. Plasma glucose, triglyceride, HDL-cholesterol, urea nitrogen, creatinine, albumin, and ALT levels remained constant among periods.

Energy and nutrient intakes

The subjects consumed similar amounts of fluid, energy and nutrients among the placebo, IO1, IO2 and post periods (Table 5). Carbohydrates, protein, and fat contributed to $59.9 \pm 0.6\%$, $13.4 \pm 0.2\%$, and $27.0 \pm 0.4\%$ of total energy, respectively, in the placebo period.

Anthropometric measurement

The body weight (kg) was similar among periods, 52.5 ± 3.0, 52.6 ± 3.0, 53.5 ± 2.8 and 52.4 ± 3.1, while the body mass index (kg/m^2) was 20.9 ± 1.1, 20.9 ± 1.1, 21.4 ± 1.1 and 20.9 ± 1.1, in the placebo, IO1, IO2 and post periods, respectively.

Discussion

The profile of fecal microflora in these older subjects were different from those observed in young adults (15). The fecal clostridia composed more than 35% of the total fecal bacteria, which is almost 2-folds the level of that observed in young constipated adults, while the bifidobacteria ratio was only slightly lower than that in young constipated adults (15). This shift in microbial profile with age agrees with the observation described by Hopkins *et al*. (6). The genus of *Clostridium* is pathogenic to humans (19). Therefore, decreasing clostridia can protect humans from diseases.

Supplementation of IO into a typical Chinese low-fiber diet was demonstrated in this study to exert beneficial time-course effects on the colonic microflora in older subjects. The profile of microflora was improved with 4-wk dietary IO supplementation, and the clostridia population was further reduced with 8-wk supplementation. This lactic-acid-bacteria promotive effect of IO suggests this oligosaccharide acts as a prebiotic in chronically-constipated older people and may help maintain their health. The microbial activity was also shown in the fecal short chain fatty acid levels. The increased fecal acetate and propionate concentrations, and the daily output of acetate, propionate and butyrate, suggest that IO was well-utilized by the colonic bacteria in our subjects. The bacteroides, major saccharolytic bacteria (20), also increased with IO supplementation, suggesting that IO can be utilized by bacteroides, which in turn increases the production of short chain fatty acids.

A previous study indicated that daily supplementation of 10 g of active IO component for 4 weeks significantly increased the defecation frequency in frail nursing-home residents, while the frequency of enemas was accordingly decreased by half (5). Our study extended the feeding period to two months and demonstrated time-dependent benefits in moderately-active older subjects. We observed a significant increase in the frequency of spontaneous defection, but no significant decrease in the frequency of enema usage with 8-wk IO supplementation, which did not agree with the previous study. The reason for the difference may be that the enema usage was not tightly controlled in this study and they could be administered on request of subjects who had relied on them for a long period of time. The effect of IO on fecal mass was first shown in the IO1 period, extending to the IO2 period, which suggests that IO can be a laxative in these older subjects. IO is fermented by colonic bacteria as shown in the

increased short chain fatty acid production in this study and a previous study (5), which could explain why the dry fecal mass only increased by 0.18 g for each gram of IO ingested. The increased fecal mass with IO supplementation could be partially due to residual IO and an increased colonic bacteria population.

The effect of IO on blood cholesterol level has rarely been studied, and results from previous studies are inconsistent. Supplementation of IO has been shown to reduce the blood cholesterol levels in healthy college men (21), but not in severely dysfunctional and constipated nursing-home residents (5). However, IO supplementation effectively reduced the plasma cholesterol level in our subjects who had a greater physical activity level and constipation was less severe than in subjects in the previous study (5). The mechanism by which IO modulates lipid homeostasis has never been investigated. The hypocholesterolemic effect of prebiotics has been proposed to be mediated by modulating bile acid metabolism in the intestine. Prebiotics may increase fecal steroid excretion and subsequently reduce the body pool of bile acids. A recent report indicated that feeding a fructo-oligosaccharide diet to rats significantly enhanced fecal bile acid output, but this study failed to prove a significant reduction in the pool size of cholate, a major component of bile acid (22). Another potential mediator for the hypocholesterolemic effect of IO could be propionate, which has been shown to inhibit hepatic cholesterol synthesis in isolated hepatocytes (23). The current study indicated decreased plasma cholesterol levels and increased fecal propionate outputs in the IO1 and IO2 periods. However, a greater increase in propionate output in the IO2 period was not associated with a greater decrease in plasma cholesterol levels. An *in vitro* study has suggested that lactic acid bacteria assimilate cholesterol from the culture medium (24). Therefore, we hypothesize that IO may increase fecal cholesterol excretion by stimulating bifidobacteria growth, which in turn reduces the blood cholesterol level.

A previous study demonstrated that short-term (28 d) ingestion of 10 g IO did not exert adverse effects on nutritional indicators (5). The current study extended the feeding period to two months and found the body weight, plasma albumin, glucose, triglyceride, urea nitrogen, creatinine and ALT levels did not change among periods. No adverse abdominal symptoms were reported, either. Therefore, this study suggests its safety for use in the elderly.

Conclusions

Beneficial effects of IO on fecal microflora profile, defecation frequency, fecal mass and blood cholesterol levels were shown with 4-wk supplementation in chronically-constipated elderly people. A longer period of supplementation (8 wk) further improved the fecal microflora profile and the bowel function. Withdrawal of this supplement for 28 day reversed the changes that resulted from the supplements.

ACKNOWLEGEMENTS

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Table 1. Seven-day cycle menu[*]

Day	Menu
Day 1	
Breakfast	Rice porridge, shredded chicken, Chinese cabbage
Lunch	Rice/porridge , fried tofu, salted egg, Chinese spinach
Snack	Canned fruit cocktail
Dinner	Fried chicken, chicken wing, corn, ham, cabbage
Snack	Milk/soy milk, brown rice powder
Day 2	
Breakfast	Rice porridge, shredded pork, bean sprouts
Lunch	Rice/porridge, sweet potato leaves, pickles, Tofu, stir-fried pork with carrot
Snack	Guava
Dinner	Rice/porridge, bok choy, Chinese cabbage, steamed egg
Snack	Milk/soy milk, brown rice powder
Day 3	
Breakfast	Rice porridge, shredded pork, canned peanut gluten, squash
Lunch	Rice/porridge, stewed pork, bamboo shoot, Chinese spinach, fish fillet, mushrooms
Snack	Banana
Dinner	Rice/porridge, egg, chicken nuggets, bok choy, Chinese mushrooms
Snack	Milk/soy milk, brown rice powder
Day 4	
Breakfast	Rice porridge, shredded pork, pickles
Lunch	Rice/porridge, roasted drumstick, bok choy, mushrooms
Snack	Apple
Dinner	Rice/porridge, pan-fried milk fish, carrot, pork slice, bamboo shoots
Snack	Milk/soy milk, brown rice powder
Day 5	
Breakfast	Rice porridge, shredded pork, seaweed sauce
Lunch	Stir-fried noodles with carrot, onion, pork, cabbage, mung bean soup
Snack	Watermelon
Dinner	Meatball, imitation chicken (soy product), sunny egg, bok choy
Snack	Milk/soy milk, brown rice powder
Day 6	
Breakfast	Rice porridge, shredded pork, pickle, stewed peanut gluten
Lunch	Dumplings, vegetable soup
Snack	Grapes
Dinner	Rice/porridge, pan-fried meat fish, bean sprouts, carrot, sunny egg
Snack	Milk/soy milk, brown rice powder
Day 7	
Breakfast	Rice porridge, shredded pork, stewed tofu
Lunch	Stir-fried noodles with carrot, onion, pork, shrimp, cabbage
Snack	Banana
Dinner	Rice/porridge, stewed fish, ground pork, bean noodles, cucumber

Snack	Milk/soy milk, brown rice powder			

[*]The weight of each food provided to the subjects was individually designed to meet their dietary habits and energy requirements.

Table 2. Microflora profile of feces collected during the last week of each period[*]

Feces	Placebo	IO1	IO2	Post
Concentration (log counts/g wet feces)				
Bifidobacterium	8.58 ± 0.06^a	8.80 ± 0.06^b	9.00 ± 0.06^c	8.83 ± 0.05^b
Lactobacillus	8.44 ± 0.04^a	8.80 ± 0.10^b	9.00 ± 0.06^c	8.75 ± 0.05^b
Bacteroides	8.48 ± 0.06^a	8.56 ± 0.09^b	8.71 ± 0.05^c	8.58 ± 0.07^b
Clostridium	9.06 ± 0.05^c	8.93 ± 0.04^b	8.79 ± 0.06^a	8.93 ± 0.05^b
Total	9.60 ± 0.04^a	9.80 ± 0.04^c	9.85 ± 0.04^c	9.75 ± 0.04^b
Distribution (% of total bacteria)				
Bifidobacterium	10.1 ± 1.3^a	10.7 ± 1.4^a	14.6 ± 1.6^b	12.3 ± 1.3^{ab}
Lactobacillus	6.8 ± 0.2^a	10.5 ± 0.5^b	14.4 ± 0.5^c	10.0 ± 0.7^b
Bacteroides	8.0 ± 1.0^a	6.2 ± 1.1^a	7.5 ± 0.7^a	6.7 ± 1.4^a
Clostridium	34.8 ± 5.3^c	15.7 ± 3.1^b	9.1 ± 1.2^a	14.6 ± 1.7^b

[*]Data are means $\pm$ SEM ($n = 13$). Data were analyzed by General Linear Model repeated measures follow by the LSD test. Means within a row without a common letter differ, $P < 0.05$.
IO1 and IO2 denote 4- and 8-wk isomalto-oligosaccharide supplementation, respectively.

Table 3. Frequencies of defecation and enema use, and fecal output during the final week of each period[*]

	Placebo	IO1	IO2	Post
Spontaneous defecation (no./wk)	1.2 ± 0.4^a	1.5 ± 0.4^a	2.0 ± 0.3^b	1.2 ± 0.3^a
Enema (no./wk)	1.9 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.8 ± 0.1
Wet fecal mass (g/wk)	268.2 ± 49.6^a	333.1 ± 51.5^b	331.5 ± 34.9^b	275.2 ± 32.0^a
Dry fecal mass (g/wk)	77.5 ± 14.3^a	89.9 ± 13.9^b	86.9 ± 9.1^b	73.5 ± 8.5^a
Fecal moisture (%)	71.1 ± 1.3	73.1 ± 1.4	73.8 ± 1.1	71.3 ± 1.4
pH	7.3 ± 0.2	7.3 ± 0.2	7.2 ± 0.2	7.2 ± 0.1

[*]Data are means $\pm$ SEM ($n = 13$). Data were analyzed by General Linear Model repeated

measures follow by the LSD test. Means within a row without a common letter differ, $P <$ 0.05. IO1 and IO2 denote 4- and 8-wk isomalto-oligosaccharide supplementation, respectively.
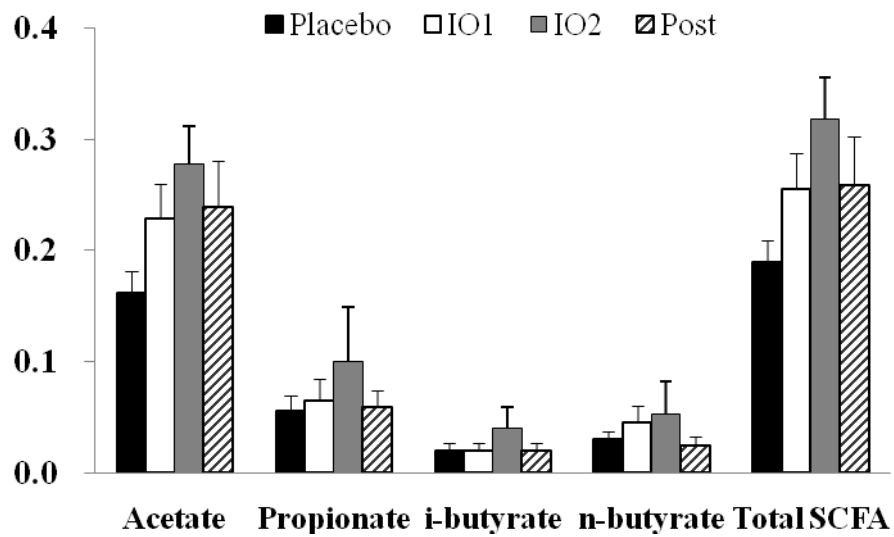

Figure legend
Fig. 1 (A) Concentrations or (B) daily output of short-chain fatty acids in feces collected during the last week of each period. Data are means ± SEM (n = 13). Data were analyzed by General Linear Model repeated measures follow by the LSD test. Means across periods without a common letter differ, $P <$ 0.05. IO1 and IO2 denote 4- and 8-wk isomalto-oligosaccharide supplementation, respectively.

Table 4. Biochemical measurement of subjects on the last day of each period[*]

	Placebo	IO1	IO2	Post
Glucose (mmol/L)	4.9 ± 0.2	4.8 ± 0.1	4.8 ± 0.4	4.5 ± 0.1
Total Cholesterol (mmol/L)	4.1 ± 0.1[b]	3.7 ± 0.1[a]	3.6 ± 0.1[a]	3.9 ± 0.1[ab]
LDL cholesterol (mmol/L)	2.4 ± 0.1[b]	2.2 ± 0.1[a]	2.1 ± 0.1[a]	2.3 ± 0.1[ab]
HDL cholesterol (mmol/L)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Triglyceride (mmol/L)	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
Urea nitrogen (mmol/L)	7.6 ± 1.3	7.5 ± 1.0	7.6 ± 0.8	7.1 ± 1.0
Creatinine (μmol/L)	106.1 ± 8.8	114.9 ± 8.8	114.9 ± 8.8	114.9 ± 8.8
Albumin (g/L)	36.0 ± 1.0	35.3 ± 0.9	35.5 ± 0.9	35.4 ± 0.9
ALT (U/L)	13.0 ± 1.5	12.3 ± 0.6	11.6 ± 1.3	12.1 ± 1.4

[*]Data are means ± SEM ($n = 13$). Data were analyzed by General Linear Model repeated measures follow by the LSD test. Means within a row without a common letter differ, $P < 0.05$.
IO1 and IO2 denote 4- and 8-wk isomalto-oligosaccharide supplementation, respectively.

Table 5. Daily fluid, energy and nutrition intakes of participants on three non-consecutive days during the last week of each period[*]

	Placebo	IO1	IO2	Post
Fluid (mL)	1355.1 ± 26.0	1355.6 ± 26.7	1349.6 ± 28.9	1360.6 ± 26.5
Calories (Kcal)	1310.4 ± 0.3	1311.8 ± 0.3	1310.2 ± 1.0	1311.2 ± 0.3
Carbohydrate (g)	196.4 ± 5.3	196.6 ± 3.7	196.6 ± 3.5	196.8 ± 3.7
Protein (g)	43.8 ± 1.3	44.2 ± 1.5	43.6 ± 2.0	43.2 ± 2.0
Fat (g)	39.3 ± 1.2	39.3 ± 1.2	39.5 ± 1.2	39.3 ± 1.2
Dietary fiber (g)	12.0 ± 0.2	12.0 ± 0.2	12.0 ± 0.2	12.0 ± 0.2

[*]Data are means ± SEM ($n = 13$). Data were analyzed by General Linear Model repeated measures follow by the LSD test. Means within a row without a common letter differ, $P < 0.05$.
IO1 and IO2 denote 4- and 8-wk isomalto-oligosaccharide supplementation, respectively.

Effects of Fructo-oligosaccharide on Potential Risk Factors of Colon Cancer in Constipated Elders

中文摘要

本實驗採飲食控制、單盲、自體比較的方法,觀察補充果寡醣後對慣性便祕老人之大腸癌發生的潛在危險因子之調節作用。九位受試者參與本實驗,實驗期間包含每天給予果糖水 100 mL 四週的控制期;接著給予四週果寡醣水(每天含 10 g 寡醣活性成分);最後為四週淨空期,期間不再補充寡醣水。在每個時期的最後一週收集七天的糞便,並用來測試大腸癌發生的危險因子,包含糞便酵素活性(β-glucosidase、β-galactosidase、β-glucuronidase 以及 mucinase)、膽酸組成、短鏈脂肪酸濃度以及糞便水對 Caco-2 細胞的毒性影響。由糞便酵素得知,在補充果寡醣四週後可顯著降低 β-glucosidase、β-glucuronidase 以及 mucinase 的活性。與控制期相比,糞便膽酸濃度雖沒有顯著變化,二級膽酸顯著下降 10.4%,淨空期的二級膽酸雖有下降趨勢但未達顯著差異。補充果寡醣四週後,糞便乙酸及總短鏈脂肪酸的濃度與控制期相比有顯著增加,丙酸、正丁酸及異丁酸雖未達顯著差異但仍有增加的趨勢。補充果寡醣四週後,可顯著降低 Caco-2 細胞與糞便水處理或合併使用 FPG-protein 誘發的 DNA 傷害與過氧化傷害,且此效果延續至淨空期。給予安養中心便秘老人果寡醣四週後,可顯著降低與直結腸癌相關的因子,包含降低糞便細菌酵素的活性,改變膽酸組成,增加短鏈脂肪酸濃度,並可能降低糞便物質對人類結腸表皮細胞的細胞毒性及基因毒性。

Abstract

This diet-controlled, single-blind study was designed to examine effects of fructo-oligosaccharides (FO) on the potential risks of colon cancer in constipated elders. Nine constipated subjects participated in this study that consisted of a 4-wk placebo period, a 4-wk FO-supplemented period (10 g active components/d), and a 4-wk post period. Fecal bacterial enzymes and bile acid profile were determined. The fecal β-glucosidase, β-galactosidase, and β-glucuronidase was significantly decreased by ~45%, while mucinase was decreased by 31.8%, in the FO period as compared to that in the placebo period. All enzyme activities were still suppressed in the post period, except for the β-glucuronidase. The ratio of secondary bile acid concentration to the total bile acid concentration significantly decreased in the FO period by 10.7%, but the effect didn't last through the post period. Therefore, this study suggests supplementation of FO decreased the potential risks of colon cancer in constipated elders.

Keyword: Fructo-oligosaccharide, elders, β-glucuronidase, mucinase, bile acids

前言

台灣地區由行政院衛生署在 2009 年公布的資料顯示,至 2008 年止,直結腸癌在國內不

論是發生率或死亡率都是十大癌症的第三位(1)。大腸癌早期並沒有明顯症狀，而 85% 都是由大腸瘜肉轉化而來，癌變時間大約 5-10 年。直結腸癌的發生約有 70-80%與環境因子有關，這些因子包括文化，社會背景、生活方式及飲食(2)，而飲食特別被指出是影響直結腸癌發生的顯著因素(3)。近年來國人因飲食西化及攝取過多動物脂肪的影響，罹患大腸癌的年齡層逐漸降低，因此飲食習慣的調整是預防腸道病變的第一步。

研究目的

　　本實驗的目的為由飲食介入果寡醣及異麥芽寡醣,並觀察寡醣對具有慣性便秘的老人其大腸癌發生的相關指標，如細菌酵素、短鏈脂肪酸濃度、膽酸濃度，以及腸道物質對 Caco-2 細胞基因毒性之影響。

文獻探討

　　直結腸癌的發生逐年且快速的提升,在西方國家男性直結腸癌佔所有癌症的發生率為 12.6%,女性則是 14.1%。以全球來看,直結腸癌的癌症死亡率為第四位,但在歐洲、北美、澳洲及紐西蘭則是第二位,且其發生率仍在攀升中(4)。而台灣地區由行政院衛生署(1)在 2010 年公布的資料顯示,至 2009 年止,直結腸癌在國內不論是發生率或死亡率都是十大癌症的第三位,每年都有四千多人死於直結腸癌,並有逐漸年輕化的趨勢。直結腸癌已是已開發國家人民的重要死因之一,說明國人應更正視直結腸癌的嚴重性。

　　直結腸癌的發生除了遺傳因素之外,約有 70-80%與環境因子有關,這些因子包括文化,社會背景、生活方式及飲食(2),而飲食特別被指出是影響直結腸癌發生的顯著因素(3)。現代人飲食西化,攝取過多紅肉、高油脂以及缺乏膳食纖維,都會增加直結腸癌的發生率(5)。在一項探討短鏈果寡醣對於腸道腫瘤影響的研究中,發現若給予六或七週齡有大腸息肉結腸腫瘤基因突變的老鼠(C57BL/6J-Min/+ mice) 5.8%果寡醣飼料,42 天之後老鼠體內大腸腫瘤的數目顯著減少了 67%,同時也增加了腸道淋巴結的數目(6)。另外有一動物實驗也證實,餵食老鼠補充 5%果寡醣三週後再注射 1,2-dimethylhydrazine (DMH),結果發現果寡醣能提高黏膜突間叢聚內凋亡細胞數至三倍,說明果寡醣有預防直結腸癌發生的功能(7)。

研究方法

1. 糞便 β-glucosidase、β-galactosidase、β-glucuronidase、mucinase 酵素活性測定方法

　　依據 Marteau 等人 (8)方法,糞便樣品以磷酸緩衝溶液(0.1M Na_2HPO_4/L、0.15 M NaCl、PH 7.4)稀釋並以超音波均質破膜。取出 0.5 mL 上清液後再加入受質反應,並於 37℃下反應 3 個時間點,之後置於沸水浴 2 分鐘停止酵素反應。酵素活性以 IU/mg protein 表示。

2. 糞便中短鏈脂肪酸的萃取及分析

　　參考 Remesy 與 Demigne 的方法(9)。利用氣相層析儀(Shimadzu GC-14B)分析樣品中各種膽酸的含量。

3. 糞便水中膽酸之測定

　　參考 Czubayko 等人及 Batta 等人(10,11)所描述的方法加以修改。利用氣相層析儀(Shimadzu GC-14B)分析樣品中各種膽酸的含量。

4. 細胞培養

　　本實驗選用的細胞株為 Caco-2 clone，屬於人類大腸腺癌細胞(Human colon adenocarcinoma, clone of Caco-2)，具有正常腸道上皮細胞的黏膜與型態，因此以這株細胞來模擬大腸上皮細胞。

5. DNA 毒性傷害分析

　　參考 Rieger 等人(12)所描述的方法加以修改。以彗星電泳法測定 tail moment 作為 DNA 傷害程度的指標，越高表示 DNA 傷害越大。

結果與討論

　　在給予果寡醣後，糞便酵素 β-glucosidase、β-galactosidase、β-glucuronidase 與 mucinase 的酵素活性與控制期相比均有下降的趨勢(Fig. 1)，β-glucosidase、β-glucuronidase 及 mucinase 分別顯著下降59.1%、33.7%及36.7% ($p < 0.05$)。在淨空期可發現 β-glucosidase 的酵素活性顯著回升並與控制期相當，而 β-glucuronidase 在淨空期雖有上升的趨勢，但並未與控制期及果寡醣期達顯著差異。淨空期的 Mucinase 活性與控制期相比達顯著差異。比較三個時期的糞便酵素活性變化，可發現補充果寡醣有顯著抑制糞便酵素 β-glucosidase、β-glucuronidase 與 mucinase 的效果，且效果延續至淨空期的有 β-glucuronidase 及 mucinase。

　　補充果寡醣前後各膽酸與總膽酸的濃度皆沒有顯著差異(Fig. 2)，在補充果寡醣四週後可顯著提升一級膽酸佔總膽酸的比例約10.40% ($p=0.036$)，並顯著下降二級膽酸佔總膽酸的比例約10.40% ($p=0.036$)。在淨空期一級/二級膽酸的比例組成與果寡醣期和控制期相比皆無顯著差異。

　　與控制期相比，補充果寡醣四週後，可顯著增加短鏈脂肪酸的總濃度 124% ($p < 0.05$)，且有增加各短鏈脂肪酸濃度的趨勢，包含乙酸、丙酸、異丁酸和正丁酸；其中乙酸在 FO 時期有顯著增加($p=0.033$)，到淨空期則有回降的趨勢(Fig. 2)。

　　DNA 傷害以 DMEM (空白試驗)最低，其次是果寡醣期、淨空期，傷害最大的為控制期，且各組間皆有顯著差異($p<0.05$)。與控制期相比，給予果寡醣可顯著降低糞便水的 DNA 傷害，且此結果可延續至淨空期(Fig. 3)。加上 FPG-protein 誘發 DNA 氧化傷害也可看出同樣結果，DNA 傷害以 DMEM（空白試驗）最低，其次是果寡醣期、淨空期，傷害最大的為控制期，且各組間皆有顯著差異（$p<0.05$）。FPG-protein lesion 為計算添加 FPG-protein 後，誘發 DNA 傷害的程度，結果顯示控制期的 FPG-protein lesion 最大，給予果寡醣可顯著降低 DNA 傷害(Fig. 3)。
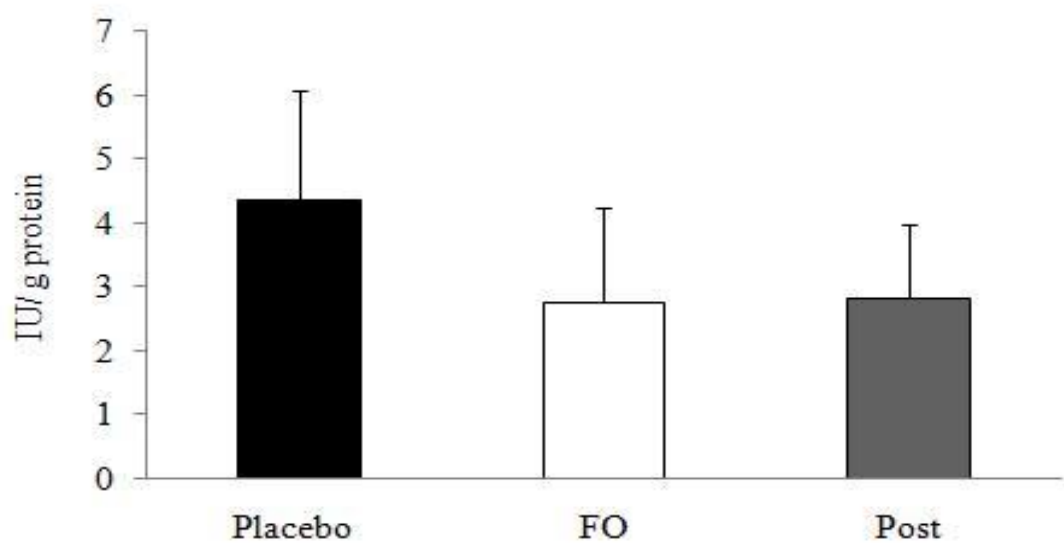
(A)
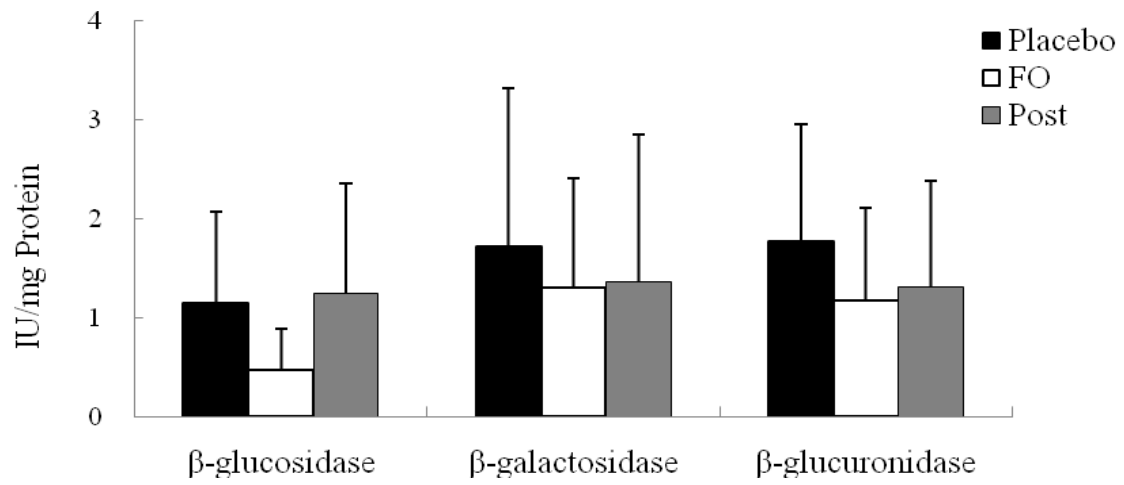
(B)

Figure 1.　Effects of fructo-oligosaccharide on human fecal bacteria enzymes (A) β-glucosidase,　galactosidase and glucuronidase (B) mucinase.　Data are expressed as means± standard deviation (n＝9).
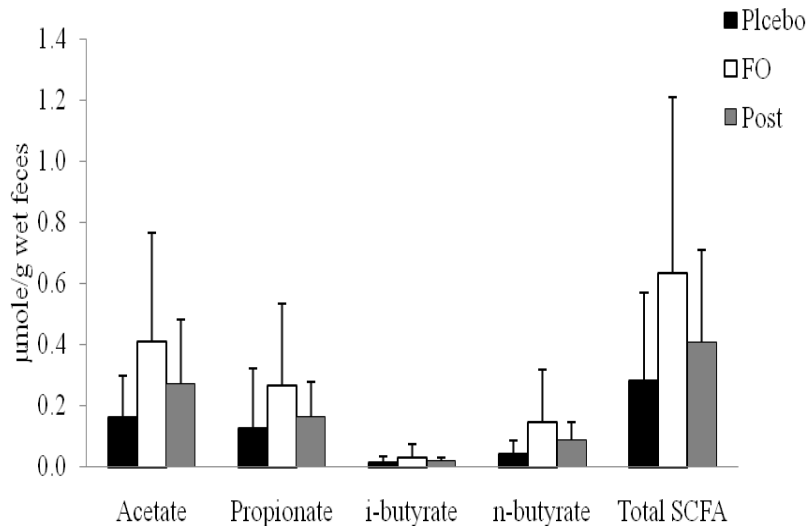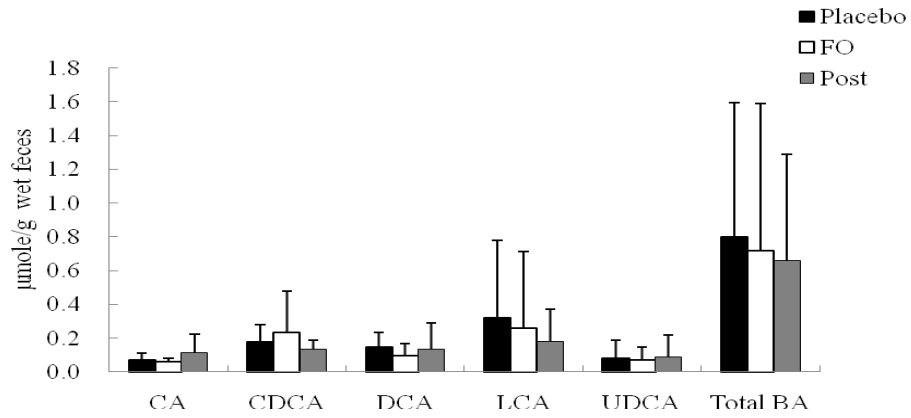
Figure 2. Effects of fructo-oligosaccharide on human fecal (A) bile acids; (B) short chain
fatty acids. Data are expressed as means ± standard deviation (n＝9)

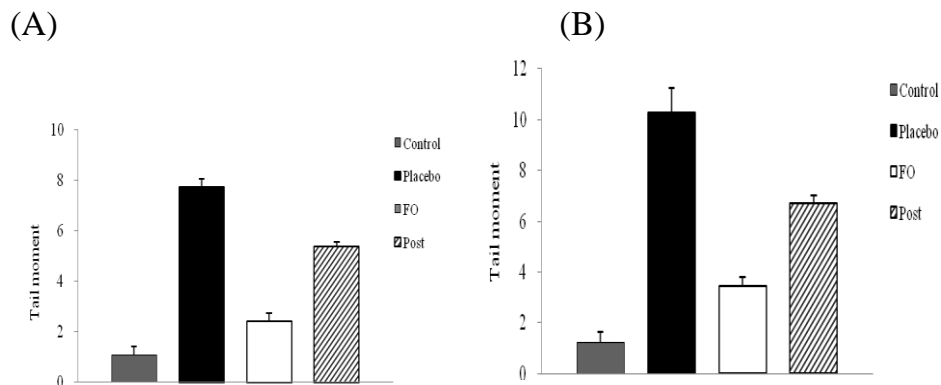(A)   (B)

Figure 3. Effects of fructo-oligosaccharide supplement on the DNA damage in Caco-2 cells
treated with (A) fecal water alone; (B) fecal water in combination with FPG-protein.

結論

　　給予安養中心便秘老人果寡醣四週後，可顯著降低與直結腸癌相關的因子，包含降低糞便細菌酵素的活性，改變膽酸組成，增加短鏈脂肪酸濃度，並可能降低糞便物質對人類結腸表皮細胞的細胞毒性及基因毒性。

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國科會補助專題研究計畫成果報告自評表

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
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） 尚未發表的部分考慮申請專利　請勿公開結果
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） 學術成就：目前已經發表兩篇 SCI 論文，其他結果會依進度撰寫。寡醣容易攝取，可廣泛應用於所有年齡層，因此研究具有應用性。本研究第 3 年探討對大腸癌預防之可能性，因此有助於維護成年及老年人健康。 技術創新: 以螢光免疫技術定量腸道細菌。 社會影響: 本研究可以一般性健康專題發表，有助於一般民眾維持健康。

國科會計畫編號: 96-2320-B-040-031-MY3

會議名稱: 2009 年全球華人保健食品大會
會期: 2009/8/12-14
出席會議及報告撰寫者:　中山醫學大學陳曉鈴

全球華人保健食品大會每兩年舉辦一次，這是第四屆，由香港主辦。會期分為兩

天半，有少數壁報展出、7 場特別演講以及 31 場口頭報告。特別演講內容包括

何其檔教授發表橘皮抗癌活性成分的比較以及如何增加這些成分的加工方式。孫

璐西教授發表以 aggregated Aβ-induced differentiated PC-12 cells 作為 Alzheimer

disease(AD)的細胞模式，探討不同溶劑萃取物的功效，並且深入以 UV、NMR、

ESI-LC/MS 鑑定活性成分。香港中文大學探討 black tea extract、apple polyphenol

這兩種抗氧化物質對 *D. melanogaster* 壽命延長的作用，並且探討 SOD、catalase

的 gene expression and enzyme activity。讓我耳目一新的是: Methuselah (Mth): G

protein-coupled receptor、insulin/IGF-1 signaling pathway 與老化有關。這些研究說

明保健食品存在於日常植物性食品中，並且將之實證。因為我近年也從事抗老化

相關動物實驗，以及老人餐食的開發，希望可以提升我國銀髮族的生活品質，因

此孫教授的細胞抗 AD 研究以及延長壽命的模式讓我受益良多。會中有數篇與癌

症預防相關研究，康乃爾的劉瑞海(Rui-hai Liu) 教授也是以 fruit phytochemicals

為研究重點，先驗證體外抗氧化性、anti-proliferative activity，*in vivo* inhibitory

effect on mammary cancer growth，而且探討分子機制，發現 PCNA 以及 NF-γB

可作為 molecular target。台北醫學大學施老師研究室發表比較 brown rice, polished rice 對 DMH-induced aberrant crypt foci 的研究，並且將 foci 是否分泌 mucin 作分類，此研究發現 brown rice 具有最好的預防大腸癌病變的作用。

這是第一次參加這個集合華人智慧的國際會議，時間及地點都很方便，有利於學術交流。感謝國科會給予此行的補助以及相關承辦人員的協助。

96 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：96-2320-B-040-031-MY3

計畫名稱：探討果寡醣、異麥芽寡醣對慣性便秘安養中心居民之大腸菌相、血液抗氧化狀態及大腸癌前危險指標之影響

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	4	4	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	3	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		顏啟華(MD, PhD)於第1-2年有參與第三年計劃並無參與
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	3	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	2	2	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。)	無

	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	

國科會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。

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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：

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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）

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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）

   學術成就：目前已經發表兩篇 SCI 論文，其他結果會依進度撰寫。寡醣容易攝取，可廣泛應用於所有年齡層，因此研究具有應用性。本研究第 3 年探討對大腸癌預防之可能性，因此有助於維護成年及老年人健康。

   技術創新：以螢光免疫技術定量腸道細菌。

   社會影響：本研究可以一般性健康專題發表，有助於一般民眾維持健康