Biological Activities of Fructooligosaccharides Produced by *Aspergillus aculeatus* in Mice Fed a High-fat Diet and Caco-2 Cell

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Abstract We investigated the effects of the dietary supplement of fructooligosaccharides (FOS) on intestinal bacteria, blood cholesterol and mineral absorption in the Balb/c mice fed a high-fat diet. Mice were divided into four groups: Normal control (Balb/c mice fed normal diet), High fat control (Balb/c mice fed 60% fat diet), HF-FOS 5% (Balb/c mice fed 60% fat and 5% FOS diet), and HF-FOS 10% (Balb/c mice fed 60% fat and 10% FOS diet). We used an *in vitro* assay to evaluate whether the direct effects of FOS on calcium and magnesium transport improve absorption in the differentiated Caco-2 cell. The dietary supplementation with FOS resulted in a significant increase in *Lactobacillus* and *Bifidobacterium* in the feces and large intestine compared with the high fat control group. We found that dietary supplementation with FOS suppressed weight gain, hypercholesterolemia and hypertriglyceridemia induction by the high-fat diet although food consumption by the HF-FOS 5% and HF-FOS 10% groups was higher compared to the high fat control. Furthermore, the blood concentrations of calcium and magnesium that were lowered by the high fat diet were significantly increased in the HF-FOS 5% and HF-FOS 10% groups even though the absorption of calcium and magnesium following FOS treatment was low in the *in vitro* assay. Overall, these results suggest that dietary supplementation with FOS can help prevent a wide range of ailments induced by chronic high fat diet intake.

Keywords: fructooligosaccharides, intestinal bacteria, high fat diet, cholesterol, mineral absorption

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1. Introduction

Gut bacteria have an important effect on the function of the mucosal immune system and on nutritional metabolism in the healthy body [1]. Non-digestible food is fermented by the intestinal bacteria and selectively stimulates the growth of healthy bacteria such as *Bifidobacterium* and *Lactobacillus* in the colon, which can improve the host health by increasing resistance to invading pathogens [2]. These non-digestible food ingredients that help in improving host health are known as prebiotics. A number of health benefits associated with dietary prebiotics have been reported, thus, there is growing interest in the study of and development of prebiotics [2,3].

Interest in the use of fructooligosaccharides (FOS), low molecular weight and non-digestible carbohydrates, as prebiotics is increasing as they can serve as a low-calorie food sweetener [4]. FOS is distributed naturally in plants such as onions, asparagus, and bananas, among many others, and can be developed from sucrose by the action of fructosyltransferase [4]. FOS contains several units including 1-Kestose, Nystose and 1f-Fructofranosylnystose that are derived from sugar through natural fermentation processes [5]. From many recent studies in humans as well as animal models, it was determined that dietary treatment of FOS induced an increase in the proliferation of healthy bacteria including *Bifidobacterium* and *Lactobacillus* [6,7].

The production of organic acids by FOS-induced bacterial fermentation in the colon can improve resistance against acid-sensitive pathogens [8]. FOS-induced generation of an acidic environment in the colon can

contribute to the absorption of minerals such as calcium, magnesium, and zinc due to the increased mineral solubility released from binding to fiber [9,10]. Some studies have reported that dietary treatment of FOS not only improved intestinal calcium absorption but also prevented the decrease of bone mineral density by maintaining calcium homeostasis [9,10]. In addition, dietary treatment of FOS has an effect on hypocholesterolemia by enhancing cholesterol excretion in fecal bile acid as bile acids bound to FOS cannot be recirculated [11,12,13]. Thus, dietary treatment of FOS can have beneficial biological effects on several nutritional metabolisms.

Many recent studies focusing on the link between the intestinal bacteria and obesity observed a change in intestinal bacteria in high-fat-induced obese animal models or in obese patients [14,15]. A high-fat diet can induce the production of endotoxins with the growth of *harmful bacteria* and the development of colonic inflammation. It was reported that chronic high-fat-induced mice exhibited metabolic endotoxemia following the infusion of LPS and that gut microflora modulated plasma LPS levels [16,17]. However, the study results showed that oligofructose-treatment in mice led to an increase in *Bifidobacterium* and improved high fat diet-induced diabetes [17]. We may therefore hypothesize that FOS also prevents changes in intestinal bacteria in high fat diet-induced metabolic diseases.

Thus, we investigated the effects of FOS dietary supplementation on intestinal bacteria, blood cholesterol and mineral absorption in Balb/c mice fed a high fat diet. In addition, we evaluated whether the direct effects of FOS on calcium and magnesium transport, independent of the acidic environment, improved absorption in the Caco-2 cell.

2. Methods

2.1. Production of Fructooligosaccharides

Raw sugar (cane sugar) (Sagay Central, Bacolod, Philippines) was stirred and melted in water at 80-90°C (Raw sugar : water = 6 : 4). The mixing sugar syrup was 55-60°Brix and was maintained at pH 6.0∼6.5. 0.5% *Aspergillus aculeatus* was added and the solution was incubated for 48 h at 55-60°C. The solution was filtered and concentrated to 75°Brix. Quantitative analysis of FOS was performed using HPLC. We used a reference standard stock solution containing 1-Kestose, Nystose and 1f-Fructofranosylnystose (Wako Pure Chemical Industries, Osaka, Japan). The HPLC conditions were YMC PolyamineⅡ, 1.0 mL/min flow rate and the mobile phase consisted of Acetonitrile 64%. The product contained glucose 19.7%, sucrose 15% and FOS 64.5% (1-Kestose, 44.6%; Nystose, 15.4%; 1f-Fructofranosylnystose, 4.5%).

2.2. Experimental Animals and Treatment

The experimental protocols described in this study were approved by the Institutional Animal Care and Use Review Committee of Chonnam National University (CNU IACUC-YB-2015-24). Balb/c mice (20-22 g, 6 weeks, male) were housed in wire mesh bottomed

individual cages, in climate-controlled quarters (24 ± 1°C, 55 ± 5% relative humidity), with a 12-h light: 12-h dark cycle. All mice were acclimatized for an adaptation period of seven days before the experiment. Mice were randomly divided into four groups (eight mice per group): Normal control (mice fed AIN93G), High fat control (mice fed 60% fat in modified AIN93G), HF-FOS 5% (mice fed 60% fat and 5% FOS in modified AIN93G) and HF-FOS 10% (mice fed 60% fat and 5% FOS in modified AIN93G). At the end of 8 weeks, all mice were euthanized by cervical dislocation.

Table 1. Composition of BL agar (pH 7.2)

Agar	15 g
L-cysteine hydrochloride	0.5 g
Glucose	10 g
Lab-Lemco	3 g
Liver extract solution [1]	150 mL
Peptone	3 g
Proteose peptone No.3	10 g
Solution A [2]	10 mL
Solution B [3]	5 mL
Soluble starch	0.5 g
Tween 80	1 g
Tryptone	5 g
Yeast extract	5 g
Distilled water	815 mL

[1] Liver extract solution ; extraction and filtration of liver powder 10 g in distilled water 170 mL for 1 h at 50~60°C
[2] Solution A ; KH_2PO_4 25 g and K_2HPO_4 25 g in distilled water 250 mL
[3] Solution B ; $FeSO_47H_2O$ 0.5 g, $MgSO_47H_2O$ 10 g , $MnSO_44H_2O$ 0.337g , NaCl 0.5 g in distilled water 250 mL.

2.3. Measurement of Fecal and Intestinal Bacteria

Fecal samples were collected for 7 weeks and intestinal samples were collected after killing the mice on the 8th week. The samples were serially diluted in anaerobic diluent (KH_2PO_4 4.5 g, Na_2HPO_4 6 g, L-cysteine hydrochloride 0.5 g, Tween80 0.5 g in distilled water 1 L). We used Reinforced Clostridial Medium (Difco, BD Biosciences, San Jose, CA, USA) containing 50 μg/mL vancomycin for *Clostridium*, Lactobacilli MRS Agar (Difco, BD Biosciences, San Jose, CA, USA) containing 50 μg/mL vancomycin for *Lactobacillus* and BL medium (Table 2) containing 5% NPNL solution (Neomycin-sulphate 0.2 g, Paramomycin 0.4 g, Nalidixic acid 0.03 g and LiCl 6 g in distilled water 100 mL, pH 7.2~7.5) for *Bifidobacterium*. All plates were incubated for 48 h at 37°C under anaerobic conditions in a jar (BBL, gaspak system, USA) located within an anaerobic chamber gas pack (BD Biosciences, San Jose, CA, USA). The result of bacterial enumeration was expressed as a percentage of the control group.

2.4. Serum Biomarker Analyses

At the end of 8 weeks, all mice were sacrificed by cervical dislocation. Serum was collected from the whole blood by centrifugation (12,000 rpm, 4°C, for 20 min). The levels of serum triglyceride, total cholesterol, free cholesterol, LDL/VLDL cholesterol and HDL cholesterol were measured using quantification kits from Biovision (Milpitas, CA, USA). Serum calcium and magnesium were respectively measured with a Magnesium

colorimetric assay kit (Biovision, Milpitas, CA, USA) and a Calcium colorimetric assay kit (Biovision, Milpitas, CA, USA) according to the manufacturer's protocols.

Table 2. Composition of experimental diets

	Normal control (AIN93G)		High fat control (60% high fat AIN93G)		HF-FOS 5%		HF-FOS 10%	
	g	kcal	g	kcal	g	kcal	g	kcal
Casein	200	800	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12	3	12
Corn Starch	397.5	1590	-	-	-	-	-	-
Maltodextrin	132	528	125	500	125	500	125	500
Sucrose	100	400	78.3	313.2	39.15	156.6	-	-
Cellulose	50	0	50	0	50	0	50	0
Soybean Oil	70	630	25	225	25	225	25	225
Lard	-	-	245	2205	245	2205	245	2205
Mineral Mix	35	0	45	0	45	0	45	0
Vitamin Mix	10	40	10	40	10	40	10	40
Choline bitartrate	2.5	0	2	0	2	0	2	0
Fructooligosaccharides	-	-	-	-	39.15	114.4	78.3	228.8
Total	1000	4000	783.3	4095.2	783.3	4053	783.3	4010.8
	4.00 kcal/g		5.23 kcal/g		5.17 kcal/g		5.12 kcal/g	

2.5. Cell culture and Cell Viability Assay

Caco-2 cells were obtained from American type culture collection (ATCC, Manassas, VA, USA). Caco-2 cells were maintained in Dulbecco's minimal essential medium (DMEM, Hyclone Laboratories, Logan, Utah, USA) containing 20% fetal bovine serum (FBS, Hyclone Laboratories, Logan, Utah, USA) and 100 mg/L penicillin-streptomycin. Cells were maintained at 37°C under a humidified atmosphere of 5% CO_2. Cell viability was measured| with a 3-(4,5-dimethylthiazol-2yl-)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma Aldrich, Sigma, St. Louis, MO, USA). Caco-2 cells were cultured with FOS at various concentrations ranging from 100-1000 µg/mL. After incubation for 24 h, 500 µg/mL MTT was added to each well. After 4 h, the supernatants were removed and 200 µL of DMSO was added to each well. The plates were read at 560 nm and the results were expressed as a percentage of the control group.

2.6. Caco-2 Cell Differentiation and Calcium and Magnesium Transport Assay

Caco-2 cells were seeded in 12 well transwell plates (Corning Costar Co., Cambridge, MA, USA) to form a monolayer. The medium was changed every day until cell differentiation took place. Formation of the Caco-2 monolayer was confirmed during the differentiation stage by measuring the transepithelial electrical resistance (TEER) using EVOM (World Precision Instruments, STX100, Sarasota, FL, USA)). A TEER value > 800 $\Omega \cdot cm^2$ on day 16 after the seeding of the cells was recorded in the epithelial layer used for transport study. 1 mM $CaCl_2$, 1 mM $MgCl_2$ and various concentrations of FOS were added to the upper compartment (Apical). After 2 h, the concentration of calcium and magnesium in the upper compartment (Apical) and the lower compartment (Basolateral) were measured with a Magnesium colorimetric assay kit (Biovision, Milpitas, CA, USA) and

a Calcium colorimetric assay kit (Biovision, Milpitas, CA, USA).

2.7. Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted with one-way ANOVA using SPSS statistical procedures for Windows (SPSS PASW Statistic 22.0, SPSS Inc. Chicago, IL, USA) and Duncan's multiple range test was used to examine the differences among groups. Statistical differences were considered significant for $p < 0.05$.

3. Results

3.1. Effect of FOS on Fecal and Intestinal Bacteria

In the high fat control group, fecal and intestinal *Lactobacillus* and *Bifidobacterium* were significantly decreased, but fecal and intestinal *Clostridium* was not significantly different when compared with the normal control group. The dietary supplementation with FOS 5% and 10% resulted in a significant decrease in fecal *Clostridium* and a significant increase in fecal *Lactobacillus* and *Bifidobacterium* compared with the high fat control group ($p < 0.05$) (Figure 1). Intestinal *Clostridium* in the mice that received dietary supplementation with FOS 10% was significantly decreased compared with that in the high fat control group. In addition, intestinal *Lactobacillus* and *Bifidobacterium* was significantly increased in the mice that received dietary supplementation with FOS 10% compared with that in the high fat control group. However, intestinal bacteria in the mice that received dietary supplementation with FOS 5% were not significantly different compared with that in the high fat control group ($p < 0.05$) (Figure 2).

3.2. Effect of FOS on FER and Organ Weights

We found that the high fat control group had a marked increase in weight gain and food efficiency rate (FER) as well as a decrease in food consumption compared with the normal control group. The groups that received FOS dietary supplementation showed significant decreases in weight gain compared with the infection control group. In addition, the mice that received dietary supplementation with FOS showed significant differences in food consumption and FER in a dose-dependent manner compared with the high fat control group. The weights of the kidney and spleen did not differ in a statistically significant manner among any of the groups. However, we observed that the high fat diet caused a significant increase in the weight of the liver compared with the normal control ($p < 0.05$) (Table 3).
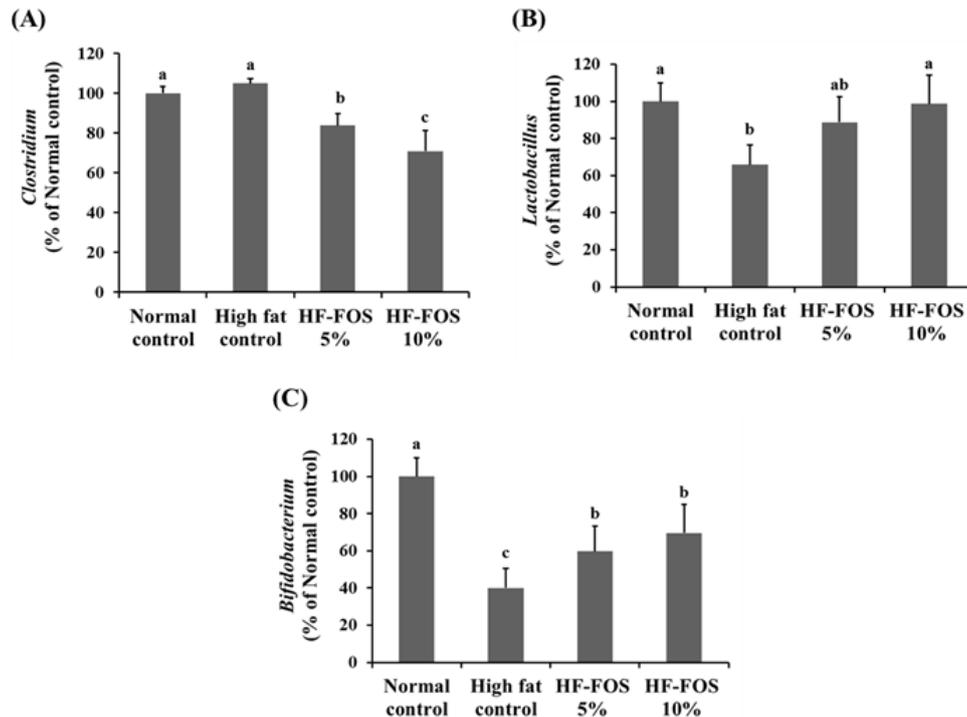

Figure 1. Effect of dietary supplementation of fructooligosaccharides on the fecal bacteria in Balb/c mice fed a high-fat diet. (A) *Clostridium* (B) *Lactobacillus* (C) *Bifidobacterium*. All data are expressed as mean ± standard deviation (n=8). Different letters show a significant difference at $p < 0.05$ as determined by Duncan's multiple range test
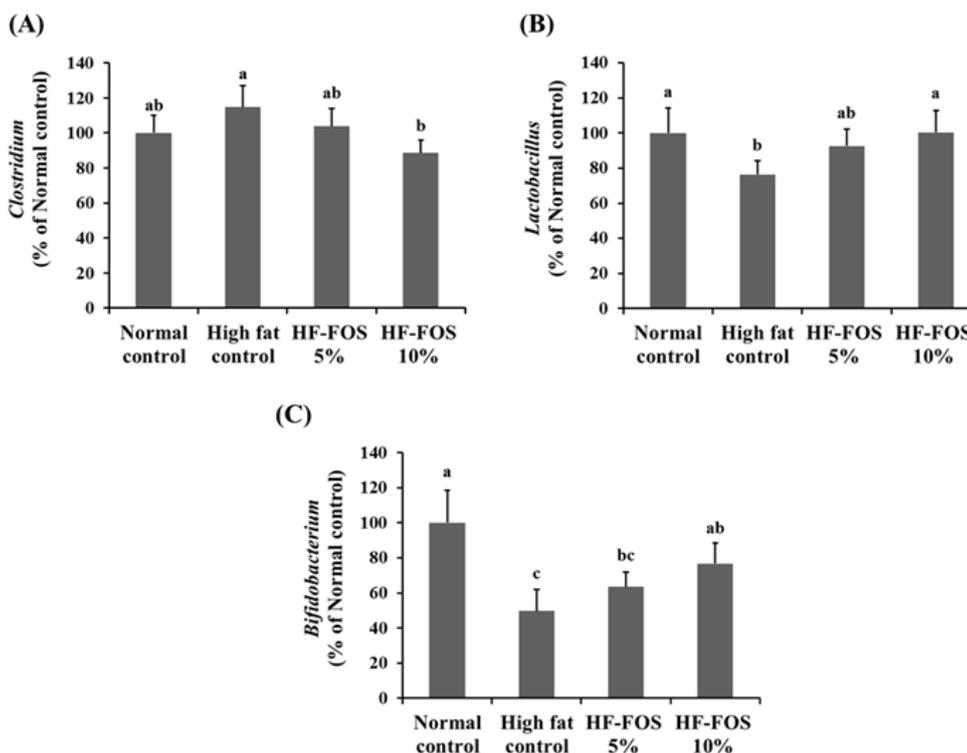

Figure 2. Effect of dietary supplementation of fructooligosaccharides on the intestinal bacteria in Balb/c mice fed a high-fat diet. (A) *Clostridium* (B) *Lactobacillus* (C) *Bifidobacterium*. All data are expressed as mean ± standard deviation (n=8). Different letters show a significant difference at $p < 0.05$ as determined by Duncan's multiple range test

Table 3. Effect of dietary supplementation of fructooligosaccharides on FER and organ weights in the C57BL/6 mice fed a high-fat diet

	Normal control	High fat control	HF-FOS 5%	HF-FOS 10%
Initial body weight (g)	21.40 ± 0.67 [NS]	21.60 ± 0.79	21.77 ± 0.42	21.54 ± 0.71
Final body weight (g)	27.14 ± 0.88 [c]	30.85 ± 1.05 [a]	29.06 ± 0.49 [b]	28.16 ± 0.91 [b]
Weight gain (g)	5.74 ± 0.92 [c]	8.98 ± 1.29 [a]	7.29 ± 0.46 [b]	6.61 ± 0.88 [bc]
Food consumption (g/8 weeks)	71.59 ± 1.89 [b]	59.63 ± 3.03 [c]	74.15 ± 1.39 [b]	87.33 ± 4.99 [a]
FER [1]	2.01 ± 0.32 [b]	2.90 ± 0.42 [a]	1.93 ± 0.12 [b]	1.50 ± 0.20 [c]
Organ weight (g/100 g b.w.)				
Liver	3.34 ± 0.33 [b]	3.76 ± 0.23 [a]	3.70 ± 0.20 [a]	3.96 ± 0.34 [a]
Kidney	1.39 ± 0.07 [NS]	1.31 ± 0.10	1.39 ± 0.08	1.34 ± 0.10
Spleen	0.29 ± 0.07 [NS]	0.28 ± 0.04	0.31 ± 0.02	0.27 ± 0.03

[1] FER (Food efficiency rate) = weight gain (g) / Total food intake (kcal) x 100
All data are expressed as mean ± standard deviation (n=8). Different letters show a significant difference at *p*<0.05 as determined by Duncan's multiple range test.

3.3. Effect of FOS on Plasma Triglyceride and Cholesterol

When compared with the normal control, the high fat control showed a significant increase in the concentrations of triglyceride, total cholesterol and LDL/VLDL cholesterol. However, there was no marked difference between the normal control group and the high fat control group in the concentration of HDL cholesterol and free cholesterol. Dietary supplementation of FOS significantly reduced the triglyceride total cholesterol compared with the high fat control. Particularly, dietary supplementation of FOS significantly reduced LDL/VLDL cholesterol in a dose-dependent manner. Consequently, we observed that dietary supplementation of FOS caused a significant decrease in the Atherogenic index (AI) and an increase in the ratio of HDL cholesterol to total cholesterol (HTR) compared with the high fat control (*p* < 0.05) (Table 4).

Table 4. Effect of dietary supplementation of fructooligosaccharides on lipid profiles in the C57BL/6 mice fed a high-fat diet.

	Normal control	High fat control	HF-FOS 5%	HF-FOS 10%
Triglyceride (mM)	1.22 ± 0.10 [c]	1.78 ± 0.11 [a]	1.43 ± 0.13 [b]	1.31 ± 0.11 [bc]
Total cholesterol (mg/dL)	97.21 ± 6.56 [b]	115.75 ± 9.45 [a]	103.64 ± 6.86 [b]	98.18 ± 7.30 [b]
HDL cholesterol (mg/dL)	53.62 ± 3.90 [NS]	56.12 ± 8.19	58.27 ± 5.00	59.32 ± 8.13
LDL/VLDL cholesterol (mg/dL)	17.18 ± 1.67 [c]	29.16 ± 2.09 [a]	21.38 ± 2.12 [b]	17.64 ± 3.27 [c]
Free cholesterol (mg/dL)	24.74 ± 1.54 [NS]	26.11 ± 1.56	25.13 ± 3.40	23.37 ± 3.79
AI [1]	0.81 ± 0.04 [b]	1.08 ± 0.14 [a]	0.78 ± 0.09 [b]	0.67 ± 0.13 [b]
HTR (%) [2]	55.15 ± 1.32 [b]	48.28 ± 3.29 [c]	56.20 ± 2.74 [ab]	60.25 ± 4.98 [a]

1) AI (Atherogenic index) = (Total cholesterol - HDL cholesterol) / HDL cholesterol
2) HTR (%) = HDL cholesterol / Total cholesterol x 100
All data are expressed as mean ± standard deviation (n=8). Different letters show a significant difference at *p*<0.05 as determined by Duncan's multiple range test.

3.4. Effect of FOS on Plasma Calcium and Magnesium

The plasma calcium and magnesium concentrations decreased significantly in the high fat control group compared with the normal control group. Dietary supplementation of FOS resulted in significant increases in the plasma calcium concentration compared with the high fat control group. In addition, dietary supplementation of FOS significantly increased the plasma magnesium concentration in a dose-dependent manner compared with the high fat control group (*p* < 0.05) (Figure 3).
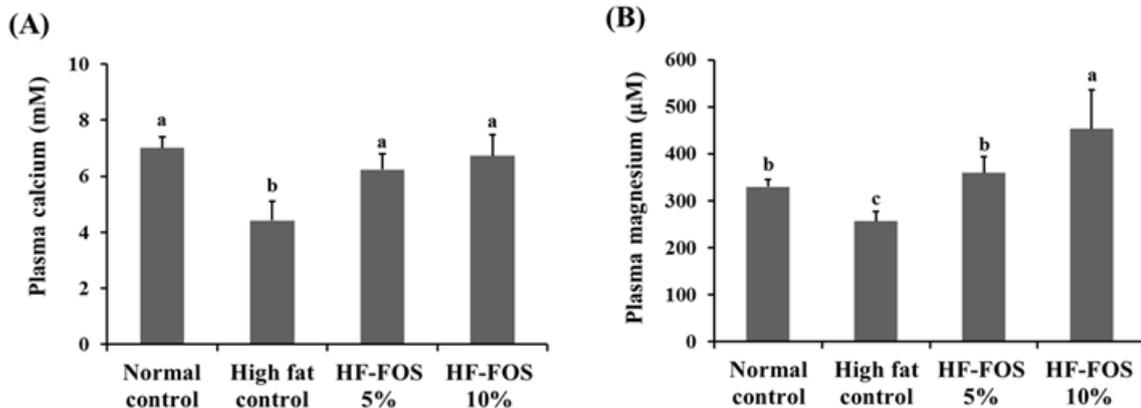

Figure 3. Effect of dietary supplementation of fructooligosaccharides on (A) the plasma calcium and (B) magnesium concentration in the Balb/c mice fed a high-fat diet. All data are expressed as mean ± standard deviation (n=8). Different letters show a significant difference at *p* < 0.05 as determined by Duncan's multiple range test

3.5. Effect of FOS on the Transport of Calcium and Magnesium in Differentiated Caco-2 cells

We first investigated the concentration dependence of the cytotoxic effects of FOS at various concentrations (100 - 1000 µg/mL) for 24 h. We found that FOS showed no signs of cytotoxicity at the indicated concentrations in Caco-2 cells (Figure 4).

Apical calcium and magnesium did not differ in a statistically significant manner among any of the groups.

Basolateral calcium concentration increased in the FOS treated differentiated Caco-2 cell groups (200 µg/mL: 2.34 ± 0.03 mM; 500 µg/mL: 2.35 ± 0.03 mM; 1000 µg/mL: 2.32 ± 0.05 mM) compared with the FOS untreated differentiated Caco-2 cells (2.19 ± 0.04 mM), but the increase in the basolateral calcium concentration was small. Basolateral magnesium concentration increased in the 1000 µg/mL FOS-treated differentiated Caco-2 cells (0.74 ± 0.04 mM), unlike the FOS untreated differentiated Caco-2 cells (0.65 ± 0.01 mM) ($p < 0.05$) (Figure 5).
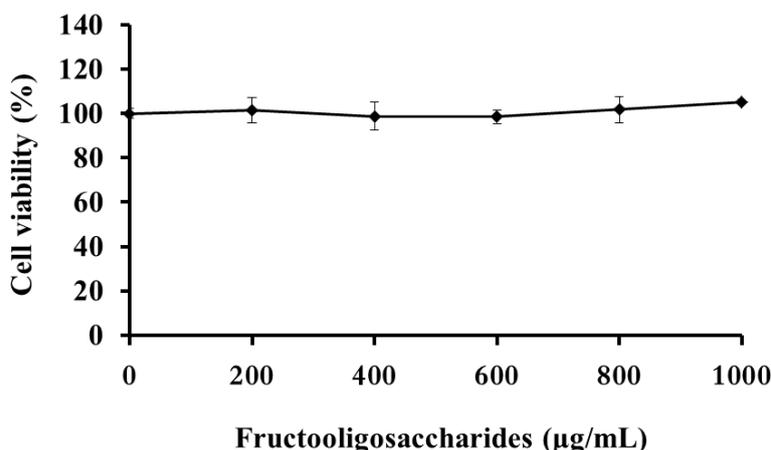

Figure 4. Viability of Caco-2 cells following 24 h of treatment with different concentrations of fructooligosaccharides. All data are expressed as mean ± standard deviation (n=3)
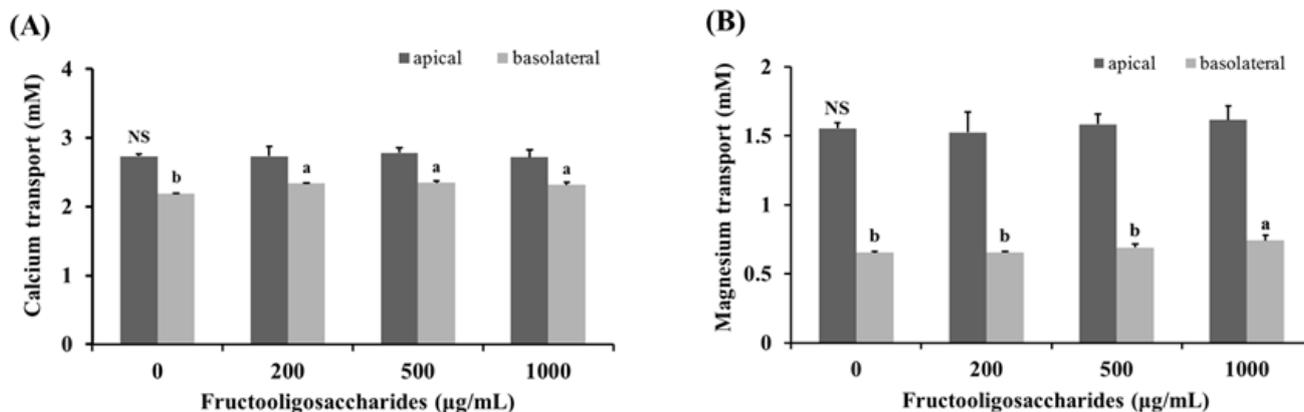

Figure 5. Effect of fructooligosaccharides on (A) the calcium and (B) magnesium transport in differentiated Caco-2 cells. All data are expressed as mean ± standard deviation (n=3). Different letters show a significant difference at $p < 0.05$ as determined by Duncan's multiple range test

4. Discussion

Accumulating evidence indicates that specific changes in the gut microbiota might trigger the development of obesity and obesity-associated inflammation induced by a high-fat diet [15,16,17]. The results of a study by Cani *et al.* showed that the number of *Bifidobacterium* in mice fed a diet containing 49.5% fat decreased markedly [17]. In addition, Dong *et al.* showed that the *Lactobacillus* counts in the colon of mice fed a high fat diet was significantly lower than that in mice fed a normal diet [18]. Several studies have reported that the supplementation of *Bifidobacterium* and *Lactobacillus* as probiotics bacteria had beneficial physiological effects including anti-obesity and anti-inflammation [19,20]. On the other hand, it has been reported that a high fat diet can increase the proportion of *Clostridium ramosum,* which can cause

pathologic infections [21]. Therefore, the proportion of specific gut bacteria may influence body health in a number of ways.

In the present study, we investigated the effects of dietary supplementation of FOS on intestinal *Clostridium*, *Bifidobacterium* and *Lactobacillus* using mice fed a diet containing 60% fat. The present study revealed marked decreases in fecal and intestinal *Lactobacillus* and *Bifidobacterium* but no significant differences in fecal and intestinal *Clostridium* in the high fat control group compared with those of the normal control group. The dietary supplementation with FOS induced a significant decrease in fecal and intestinal *Clostridium* and a significant increase in fecal and intestinal *Lactobacillus* and *Bifidobacterium* compared with the high fat control group ($p < 0.05$). Many studies have documented that dietary treatment of FOS induced an increase in intestinal *Bifidobacterium* and *Lactobacillus* [6,7]. A study by Xu *et*

al. found that *Bifidobacterium* and *Lactobacillus* were significantly increased in the small intestinal digesta of broilers fed diets with 4.0 g/kg FOS compared with the control whereas *Escherichia coli* were significantly reduced [6]. Hsu *et al.* documented that dietary treatment of FOS inhibited the development of precancerous lesions through the growth of *Bifidobacterium* and lowered the cecal pH in rats. They suggested that dietary treatment of FOS may be beneficial to gastrointestinal health [7].

In addition, there have been several reports showing that dietary treatment of FOS has an inhibitory effect on fat accumulation and hypercholesterolemia [11,12,13]. Nakamura *et al.* reported that the body weight and percent body fat were significantly lower while fecal excretion of lipids was markedly enhanced in mice fed FOS compared with the control group [11]. We also found that the dietary supplementation of FOS significantly reduced weight gain and the concentration of triglyceride compared with the high fat control ($P < .05$). Interestingly, even though the mice fed 10% FOS had the highest food consumption among mice fed the high fat diet, they had the lowest weight gain. In addition, we found in this study that dietary supplementation of FOS significantly reduced total cholesterol and LDL/VLDL cholesterol compared with the high fat control ($p < 0.05$).

The effect of FOS on hypercholesterolemia can be hypothesized to occur through several mechanisms [22,23,24,25,26]. The dietary supplementation of FOS can induce cholesterol excretion via precipitation with bile acids and the excretion of bile acids in the colon [23,24]. In addition, FOS-induced bacterial growth in the colon can assimilate the cholesterol [25]. A study on the cholesterol-lowering effect of *Lactobacillus acidophilus* P47 by Gilliland *et al.* found that consumption of *Lactobacillus* acidophilus P47 assimilated cholesterol within the laboratory medium and significantly inhibited increases in the serum cholesterol levels of pigs fed a high-cholesterol diet [26]. These reports and this present study suggest that dietary treatment of FOS can help prevent a wide range of ailments including hypercholesterolemia through the proliferation of *Bifidobacterium* and *Lactobacillus* in the gut.

Several studies have documented the association between mineral metabolism and the development of obesity. The results of studies by Foldes *et al.* [27] and Zamboni *et al.* [28] showed that obesity induced hypercalciuria, hypermagnesemia and a decrease in serum calcium and phosphorus. In addition, Zemel *et al.* reported that increasing dietary calcium enhanced the efficacy of an energy-restricted diet in weight control and had a particularly beneficial effect on central obesity [29]. In the present study, we found that the levels of plasma calcium and magnesium decreased significantly in the high fat control group compared with the normal control group ($p < 0.05$). This result may be attributed to the chronic supplementation of a high fat diet inhibiting mineral absorption. However, supplementation of FOS with a high fat diet caused significant increases in the levels of plasma calcium and magnesium compared with the high fat control group ($p < 0.05$). On the other hand, we found in the *in vitro* assay that the increase in the absorption of calcium and magnesium following FOS treatment was small in differentiated Caco-2 cells. Thus, we can assume that the effect of FOS on mineral absorption is not direct.

The supplementation of FOS has been demonstrated to have a positive effect on the absorption of several minerals including calcium, magnesium and iron [9,10]. During fermentation of the FOS by intestinal bacteria, minerals are released from the bound fiber and become available for absorption in the colon [30]. This absorption of minerals in the colon is induced by the reduction of pH and the high concentration of short chain fatty acids through FOS fermentation by intestinal bacteria [9,10]. Scholz-Ahrens *et al.* reported that insulin-type fructans including oligofructose and FOS can stimulate mineral absorption and bone mineralization when combined with the probiotic *Lactobacillus* [31]. In addition, Takahara *et al.* reported that the increase in calcium and magnesium absorption by the supplementation of FOS in rats leads to the enhancement of femoral bone volume and mineral concentrations [32]. Therefore, the supplementation of FOS can prevent osteoporosis as increased mineral absorption induces an increase in bone density and a subsequent decrease in the risk of osteoporosis.

5. Conclusions

In summary, FOS is fermented by the intestinal bacteria and stimulates the growth of healthy bacteria such as *Bifidobacterium* and *Lactobacillus* in the colon. The proliferation of *Bifidobacterium* and *Lactobacillus* with FOS supplementation can have effects such as a decrease in the cholesterol level and an increase in the absorption of minerals. In the present study, we found that supplementation of FOS products from *Aspergillus aculeatus*-treated raw sugar increased intestinal *Bifidobacterium* and *Lactobacillus* in Balb/c mice fed a high fat diet. In addition, the results of this study showed that dietary supplementation of FOS reduced weight gain, the triglyceride level, the total cholesterol and the LDL/VLDL cholesterol. It also increased the levels of plasma calcium and magnesium even though the absorption of calcium and magnesium following FOS treatment was low in the *in vitro* assay. In conclusion, we suggest that FOS can help prevent a wide range of ailments induced by chronic high fat diet intake and is beneficial to health.

Acknowledgements

Conflicts of Interest

The authors declare no conflicts of interest.

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