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ORIGINAL ARTICLE

Effect of probiotic-fermented, genetically modified soy milk on hypercholesterolemia in hamsters

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Background/Purpose: The rapid progress of biotechnology and molecular biology has led to genetically modified (GM) crops becoming a part of agricultural production. There are concerns that the issues of the functional ingredients in GM products have not been addressed, such as the bioactivities of soy proteins and isoflavones. This study aimed to investigate the effects of probiotic-fermented GM soy milk on hypercholesterolemia, and atherosclerotic risks in hamsters.
Methods: One hundred and twelve male Golden Syrian hamsters (*Mesocricetus auratus*) were randomly assigned into 14 groups of 8 animals each. Normal- and high-cholesterol experimental diets were supplemented with GM or non-GM soy milk with or without probiotic-fermentation for 8 weeks. Serum and fecal lipid levels were measured. Moreover, aortic plaque in artery were stained, and thiobarbituric acid reactive substance content, super oxide dismutase activity and caralase activity were determined.
Results: GM or non-GM soy milk with or without probiotic-fermentation significantly decreased ($p < 0.05$) serum TC levels, compared with a high-cholesterol diet group. TC levels in hamsters fed GM soy milk were not significantly different from TC levels in the non-GM soy milk group ($p > 0.05$). GM soy milk groups can reduce risk of developing atherosclerosis through lowered oxidative stress and reduced atherosclerotic plaque formation in the aorta, and are thus at least equivalent to non-GM soy milk.
Conclusion: GM soy milk with or without probiotic-fermentation can improve hypercholesterolemia and reduce the risk of atherosclerosis, and is considered substantially equivalent to non-GM soy milk in terms of these bioactive functions.

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Introduction

The rapid progress of biotechnology and molecular biology has led to genetically modified (GM) crops becoming a part of agricultural production. From 1996 to 2010, 964 approvals for GM crops were granted worldwide. Soybean and corn are the top two most widely grown GM crops.[1] Soybean-related products, such as soy milk, are traditional cuisine consumed in Asia. Soybean provides high-quality proteins, fats, and carbohydrates and contains no cholesterol or lactose. It is a plentiful and inexpensive source of nutrition for lactose-intolerant individuals, vegetarians, and milk-allergy patients.[2] The undigested pepsin fraction of soybean protein effects the fecal excretion of steroids or bile acids, which may influence cholesterol metabolism.[3] Also, the mechanism for the hypocholesterolemic effect of soy milk is indicated that non-absorbed nitrogen-containing substances are correlated with the lipid-lowering effect.[4] Isoflavones in soybeans and unfermented soy foods exist in their glucoside form, but rarely in the aglycone form. Several studies indicate that increasing the bioavailability of these glucosides requires hydrolysis of the sugar moiety by intestinal β-glucosidase, which increases the absorptivity of aglycone isoflavones above that of the glucoside form in healthy adults.[5,6] In previous studies, soy milk fermented with probiotics to produce high levels of β-glucosidase improved the nutrition and bioactivity value of the foods.[7]

Development of the Roundup Ready soybean required the identification of a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) variant that was not affected by glyphosate. Although this CP4 EPSPS differs slightly from its natural counterpart in the soy plant, the difference is similar in degree to those found among food plants generally.[8] In a previous study, compositional analyses of meals from GM soybeans were undertaken, and defatted GM soybean meals were found to be similar to the control meal in total protein, fat, fiber, carbohydrates, and other components.[9] Compounds with hormone-like (e.g., estrogen) activities are also of interest to consumers due to their potential effects on cellular functions.

Based on the information described in previously published studies, GM soybeans are equivalent to non-GM soybeans. However, as knowledge and experience with GM foods (GMFs) increases, new questions may arise.[10] Moreover, Chen[11] indicate that natural content is observed to have a negative impact on consumers' attitudes toward GM foods in Taiwan. Soybean-related products fermented with microorganisms, including miso and soy sauce, are popular foods in Asia. Soy milk is also a common beverage in Taiwan. In our previous study, fermented milk and soy milk were proven to have potential hypocholesterolemic effects, which are related to the levels of soy proteins and isoflavones in such products.[12] There have been concerns that the functional issues underlying improvement of the hypercholesterolemic effects in GM and fermented soy products were not addressed. It is of interest to study the potential effects associated with consuming the GM soy-derived product soy milk. Therefore, to clarify the uncertainties of functional issues regarding consumption of GM soy products, hypocholesterolemia and improvement of atherosclerotic risks in hamsters by feeding GM soy milk and probiotic-fermented GM soy milk were evaluated in this study.

Methods

Chemicals and media

Thiobarbituric acid (TBA), malondialdehyde (MDA), and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lactobacilli MRS broth and Bacto-agar were purchased from Difco Co. (Detroit, MI, USA). GM soybean was purchased from supermarkets (Taipei, Taiwan) and non-GM soybean [*Glycine max* (L.) *Merrill* BB50] was obtained from Chuan Gui Bio-Organic Co. (Taoyuan, Taiwan).

Analysis and preparation of soy milk and fermented soy milk with lactic acid bacteria

The transgenes in purchased soybeans were analyzed by nested polymerase chain reaction (PCR) according to our previous study.[13] The preparation of soy milk was carried out with the substrate of GM and non-GM soybeans. Briefly, soybean was soaked in deionized water for 8 hours at 25°C. The swollen bean was ground into a homogenate with eight times water of soybean dry weight using a blender, and the homogenate was then filtered through a defatted cotton sheet. The resultant slurry was filtered through a sieve and then heated in a water bath at 90°C for 1 hour. The bacterial strain used in this study was *Lactococcus lactis* subsp. *lactis* BCRC 14016, which was purchased from Bioresource Collection and Research Center, Food Industry Research and Development Institute (Hsinchu, Taiwan). The culture strain was inoculated 1% (v/v) to soy milk. The cultured soy milk was incubated in flasks at 37°C for 72 hours. At the end of preparation, all samples were dried by freeze dryer (SDF-25, Chang Jung Business Co., Feng-Jen, Taiwan) for analysis and feeding hamsters. Standard methods of the Association of Official Analytical Chemists[14] were used to determine the crude protein and crude fat content. And the phenol–sulfuric acid method was used to estimate total carbohydrates.[15] Crude protein (total nitrogen (%) × 6.25) was determined by the Kjeldahl method using 2 g samples. Crude fat was obtained by exhaustively extracting 5 g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40–60°C) as the extractant. The glycoside and aglycone isoflavones were analyzed by high performance liquid chromatography (HPLC; Jasco Co., Tokyo, Japan) according to Lin and colleagues.[16]

Diets and experimental eesign

One hundred and twelve male Golden Syrian hamsters (*Mesocricetus auratus*) weighing 100 to 120 g were housed in individual cages and subjected to a 12-hour light/dark cycle with a maintained relative humidity of 50%–60%, and

a temperature at 25 ± 2°C. The animals were given free access to regular rodent feed and water for 4 weeks to adapt the new environment. Hamsters were randomly assigned into 14 groups of eight animals each before the animal experiment. The dose of soy milk was calculated in accordance with Boyd's Formula of body surface area as recommended by the U.S. Food and Drug Administration (FDA).[17] In previous study, 15 g of total proteins contents in non-GM soy milk was used as the reference dosage of adult as hypocholesterolemic effects.[4,12,18] All test samples were prepared according to the reference dosage and included in the daily total energy requirement. Samples were mixed with experimental diets to and orally administrated to the hamsters for 4 weeks and 8 weeks, respectively. Composition of experimental diets and samples were shown in Table 1 and provided in accordance with AIN-76 diet formulation with modification. The food intake was recorded daily, and animals were weighed weekly. The N group was fed a normal diet via AIN-76 formulation, and the HC group was given a high cholesterol diet that contained 0.2% cholesterol. GM, NGM, FGM and FNGM groups were fed the high cholesterol diet and orally given GM soy milk, non-GM soy milk, fermented GM soy milk and fermented non-GM soy milk with *L. lactis* subsp. *lactis* BCRC 14016 (4.5 g/kg body weight per day, including 15 g total proteins), respectively. In addition, the HM group, a positive control group, was fed the high cholesterol diet and given orally red mold rice powder (100 mg/kg body weight per day).[18] Twenty four hours before sacrifice, all food was removed. Animals were anesthetized by carbon dioxide inhalation and sacrificed. Blood was collected by cardiac puncture and centrifuged at 5000*g* for 10 minutes at 4°C to prepare plasma that was frozen at −20°C until analysis. Whole blood was collected for the assay of superoxide dismutase (SOD) and catalase (CAT) activities. Serum samples were obtained by drawing the blood into a serum separated tube, allowing it to clot and centrifuging for 10 minutes at 3000*g* to separate serum. Liver tissue was cleaved and rinsed frequently with sterile phosphate buffered saline (PBS) to eliminate blood and immersed in the liquid nitrogen, and then stored at −80°C. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taiwan University.

Table 1 Composition of the experimental diet (g/kg diet)

Composition	N	HC	HM	GM	NGM	FGM	FNGM
Casein	200	138	138	126	119	126	122
Corn starch	650	680	680	620	587	621	603
Cellulose	50	50	50	50	50	50	50
Soybean oil	50	80	80	73	69	73	71
Mineral	35	35	35	35	35	35	35
Vitamin	10	10	10	10	10	10	10
L-cysteine	3	1	1	1	1	1	1
Choline bitartrate	2	2	2	2	2	2	2
Cholic acid	—	2	2	2	2	2	2
Cholesterol	—	2	2	2	2	2	2
Soy milk powder	—	—	—	83	121	81	104
Monascus powder	—	—	1.67	—	—	—	—

The experimental diet is based on the AIN-76 formula.
FGM = fermented genetically modified soy milk and high-cholesterol diet; FNGM = fermented non-genetically modified soy milk and high-cholesterol diet; GM = genetically modified soy milk and high cholesterol diet; HC = high cholesterol diet; HM = *Monascus*-fermented powder and high cholesterol diet; N = normal diet; NGM = non-genetically modified soy milk and high cholesterol diet.

Serum and liver lipid analysis

Serum and fecal total cholesterol (TC), triglyceride (TG), and serum high density lipoprotein cholesterol (HDL-C) levels were measured in triplicate using commercial enzymatic kits. These kits were as follows: TC assay kit (CH 200, Randox Laboratories Ltd., Antrim, UK), TG assay kit (TR-210, Randox Laboratories Ltd), and the HDL-C assay kit (CH-203, Randox Laboratories Ltd). Serum low density lipoprotein cholesterol (LDL-C) levels was gained via the follow calculation,[19] LDL-C (mg/dL) = TC − TG/5 − HDL-C. Liver tissue (0.5 g) was ground in 10 mL of ice-cold Folch solution (chloroform: methanol = 2:1; v/v) and incubated for 30 minutes at room temperature. The aqueous layer was aspirated and discarded, and the fixed volume of organic layer was then evaporated to dryness. The dried lipid layer were dissolved with equal volume of dimethyl sulfoxide (DMSO) and then used to determine the TC and TG levels using commercial enzymatic kits.

Determination of thiobarbituric acid reactive substance content, SOD activity, and CAT activity

According to the procedure of previous study, thiobarbituric acid reactive substance (TBARS) levels of serum were determined by the method of thiobarbituric acid (TBA) colorimetric analysis, and the optical density (OD) value was measured at 532 nm.[20] The SOD activity of erythrocyte from whole blood was examined by using the commercial kit (Ransod, Randox Laboratories Ltd). The results were expressed for erythrocytes as U/g Hb.[21] The CAT activity of erythrocyte from whole blood was determined as previously described.[22] CAT activity was measured as consuming 10 mM H_2O_2 by measuring the changes in absorbance at 240 nm for 3 minutes.

Stain of aortic plaque in artery

The thoracic aorta was cut open longitudinally along the anterior side, the lipid-rich lesions on the surface of the aorta were stained with 2% Sudan IV, and then washed with gradient concentration of methanol (100%, 90%, 80%, 70%, 60%) and PBS. Whole surface area of thoracic aorta was stained by Sudan IV and photographed using digital camera. The aortic surface area and its stained plaque area (red) were selected and quantitated by the Posterize program of Photoshop 7.0 software (Adobe Systems Incorporated, San Jose, CA, USA). The selected pixels of plaque area and whole aorta were used to calculated percent area of the aortic plaque, as follows:

$$\text{Aortic plaque percent}\,(\%) = (\text{pixel of stained plaque area}/\text{pixel of whole aorta}) \times 100\%$$

Table 2 Isoflavone content (μg/g) in GM soy milk, NGM soy milk, FGM soy milk, and FNGM soy milk

Soy milk	Isoflavone content (μg/g)			
	Genistin	Daidzin	Genistein	Daidzein
GM	88.2 ± 2.7^{c}	283.4 ± 7.3^{d}	51.7 ± 3.7^{b}	67.0 ± 2.3^{b}
NGM	70.6 ± 0.8^{b}	241.0 ± 3.3^{c}	10.3 ± 0.8^{a}	15.6 ± 0.6^{a}
FGM	3.9 ± 0.2^{a}	1.2 ± 0.8^{a}	240.8 ± 16.8^{d}	256.8 ± 22.6^{d}
FNGM	3.2 ± 0.2^{a}	9.6 ± 0.0^{b}	170.3 ± 3.9^{c}	210.8 ± 0.6^{c}

FGM = fermented genetically modified soy milk; FNGM = fermented non-genetically modified soy milk; GM = genetically modified soy milk; NGM = non-genetically modified soy milk.
Data are presented as mean ± SD, and all values represent triplicate results. Values bearing different superscript letters in the same column are significantly different in accordance with Duncan's multiple range test where significance is defined as $p < 0.05$.

Statistics

Data are expressed as the mean ± standard deviation (SD). The statistical significance in the biochemical effects was determined by one-way analysis of variance (ANOVA) followed by the Duncan's multiple range tests. Values bearing different superscript letters in the same column are significantly different in accordance with Duncan's multiple range test where significance is defined as $p < 0.05$.

Results

Analysis of the transgenes and isoflavones in soy milk

A nested PCR reaction was performed to confirm the transgene EPSPS in the soybean, and all samples used in this study were found to have no cross contamination during the experimental period. Glycoside isoflavones (genistin and daidzin) and aglycone isoflavones (genistein and daidzein) were analyzed after fermentation with lactic acid bacteria. As in Table 2, genistein and daidzein increased 15.5-fold (from 10.3 ± 0.8 μg/g to 170.3 ± 3.9 μg/g) and 12.5-fold (from 15.6 ± 0.6 μg/g to 210.8 ± 0.6 μg/g), respectively, following 16-hour fermentation with *L lactis* subsp. *lactis* BCRC 14016. Genistin and daidzin decreased by 95.5 and 96.0%, respectively, during this period of fermentation.

Changes in body weight and daily food intake

There was no significant difference ($p > 0.05$) between body weight and daily food intake among the various groups (data not shown). Body weights increased normally during the period of the practical experiment. In addition, the behavior and health of all experimental animals were normal.

Hypolipidemic effects in the serum and liver

As shown in Table 3, high-cholesterol diet increased the serum TC levels of hamsters in the HC group by 37.1 and 40.4% ($p < 0.05$), respectively, after the 4- and 8-week feeding regimes as compared with the N group. The TC levels of other high-cholesterol diet groups that were administered *Monascus*-fermented powder (HM), GM soy milk (GM) and non-GM soy milk (NGF) decreased significantly by 19.9, 20.0 and 26.5%, respectively, after 4 weeks and by 20.2, 9.1, and 14.8%, respectively, after 8 weeks of feeding compared with the HC group ($p < 0.05$). The serum

Table 3 Effect of GM and non-GM soy milk on serum and liver TC levels

Group*	Serum TC (mg/dL)		Liver TC (mg/dL)	
	4 wks	8 wks	4 wks	8 wks
N	59.8 ± 6.3^{a}	109.8 ± 6.2^{a}	55.4 ± 14.8^{a}	93.9 ± 36.8^{b}
HC	82.0 ± 10.1^{b}	154.2 ± 22.8^{d}	78.4 ± 12.3^{b}	139.7 ± 30.8^{c}
HM	65.7 ± 6.4^{a}	123.0 ± 10.8^{b}	54.1 ± 11.2^{a}	55.5 ± 12.7^{a}
GM	65.6 ± 9.0^{a}	140.1 ± 4.9^{c}	80.1 ± 15.8^{b}	84.7 ± 13.7^{b}
NGM	60.3 ± 8.9^{a}	131.4 ± 12.7^{bc}	88.0 ± 18.7^{b}	102.5 ± 35.2^{b}
FGM	59.7 ± 9.3^{a}	134.7 ± 11.3^{bc}	85.3 ± 9.2^{b}	91.8 ± 6.8^{b}
FNGM	56.6 ± 9.4^{a}	127.5 ± 9.8^{bc}	83.3 ± 8.1^{b}	84.5 ± 23.8^{b}

Data are presented as mean ± SD ($n = 8$), and all values represent triplicate results. Values bearing different superscript letters in the same column are significantly different in accordance with Duncan's multiple range test where significance is defined as $p < 0.05$.
FGM = fermented genetically modified soy milk and high-cholesterol diet; FNGM = fermented non-genetically modified soy milk and high-cholesterol diet; GM = genetically modified soy milk and high cholesterol diet; HC = high cholesterol diet; HM = *Monascus*-fermented powder and high cholesterol diet; N = normal diet; NGM = non-genetically modified soy milk and high cholesterol diet; TC = total cholesterol.

Table 4 Effect of GM and non-GM soy milk on serum HDL-C and LDL-C levels

Group*	HDL-C (mg/dL)		LDL-C (mg/dL)		LDL-C/HDL-C ratio	
	4 wks	8 wks	4 wks	8 wks	4 wks	8 wks
N	31.0 ± 5.4^{a}	57.4 ± 6.7^{a}	9.74 ± 7.2^{ab}	33.2 ± 7.8^{a}	0.3 ± 0.3^{ab}	0.6 ± 0.1^{a}
HC	35.1 ± 4.1^{a}	65.6 ± 7.3^{ab}	28.0 ± 8.1^{c}	69.7 ± 18.6^{c}	0.8 ± 0.2^{c}	1.1 ± 0.3^{c}
HM	34.0 ± 4.6^{a}	61.7 ± 8.4^{ab}	16.6 ± 7.5^{b}	43.1 ± 11.7^{ab}	0.5 ± 0.2^{b}	0.7 ± 0.2^{ab}
GM	34.1 ± 5.9^{a}	63.7 ± 6.5^{ab}	8.1 ± 8.4^{a}	55.4 ± 7.7^{b}	0.2 ± 0.3^{a}	0.9 ± 0.1^{bc}
NGM	33.0 ± 4.6^{a}	59.5 ± 7.7^{a}	7.3 ± 6.7^{a}	54.6 ± 8.7^{b}	0.2 ± 0.2^{a}	0.9 ± 0.1^{c}
FGM	36.2 ± 5.3^{a}	69.6 ± 6.5^{b}	5.9 ± 6.9^{a}	35.3 ± 19.0^{a}	0.2 ± 0.2^{a}	0.5 ± 0.3^{a}
FNGM	35.6 ± 5.4^{a}	65.1 ± 7.4^{ab}	5.7 ± 6.3^{a}	44.2 ± 4.5^{a}	0.2 ± 0.2^{a}	0.7 ± 0.1^{ab}

Data are presented as mean ± SD ($n = 8$), and all values represent triplicate results. Values bearing different superscript letters in the same column are significantly different in accordance with Duncan's multiple range test where significance is defined as $p < 0.05$.
FGM = fermented genetically modified soy milk and high-cholesterol diet; FNGM = fermented non-genetically modified soy milk and high-cholesterol diet; GM = genetically modified soy milk and high cholesterol diet; HC = high cholesterol diet; HDL = high-density lipoprotein; HM = *Monascus*-fermented powder and high cholesterol diet; LDL = low-density lipoprotein; N = normal diet; NGM = non-genetically modified soy milk and high cholesterol diet.

TC of the FGM and FNGM groups, which were fed with fermented GM and non-GM soy milk, was also significantly lower than that of the HC group ($p < 0.05$). Furthermore, the serum TC levels of hamsters fed GM soy milk (GM and FGM) were not significantly different from those of the non-GM soy milk (NGM and FNGM) groups ($p > 0.05$). Regarding serum TG levels, all experimental groups fed with the high-cholesterol diet showed no significant difference with the HC group ($p > 0.05$; data not shown). In addition, the soy milk, whether GM or non-GM, did not improve the hypertriglyceridemic symptoms of hypercholesterolemic hamsters.

The effects of consuming GM or non-GM soy milk, with or without fermentation, on lowering TC levels in the liver are shown in Table 3. As expected, hamsters treated with a high-cholesterol diet for 4 weeks and 8 weeks showed a remarkable increase in liver TC levels as compared with the N group ($p < 0.05$). In all treated groups, the liver TC levels decreased significantly ($p < 0.05$) after the 8-week feeding pattern compared with the HC groups. Nevertheless, there was no significant difference between GM and non-GM soy milk, with or without fermentation.

Effect of feeding on serum HDL-C, LDL-C, and LDL-C/HDL-C levels

As shown in Table 4, feeding the hamsters with a high-cholesterol diet (HC group) resulted in significantly higher LDL-C levels than those in the N group ($p < 0.05$). LDL-C levels significantly decreased in all treated groups ($p < 0.05$) after feeding with soy milk compared with the HC group. Additionally, there was no significant difference between the groups fed with GM and non-GM soy milk, with or without fermentation, in terms of lowering of LDL-C levels after 4 weeks of feeding. In the HDL-C analysis, the high-cholesterol diet did not increase the levels of HDL-C in serum compared with the N group after 4- and 8-week feeding patterns. However, the present study indicated that a high-cholesterol diet with soy milk would lead to a significant decrease in the ratio of LDL-C to HDL-C compared with the HC group ($p < 0.05$) after 4 weeks of feeding and that there was no difference between GM and non-GM soy milk in this regard. The results obtained by a statistical analysis showed that the ratio of LDL-C to HDL-C was less by 75.0% in the non-

Table 5 Effect of GM and non-GM soy milk on blood catalase levels, SOD activity, and TBARS

Group	Catalase activity (units/g Hb)		SOD activity (units/g Hb)		TBARS (μM MDA)	
	4 wks	8 wks	4 wks	8 wks	4 wks	8 wks
N	2.6 ± 1.2^{a}	7.6 ± 1.3^{c}	187.7 ± 34.6^{a}	104.6 ± 8.6^{a}	2.2 ± 0.3^{abc}	2.0 ± 0.3^{c}
HC	2.2 ± 0.7^{a}	5.0 ± 1.3^{a}	244.5 ± 72.6^{a}	101.7 ± 18.3^{a}	2.2 ± 0.9^{bc}	2.1 ± 0.3^{c}
HM	2.3 ± 0.9^{a}	6.7 ± 1.6^{bc}	208.3 ± 34.9^{a}	104.1 ± 18.3^{a}	2.8 ± 0.4^{d}	1.6 ± 0.3^{ab}
GM	1.2 ± 0.5^{a}	5.9 ± 0.9^{ab}	327.3 ± 96.7^{b}	121.1 ± 23.7^{ab}	2.6 ± 0.5^{cd}	2.6 ± 0.3^{d}
NGM	2.6 ± 1.4^{a}	7.0 ± 1.7^{bc}	412.4 ± 67.2^{c}	110.5 ± 14.6^{ab}	1.9 ± 0.5^{ab}	2.3 ± 0.4^{cd}
FGM	2.7 ± 1.4^{a}	6.6 ± 1.7^{bc}	429.2 ± 43.5^{c}	126.4 ± 20.8^{b}	1.7 ± 0.6^{ab}	1.9 ± 0.6^{bc}
FNGM	4.2 ± 2.4^{b}	7.1 ± 0.8^{bc}	448.1 ± 32.0^{c}	114.6 ± 12.0^{ab}	1.5 ± 0.3^{a}	1.4 ± 0.5^{a}

Data are presented as mean ± SD ($n = 8$) and all values are based on results of triplicates. Values bearing different superscript letters in the same column are significantly different in accordance with Duncan's multiple range test where significance is defined as $p < 0.05$.
FGM = fermented genetically modified soy milk and high-cholesterol diet; FNGM = fermented non-genetically modified soy milk and high-cholesterol diet; GM = genetically modified soy milk and high cholesterol diet; HC = high cholesterol diet; HM = *Monascus*-fermented powder and high cholesterol diet; MDA = malondialdehyde; N = normal diet; NGM = non-genetically modified soy milk and high cholesterol diet; SOD = superoxide dismutase; TBARS = thiobarbituric acid reactive substance.

fermented groups (GM and NGM) and fermented soy milk groups (FGM and FNGM) than in the HC group ($p < 0.05$) after 4 weeks of feeding. Moreover, fermented GM and non-GM soy milk, fed for 8 weeks, had a significant effect ($p < 0.05$) on lowering the ratio of LDL-C to HDL-C; however, unfermented milk did not ($p > 0.05$) when compared with the HC group.

Analysis of the atherosclerotic plaques

As shown in Table 5, feeding with GM or non-GM soy milk, with or without fermentation, significantly enhanced SOD activities by 33.87 ($p < 0.05$), 68.67 ($p < 0.05$), 75.54 ($p < 0.05$) and 83.27% ($p < 0.05$), respectively, after 4 weeks of feeding. CAT activity assays were performed in

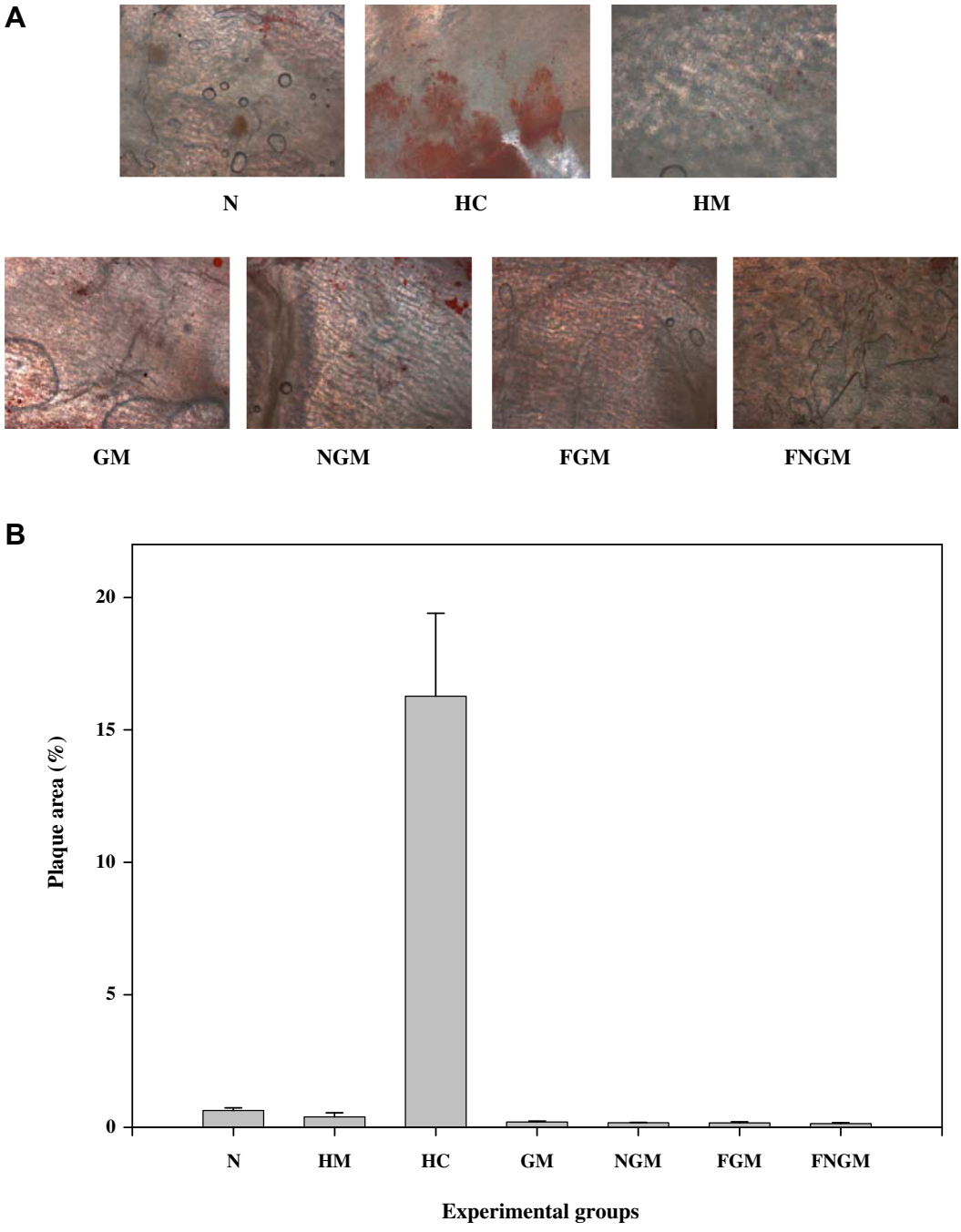

Figure 1. Effects of GM and non-GM soy milk, with or without fermentation, on atherosclerotic plaques in the thoracic aortas of hypercholesterolemic hamsters. (A) Atherosclerotic plaques are indicated by the red dye in the graph; (B) the proportion of the area taken up by the atherosclerotic plaques in the aorta. Abbreviation meaning of each group is shown in Table 3. Data are presented as mean ± SD ($n = 8$), and values bearing different letters are significantly different in accordance with Duncan's multiple range test where significance is defined as $p < 0.05$.

the present study, and the results revealed that the activity of CAT in the non-GM soy milk, with or without fermentation (NGM and FNGM), and in the GM-fermented soy milk groups (FGM) increased significantly by 40.0 ($p < 0.05$), 42.0 ($p < 0.05$) and 32.0% ($p < 0.05$), respectively, after feeding for 8 weeks. A TBARS assay was used to evaluate lipid peroxidation. As the results demonstrate, fermented non-GM soy milk significantly decreased the TBARS levels of serum in hamsters ($p < 0.05$) after 4 and 8 weeks of feeding, compared with the HC group. In summary, soy milk, especially fermented non-GM soy milk, improves antioxidant activities in hypercholesterolemic hamsters.

Atherosclerotic plaques in the aorta are caused by oxidative stress and lipid accumulation, which causes the formation of atheromatous lesions in atherosclerosis. As illustrated in Fig. 1, the lipid plaques accumulate significantly in the aorta of hypercholesterolemic hamsters, taking up 16.27% of the total area on average. The formation of lipid plaques in the aorta was significantly decreased ($p < 0.05$) compared with the HC group, after feeding with GM and non-GM soy milk, with or without fermentation. In the GM and non-GM soy milk groups, the areas of lipid plaques in the aorta were 0.19 and 0.17%, respectively. Lipid plaques formed over 0.16 and 0.14% of the aortic area, respectively, in the fermented GM and non-GM soy milk groups. All soy milk preparations that were tested appeared to significantly improve the accumulation of atherosclerotic plaques in the hamster aorta compared with the HC group.

Discussion

According to the information from the GM crop database in the Center Environmental Risk Assessment, 144 GM crops have been approved for use in markets around the world.[23] With the development of GM crops, there has been a growing interest in the approaches available to assess the potential safety of novel gene products. In this current study, two types of soy milk, GM and non-GM soy milk, were manufactured by our laboratory, and the transgene in these products was carefully determined in different batches of soy milk. A cholesterol-rich diet was associated with an increased risk for the development of dyslipidemia. This profile, elicited by an unhealthy dietary pattern, was reversed when GM or non-GM soy milk, either with or without fermentation, was consumed. Over a 4-week feeding pattern, there was no statistically significant difference between animals given food containing GM or non-GM soy milk, whether fermented or not, on reduction in cholesterol levels or the ratio of LDL-C and HDL-C in the serum and liver. Moreover, unfermented soy milk did not decrease the LDL-C/HDL-C ratio in long-term experiments in either the GM or non-GM groups over 8 weeks. High-cholesterol diets increased the accumulation of LDL-C over a long-term feeding period. Previous studies indicated that some kinds of microorganisms, such as *Lactobacillus* spp., *Bacteroides* spp., and *Bifidobacterium* spp., possess endogenous β-glucosidases, which play a role in the hydrolysis of isoflavone glycosides.[24] In the current study, the lactic acid bacteria *L lactis* subsp. *lactis* BCRC 14016 was used to prepare the GM and non-GM fermented soy milk, and the aglycone isoflavones were bioconverted by fermentation. These aglycone isoflavones were absorbed faster and in greater amounts than glucosides when ingested in a beverage form such as soy milk,[5] and, in long-term experiments, the LDL-C/HDL-C ratio was reduced after feeding with either GM or non-GM fermented soy milk. In this context, the consumption of GM soy milk is no different from that of non-GM soy milk for improving hypercholesterolemia in hamsters, and GM soy milk is considered substantially equivalent to non-GM soy milk in terms of these bioactive functions.

In this study, the consumption of GM and non-GM probiotic-fermented soy milk was also compared to evaluate the reduction in oxidative stress and atherosclerotic plaque formation. The causal relationship between blood cholesterol levels and atherosclerosis is widely accepted; there is already considerable interest in the possibility of using drugs to decrease cholesterol levels.[25] Also, oxidative stress is an important risk factor in the pathogenesis of atherosclerosis.[26] The antioxidative enzymes in red blood cells and serum include SOD, seleno-dependent glutathione peroxidase, glutathione reductase, and CAT.[27] In our previous study, increase in total antioxidant status and SOD activity and decrease in lipid oxidation was observed in the blood of hypercholesterolemic hamsters. Formation of atherosclerotic plaques was repressed by the milk-soy milk fermented by lactic acid bacteria and red mold rice.[12,18] In addition, previous studies have indicated that aglycone isoflavones exhibit free radical-scavenging action and that soy is a rich source of antioxidants.[28] Je and colleagues[29,30] also demonstrated that peptides with antioxidant activity can be obtained from enzyme hydrolyzed protein. A soy-enriched diet offered to rats could help repress the oxidative stresses and preserve their heart function after myocardial ischemia.[22] In current study, the increase of peptides which were digested by the enzymes in digestive tract exerted antioxidant effect by feeding with non-fermented soy milk and provided the similarity effect on antioxidant activity with probiotic-fermented soy milk. The changes in SOD activities in hypercholesterolemic hamsters were elevated by feeding them with GM and non-GM soy milk, with or without fermentation. Additionally, increased CAT activity in the blood of hypercholesterolemic hamsters was observed after feeding them with non-GM soy milk and fermented GM and non-GM soy milk. In summary, oxidative stress was reduced by feeding hamsters with soy milk, especially fermented non-GM soy milk. Moreover, the formation of atherosclerotic plaques was reduced after feeding with GM and non-GM soy milk. All types of soy milk that are made from GM or non-GM soy reduce the symptoms of hypercholesterolemia and the risk of atherosclerosis in hamsters.

This study is the first to demonstrate an improvement in hypocholesterolemic symptoms and anti-atherosclerotic effects in hamsters fed with GM and non-GM soy milk, with or without fermentation by lactic acid bacteria. GM soy-derived products can improve hypercholesterolemia and reduce the risk of atherosclerosis. Thus, GM fermented soy milk is considered substantially equivalent to non-GM soy milk in terms of these bioactive functions.

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