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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 (%) $\times$ 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

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