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Lactobacillus casei-fermented milk improves serum and hepatic lipid profiles in diet-induced hypercholesterolaemic hamsters

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ABSTRACT

The hypocholesterolaemic effects of milk fermented by *Lactobacillus casei* F0822 with bile salt hydrolase activity were investigated in hamsters fed a cholesterol-rich diet. Fermented milk (FM) exhibited dose-dependent hypocholesterolaemic activity. Sterilized and low-dose FM did not significantly affect the lipid profile of hamsters, whereas high-dose FM significantly reduced the levels of serum total cholesterol (TC) and non-high-density lipoprotein cholesterol (non-HDL-C), as well as hepatic TC and esterified cholesterol (EC). The daily faecal total bile acid excretion was significantly negatively correlated with the serum TC and non-HDL-C, as well as with the hepatic TC and EC. High-dose FM significantly enhanced daily faecal total bile acid excretion, which attributed to the hydrolysis of conjugated bile acids and the binding of free bile acids by the cells of strain F0822. These data suggest that the FM may serve as a functional food and has the potential to regulate cholesterol metabolism in humans.

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1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, and its incidence is rapidly increasing in low- and middle-income countries (DiRienzo, 2014). In China, 40% of deaths are CVD-related, with one-third of these cases caused by coronary heart disease (CHD) (National Center for Cardiovascular Diseases, China, 2014). Epidemiological and clinical investigations have indicated that high concentrations of serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) correlate highly with the incidence of CHD (Lipid Research Clinics Program, 1984; Scandinavian Simvastatin Survival Study Group, 1994). Thus, considerable research has been conducted to determine factors that are effective in lowering the serum cholesterol concentration, including dietary modifications and pharmacological agents.

The current dietary strategies for CHD prevention advocate adherence to low-fat diets (Lichtenstein et al., 2006). Although, under experimental conditions, low-fat diets offer an effective means of reducing serum cholesterol concentrations on the population level, they appear to be less effective in practice because of poor compliance, which is attributed to the low palatability and acceptability of such diets by

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consumers (Pereira & Gibson, 2002). As such, researchers have attempted to identify other dietary components that can reduce serum cholesterol levels.

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Chiu, Lin, Tsai, & Lin, 2014). Probiotics and probiotic dairy products are safe for large-scale consumption. Several animal (Ivanovic et al., 2015; Li et al., 2014) and human studies (Fuentes, Lajo, Carrioon, & Cune, 2013; Jones, Martoni, Parent, & Prakash, 2012) have indicated that the consumption of certain probiotics or probiotic dairy products reduces the concentrations of serum cholesterol. However, not all probiotic strains or probiotic dairy products are active in this regard (de Roos, Schouten, & Katan, 1999).

In our previous study, *Lactobacillus casei* F0822 showed hypocholesterolaemic activity in rats fed with a cholesterol-rich diet through an alteration in the enterohepatic circulation of bile acids (Guo & Li, 2013). Rats possess a different bile acid metabolic profile than humans, whereas hamsters synthesize and excrete bile acids in a manner more similar to that of humans than that of rats (Zhang et al., 2009). Thus, choosing a hamster as a model for studying the cholesterol-lowering activity of *L. casei* F0822 enables us to draw a conclusion that can be extrapolated to humans.

This study aimed to evaluate the hypocholesterolaemic activity of milk fermented by *L. casei* F0822 in hamsters fed a cholesterol-rich diet. The underlying functional mechanisms were also investigated both *in vivo* and *in vitro*.

2. Materials and methods

2.1. Source and maintenance of culture

L. casei F0822 was isolated from a faecal sample obtained from a healthy adult volunteer in a previous study (Guo & Li, 2013). The culture was maintained by subculturing in de Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, Hampshire, UK) supplemented with 0.05% (w/v) L-cysteine hydrochloride (Sigma–Aldrich, St. Louis, MO, USA) (MRSC broth) using a 1% inoculum and 18-h anaerobic incubation at 37 °C. The organism was successively activated three times in the sterile MRSC broth prior to use.

2.2. Preparation of fermented and sterilized fermented milk

Fermented milk was produced by inoculating 1% (v/v) strain F0822 into 10% (w/v) reconstituted skim milk (Nestlé, Harbin, Heilongjiang, China). The mixture was subsequently incubated at 37 °C for 16 h. The fermented milk had an acidity of 0.9% and a viable count of approximately 8.0×10^8 CFU/ml. Sterilized fermented milk was prepared by autoclaving the fermented milk at 115 °C for 15 min.

2.3. Animal feeding and grouping

Thirty male Golden Syrian hamsters, weighing 110–120 g, were purchased from Vital River Laboratories (Beijing, China). The animals were housed individually in plastic cages under controlled environmental conditions (22 ± 2 °C, 55 ± 5% humidity) with a 12-h light/dark cycle. After 3 d of adaptation, the hamsters were randomly assigned into five groups of six hamsters each. The first group was given a cholesterol-free diet and then intragastrically administered with 1.25 ml of sterilized water (normocholesterolaemic control). The four other groups were given a cholesterol-rich diet and then intragastrically administered with 1.25 ml of sterilized water (hypercholesterolaemic control), 1.25 ml of sterilized fermented milk, 0.125 ml of fermented milk combined with 1.125 ml of sterilized water (low-dose FM), or 1.25 ml of fermented milk (high-dose FM). During the 4-week experimental period, food and water were available ad libitum to all animals. The composition of the cholesterol-free diet was based on the AIN 93M recommendation of a protein content of 14%, as described previously (Guo & Li, 2014). A cholesterol-rich diet was prepared by adding 0.4% cholesterol (Aladdin, Shanghai, China) into the cholesterol-free diet.

2.4. Assay for serum lipids

At the end of the experiment, the rats were fasted overnight and anaesthetized through an intraperitoneal injection of sodium pentobarbital (75 mg/kg BW, Sigma–Aldrich). Blood samples were collected from the femoral artery, and serum was separated from the blood by centrifugation at 3000 *g* for 10 min. Serum TC and high-density lipoprotein cholesterol (HDL-C) concentrations were measured enzymatically using a Hitachi 7180 automated biochemical analyzer (Hitachi, Tokyo, Japan) with commercial kits (BioSino Biotechnology and Science Inc., Beijing, China). The non-HDL-C level was calculated as the difference between the TC and HDL-C.

2.5. Assay for hepatic lipids

After the animals were euthanized, the viscera were opened, and the liver was quickly removed, rinsed with sterile physiological saline solution, blotted dry with sterile filter paper, and then stored at –80 °C until analysis. Approximately 0.5 g of the liver sample was homogenized, and the lipids were extracted three times using a mixture of chloroform/methanol (2:1, v/v). The TC concentration in the extracts was quantified using a capillary gas chromatography on an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5 ms fused-silica capillary column (30 m × 0.25 mm i.d. and film thickness of 0.25 μm; J&W Scientific, Folsom, CA, USA), as described previously (Fletouris, Botsoglou, Psomas, & Mantis, 1998). The free cholesterol (FC) concentration was determined by the same method except that hydrolysis was not performed. The esterified cholesterol (EC) concentration was calculated as the difference between the TC and FC of a sample. The cholesterol levels in the homogenates are expressed as μmol cholesterol/g liver.

2.6. Assay for faecal sterols

Faecal samples were collected over a 3-d period at week 4. The samples were freeze dried, weighed, and then stored at –80 °C until analysis. The individual faecal neutral sterols and bile acids

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