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Lactobacillus acidophilus CHO-220 and inulin reduced plasma total cholesterol and low-density lipoprotein cholesterol via alteration of lipid transporters

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ABSTRACT

This randomized, double-blind, placebo-controlled, and parallel-designed study was conducted to investigate the effect of a synbiotic product containing *Lactobacillus acidophilus* CHO-220 and inulin on lipid profiles of hypercholesterolemic men and women. Thirty-two hypercholesterolemic men and women with initial mean plasma cholesterol levels of 5.7 ± 0.32 mmol/L were recruited for the 12-wk study. The subjects were randomly allocated to 2 groups; namely the treatment group (synbiotic product) and the control group (placebo), and each received 4 capsules of synbiotic or placebo daily. Our results showed that the mean body weight, energy, and nutrient intake of the subjects did not differ between the 2 groups over the study period. The supplementation of synbiotic reduced plasma total cholesterol and low-density lipoprotein (LDL)-cholesterol by 7.84 and 9.27%, respectively, compared with the control over 12 wk. Lipoproteins were subsequently subfractionated and characterized. The synbiotic supplementation resulted in a lower concentration of triglycerides in the very low, intermediate, low, and high-density lipoprotein particles compared with the control over 12 wk. The concentration of triglycerides in lipoproteins is positively correlated with an increased risk of atherosclerosis. Our results showed that the synbiotic might exhibit an atheropreventive characteristic. Cholesteryl ester (CE) in the high-density lipoprotein particles of the synbiotic group was also higher compared with the control, indicating greater transport of cholesterol in the form of CE to the liver for hydrolysis. This may have led to the reduced plasma total cholesterol level of the synbiotic group. The supplementation of synbiotic also reduced the concentration of CE in the LDL particles compared with the control, leading to the formation of smaller and denser particles that are more easily removed from blood. This supported the reduced LDL-cholesterol level of the synbiotic group compared with the control. Our present study showed that the synbiotic product improved plasma total- and LDL-cholesterol levels by modifying the interconnected pathways of lipid transporters. In addition, although *Lactobacillus acidophilus* CHO-220 could deconjugate bile, our results showed a statistically insignificant difference in the levels of conjugated, deconjugated, primary, and secondary bile acids between the synbiotic and control groups over 12 wk, indicating safety from bile-related toxicity.

Key words: *Lactobacillus acidophilus*, inulin, lipoprotein, bile

INTRODUCTION

Probiotics are "living microorganisms which when administered in adequate amounts and sufficient concentrations could beneficially affect the host by improving microflora in the gastrointestinal tract thus contributing to various health benefits" (FAO-WHO, 2001). Probiotics are usually "friendly bacteria" such as bifidobacteria and lactobacilli, and can be found in the human gut. Prebiotics are "indigestible fermented food substrates that selectively stimulate the growth, composition, and activity of microflora in gastrointestinal tract and thus improve hosts' health and well-being" (Roberfroid, 2007). Probiotics and prebiotics have been well documented for their roles in enhancing gastrointestinal health. However, recent advances in research have documented new potentials of probiotics and prebiotics in other aspects of human health. This includes hypocholesterolemic effects and the prospect of establishing probiotics and prebiotics as nondrug alternatives for hypercholesterolemia.

Past studies have shown that administrations of probiotics and prebiotics are effective in improving lipid profiles such as the reduction of serum total cholesterol, triglycerides, and low-density lipoprotein (**LDL**)-cholesterol. Probiotic strains such as *Lactobacillus acidophilus* (Fukushima et al., 1999; Lubbadeh et al., 1999), *Lactobacillus plantarum* (Naruszewicz et al., 2002; Ha et al., 2006; Jeun et al., 2010), *Bifidobac-*

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terium longum (Xiao et al., 2003; Abd El-Gawad et al., 2005), *Lactobacillus casei* (Bertazzoni-Minelli et al., 2004), *Enterococcus faecium*, and *Streptococcus thermophilus* (Agerholm-Larsen et al., 2000) have been found to positively improve blood lipid profiles, especially total cholesterol and LDL-cholesterol. Prebiotics such as inulin (Causey et al., 2000; Letexier et al., 2003) and fructooligosaccharides (Alles et al., 1999) have also been shown to positively modulate lipid profiles. Considering that prebiotics often enhance the growth of probiotics, many efforts have emphasized the use of synbiotics (probiotics and prebiotics in combination) to augment a cholesterol-lowering effect (Schaafsma et al., 1998; Kießling et al., 2002; Liong et al., 2007). However, to our knowledge, the exact mechanisms of probiotics, prebiotics, and synbiotics in lowering cholesterol remain unclear. Most of the documented work has emphasized proving a hypocholesterolemic effect and little is known on the underlying mechanisms.

Human lipid profiles are affected by the interrelated metabolisms of blood lipoproteins, particles containing proteins and lipids that play a role in the transportation of water-insoluble lipids and cholesterol in the blood circulation (Musunuru et al., 2009). Very low density lipoprotein (**VLDL**)-cholesterol is synthesized by the liver to modulate the movement of fats and cholesterol within the bloodstream and carry triglycerides to adipose tissue and muscle (Jong et al., 2000). The triglycerides in VLDL are removed by lipoprotein lipase in the blood capillaries, and VLDL particles return to the circulation as intermediate-density lipoprotein (**IDL**) particles (Lundahl et al., 2006). Some of the IDL particles are rapidly taken up by the liver, whereas others remain in the circulation where they undergo further triglyceride hydrolysis and are converted to LDL (Chang et al., 2009). The LDL particles carry cholesterol and triglycerides from the liver to peripheral tissues; LDL can be retained in the arteries leading to the formation of plaques and increased risk of cardiovascular disease (Arsenault et al., 2009). High-density lipoprotein (**HDL**) is the smallest lipoprotein particle that transports cholesterol from the arteries to the liver for excretion (Harel et al., 2010). High concentrations of HDL have been found to protect against cardiovascular heart diseases (Real et al., 2001), whereas low concentrations increase the risk for atherosclerotic diseases (Goldbourt et al., 1997).

Bile acids are produced from cholesterol in the liver (Xu et al., 2007). Bile can be hydrolyzed to form deconjugated bile acids, such as cholic acid, that are converted by intestinal microorganisms into secondary bile acids (Matheson and Story, 1994). Many strains of probiotics have been found able to deconjugate bile acids via the production of bile-salt hydrolase (**BSH**). The deconjugation of bile by probiotics could increase the accumulation of cholic acid, which could be subsequently transformed into detrimental secondary bile acids by intestinal microflora. The accumulation of potentially cytotoxic secondary bile acids in the enterohepatic circulation could increase the risk of gastrointestinal diseases such as cholestasis and colorectal cancer (Tan et al., 2007).

In past studies, the combination of a probiotic and prebiotic (synbiotic) was developed and showed promising hypocholesterolemic effect in vivo in animal models. However, transferability of a similar effect in humans has yet to be evaluated. Thus, the aim of this study was to investigate the effect of a synbiotic product on plasma lipid profiles of hypercholesterolemic human subjects. The effects of the synbiotic product on plasma lipid transporters and the possible mechanisms involved were also evaluated. In addition, the safety of the synbiotic product on bile conversion was assessed.

MATERIALS AND METHODS

Source of Probiotic Culture and Prebiotic

Probiotic *Lactobacillus acidophilus* CHO-220 is a human-derived strain that was obtained from the Bioprocess Technology Culture Collection Center (Universiti Sains Malaysia, Penang, Malaysia). The stock culture was stored at −20°C in 40% (vol/vol) sterile glycerol. The probiotic culture was successively activated 3 times in sterile de Man, Rogosa, Sharpe broth (Hi-Media, Mumbai, Maharashtra, India) supplemented with 0.15% (wt/vol) L-cysteine hydrochloride (Hi-Media) before experimental use. The activation was performed using a 1% (vol/vol) inoculum of *L. acidophilus* CHO-220, and the culture was incubated for 24 h at 37°C before use and stored at 4°C between transfers. A freeze-dried culture (containing approximately 9 log cfu/g) was used in the present study. For freeze-drying, the cell pellet of *L. acidophilus* CHO-220 obtained from harvesting the fermentation broth was suspended in 2.0% (wt/vol) of food-grade cryoprotectant pectin (Unipectine RS 150, Specialty Point Sdn. Bhd., Selangor, Malaysia) at a ratio of 1:1, frozen at −20°C, and freeze-dried at −40°C and 13.3 Pa. A commercially available prebiotic, inulin (Raftiline ST, Orafti Pty. 153 Ltd., Tienen, Belgium), was used. The probiotic culture was produced and certified under Good Laboratory Practice and Good Manufacturing Practice (Malaysian Pharmaceutical Industries Sdn. Bhd., Penang, Malaysia).

Production of Synbiotic Capsules

Synbiotic capsules were produced by Polens (M) Sdn. Bhd. (Shah Alam, Malaysia) under Good Manufactur-