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

L.-G. Ooi,* R. Ahmad,* K.-H. Yuen,† and M.-T. Liong*¹

*School of Industrial Technology, and

†School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

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; Lubbadah et al., 1999), *Lactobacillus plantarum* (Naruszewicz et al., 2002; Ha et al., 2006; Jeun et al., 2010), *Bifidobac-*

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¹Corresponding author: mintze.liong@usm.my

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 $9 \log \text{cfu/g}$) 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 (Unipektine 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-

ing Practice conditions. The capsules produced were subsequently bottled, sealed, and stored at 4°C. The capsules were cultured and tested for the viability of *L. acidophilus* CHO-220 before and at the end of the trial. Each synbiotic capsule contained 9 log cfu of *L. acidophilus* CHO-220 and 0.20 g of inulin, whereas the placebo capsules contained rice starch (Alagappa Flour Mills Sdn. Bhd., Penang, Malaysia).

Selection of Subjects

Subjects were screened and requested to complete a 3-d dietary diary. The dietary data and screening laboratory results were assessed against the exclusion criteria and subjects who met the inclusion criteria were randomized to 1 of 2 treatment arms; namely the treatment group (synbiotic product) or the control group (placebo).

Subjects were recruited based on the following inclusion criteria: total plasma cholesterol concentration of ≥ 5.20 mmol/L and ≤ 6.20 mmol/L and an LDL level of ≤ 4.2 mmol/L; without taking lipid-lowering medication; without myocardial infarction, angioplasty, or stroke within the last 3 mo; without diabetes and/or were not on diabetic medication; without glucose intolerance; without hepatic and/or renal failure and/or not on medications related to heart disease, hepatic, or renal disease; were not treated with anticoagulants, immunosuppressant, corticosteroids, or estrogens; and had no history of thyroid replacement.

Exclusion criteria include medical (including known history of major hematological, renal, cardiovascular or hepatic abnormalities) or physiological condition; social circumstances that would impair reliable participation in the trial; increased risk to oneself or others by participating; female subjects who were pregnant, nursing, or with a positive urine pregnancy test, or who were intending to become pregnant within 3 mo after completion of the trial; age below 18 years; recent history of alcohol abuse; immunocompromised individuals; and subjects who, in opinion of the investigator, were not likely to complete the trial for whatever reason.

Study Protocol

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Joint Ethics Committee of School of Pharmaceutical Sciences USM – Hospital Lam Wah Ee (Penang, Malaysia). Written informed consent was obtained from all subjects. Subjects ($n = 32$) were randomly divided into 2 groups of 16 subjects each. The treatment group was given synbiotic capsules and the control group was

given placebo capsules. Each subject took 4 capsules daily (2 in the morning and 2 in the evening) of either synbiotic or placebo for a period of 12 wk.

Fasting blood samples were collected at wk 0, 6, and 12 of the trial into tubes containing lithium heparin, and plasma was separated by centrifugation for 15 min at $550 \times g$ at 15°C. The plasma sample was then stored frozen at -20°C until analyses. Assessment of BW was conducted at wk 0, 6, and 12 of the trial, and subjects were assessed for any adverse effects via phone weekly. All subjects maintained their usual dietary habits (except probiotic-containing foods and cholesterol-lowering medication), and food intake was monitored using a 3-d food diary.

Analyses

Plasma samples at wk 0, 6, and 12 of the trial were analyzed for total cholesterol, LDL-cholesterol, triglycerides, and HDL-cholesterol levels using commercial enzymatic kits (bioMérieux Corp., Marcy l'Etoile, France).

A density gradient ultracentrifugal procedure was used to fractionate plasma lipoproteins as described previously (Argmann et al., 2006). Chylomicron-free plasma was adjusted with NaCl-KBr solution (1.346 g/mL) to desired densities of 1.006, 1.019, 1.063, and 1.210 g/mL. Gradients were centrifuged at $500,000 \times g$ for 3 h at 15°C without braking (Beckman Coulter Optima MAX-XP ultracentrifuge, Fullerton, CA). The centrifuged samples were allowed to return to room temperature and the subfractionated layers were collected by downward aspiration using a Pasteur pipette. The subfractions VLDL-cholesterol, IDL-cholesterol, LDL-cholesterol, and HDL-cholesterol were isolated and obtained at densities of 1.006, 1.006–1.019, 1.019–1.063, and 1.063–1.21 g/mL, respectively.

All lipid subfractions were then analyzed for triglycerides, protein, phospholipids, cholesteryl esters (CE), and free cholesterol concentrations. Triglycerides, phospholipids, and total cholesterol were analyzed using commercial kits (bioMérieux Corp.). Cholesteryl esters were determined using the Amplex Red Reagent Kit (Molecular Probes, Eugene, OR), and free cholesterol was determined as the difference between total cholesterol and CE (Liong et al., 2007). The protein content was determined with BSA as the standard (Bradford, 1976).

Plasma bile acids were quantified using gas chromatography (Varian Chrompack Application Note 215-GC, https://www.varianinc.com/media/sci/tech_scan/A00215.pdf; Batta and Salen, 1999). The GC (GC-7890A, Agilent Technologies, Santa Clara, CA) was equipped with a capillary column (50 m $\times$ 0.53 mm

Table 1. Mean age and sex (male, M, or female, F) of hypercholesterolemic subjects (n = 32; means $\pm$ SD) recruited for this study

Treatment	Age (yr)	Sex
Control	36.9 $\pm$ 13.4 ^A	10 M and 6 F
Synbiotic	31.5 $\pm$ 10.4 ^A	8 M and 8 F

^AMeans in the same column with different superscripts are significantly different ($P < 0.05$).

inside diameter $\times$ 0.70 mm outside diameter; Varian CP-Sil 5CB, Walnut Creek, CA). The oven temperature was held at 185°C after sample injection and increased to 260°C at a rate of 25°C/min. The injector and detector temperature were 285°C and 290°C, respectively. Purified nitrogen was used as the carrier gas (1.5 mL/min), split ratio was 10:1, and the injection volume was 1 μ L. The concentrations of plasma bile were determined using standards of bile salts such as cholic acid, lithocholic acid, chenodeoxycholic acid, and deoxycholic acid (Acros Organics, Geel, Belgium).

Statistical Analysis

The determination of sample size was based on total cholesterol. To demonstrate a difference in population means of 1.26 mmol/L, standard deviation of 0.93 mmol/L, an α error of 5%, and a power of 95% (Liong et al., 2007), a minimum of 30 subjects was required for this study. Data analysis was performed using a split-plot design. Factorial design with block-treatment confounding was used for time-based analyses. The layout of the type SPF-2.4 design was used to evaluate the significant differences between sample means, with the significance level at $\alpha = 0.05$. Mean comparisons were also assessed by the same factorial design using ANOVA for simple effects (Kirk, 1968).

RESULTS AND DISCUSSIONS

BW and Dietary Intake

All 32 subjects completed the treatment protocol and did not show any adverse effects. Their entry characteristics at baseline are summarized in Table 1. No

significant ($P > 0.05$) difference in BW or body mass index was observed between the treatment and control groups over the 12-wk period (Table 2). Energy and nutrient intake, as calculated based on the *Nutrient Composition of Malaysian Foods* (Tee et al., 1997), was also constant and did not change significantly ($P > 0.05$) between the 2 groups over the study (Table 3). No changes in consumption of carbohydrate, protein, fat, or cholesterol in either group occurred between baseline and the end of the study ($P > 0.05$).

Plasma Lipid Profile

Hypercholesterolemia has been associated with higher than normal total cholesterol (≥ 5.2 mmol/L) and LDL-cholesterol (≥ 2.6 mmol/L). In the present study, the baseline plasma total cholesterol, LDL-cholesterol, triglycerides, and HDL-cholesterol were not significantly ($P > 0.05$) different between the synbiotic and control groups (Table 4). The plasma total cholesterol and LDL-cholesterol decreased significantly ($P < 0.05$) in the synbiotic group after 6 wk of treatment and remained constant until 12 wk. Synbiotic supplementation resulted in a significant decrease of 0.45 mmol/L (7.84%) and 0.33 mmol/L (9.27%) in plasma total cholesterol and LDL-cholesterol, respectively over 12 wk ($P < 0.05$), whereas the control showed insignificant changes. Past studies showed that reductions of 1 and 1.8 mmol/L of LDL-cholesterol contributed to reduction of risk of stroke of 10 and 17%, respectively (Law et al., 2003), and every 1% reduction of total cholesterol level could decrease the risk of coronary heart disease by 2% (Ahmed et al., 1998). Thus, this synbiotic product may have a hypocholesterolemic potential for those favoring a nondrug approach for lipid management. However, no significant ($P > 0.05$) changes in triglycerides and HDL-cholesterol were detected between the synbiotic and the control group at the end of the study.

Lipid Subfractions

The triglycerides of VLDL-cholesterol for the synbiotic group decreased significantly ($P < 0.05$) over 12 wk compared with the control (Figure 1). Very low density

Table 2. Effect of the synbiotic on BW and body mass index (BMI) of hypercholesterolemic subjects (n = 32) for 12 wk¹

Item	Treatment	Week 0	Week 6	Week 12
Weight (kg)	Control	61.25 $\pm$ 13.62 ^{A,a}	61.06 $\pm$ 13.06 ^{A,a}	60.94 $\pm$ 13.23 ^{A,a}
	Synbiotic	60.78 $\pm$ 18.86 ^{A,a}	61.13 $\pm$ 18.97 ^{A,a}	61.09 $\pm$ 19.45 ^{A,a}
BMI (kg/m ²)	Control	23.16 $\pm$ 4.59 ^{A,a}	23.01 $\pm$ 4.42 ^{A,a}	23.04 $\pm$ 4.41 ^{A,a}
	Synbiotic	22.76 $\pm$ 5.68 ^{A,a}	22.90 $\pm$ 5.71 ^{A,a}	22.86 $\pm$ 5.82 ^{A,a}

^AMeans in the same column with different uppercase superscripts are significantly different ($P < 0.05$).

^aMeans in the same row with different lowercase superscripts are significantly different ($P < 0.05$).

¹Data are presented as mean values $\pm$ SD.

Table 3. Energy and nutrient intake of hypercholesterolemic subjects (n = 32) for 12 wk¹

Item	Treatment	Week 0	Week 6	Week 12
Energy (kcal/d)	Control	2,001.70 ± 615.78 ^{A,a}	2,003.31 ± 612.86 ^{A,a}	2,004.19 ± 617.45 ^{A,a}
	Synbiotic	2,201.74 ± 639.95 ^{A,a}	2,192.49 ± 632.12 ^{A,a}	2,196.69 ± 639.80 ^{A,a}
Carbohydrates (g/d)	Control	262.25 ± 82.46 ^{A,a}	264.88 ± 85.50 ^{A,a}	265.64 ± 87.67 ^{A,a}
	Synbiotic	282.39 ± 87.68 ^{A,a}	283.36 ± 87.61 ^{A,a}	284.56 ± 86.45 ^{A,a}
Protein (g/d)	Control	60.39 ± 26.23 ^{A,a}	60.83 ± 26.86 ^{A,a}	60.97 ± 25.76 ^{A,a}
	Synbiotic	65.89 ± 23.78 ^{A,a}	66.90 ± 24.42 ^{A,a}	66.21 ± 23.08 ^{A,a}
Fat (g/d)	Control	129.24 ± 72.92 ^{A,a}	128.21 ± 74.54 ^{A,a}	128.56 ± 76.52 ^{A,a}
	Synbiotic	139.85 ± 71.49 ^{A,a}	137.93 ± 70.02 ^{A,a}	137.42 ± 70.08 ^{A,a}
Cholesterol (mg/d)	Control	113.92 ± 89.23 ^{A,a}	113.67 ± 91.97 ^{A,a}	113.29 ± 91.74 ^{A,a}
	Synbiotic	110.31 ± 72.73 ^{A,a}	111.04 ± 71.08 ^{A,a}	111.28 ± 74.49 ^{A,a}

^AMeans in the same column with different uppercase superscripts are significantly different ($P < 0.05$).

^aMeans in the same row with different lowercase superscripts are significantly different ($P < 0.05$).

¹Data are presented as mean values ± SD.

lipoprotein-cholesterol is synthesized in the liver and contains approximately 50 to 65% of triglycerides and 10 to 15% of CE (Ruiz-Gutiérrez et al., 1998). The triglycerides are packed into VLDL particles and delivered to various tissues such as adipose tissues and muscle for energy production. Remnants of VLDL are responsible for converting VLDL to IDL particles. The VLDL particles are the precursors for IDL particles, whereby the conversion of VLDL to IDL occurs upon the transfer of triglycerides in VLDL to the core of IDL-cholesterol particles (Marzetta et al., 1990). The present study showed that supplementation of the synbiotic product reduced the triglycerides in VLDL particles ($P < 0.05$), suggesting greater conversion of VLDL-cholesterol to IDL-cholesterol. In addition, free cholesterol and CE of the VLDL particles for the synbiotic group increased significantly ($P < 0.05$) by 46.75 and 17.98%, respectively, over 12 wk, whereas the control group showed nonsignificant changes. Past studies have found that CE is a metabolically active compound (Mazzone et al., 1995) that undergoes a continuous cycle of intracellular hydrolysis and re-esterification to form free cholesterol in the VLDL particles (Schwartz et al., 2004). In addition, CE could be transferred from LDL to VLDL,

resulting in the doubling of CE concentration in the VLDL particles (Barter et al., 1980). This is also supported by our present data, which showed a decrease in CE of LDL particles by 5.14% of the synbiotic group over 12 wk ($P < 0.05$), whereas the control showed an insignificant difference (Figure 2).

The synbiotic group also showed a significantly lower ($P < 0.05$) amount of triglycerides in IDL-cholesterol particles over 12 wk compared with control (Figure 3). The supplementation of synbiotic also contributed to a significant ($P < 0.05$) reduction of CE in IDL-cholesterol. The IDL particles consist of 30% triglycerides and approximately 22% of CE (Attman et al., 1996). Intermediate-density lipoprotein-cholesterol is converted into LDL when more triglycerides are removed via hydrolysis by hepatic lipase to form LDL particles (Dammerman and Breslow, 1995). Cholesteryl esters are transported from IDL-cholesterol to LDL-cholesterol (Matthan et al., 2008). Our current results showed that the administration of the synbiotic product contributed to reduced triglycerides and CE of IDL particles, suggesting a higher conversion of IDL to LDL. In addition, our present study showed that the synbiotic group significantly ($P < 0.05$) reduced total cholesterol by

Table 4. Effect of the synbiotic on lipid profiles of the hypercholesterolemic subjects (n = 32) for 12 wk¹

Lipid parameter	Treatment	Week 0	Week 6 ²	Week 12 ²
Total cholesterol (mmol/L)	Control	5.67 ± 0.35 ^{A,a}	5.64 ± 0.48 ^{A,a} (−0.53)	5.77 ± 0.54 ^{A,a} (+1.76)
	Synbiotic	5.74 ± 0.28 ^{A,a}	5.28 ± 0.49 ^{B,b} (−8.01)	5.29 ± 0.41 ^{B,c} (−7.84)
LDL-cholesterol (mmol/L)	Control	3.68 ± 0.36 ^{A,a}	3.74 ± 0.50 ^{A,a} (+1.63)	3.76 ± 0.57 ^{A,a} (+2.17)
	Synbiotic	3.56 ± 0.47 ^{A,a}	3.28 ± 0.59 ^{B,a} (−7.87)	3.23 ± 0.49 ^{B,c} (−9.27)
HDL-cholesterol (mmol/L)	Control	1.40 ± 0.35 ^{A,a}	1.31 ± 0.35 ^{A,a} (−6.43)	1.40 ± 0.45 ^{A,a} (0)
	Synbiotic	1.62 ± 0.55 ^{A,a}	1.45 ± 0.32 ^{A,a} (−10.49)	1.49 ± 0.47 ^{A,a} (−8.02)
Triglycerides (mmol/L)	Control	1.32 ± 0.67 ^{A,a}	1.29 ± 0.50 ^{A,a} (−2.27)	1.34 ± 0.45 ^{A,a} (+1.52)
	Synbiotic	1.20 ± 0.60 ^{A,a}	1.24 ± 0.75 ^{A,a} (+3.33)	1.23 ± 0.89 ^{A,a} (+2.50)

^{A,B}Means in the same column with different uppercase superscripts are significantly different ($P < 0.05$).

^{a-c}Means in the same row with different lowercase superscripts are significantly different ($P < 0.05$).

¹Data are presented as mean values ± SD.

²Positive (+) values in parentheses indicate percentage increase in each lipid profile compared with wk 0; negative (−) values indicate percentage reduction.

Figure 1. Subfraction of very low density lipoprotein (VLDL)-cholesterol of hypercholesterolemic subjects supplemented with the control and synbiotic for 12 wk. CE = cholesteryl ester.

5.37% in the IDL particles, whereas the control showed insignificant changes. Intermediate-density lipoprotein-cholesterol has been related to atherogenicity and the progression of arterial lesions (Sutherland et al., 1998). Nordestgaard et al. (1995) demonstrated that IDL facilitated the transfer of cholesterol into arterial cells of hypercholesterolemic animals, which was associated with atherosclerotic plaques, whereas Guerin et al. (2008) found that cholesterol-enriched IDL was

associated with increased risks of atherosclerosis in hypercholesterolemic subjects. In the present study, the supplementation of the synbiotic product reduced such a risk by reducing the total cholesterol content in the IDL particles, leading to their degradation or the formation of new LDL particles.

Low-density lipoprotein particles contain 35 to 40% of CE and 7 to 10% of triglycerides (Kasim et al., 1993). The 12-wk supplementation of synbiotic contributed to

Figure 2. Subfraction of low-density lipoprotein (LDL)-cholesterol of hypercholesterolemic subjects supplemented with the control and synbiotic for 12 wk. CE = cholesteryl ester.

Figure 3. Subfraction of intermediate-density lipoprotein (IDL)-cholesterol of hypercholesterolemic subjects supplemented with the control and synbiotic for 12 wk. CE = cholesteryl ester.

a significant ($P < 0.05$) decrease in triglycerides and CE of LDL particles, whereas the control showed constant concentrations (Figure 2). The depletion of triglycerides and CE in LDL would produce smaller and denser LDL particles (Kwiterovich, 2002). Low-density lipoprotein-cholesterol is the principal plasma carrier of cholesterol for delivery from the liver to peripheral tissues such as adipose tissues and adrenals (Mudd et al., 2007). Some studies showed that smaller and denser LDL particles are more atherogenic than larger LDL particles because of their high susceptibility to oxidation (Carr et al., 2000). These abnormal LDL particles are also increasingly removed by plasma compared with normal and larger LDL particles. Results from the present study showed that the supplementation of the synbiotic product reduced plasma LDL concentration via increased depletion of triglycerides and CE from LDL particles, leading to the formation of small dense particles that are more prone to hydrolysis. The present study also showed that the synbiotic group had significantly ($P < 0.05$) lowered total cholesterol by 7.25% in the LDL particles over 12 wk, whereas the control showed insignificant changes. Total cholesterol in the core of LDL could be drawn from the plasma back to the liver for excretion (Ramjiganesh et al., 2002), a mechanism mediated by upregulation of the LDL receptors (Gill et al., 2003). Such an occurrence has reportedly been induced by low-cholesterol diets (Daumerie et al., 1992) and some cholesterol-lowering drugs targeted at decreasing the cholesterol content of LDL particles

(Ast and Frishman, 1990). The removal of cholesterol from LDL particles stimulates LDL catabolism, leading to the increased hydrolysis of LDL particles and their subsequent removal from plasma (Carden, 2001).

High-density lipoprotein-cholesterol is secreted by the liver and intestines and contains little triglyceride (approximately 3%) and 12% of CE. The concentration of triglycerides in the HDL-cholesterol was reduced significantly ($P < 0.05$) by 31.51% in the synbiotic group over 12 wk, whereas the control showed insignificant changes (Figure 4). This subsequently increased the concentration of CE in the HDL particles of the synbiotic group. High-density lipoprotein-cholesterol is inversely correlated with the risk of cardiovascular heart diseases and is important in determining the flow of cholesterol in the body (Kwong and Wasan, 2002). High-density lipoprotein-CE directs cholesterol to the liver via the reverse cholesterol transport system, where CE is selectively taken up by HDL receptors (Tsompanidi et al., 2010). Studies have indicated that hypercholesterolemic individuals often experience a high activity of cholesteryl ester transfer protein (CETP) and low activity of lecithin-cholesterol acyltransferase (LCAT), leading to increased concentrations of blood total cholesterol levels (Lottenberg et al., 1996). Lecithin-cholesterol acyltransferase is responsible for catalyzing the transfer of 2-acyl groups from lecithin to free cholesterol (McPherson et al., 2007). This free cholesterol is then transported into HDL particles and subsequently esterified to generate CE and lysolecithin. Cholesteryl ester is produced by

Figure 4. Subfraction of high-density lipoprotein (HDL)-cholesterol of hypercholesterolemic subjects supplemented with the control and synbiotic for 12 wk. CE = cholesteryl ester.

LCAT from free cholesterol on the surface of HDL and resides in the core of the HDL particles (Ishikawa et al., 2001). Thus, the activity of LCAT is crucial for a normal maturation process of HDL-cholesterol (Yang et al., 2005), which contains a larger core for greater transportation of CE and cholesterol to the liver for subsequent hydrolysis (Nofer et al., 2002). The present study showed that supplementation of synbiotic significantly ($P < 0.05$) increased concentration of CE and total cholesterol in HDL particles, accompanied by a decrease in plasma total cholesterol of the synbiotic group. Our results indicated that the synbiotic product reduced plasma cholesterol via enhanced transportation of CE and total cholesterol by HDL particles.

Plasma Bile Acids

In our in vitro preliminary experiment, *L. acidophilus* CHO-220 was evaluated for its ability to produce BSH, an enzyme catalyzing the deconjugation of bile. Cell pellet of *L. acidophilus* CHO-220 was sonicated at 4°C to release the cellular extract containing intracellular enzyme. This spectrophotometry assay was based on the liberation of cholic acids from bile such as sodium glycocholate, sodium taurocholate, and bile mixture containing glycocholic acid, glycochenodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid, and taurodeoxycholic acid. Upon incubation of the cellular extract from *L. acidophilus* CHO-220 with bile, we found that sodium glycocholate, sodium taurocholate, and bile mixture were deconjugated, releasing 4.3 ± 0.2 ,

3.3 ± 0.3 , and 2.8 ± 0.3 mM of cholic acid, respectively. In addition, *L. acidophilus* CHO-220 exhibited specific BSH activities of 1.1 ± 0.2 , 1.0 ± 0.3 , and 1.3 ± 0.2 U/mL for sodium glycocholate, sodium taurocholate, and bile mixture, respectively. This is often a desired property of a probiotic strain, because the deconjugation of bile has been postulated to exert a hypocholesterolemic effect. Lye et al. (2010) found that several strains of probiotics including *Lactobacillus acidophilus* ATCC 314, *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411, *Lactobacillus bulgaricus* FTDC 1311, and *Lactobacillus casei* ATCC 393 could deconjugate sodium taurocholate, sodium glycocholate, oxgall, and a mixture of sodium glycocholate and sodium taurocholate via production of BSH. The deconjugation of bile results in the production of deconjugated bile, which is less absorbed in the intestine and thus secreted in the feces. New bile is formed using cholesterol as the precursor to replace the lost bile, leading to a reduced plasma cholesterol level.

However, the deconjugation of bile produces cholic acid, a primary bile acid that could be converted by intestinal microorganisms into secondary bile acids such as deoxycholic acid (Matheson and Story, 1994). Secondary bile acids have been associated with increased toxicity leading to various gastrointestinal diseases, including cholestasis, and promotion of carcinogenesis along the intestinal tract (Tan et al., 2007). Lithocholic acid was found to induce intrahepatic cholestasis (Beilke et al., 2008), and deoxycholic acid was found to promote liver carcinogenesis (Stalker et al., 1994). Thus, it is of

Table 5. Effect of the synbiotic product on conjugated, deconjugated, primary, and secondary bile of hypercholesterolemic subjects (n = 32) for 12 wk¹

Item	Treatment	Week 0	Week 6	Week 12
Cholic acid (mg/mL)	Control	1.53 ± 0.25 ^{A,a}	1.50 ± 0.35 ^{A,a}	1.54 ± 0.31 ^{A,a}
	Synbiotic	1.60 ± 0.13 ^{A,a}	1.67 ± 0.17 ^{A,a}	1.68 ± 0.15 ^{A,a}
Lithocholic acid (mg/mL)	Control	1.73 ± 0.69 ^{A,a}	1.72 ± 0.41 ^{A,a}	1.76 ± 0.51 ^{A,a}
	Synbiotic	1.94 ± 0.14 ^{A,a}	1.87 ± 0.19 ^{A,a}	1.92 ± 0.19 ^{A,a}
Chenodeoxycholic acid (mg/mL)	Control	1.86 ± 1.37 ^{A,a}	1.85 ± 0.53 ^{A,a}	1.80 ± 0.35 ^{A,a}
	Synbiotic	1.96 ± 0.26 ^{A,a}	1.94 ± 0.14 ^{A,a}	1.96 ± 0.14 ^{A,a}
Deoxycholic acid (mg/mL)	Control	1.96 ± 0.18 ^{A,a}	1.99 ± 0.26 ^{A,a}	1.92 ± 0.23 ^{A,a}
	Synbiotic	2.07 ± 0.18 ^{A,a}	2.07 ± 0.15 ^{A,a}	1.96 ± 0.17 ^{A,a}

^AMeans in the same column with different uppercase superscripts are significantly different ($P < 0.05$).

^aMeans in the same row with different lowercase superscripts are significantly different ($P < 0.05$).

¹Data are presented as mean values ± SD.

utmost importance that the increase in toxicity from bile conversion is evaluated, especially in the presence of a probiotic strain capable of deconjugating bile.

The present study showed nonsignificant ($P > 0.05$) differences in the levels of conjugated, deconjugated, primary, and secondary bile acids between the synbiotic and control group over 12 wk (Table 5). In addition, the levels of primary and secondary bile acids were constant in the synbiotic group over 12 wk. These observations may be attributed to 2 main possibilities: the *in vitro* deconjugation of bile by *L. acidophilus* CHO-220 was not exhibited in *in vivo* models or the deconjugating activity of *L. acidophilus* CHO-220 did occur but was too minimal to produce a significant *in vivo* effect (du Toit et al., 1998).

CONCLUSIONS

Although studies to date have shown that probiotics and prebiotics could improve lipid profiles, reports have also shown contradicting results. Results from our present study provided the clinical evidence to support the hypocholesterolemic claims of synbiotic. In addition, our results suggested that the synbiotic exhibited such cholesterol-lowering effects by altering the interrelated pathways of lipid transporters such as VLDL, LDL, and HDL. Although *L. acidophilus* CHO-220 could deconjugate bile *in vitro*, such an effect was not manifested in *in vivo* models. This establishes the potential of this synbiotic as a safe and nondrug alternative in the management of hypercholesterolemia.

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