Special Article

NUTRITION REVIEWS

Effect of probiotics on biomarkers of cardiovascular disease: implications for heart-healthy diets

Douglas B DiRienzo

The objectives of this article are to review clinical trials that have examined the effects of probiotics on low-density lipoprotein cholesterol (LDL-C) and to assess the potential of probiotic intake as a therapeutic lifestyle change (TLC) dietary option. Twenty-six clinical studies and two meta-analyses are reviewed. Significant LDL-C reductions were observed for four probiotic strains: Lactobacillus reuteri *NCIMB 30242,* Enterococcus faecium*, and the combination of* Lactobacillus acidophilus *La5 and* Bifidobacterium lactis *Bb12. Two synbiotics,* L. acidophilus *CHO-220 plus inulin and* L. acidophilus *plus fructo-oligosaccharides, also decreased LDL-C. Of the probiotics examined,* L. reuteri *NCIMB 30242 was found to best meet TLC dietary requirements by 1) significantly reducing LDL-C and total cholesterol, with robustness similar to that of existing TLC dietary options, 2) improving other coronary heart disease risk factors, such as inflammatory biomarkers, and 3) having "generally recognized as safe" (GRAS) status. Based on these results, the probiotic* L. reuteri *NCIMB 30242 is a viable candidate both for future TLC dietary studies and as a potential option for inclusion in TLC dietary recommendations.*

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death worldwide and is rapidly increasing in low- and middle-income countries.[1] In the United States, one in three deaths occurs as a result of CVD, with half of these due to coronary heart disease (CHD). Estimated costs associated with CVD and stroke in the United States reached nearly $300 billion in 2008 and are projected to triple by 2030.[2] CVD is a progressive disease that begins in childhood and is clinically manifested in adults of all ages. A number of modifiable risk factors have been identified; adopting healthy and therapeutic lifestyle habits is an important part of managing CVD risk and reducing costs associated with the disease.[3–6] Healthy lifestyle habits include maintaining a healthy body weight, engaging in regular physical activity, consuming a healthy diet, reducing intakes of saturated fats, *trans* fatty acids, and cholesterol, avoiding the use of tobacco products, and undergoing routine medical check-ups for blood pressure and cholesterol.[3–6] While sharing the same features as a "healthy lifestyle," dietary therapeutic lifestyle changes (TLCs) for CHD can also include the regular consumption of specific clinically tested foods or supplements, such as phytosterols, viscous soluble fibers, and – potentially – certain probiotics, shown to lower low-density lipoprotein cholesterol (LDL-C).[3,5,7]

Elevated LDL-C is a major risk factor for CHD and is the primary target of lipid-lowering therapy.[3,4,8] LDL-C initiates and promotes the progression of CHD, including the formation, growth, destabilization, and rupture of atherosclerotic plaques.[1,8] Additional risk factors for CHD include elevated levels of serum triacylglycerol (TAG) and triglyceride-rich lipoproteins, low levels of high-density lipoprotein cholesterol (HDL-C), and non-lipid-related risk factors, such as markers for inflammation. These risk factors are important, but targeting them for therapy is secondary to targeting LDL-C.[3]

Affiliations: *DB DiRienzo* is with Quadrant Nutrition, LLC, Hendersonville, North Carolina, USA.

Correspondence: *DB DiRienzo*, 82 Sky Vista Ln., Hendersonville, NC 28792, USA. E-mail: Ddirienzo@quadrantnutrition.com, Phone: +1-828-595-9086.

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The benefits of statins for lowering LDL-C and reducing CHD risk are well documented.[4] Recently, the 2012 Cholesterol Treatment Trialists' Collaboration reported that, "In individuals with 5-year risk of major vascular events lower than 10%, each 1 mmol/L reduction in LDL-C produced an absolute reduction in major vascular events of about 11 per 1,000 over 5 years. Under present guidelines, such individuals would not typically be candidates for LDL-lowering statin therapy."[9] Moreover, for individuals on statin therapy, combined use of statins with lipid-lowering dietary interventions may reduce statin requirements.[4] The use of safe, inexpensive, and nonpharmacologic TLCs, along with other healthy lifestyle habits, may significantly lower LDL-C in low-risk individuals.[4,5,8,10]

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[11] Probiotics and fermented foods have played important roles in human diets for thousands of years and are safely consumed on a large scale.[7,11] The human digestive tract is a large microbial reservoir that has an important influence on human health and well-being, affecting glucose and lipid metabolism, predisposition to obesity, and inflammatory conditions, among others.[12–14] When considering the clinical effects of probiotics, it is important to understand that different probiotic strains may have unique clinical effects.

Guidelines for the evaluation and testing of probiotics for food use have been developed.[15] The following three factors are important considerations in probiotic clinical research: 1) appropriate identification, characterization, and maintenance of probiotic strains; 2) maintenance of probiotics in a live condition during the course of a study to ensure that potency is preserved; and 3) assurance that probiotics arrive alive to the site of action. This last characteristic varies for different probiotic applications, but withstanding transit through the gastrointestinal tract is often necessary. It is recommended that at least two independent double-blinded placebo-controlled clinical trials are conducted and that the probiotic being tested is assessed for safety.

Low cholesterol levels and low incidence of CHD in the Maasai and Samburu tribes of northern Africa, despite high intakes of full-fat dairy products and beef in these populations, raised interest in the effects of fermented milk and other dairy products on cholesterol reduction.[16,17] Since that time, numerous in vitro, animal, and human clinical studies have been conducted to characterize the ability of different probiotics to reduce LDL-C and lower the risk of CHD. The objective of this article is to review the existing clinical trials that have examined the effects of probiotics on LDL-C and to assess the potential of probiotic intake as a dietary TLC option for reducing CHD risk.

METHODS

The literature was initially searched using PubMed (National Library of Medicine, Bethesda, MD), with the term "probiotics" in combinations with the terms "cholesterol," "LDL," and "blood lipids." The following filters were utilized during these searches: human, clinical trial, RCT, and no date restriction. Subsequent searches were conducted utilizing the term "cholesterol" along with the following individual probiotic names: *L. acidophilus, B. longum, L. fermentum, L. paracasei, L. plantarum,* and *L. reuteri*. The same filters listed above were used, excluding RCT, to ensure that term did not overly restrict the initial search results. Using the same filters again, a search was also conducted for the term "meta-analysis" combined with terms "probiotics" and "cholesterol." A total of 113 references were retrieved by the searches and further reduced to 53 original studies by removal of duplications. References listed in articles of interest obtained from the above searches, from previously published reviews on probiotics and health, or utilized during the development of this review were then scrutinized to identify other relevant clinical studies. Studies that were randomized, placebo-controlled trials conducted in an adult population whose primary endpoints included LDL-C and other plasma lipids were summarized and included in the tables. Studies not published as full reports, such as conference abstracts or those not published in English, were excluded.

ASSESSMENT OF PROBIOTIC EFFECTS ON LDL-C AND OTHER BLOOD LIPID MARKERS FOR CHD

Two meta-analyses have examined the effect of probiotic consumption on CHD. Guo et al.[18] conducted a meta-analysis to evaluate the LDL-C- and total cholesterol (TC)-lowering properties of 10 different probiotic strains. The analysis included 13 randomized, controlled trials in subjects with normal and high cholesterol levels.[19–29] The dose of probiotics provided to subjects in these studies ranged from 2×10^7 colony-forming units (CFU) per day to 2×10^{13} CFU/day. Feeding duration ranged from 4 weeks to 10 weeks, with exception of one study in which 2-week test periods per dose were used. Results from a combined 485 subjects found that, when compared with placebo, probiotic consumption significantly lowered LDL-C by 4.9 mg/dL ($P < 0.01$), TC by 6.4 mg/dL ($P < 0.001$), and TAG by 3.95 mg/dL ($P < 0.05$) but had no effect on HDL-C (0.11 mg/dL, $P > 0.05$). This analysis concludes that probiotics "have beneficial effects on TC and LDL-C for subjects with high, borderline high and normal cholesterol levels."[18] Thus, probiotic use holds promise as a nonpharmaceutical approach to help manage CHD risk. However, this meta-analysis is limited

by the lack of investigation of the effects of specific probiotic strains on blood lipids. Because effects on blood lipids may be strain specific, a single-strain meta-analysis like the one conducted by Agerholm-Larsen et al.[30] on *Enterococcus faecium* may be a stronger approach to examine probiotic efficacy for therapeutic use. Results of this meta-analysis will be discussed later in this review.

The results of 26 randomized, placebo-controlled trials reported in the literature that examined the effects of probiotics on TC, lipoproteins, and inflammatory biomarkers in subjects given a fermented dairy product (Table 1), a capsule (Table 2), or a synbiotic (Table 3) have been summarized. In these trials, significant lowering of LDL-C was observed for four probiotic strains that were compared with placebo: *L. reuteri* NCIMB 30242,[31,32] *E. faecium*,[19,21] and the combination of *L. acidophilus* La5 *and Bifidobacterium lactis* Bb12.[33] A synbiotic combines a probiotic and a prebiotic. Two synbiotics that were found to decrease LDL-C included *L. acidophilus* CHO-220 plus inulin[34] and *L. acidophilus* (2 strains undefined) plus fructo-oligosaccharides.[35]

L. reuteri NCIMB 30242

Two randomized, placebo-controlled, double-blind, parallel-arm, multicenter studies provide support for the ability of *L. reuteri* NCIMB 30242 in both yogurt and capsules to significantly lower LDL-C and TC compared with placebo. In the yogurt study, 114 hypercholesterolemic men and women consumed 250 mL of yogurt containing this probiotic (2.8×10^9 CFU/day, microencapsulated) for 6 weeks and attained significant reductions in LDL-C of 8.9%, in TC of 4.8%, and in non-HDL-C of 6.0% over placebo (Table 1).[36] Serum concentrations of TAG and HDL-C were unchanged over the course of the study. In the study using capsules, 127 hypercholesterolemic men and women consumed two capsules (200 mg; 4×10^9 CFU/day) for 9 weeks and achieved significant reductions in LDL-C of 11.64%, in TC of 9.14%, in non-HDL-C of 11.30%, and in apoB-100 of 8.41% relative to placebo (Table 2).[32] The ratios of LDL-C/HDL-C and apoB-100/apoA-1 were reduced by 13.39% and 9.0%, respectively, relative to placebo. Concentrations of TAG and HDL-C were unchanged. In the current literature, *L. reuteri* NCIMB 30242 capsules are the only probiotic capsules shown to significantly lower LDL-C.

E. faecium

A meta-analysis of five randomized, controlled studies with *E. faecium* in milk products involving about 400 subjects of both genders and different initial LDL-C levels found a significant decrease in LDL-C and TC by 5% ($P < 0.05$) and 4% ($P < 0.05$) versus placebo, respectively.[30] Outcomes from individual randomized, placebo-controlled, double-blind trials (Tables 1 and 2) were mixed, with studies showing decreased LDL-C[19] or no effect.[37,38] A second study reports a reduction in LDL-C, but the lack of a washout period in the crossover design as well as design inconsistencies during the conduct of this confirmatory study raises questions about its validity.[21] Richelsen et al.[38] did find a reduction of LDL-C in the first 3 months of the study, yet by 6 months there were no differences between test and control yogurts. One possible reason was a significant drop in the dose of *E. faecium* from 10^8 CFU/mL to $10^{4–5}$ CFU/mL, which occurred 2 to 3 months into the study. In a study using capsules, 43 hypercholesterolemic men and women consumed *E. faecium* M-74 (2×10^9 CFU/day) for 60 weeks. Changes from baseline in LDL-C and TC, but not HDL-C or TAG, were reported.[39]

L. acidophilus La5 and B. *lactis* Bb12 mixture

There are two randomized, placebo-controlled, double-blind, parallel-arm studies evaluating the LDL-C-lowering effects of *L. acidophilus* La5 *and B. lactis* Bb12 (Table 1). One trial in 60 people with type 2 diabetes who consumed 300 g of yogurt per day (1×10^6 CFU) for 6 weeks reported a significant reduction in LDL-C and TC of 7.5% and 4.5%, respectively.[33] A similarly designed trial in 59 normocholesterolemic women who consumed 300 g of yogurt per day (3.9×10^7 CFU) for 6 weeks reported no changes in LDL-C and TC.[29] Thus, a mixture of *L. acidophilus* La5 and *B. lactis* Bb12 may be a candidate for a therapeutic dietary option to help people with type 2 diabetes manage their LDL-C and TC levels, but more clinical research is needed.

Synbiotic studies

Two randomized, placebo-controlled double-blind synbiotic studies were shown to decrease LDL-C (Table 3). The first study was a parallel-armed study in 32 hypercholesterolemic men and women and examined the combination of *L. acidophilus* CHO-220 plus inulin.[34] Subjects consumed four capsules per day containing 1×10^9 CFU of *L. acidophilus* CHO-220 and 0.2 g of inulin for 12 weeks and achieved reductions in LDL-C (9.3%) and TC (7.8%) over placebo, with no change in HDL-C or TAG. The combination of *L. acidophilus* (strains undefined) plus fructo-oligosaccharides was examined in 30 normocholesterolemic men in a crossover study.[35] Subjects consumed 125 mL of a fermented milk product containing 10^7–10^8 CFU/g of *L. acidophilus* and a 2.5% fructo-oligosaccharide mixture daily for 3 weeks at breakfast, lunch, and dinner. Significant reductions in LDL-C (5.4%), TC (4.4%), and LDL-

Table 1 **Clinical effects of probiotics on lipoproteins, lipids, and inflammatory biomarkers: yogurt/fermented milk.**

Probiotic strain(s)	Probiotic intake	Trial design	Subject profile	Results	Reference
Significant reduction in LDL-C versus placebo					
L. reuteri NCIMB 30242 (microencapsulated)	250 g (providing 2.8×10^9 CFU/day in 2 divided doses at breakfast and dinner) for 6 weeks	Randomized, placebo-controlled, double-blind, parallel, multicenter study	114 hypercholesterolemic men and women with a mean BMI of 26 and a mean age of 50 yrs	LDL-C: −8.9% ($P = 0.016$). TC: −4.8% ($P = 0.031$). Non-HDL-C: −6.0% ($P = 0.029$). No difference: HDL-C, TAG, and LDL-C:HDL-C	Jones et al. (2012)[31]
L. acidophilus La5 and *B. lactis* Bb12	300 g/day (containing ≈4×10^6 CFU/g for each probiotic strain) for 6 weeks	Randomized, placebo-controlled, double-blind, parallel study	60 type 2 diabetic men and women with a mean BMI of 29 and a mean age of 51 yrs	LDL-C: −7.5% ($P < 0.01$). TC: −4.5 ($P < 0.01$). TC:HDL-C: −5.4% ($P < 0.05$). LDL-C:HDL-C: −8.6% ($P < 0.05$). No difference: HDL-C and TAG	Ejtahed et al. (2011)[33]
E. faecium	200 g/day (containing 10^5–10^9 CFU/mL) for 8 weeks	Randomized, placebo-controlled, double-blind, crossover study	32 hypercholesterolemic men and women with a mean BMI of 25 and a mean age of 56 yrs	LDL-C: −6.2% ($P = 0.012$.) TC: −5.3% ($P = 0.004$). No difference: HDL-C and TAG	Bertolami et al. (1999)[21]
E. faecium	200 mL/day (containing 2×10^8 CFU/mL) for 6 weeks	Randomized, placebo- controlled, double-blind, parallel study	57 normocholesterolemic men, all aged 44 yrs, with a mean BMI of 24	LDL-C: −10% ($P < 0.01$). TC: −6% ($P < 0.05$). No difference: HDL-C and TAG	Agerbaek et al. (1995)[19]
Nonsignificant reduction in LDL-C versus placebo					
L. acidophilus La5 and *B. lactis* Bb12	300 g/day (containing 3.9×10^7 CFU/g) for 6 weeks	Randomized, placebo controlled, double blind, parallel study	59 normocholesterolemic, women with a mean BMI of 24 and a mean age of 34 yrs	No difference: LDL-C, TC, HDL-C, TAG, and TC:HDL-C	Sadrzadeh-Yeganeh et al.(2010)[29]
L. acidophilus and *B. lactis*	300 g/day (containing 10^6 CFU/g) for 6 weeks	Randomized, placebo-controlled, double-blind, crossover study	14 healthy hypercholesterolemic men and women with a mean BMI of 26 and a mean age of 51 yrs	TC: −0.68 mmol/L net change ($P = 0.041$). No difference: LDL-C, HDL-C, TAG, and LDL-C:HDL-C	Ataie-Jafari et al. (2009)[28]
L. paracasei subsp. *paracasei*	100 g daily for 2 weeks and 200 g daily for 2 weeks, each gram containing 3.6×10^8 CFU	Randomized, placebo-controlled, double-blind, parallel study	33 healthy normocholesterolemic, women with a mean BMI of 21 and a mean age of 24 yrs	No difference: LDL-C, TC, HDL-C, TAG, TC:HDL-C, and LDL-C:HDL-C	Fabian & Elmadfa (2006)[26]
B. longum BL1	300 mL/day (containing 10^8 CFU/mL) for 4 weeks	Randomized, placebo-controlled, single-blind, parallel study	32 hypercholesterolemic, nonobese men and women with a mean age of 44 yrs	No difference: LDL-C, TC, HDL-C, and TAG	Xiao et al. (2003)[25]
L. plantarum 299v	400 mL/day (containing 5×10^7 CFU/mL) for 6 weeks	Randomized, placebo-controlled, double-blind, parallel study	36 hypercholesterolemic men and women with a mean BMI of 25 and a mean age of 42 yrs	Fibrinogen: −21% ($P < 0.001$) in test group vs. NC in placebo group. No difference: LDL-C, TC, HDL-C, TAG, Lp(a), and homocysteine	Naruszewicz et al. (2002)[24]
Three combinations of probiotics: *E. faecium L. acidophilus L. rhamnosus*	450 mL/day fed for 8 weeks: *E. faecium* ($n = 16$): 6×10^7 CFU/mL; *L. acidophilus* ($n = 16$): 2×10^7 CFU/mL; *L. rhamnosus* ($n = 14$): 2×10^8 CFU/mL	Randomized, placebo-controlled, double-blind, 5-arm parallel study; controls included placebo yogurt	60 normocholesterolemic, men and women with a mean BMI of 30 and a mean age of 39 yrs	No difference: LDL-C, TC, HDL-C, TAG, and fibrinogen (for *E. faecium* only, LDL-C decreased [$P = 0.03$] after adjustment for BW change)	Agerholm-Larsen et al. (2000)[23]
L. acidophilus L1	200 g yogurt/day, each gram containing 4.2×10^6 CFU, for 4 weeks	Randomized, placebo-controlled, double-blind, crossover study (study 2)	40 hypercholesterolemic men and women with BMI and age not disclosed	Mixed results: reductions in LDL-C (3.2%) and TC (2.4%) reported for first crossover treatment period, but no differences found in second treatment period. Overall study outcome statistics not provided	Anderson & Gilliland (1999)[20]
L. acidophilus L1	500 mL yogurt/day, each mL containing 5×10^6 CFU, for 6 weeks	Randomized, placebo-controlled, parallel study	78 normocholesterolemic men and women with a mean BMI of 24 and a mean age of 40 yrs	No difference: LDL-C, TC, HDL-C, and TAG	De Roos et al. (1999)[22]
E. faecium	200 mL daily for 12 weeks. Probiotic levels not provided	Randomized, placebo-controlled, double-blind, parallel, multicenter study	160 hypercholesterolemic men and women aged 30–55 yrs with a BMI of < 30	No difference: TC and apoB	Sessions et al. (1998)[37]
E. faecium	200 mL daily, each mL containing 10^5–10^9 CFU for 6 months	Randomized, placebo-controlled, double-blind, parallel study	87 normocholesterolemic men and women with a mean BMI of 25 and a mean age of 59 yrs	No difference: LDL-C, TC, HDL-C, and TAG	Richelsen et al. (1996)[38]

Abbreviations: apoA-1, apolipoprotein A-1; apoB-100, apolipoprotein B-100; BMI, body mass index; BW, body weight; CFU, colony-forming units; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); NC, no change; non-HDL-C, non-HDL cholesterol; TAG, triacylglycerides; TC, total cholesterol.

Table 2 **Clinical effects of probiotics on lipoproteins, lipids, and inflammatory biomarkers: capsules.**

Probiotic strain(s)	Probiotic intake	Trial design	Subject profile	Results (compared with placebo)	Reference
L. reuteri NCIMB 30242	2 capsules (total 200 mg) providing 4×10^9 CFU/day during breakfast and dinner for 9 weeks	Randomized, placebo-controlled, double-blind, parallel, multicenter study	127 healthy hypercholesterolemic men and women with a mean BMI of 27 and a mean age of 50 yrs	LDL-C: −11.6% ($P = 0.001$). TC: −9.1% ($P < 0.001$). Non-HDL-C: −11.3% ($P < 0.001$). ApoB-100: −8.4% ($P = 0.002$). ApoB-100/apoA-1: −9.0% ($P = 0.026$). hs-CRP: −1.05 mg/L ($P = 0.005$). Fibrinogen: −14.25% ($P = 0.004$). No difference: HDL-C and TAG	Jones et al. (2012)[32]
L. rhamnosus strain LC705 and *P. freudenreichii* ssp. *shermanii* strain JS	2 capsules containing 2×10^{10} CFU of each strain, given daily with first meal of the day for 4 weeks	Randomized, placebo-controlled, double-blind, two-period crossover study	38 healthy hypercholesterolemic men with a mean BMI of 25 and a mean age of 42 yrs	No difference: LDL-C, TC, HDL-C, TAG, and HDL-C:TC	Hatakka et al. (2008)[42]
L. fermentum	2 capsules, each containing 2×10^9 CFU, twice daily with food for 10 weeks	Randomized, placebo-controlled, single-blind, parallel study	44 healthy hypercholesterolemic men and women with a mean BMI of 26 and a mean age of 51 yrs	No difference: LDL-C, TC, HDL-C, TAG, and CRP	Simons et al. (2006)[27]
L. acidophilus La1	2 capsules, each containing 3×10^{10} CFU, three times daily with food for 6 weeks	Randomized, placebo-controlled, double-blind, crossover study	80 healthy hypercholesterolemic men and women with a mean BMI of 28 and a mean age of 47 yrs	No difference: LDL-C, TC, HDL-C, TAG, and HDL-C:LDL-C	Lewis & Burmeister (2005)[43]
E. faecium M-74	1 capsule containing 2×10^9 CFU, daily for 60 weeks	Randomized, placebo-controlled, double-blind, parallel study	43 hypercholesterolemic men and women with a mean BMI of 29 and a mean age of 76 yrs	Differences from baseline only tested	Hlivak et al. (2005)[39]
L. acidophilus (ATCC 4962) and *L. bulgaricus* (ATCC 33409)	1 tablet containing 2×10^6 CFU, 4 times daily for 6 weeks	Randomized, placebo-controlled, double-blind, crossover study	334 normo- and hypercholesterolemic subjects, no BMI or age indicated	No difference: LDL-C and TC	Lin et al. (1989)[44]

Abbreviations: apoA-1, apolipoprotein A-1; apoB-100, apolipoprotein B-100; BMI, body mass index; CFU, colony-forming units; HDL-C, high-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein; non-HDL-C, non-HDL cholesterol; TAG, triacylglycerides; TC, total cholesterol.

Table 3 **Clinical effects of synbiotics on lipoproteins, lipids, and inflammatory biomarkers.**

Synbiotic	Probiotic intake	Trial design	Subject profile	Results	Reference
L. acidophilus CHO-220 plus inulin	4 capsules (2 in morning and 2 in the evening) each containing 1×10^9 CFU of *L. acidophilus* CHO 220 and 0.20 g of inulin, daily for 12 weeks	Randomized, placebo-controlled, double-blind, parallel study	32 hypercholesterolemic men and women with a mean BMI of 23 and a mean age of 34 yrs	LDL-C: −9.3% ($P < 0.05$). TC: −7.84% ($P < 0.05$). No difference: HDL-C and TAG	Ooi et al. (2010)[34]
L. acidophilus DDS-1 and *B. longum* UABL-14 plus fructo-oligosaccharide	3 capsules each containing a total of 1.25×10^9 CFU of each probiotic and 10–15 mg fructo-oligosaccharide, daily for 8 weeks	Randomized, placebo-controlled, single-blind, parallel study	55 healthy normocholesterolemic men and women with a mean BMI of about 24 and a mean age of 27 yrs	No difference: LDL-C, TC, HDL-C, and TAG	Greany et al. (2008)[40]
L. acidophilus 145 and *B. longum* 913 plus oligofructose	300 g yogurt/day containing $10^{6–8}$ CFU *L. acidophilus* 145, $10^{3–5}$ CFU *B. longum* 913, and 1% oligofructose for 7 weeks	Randomized, placebo-controlled, blinded, crossover study	29 healthy women with a mean BMI of 24 and a mean age of 34 yrs: 15 were normocholesterolemic and 14 were hypercholesterolemic	HDL-C: 0.3 mmol/L ($P = 0.0002$). LDL-C:HDLC: 0.8 mmol/L. No difference: LDL-C, TC, and TAG	Kiessling et al. (2002)[41]
L. acidophilus (2 strains not defined) plus fructo-oligosaccharides	125 mL fermented milk daily at breakfast, lunch, and dinner for 3 weeks. Each gram contained 10^7–10^8 CFU *L. acidophilus* and 2.5% fructo-oligosaccharides	Randomized, placebo-controlled, double-blind, crossover study	30 normocholesterolemic men with an average body weight of 81.4 kg and mean age of 48 yrs	LDL-C: −5.4% ($P < 0.005$). TC: −4.4% ($P < 0.001$). LDL-C:HDL-C: −5.3% ($P < 0.05$). No differences: HDL-C and TAG	Schaafsma et al. (1998)[35]

Abbreviations: BMI, body mass index; CFU, colony-forming units; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; TAG, triacylglycerides; TC, total cholesterol.

C:HDL-C (5.3%) were achieved, compared with placebo. No differences in HDL-C or TAG were found. Since the effect of synbiotics on blood lipids can result from probiotics, prebiotics, or both, it is difficult to determine the direct effects of the probiotic used in these studies on LDL-C.

The remaining studies listed in Tables 1–3 found no effect of probiotics or synbiotics consumed on LDL-C or effects on other blood lipids examined.[20,22–26,28,29,37,38,40–44] The variable findings from the above studies may be due to probiotic strain or dose, viability of the probiotic during the course of the study, and/or differences in experimental design. To strengthen the science on probiotics, guidelines for industry on statistical principles for clinical trials have been developed and their use in future studies to examine and confirm the efficacy of probiotics on LDL-C or other health endpoints is strongly encouraged.[45] For example, as stated in 2.1.2 of this document, "a confirmatory trial is an adequately controlled trial in which the hypotheses are stated in advance and evaluated. As a rule, confirmatory trials are necessary to provide firm evidence of efficacy or safety. In such trials the key hypothesis of interest follows directly from the trial's primary objective, is always predefined, and is the hypothesis that is subsequently tested when the trial is complete. In a confirmatory trial, it is equally important to estimate with due precision the size of the effects attributable to the treatment of interest and to relate these effects to their clinical significance. Confirmatory trials are intended to provide firm evidence in support of claims; hence adherence to protocols and standard operating procedures is particularly important . . ."

The randomized, controlled, multicenter clinical trials examining *L. reuteri* NCIMB 30242 include a predefined primary endpoint in the protocol as well as sufficient statistical power to detect an expected change in the primary endpoint. This is in contrast to other published probiotic clinical studies, in which at times it is unclear whether these guidelines were followed and, therefore, whether firm evidence of efficacy is provided.

CHOLESTEROL-LOWERING MECHANISM

Research in the 1960s indicated differences between germ-free and conventional rats in their ability to metabolize cholesterol. Germ-free animals, which lack intestinal microflora, were found to have both lower amounts and different compositions of fecal steroids, higher absorption of dietary cholesterol, and greater accumulation of cholesterol, particularly in the liver, indicating a role for intestinal microbes in cholesterol regulation.[46–48] Since then, in vitro and in vivo studies have examined how intestinal microbes and probiotics could affect cholesterol metabolism. However, the exact mechanism(s) of action of the limited number of probiotic strains that have been clinically shown in humans to significantly decrease LDL-C levels versus placebo is not completely known. Several potential mechanisms whereby probiotics may reduce circulating cholesterol levels have been proposed: 1) binding of cholesterol by the cellular surfaces and membranes of the probiotics; 2) assimilation of cholesterol particles into growing probiotic cells; 3) microbial deconjugation of bile via bile salt hydrolase (BSH), resulting in increased fecal excretion of deconjugated bile salts with a compensatory increase in the use of cholesterol to produce new bile acids; 4) short-chain fatty acid production from fermentation of carbohydrate, leading to decreased levels of blood lipids and reduced production of endogenous cholesterol by the liver; and 5) a reduction in cholesterol absorption, perhaps through BSH activity and deconjugation of biliary salts in the small intestine.[31,49,50]

INFLAMMATORY MARKERS

Increasing recognition that atherosclerosis involves a chronic inflammatory process has created interest in arterial acute-phase inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-CRP) and fibrinogen, as risk factors for CHD.[51–54] *L. reuteri* NCIMB 30242 was shown to lower both hs-CRP by 1.05 mg/L and fibrinogen by 14.25% relative to control, respectively.[32] One *E. faecium* study found a significant increase in fibrinogen by 11.4% versus the control, but it should be noted that the increased fibrinogen levels still remained in the normal range.[23] Persistent elevation of fibrinogen may be associated with an increased risk of early atherosclerosis.[51] The authors could not exclude the potential of a transient colonic inflammation caused by *E. faecium* bacteria strains as a possible reason for the increase in fibrinogen in subjects. The studies using *L. acidophilus* La5 *and B. lactis* Bb12 did not provide data on inflammatory biomarkers.

Probiotics may attenuate acute-phase inflammation, which may modify the risk of chronic diseases such as CHD, type 2 diabetes, and obesity by changing the intestinal inflammatory barrier, altering the bacterial colonies present in the intestinal flora, and/or modulating the gut-associated lymphoid tissue.[55] In addition, some probiotics may affect the inflammatory process by their actions on bile acid metabolism directly or indirectly via their effect on other intestinal microflora. Further, bile acids function as signaling molecules that can activate a variety of nuclear receptors. These bile receptors, mainly expressed in enterohepatic tissues, can affect glucose tolerance, lipid and energy expenditure, and immune function, thus potentially affecting the risk of CVD and metabolic syndrome.[56,57] There is interest in the role of the G-protein-coupled bile acid receptor (TGR5), which is also expressed in human monocytes.[58] Recently, the activation

of TGR5 has been shown to inhibit macrophage inflammation, oxidized LDL uptake, and the development of atherosclerosis.[59] Bile acid receptors and their actions have been reviewed in detail by others.[56,57,60] Other researchers have suggested that probiotics may affect inflammation via fermentation byproducts produced from milk.[61] While this may be a possibility, it would not explain the effect of encapsulated probiotics on inflammation, such as that reported by Jones et al.,[32] who used *L. reuteri* NCIMB 30242.

IMPLICATIONS FOR PUBLIC HEALTH

The National Cholesterol Education Programs Adult Treatment Panel recommends dietary alterations, increased exercise, and other lifestyle changes to reduce CHD risk. In addition to reducing intakes of saturated fats, *trans*-fatty acids, and cholesterol, TLC dietary options for lowering LDL-C, such as plant stanols/sterols, soy protein, and viscous fiber, are recommended.

The combination of plant sterols, viscous fiber, soy protein, and nuts, in addition to a diet low in saturated fat, has been clinically tested in an outpatient metabolic study.[62] After 1 month, subjects on this diet had an LDL-C reduction of 28.6% ($P < 0.001$), which was similar to the 30.9% reduction ($P < 0.001$) achieved in subjects who received lovastatin and adhered to a diet low in saturated fat. Both treatments resulted in a significantly greater reduction in LDL-C ($P < 0.005$) compared with an LDL-C reduction of 8% ($P = 0.02$) for subjects on a low-saturated-fat control diet. These results demonstrate a significant LDL-C-lowering effect of a TLC diet (with multiple dietary targets) that is as effective as a low dosage of a first-generation statin drug. A follow-up 1-year study in free-living subjects showed a more modest LDL-C reduction of 13%, which may be attributable to lower compliance with the diet under free-living conditions.[63] Adding 200 mg/day *L. reuteri* NCIMB 30242 to the diet has been shown to reduce LDL-C by 9–12% in short-term studies in hypercholesterolemic individuals.[31,32] This effect on LDL-C is similar to that of 2 g of phytosterol/stanols per day[64–68] and might be greater than that of 5–10 g of viscous fiber per day[69–74] or 25 g of soy per day.[75,76] All of these therapeutic dietary options impede dietary cholesterol absorption and/or enterohepatic recirculation of cholesterol. Probiotics can be added to food products such as yogurt and can also be consumed as capsules. Thus, dietary compliance may be improved by the use of probiotics, leading to greater lowering of LDL-C.

The importance of lowering LDL-C in individuals to reduce major vascular events was highlighted in the Cholesterol Treatment Trialists' Collaboration.[9] Although statin drugs are widely prescribed to lower cholesterol, they may have side effects that prevent their continued use in some patients. Moreover, some individuals do not need marked LDL-C reduction or prefer nonpharmacologic alternatives to statin therapy.[77] TLC dietary options cannot always match the potent effects of pharmaceutical agents, but they still lower LDL-C, sometimes markedly, and may have fewer side effects than pharmaceuticals. Moreover, since doubling a statin dose typically results in only about a 6–9% further reduction in LDL-C, combining the TLC diet and other nonpharmacologic lifestyle practices with cholesterol-lowering pharmacotherapy may achieve the greatest LDL-C-lowering effect.[78,79] Therapeutic dietary approaches that include probiotics such as *L. reuteri* NCIMB 30242 can be among the potential options to manage LDL-C; the use of probiotic-containing diets as a complement to statin therapy should be studied further. In addition to a diet low in saturated fat and cholesterol, the consumption of specific probiotics in the right quantities may produce results comparable to the LDL-C-lowering effect of a low-dose statin drug.

SAFETY

In addition to demonstrating health benefits, therapeutic dietary options such as probiotics must be established as safe for human consumption. In the United States, determination of a new food ingredient as "generally recognized as safe" (GRAS) can be obtained via scientific procedures and with the concurrence of an expert panel.

The probiotic *L. reuteri* NCIMB 30242 recently was determined to be GRAS for use in a wide variety of foods at a total intake of up to 1×10^{10} CFU/day.[80] An expert panel examined data on the history of exposure to *L. reuteri* (background exposure in foods and as a commensal organism), bioinformatic and in vitro data characterizing the metabolic phenotypes of the strain, and data on strain-specific safety provided by repeat-dose studies in humans.

L. reuteri is commonly used by the food industry for its fermentation properties and is one of the most widely used microorganisms for the production of sourdough bread. Wolf et al.[81] demonstrated the safety and tolerance of 1×10^{11} CFU/day *L. reuteri* in humans. Several regulatory agencies, such as the US Food and Drug Administration (FDA), the European Food Safety Authority, and the Therapeutic Goods Administration of Australia, have recognized *L. reuteri* for human consumption.[82–84] Moreover, the use of *L. reuteri* in infant formula has been shown to be safe and well tolerated and to support normal growth.[80,85,86]

It should not be assumed that conclusions about the safety of a species of microorganism can be applied to all strains of that species. Thus, Branton et al.[87] characterized the *L. reuteri* NCIMB 30242 strain for antimicrobial resistance, production of antimicrobial compounds, the

presence of virulence genes in the genome, and production of potentially harmful metabolites. They found no evidence of characteristics that present food safety concerns.

L. reuteri NCIMB 30242 also was shown to be well tolerated in two clinical trials investigating 251 hypercholesterolemic but otherwise healthy subjects.[36,88] Compared to subjects who consumed placebo, subjects who consumed 2.9×10^9 to 5×10^{10} CFU/day as either yogurt or capsules over 6–9 weeks showed no significant changes in blood chemistry, hematological parameters, body weight, body mass index, or vital signs. Adverse effects in the probiotic and placebo groups were similar.

The FDA has concluded that a GRAS determination for *L. reuteri* NCIMB 30242 (GRN000440) presents no safety questions, as indicated in its letter to the manufacturer.[89]

The probiotic *B. lactis* Bb12 (in combination with *Streptococcus thermophilus* Th4) has been determined to be GRAS for use in infant formula.[90]

As noted by Ogier and Serror[91] and Franz et al.,[92] the *Enterococcus* genus presents safety questions for human consumption. While there are some enterococci with a long history of safe use, others can be opportunistic pathogens. Franz et al.[92] also point out that enterococci present a potential reservoir of antibiotic resistance and virulence genes and conclude that "... enterococci in foods, as commercial starters and probiotic strains, should be possible on the basis of case-by-case studies establishing their innocuity or at least the lack of acquired antibiotic resistance genes and proven virulence factors." However, in a draft guidance document from the FDA to industry regarding the safety evaluation of new dietary ingredients for dietary supplements, it is stated that *E. faecium* (among others) should not be used as a dietary ingredient because FDA regards all members of a species that contains human pathogens as potentially harmful to human health. FDA believes there is an absence of consensus regarding valid scientific ways to distinguish between pathogenic and nonpathogenic members of a single species (FDA, 2011).[93] As of December 2012, there are no notices on the FDA GRAS Notice Inventory concerning *E. faecium*.

CONCLUSION

Probiotics are increasingly being used by consumers and are advocated by many healthcare professionals. CVD remains a leading cause of death worldwide, and a reduction in LDL-C remains the primary target for intervention. Recommendations for TLC targeting LDL-C lowering include regular consumption of clinically tested foods or food ingredients, such as phytosterols/stanols and viscous soluble fibers. In recent decades, a number of probiotic strains have been evaluated for their ability to reduce LDL-C and other risk factors for CHD. Probiotics found to lower LDL-C when compared with placebo include *L. reuteri* NCIMB 30242 (8.9–11.6%) and *E. faecium* (5%). The ability of *L. reuteri* NCIMB 30242 to lower blood lipids was established in two multicenter clinical trials in which this probiotic was provided to subjects via food or as supplements. The proposed mechanism of action involves a reduction in cholesterol absorption as a result of deconjugation of bile salts in the small intestine due to BSH activity. *L. reuteri* NCIMB 30242 has GRAS status, which was obtained through scientific procedures and confirmed by an expert panel on its use as an ingredient in food and beverages. Its effects on LDL-C are similar to those of phytosterols/stanols.

A meta-analysis of five studies provides support for the ability of *E. faecium* to lower LDL-C and other blood lipids via its use in fermented dairy products. However, the FDA has raised concern about the use of *E. faecium* as a food ingredient. Mixed results were obtained by a combination of *L. acidophilus* La5 and *B. lactis* Bb12, indicating that this mixture has potential, but more research is needed. Based on these results, the probiotic *L. reuteri* NCIMB 30242 is a viable candidate to consider in recommendations for future TLC dietary studies and as a potential option for inclusion in TLC dietary recommendations. To strengthen the science on probiotics, guidelines for industry on statistical principles for clinical trials have been developed, and their use in future studies to examine and confirm the efficacy of probiotics on LDL-C or other health endpoints is strongly encouraged.

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A roundtable of leading global experts in heart health, probiotics, and nutrition convened once in person to discuss the development of a scientific review paper examining a new role for probiotics in cardiovascular health. Prior to manuscript submission, the author invited members of the roundtable to review the manuscript and provide comments. Roundtable members were then contacted individually by phone conference to review their comments and address questions by the author. The author expresses his appreciation for the expert comments provided. Expert reviewers were as follows: Richard J. Deckelbaum, MD, CM, FRCPC; Robert R. Williams, Professor of Nutrition, Professor of Pediatrics, Professor of Epidemiology, and Director, Institute of Human Nutrition, College of Physicians and Surgeons, Columbia University Medical Center, New York; Jacques Genest, MD FRCP(C), Professor, Faculty of Medicine, McGill University, Novartis Chair in Medicine, McGill University, and Scientific Director, Center for Innovative Medicine, McGill University Health Center/Royal Victoria Hospital,

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