Review Article

Potential of Probiotics in Hypercholesterolemia: A Meta-analysis

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Summary

Human studies on the effects of probiotics on lowering blood cholesterol levels have not yielded conclusive results. A meta-analysis of randomized controlled trials evaluating the effect of probiotics on lipid profile was conducted. Articles were reviewed systematically from web search bases; PubMed, Cochrane Clinical Trial Registry. Those studies which were meeting the inclusion criteria-providing matching placebo, at least single blind for probiotics and placebo, providing mean and standard deviations and not involving the use of probiotics were selected. 14 studies from 12 randomized controlled trials were analyzed providing information of 606 normo/hypercholesterolemic patients using Review Manager 5.3 (The Cochrane Collaboration, Oxford, UK). The pooled mean net change for total cholesterol (TC) is −8.40 mg/dl (−13.63, −3.61), for low-density lipoprotein (LDL) is −6.63 mg/dl (−10.63, −2.63), for high-density-lipoprotein-cholesterol is 0.59 mg/dl (−0.92, 2.09), and for triglycerides is −1.32 mg/dl (−6.49, 3.85). The findings of the analysis conclude that probiotics supplementation does lower serum TC and LDL-cholesterol levels significantly and hence a reduction in the risk factor of developing coronary heart disease.

Keywords: Coronary heart disease, functional foods, hypercholesterolemia, probiotics

Introduction

It is well known higher levels of serum cholesterol are generally considered to be a risk factor for coronary heart disease (CHD).[1] Results from several epidemiological and clinical studies indicate a positive correlation between elevated total serum cholesterol levels, mainly reflecting the low-density lipoprotein (LDL)-cholesterol fraction and risk of emergence of CHD.[2] The WHO has predicted that by 2030, CHD or diseases of heart will remain the leading cause of death, affecting approximately 23.6 million people worldwide. South Asians around the globe have the highest rates of coronary artery disease.[3]

Currently, there is extensive interest in the dietary management of serum cholesterol and triglyceride (TG) levels. This is largely driven by the large numbers of individuals affected, the expense of drug therapy; statins, fibrates, niacin, cholesterol absorption inhibitors, and bile acid sequestrants, and unwanted side effects of such treatments which have concerned long-term use. For this reason, a number of nonpharmacological approaches such as lifestyle modification, inclusion of potential cholesterol-lowering products such as plant stanols, soy, cinnamon, and also probiotic strains resulting in serum cholesterol reduction are continuously being developed and tested.[4] Probiotics are defined as "living microbial supplements that beneficially affect the host animals by

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improving its intestinal microbial balances" or as live microorganisms that when administered in adequate amounts, confer a health benefit on the host.[5]

The virtues of probiotics are already well recognized for general gut health and immunity; various studies have indicated that probiotics may alleviate lactose intolerance, have a positive influence on the intestinal flora of the host,[6] reduce inflammatory or allergic reactions,[7] possess anti-colon cancer effects,[8] antihypertensive effects,[9] and reduce clinical manifestations of atopic dermatitis, Crohn's disease, diarrhea, constipation, candidiasis, and urinary tract infections.[10-12] More recently, probiotics have also been studied for their cholesterol-lowering effects.[13-15] A number of animal studies using a variety of strains have demonstrated this effect.[16-21] Human studies, on the other hand, have yielded inconsistent results with some showing positive significant effects while others with no effects.[22-26] With more such conflicting results, it was considered worthwhile to conduct a meta-analysis of randomized controlled trials evaluating the effect of probiotics on serum lipid levels.

Materials and Methods

PubMed was searched through for randomized controlled trials for evaluating the effect of probiotics on lipid profile using keywords - (cholesterol OR lipid profile OR HDL-cholesterol OR LDL-cholesterol OR TGs) AND (probiotics OR fermented milk products). Those studies were selected which met inclusion criteria: (1) randomized controlled trials which were at least single blinded for participants, both probiotics and placebo; (2) study designs which used a matched control group, i.e., the use of yogurt against yogurt and capsules against capsule; (3) studies which provided values of mean and standard deviation for total cholesterol (TC), LDL-cholesterol, high-density lipoprotein (HDL)-cholesterol and TG levels for both intervention and control groups; (4) use of prebiotics/plant stanols/nutrient apart from probiotics; and (5) publications in English language.

Data abstraction was performed by the author (Smriti Sharma). As presented in Table 1, information related to articles such as first authors' name, year of publication, probiotics type used placebo type, sample size, study design, study duration, dosage, inclusion criteria, age, sex, sample size, baseline values of serum TC; LDL-cholesterol, HDL-cholesterol, and TGs levels

separately for intervention and control arm were abstracted from the articles reviewed. We assessed the methodological quality of the included clinical trials using the modified Jadad scale. The modified Jadad scale scores range from 1 (very low) to 5 (very high). The 5-point quality scale assigns points for randomization (described as randomized, 1 point; described appropriate randomization method, additional point), double blinding (described as double blind, 1 point; described appropriate blinding method, additional point), and follow-up (stated the number and reasons for withdrawal in each group, 1 point) in the report of each trial. All trials were classified into two groups based on scores of <3 as low-quality or ≥3 as high-quality studies.[27]

Statistical analysis

For the meta-analysis, changes in mean and standard deviation of lipid parameters (TC, LDL-cholesterol, HDL-cholesterol, and TGs) were of interest. The values were therefore appropriately modified from mmol/L to mg/dl, and standard errors were converted to standard deviation. The conversion factor used was 1 mg/dl = 0.0259 mmol/L for TC, LDL-cholesterol and HDL-cholesterol and 1 mg/dl = 0.0113 mmol/L for TG values wherever applicable.[27]

As most articles provide the mean and standard deviations at baseline and endpoint, the net change was calculated. For parallel trials, mean net changes for the outcomes listed above were calculated as the difference (probiotic diet minus control diet) of the changes (baseline minus endpoint) in mean values. For crossover trials, mean net changes for the outcomes were calculated as the difference (probiotic diet minus control diet) in values at the end of the intervention and control phases. For computing the change in standard deviations for one taken at baseline and at the end of treatment, the variance of the difference expressed as:

$$\text{Var}\ (\Delta) = 2\ \sigma^2\ (1 - r).$$

was used where r is the observed tracking correlation between cholesterol measures at baseline and follow-up.[28,29] The analysis was conducted using Review Manager 5.3 (The Cochrane Collaboration, Oxford, UK). Heterogeneity between studies was assessed using the Chi-square test and I^2 statistic with values ranging from 0% to 100%. Statistical significance for the test of heterogeneity was set at 0.05. If significant heterogeneity existed, it would have been inappropriate to combine the data for further analysis using a fixed-effects model. In such cases, the random-effect model was used for calculation.

Table 1: Baseline characteristics of the studies selected

Author, Year	Probiotics	Dosage	Study Design	Study Duration	Subjects Characteristics (mg/dl) (avg of both groups)				Subjects (M/F)	Mean Age, y	Sample Size	Jadad Score
					TC	LDL	HDL	TG				
Agerholm-Larsen a, 2000[14]	Lb. acidophilus	450 ml of yogurt/day, Lb. acidohphilus ~ 2×107 cfu/ml	Parallel	8 weeks	I 198 / C 205	123 / 121	50 / 50	126.5 / 177	9/21	39	30	5
Agerholm Larsen b, 2000[14]	E. faecium	450 ml of yogurt/day, E. faecium ~ 6×107 cfu/ml	Parallel	8 weeks	I 195 / C 205	116.2 / 121	50 / 50	146 / 177	9/21	39	30	5
Agerholm Larsen c, 2000[14]	Lb. rhamnosus	450 ml of yogurt/day, Lb. rhamnosus ~ 2×108 cfu/ml	Parallel	8 weeks	I 199 / C 205	119 / 121	52.5 / 50	138 / 177	9/19	39	28	5
Ejitahed et al., 2011[23]	B. lactis BB12 and Lb. Acidophilus LA5	300 g yogurt/day, BB12-7.23×106 LA5-6.04×106 cfu/g	Parallel	6 weeks	I 200 / C 189	122 / 112	48 / 51	151 / 134.5	23/37	51	60	5
Asemi et al., 2012[25]	Lb. acidophilus LA5, B. annimalis BB12	200g yogurt/day, 107 cfu/g	Parallel	9 weeks	I 264 / C 230	140 / 107	74 / 76	250 / 236	0/70	Age group 18-30	70	2
Simons et al., 2006[26]	Lb. fermentum	2 capsules twice/day, 2×109 cfu	Parallel	10 weeks	I 239 / C 243	143 / 151	58 / 69.5	142 / 124	16/28	51.5	44	3
Ataie-Jafari et al., 2009[30]	Lb. acidophilus and B. Lactis (ABY-1, Chr Hansen)	300 g yogurt/day, >106 cfu/g	Crossover	4 weeks	I 224 / C 217	144 / 132	42 / 39	194 / 172	4/10	50.5	14	2
Bertolami et al., 1999[31]	E. faecium	200 g of yogurt/day, 105-109 cfu/g	Crossover	8 weeks	248	172	52	119	11/21	56	32	5
de Roos et al., 1999[32]	Lb. acidophilus L-1	500 ml of yogurt/day, 4.8×109 to 2.7×1010 cfu/500 ml	Parallel	6 weeks	I 198 / C 200	123.5 / 120.5	54 / 58	100 / 108	22/56	40	78	2
Fabian and Elmadfa, 2006[33]	Lb. casei subsp. casei	100 g of yogurt/d for initial 2 weeks followed by 200 g of yogurt/d for 2 weeks, 3.6×108 cfu/g	Parallel	4 weeks	I 166 / C 174	96.5 / 104	62 / 66	59 / 57.5	0/33	24	33	2
Fuentes et al., 2013[34]	Lb. plantarum CECT 7527, 7528 and 7529	1 capsule/d, 1.2×109 cfu (each strain in same proportion)/capsule	Parallel	12 weeks	I 247 / C 253	167 / 168	44 / 46	180 / 190	NS	Age group-18-65	30	3
Naruszweich et al., 2002[35]	Lb. plantarum 299v	400 ml drink/day, 5×107 cfu/ml	Parallel	6 weeks	I 216 / C 213	138 / 136	45 / 46	121 / 124	18/18	42	36	4
Xaio et al., 2003[36]	B. longum BL1	300 ml yogurt/day, 3.7±1.1×108 cfu/g	Parallel	4 weeks	I 244 / C 244	154 / 162	62 54	139 / 139	32/0	44	32	2
Sadrzadeh-Yeganah et al., 2010[37]	B. lactis BB12 and Lb. Acidophilus LA5	300g yogurt/day, 3.9×107 cfu/g	Parallel	6 weeks	I 186 / C 174	110 / 101	49 / 51	98 / 79	0/59	34	59	4

L. acidophilus = Lactobacillus acidophilus, E. faecium = Enterococcus faecium, L. rhamnosus = Lactobacillus rhamnosus, B. animalis = Bifidobacterium animalis, B. lactis = Bifidobacterium lactis, L. casei = Lactobacillus casei, L. plantarum = Lactobacillus plantarum, L. fermentum = Lactobacillus fermentum, B. longum = Bifidobacterium longum, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, TG = Triglyceride, TC = Total cholesterol, NS = Not significant, I = Intervention, C = Control

To explore the influence of low-quality studies, subgroup analysis was performed. The subgroup was selected based on their Jadad score (<3).

Results

Description of studies

In this meta-analysis, overall 12 articles were selected meeting our criteria.[14, 23, 25-26, 30-37] Of these, study conducted by Agerholm-Larsen *et al*.[14] considered three types of yogurts manufactured using three different probiotic strains and compared to the control and was considered as three different trials compared with one same control while performing the analysis.[14] Hence, 14 studies from 12 randomized controlled trials were included in this meta-analysis. Characteristics of the studies selected are summarized in Table 1; includes 2 crossover and 12 parallel trials using various probiotics strains of *Lactobacillus, Bifidobacterium,* and *Enterococcus* general, a pooled total of 606 subjects participated in the trials, age ranging from 18 to 65 years and study duration varying from 4 to 12 weeks.[14, 23, 25-26, 30-37] A staggering 67.32% were women participants, pooled of all the trials included. Subject's baseline mean serum TC values varied from 162.5 mg/dl to 264 mg/dl and mean LDL-cholesterol levels varied from 96.5 mg/dl to 172 mg/dl. Mean baseline HDL-cholesterol levels ranged from 39 mg/dl to 76 mg/dl and mean baseline of TG levels ranged from 57.5 mg/dl to 250 mg/dl. None of the subjects were on cholesterol-lowering drugs.

All studies were at least single-blinded, randomized controlled trials; people with Type 2 diabetes mellitus was the main inclusion criteria in the trial conducted by Ejtahed *et al*.[23] and pregnant women of the third trimester in trial conducted by Asemi *et al*.[25] Two trials selected in the meta-analysis used capsule[26,34] as a carrier for probiotics strains and placebo.

Outcomes of the meta-analysis
Quality assessment
To assess the methodological quality, Jadad scale was used. Using the scale, 58.33% of the studies had a score of 3 or higher and were considered high-quality studies. Most studies were reported as randomized and double blind, but did not describe the appropriate methods for the same, reasons for low score and attributing to low quality studies. Its impact overall was however addressed in subgroup analysis on the basis of Jadad score (<3 or ≥3) for any significant difference.

Effect of probiotics on lipid profiles
The pooled mean net change for the effects of probiotics on serum TC was found to be − 8.40 mg/dl (95% confidence interval [CI]: −13.63 −−3.16) with $P = 0.002$ and I^2 value of 60% on random effects as shown in Figure 1. This shows that probiotic interventions significantly decreased serum TC levels. For LDL-cholesterol levels, the pooled mean net change by the effect of probiotics is − 6.63 mg/dl (95% CI: −10.63−−2.63) on random effects with $P = 0.01$ and I^2 value of 51% [Figure 2]. Probiotic interventions thereby also showed a significant decrease in LDL-cholesterol levels.

For HDL-cholesterol levels, the pooled mean net change by the effect of probiotics was 0.59 mg/dl (95% CI: −0.92–2.09) on fixed effects model with $P = 0.84$ and I^2 value of 0%. For TG-cholesterol levels, the pooled mean net change by the effect of probiotics − 1.32 mg/dl (95% CI: −6.49–3.85) on fixed effects model with
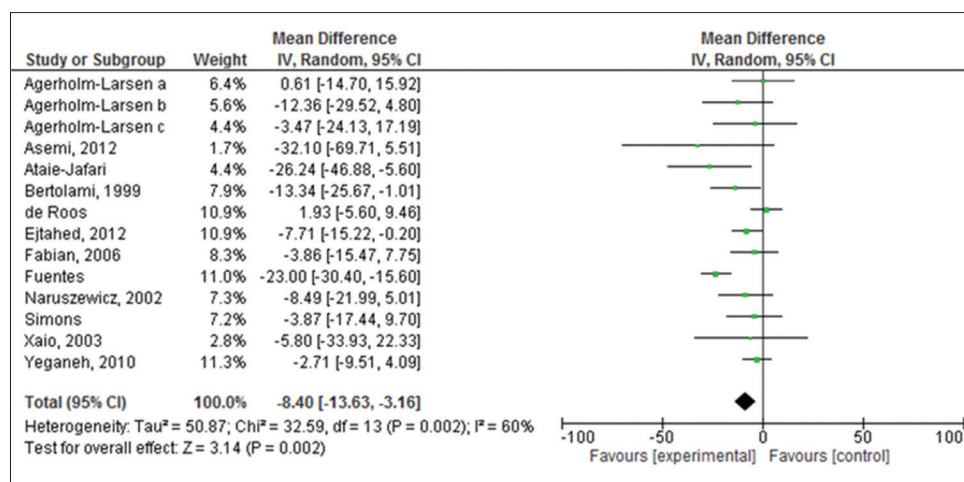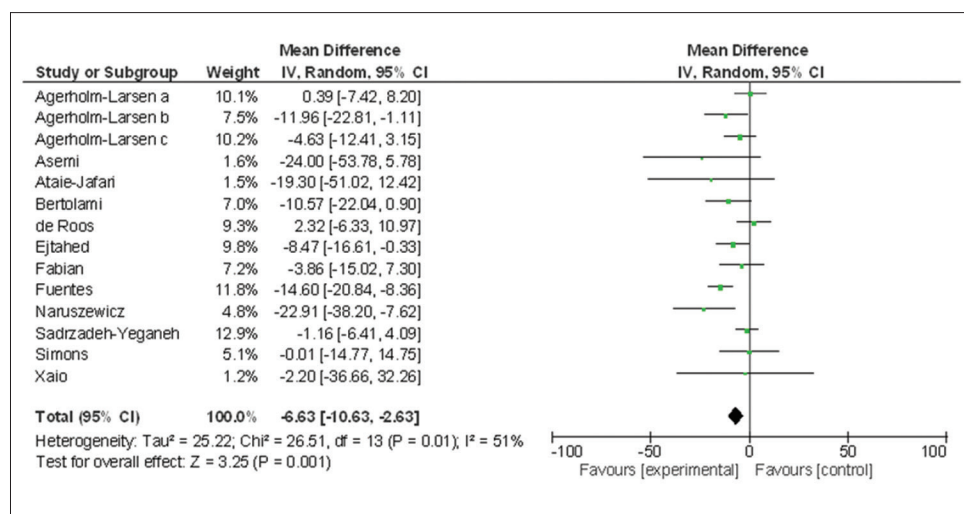

Figure 1: Forest plot depicting effect of probiotics on total cholesterol levels

Figure 2: Forest plot depicting effect of probiotics on LDL-cholesterol levels

$P = 0.51$ and I^2 value of 0%. No statistical significance was observed on the effects of probiotics interventions on HDL-cholesterol and TG levels.

Subgroup analysis

In subgroup analysis, the influence of low-quality studies categorized on the basis of Jadad score on the changes in serum TC and LDL-cholesterol was studied. The effect of probiotics was found to be significant in lowering serum TC levels by excluding studies having a Jadad score <3 with mean difference of −8.97 (95% CI: −14.89–−3.05; $P < 0.003$). However, the overall effect on TC is comparable to the effect obtained when the analysis was restricted to the good quality studies (−8.40 mg/dl [95% CI: −13.63–−3.16]).

The effect of probiotics was found to be significant in lowering LDL-cholesterol levels by excluding the studies having a Jadad score of <3 with mean difference of −7.46 (95% CI: −12.14–−2.79; $P < 0.002$). The overall effect on LDL-cholesterol was also found to be comparable to the effect obtained when the analysis was restricted to the good quality studies.

Publication bias

Funnel plots obtained were interpreted visually by the method described by Sterne *et al.*[38] Visual analysis of the funnel plots obtained for effect on TC and effect on LDL-cholesterol levels does not show evidence for publication bias.

Discussion

The present meta-analysis of 14 randomized clinical trials, with normo-, borderline-, and borderline high-baseline

cholesterol levels there was a significant decrease in the serum TC (−8.40 mg/dl) and LDL-cholesterol levels (−6.63 mg/dl) with the consumption of probiotics as compared to control. A trend toward reduction was observed for both serum TC and LDL-cholesterol levels in 13 out of 14 trials while only six trials showed statistically significant decrease in serum TC levels and four trials in LDL-cholesterol levels respectively with the use of probiotics. However, the use of probiotics does not seem to change levels of HDL-cholesterol and TG levels significantly. Results of present meta-analysis are similar to the ones previously conducted.[39-42] A meta-analysis conducted by Shimizu *et al.* including 11 studies and 26 trials also showed significant reductions in TC and LDL-cholesterol levels. However, our inclusion criteria were limited to the use of probiotics only and not synbiotics to observe and concentrate effect of probiotics in lowering cholesterol levels.

While selecting studies, it was observed that some either used capsule or yogurt as probiotics carrier along with a matching placebo. This added significant calories in the latter while keeping their diets usual as a criterion in most of them. Hence, this difference in data could have influenced the results while comparing to other systematic reviews and under-estimated the effect of probiotics on blood lipids.

The probiotic strains analyzed in this meta-analysis were *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium animalis,* and *Enterococcus faecium.* The cholesterol-lowering effect of probiotics, however,

should be noted that varies from strain to strain. The use of probiotics evaluated as cholesterol-lowering adjunct has its share of limitations. First, all the studies conducted have used dosages and amounts too high to be used on the regular daily basis, the use of small daily amounts of yogurt over a long period needs to be tested and verified. Hence, a long follow-up with feasible amounts is the thrust area and must be examined using proven strains on human subjects in future studies. Second, probiotic food additive used need to ensure that they reach the colon alive and in recommended viable numbers (1×10^7 cfu/g).[43] The viability, however, is subjective to the handling of the product (cold temperatures required) and hence the effects could be varied. Third, the mechanism suggested so far few being probiotics assimilate cholesterol in the growing cells, production of bile salt hydrolase enzyme and the incorporation in the cellular membrane to inhibit the formation of cholesterol micelles by the probiotics bacteria's will bring about a small effect compared to the cholesterol-lowering drugs.[44,45] Hence, cholesterol-lowering drugs such as statins are much a preferred choice over other lifestyle initiatives by therapists. Moreover, not all patients respond equally well to the intervention treatment and hence should be kept on drugs along with lifestyle modifications.

However, many patients prefer nondrug treatments being wary of the side effects of cholesterol-lowering drugs, hence the preference for natural alternatives and self-motivation to avert the levels the natural way are also on the rise. In the end, this meta-analysis concludes with above considerations in mind that probiotics do lower serum cholesterol levels significantly and hence a reduction in the risk factor of developing CHD.

Conflicts of interest

There are no conflicts of interest.

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