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Effect of Probiotics on Blood Lipid Concentrations
A Meta-Analysis of Randomized Controlled Trials

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Abstract: Previous clinical studies have reported mixed results regarding the effect of probiotics on lipid metabolism. Therefore, we conducted a meta-analysis of randomized controlled trials to quantify the direction and magnitude of the potential effect of probiotics on blood lipid concentrations.

Eligible studies were randomized, placebo-controlled trials whose interventions were probiotic products containing live bacteria. The studies reported net changes in lipid profiles (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) and their associated standard deviations (or the data to calculate them). The probiotic products did not contain prebiotics or other active ingredients, and the full article was accessible in English.

The pooled mean net change in lipid profiles and 95% confidence intervals (95% CIs) were calculated. Q statistics and I^2 were calculated to examine heterogeneity. Potential sources of heterogeneity were investigated via subgroup and sensitivity analyses, and publication biases were estimated.

A total of 30 randomized controlled trials with 1624 participants (828 in intervention groups and 796 in placebo groups) were included in this analysis. Subjects treated with probiotics demonstrated reduced total cholesterol and LDL cholesterol compared to control subjects by 7.8 mg/dL (95% CI: −10.4, −5.2) and 7.3 mg/dL (95% CI: −10.1, −4.4), respectively. There was no significant effect of probiotics on HDL cholesterol or triglycerides. The effect of probiotics on total cholesterol and LDL cholesterol depended on a variety of factors. The significant effects were greater for higher baseline total cholesterol levels, longer treatment durations, and certain probiotic strains. In addition, these associations seem stronger in studies supported by probiotics companies.

The studies included in this meta-analysis showed significant heterogeneity as indicated by the Q statistics and I^2. In addition, industry sponsorship may affect study findings.

These results suggest that the use of probiotics may improve lipid metabolism by decreasing total and LDL cholesterol concentrations. However, both the efficacy of probiotics for cholesterol lowering and safety should be investigated further in well-designed clinical trials.

INTRODUCTION

Cardiovascular disease is a leading cause of death worldwide. Abnormal levels of blood lipids, particularly higher concentrations of total and LDL cholesterol, are a major determining factor for cardiovascular disease.[1,2] Because many individuals seek natural or complementary medicine products to improve lipid metabolism, the cholesterol-lowering effect of probiotics has raised much interest. Probiotics are defined as living microorganisms, which when administered in adequate amounts, confer a health benefit on the host.[3] Probiotics are regarded as safe for human consumption, and numerous products are available in the marketplace.

A number of previous studies have been conducted to investigate the role of probiotics in lipid metabolism.[4] Studies using in vitro and animal model data have supported the hypocholesterolemic effect of probiotics.[5,6] However, human clinical studies have yielded mixed results, possibly due to differences in experimental designs, in statistical power due to inadequate sample sizes, in strains and doses of probiotics, or in clinical characteristics of the participants (eg, variation in baseline levels of blood lipids), which increase the difficulty of evaluating the results. A previous meta-analysis by Guo et al[4] including 13 clinical trials also investigated the role of probiotics on lipid profiles and reported an effect of probiotics on total and LDL cholesterol, but this meta-analysis did not investigate other factors that may affect the role that probiotics play in lipid concentrations, such as baseline lipid levels, study design, and bacterial strain.

Understanding the role of probiotics on lipid profiles may provide ideas for new prevention strategies. If applicable, probiotic supplementation may provide a nonpharmacologic alternative for managing cardiovascular disease risk factors. Therefore, we conducted a meta-analysis to provide the most updated and comprehensive evaluation of the results of the previous randomized controlled trials. We aimed to quantify the direction and magnitude of the potential effect of probiotics on blood lipid concentrations. In addition, we evaluated whether these effects differed by factors such as baseline lipid values, study location, study design, intervention duration, probiotic strains, delivery method, and industry sponsorship.

METHODS

Search Strategy and Study Selection

We searched for electronically available research in the PubMed, Embase, and Cochrane Library (Central) databases to identify relevant reports published before February 2, 2015 and

reviewed the reference lists of original studies and review articles investigating the effect of probiotics on blood lipid concentrations. The following keywords were used: (Cholesterol OR "plasma lipids" OR triglycerides OR HDL OR LDL OR "serum lipids") and (probiotic* OR streptococcus* OR lactobacill* OR saccharomyces* OR enterococcus* OR lactococcus* OR bifidobacter* OR VSL#3 OR yogurt OR yoghurt OR "fermented milk" OR "sour milk"). The searches were limited to human studies written in English and clinical trials.

In this meta-analysis, the following inclusion criteria were used for study selection: randomized, placebo-controlled trials; probiotic products containing live bacteria as the intervention; reported net changes in lipid profiles (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) and their associated standard deviations (or data to calculate them); probiotic products not containing prebiotics or other active ingredients; and full article accessible in English. Duplicated previous research was excluded. We assessed the relevance of all the studies using a hierarchical approach based on the title, abstract, and full article. Related reference and review articles were searched to identify other relevant publications.

Data Extraction and Quality Assessment

Two investigators independently extracted the data from all eligible publications using the selection criteria listed previously. Any disagreement was resolved by discussion. Interrater reliability for inclusion decisions was quantified using Cohen κ statistic. We extracted the following information from each study when available: first author's name, year of publication, study location (country), probiotic strains, probiotic delivery method, study design, intervention duration, sample size, participant age, baseline total cholesterol, participant health status, and financial sponsorship.

We assessed the methodological quality of the included clinical trials using the modified Jadad scale[7] and Cochrane risk of bias tool.[8,9] 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 2 groups based on scores of <4 or ≥4. The risk of bias assessment appraises a study in 6 domains.[8] adequate sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. Each domain can be rated as "yes" (low risk of bias), "no" (high risk of bias), or "unclear" (uncertain risk).

Data Analysis

The effect of probiotic use on lipid concentrations was defined as the mean difference between the intervention groups and control groups for pre–post changes in total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. We performed a meta-analysis and calculated the mean differences and 95% confidence intervals (95% CIs).

Before conducting the meta-analysis, the lipid levels in mmol/L were converted to mg/dL. The conversion factor was 1 mmol/L = 38.67 mg/dL for cholesterol and 1 mmol/L = 88.57 mg/dL for triglycerides. The mean net changes (mean values ± standard deviation) in the total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides for each study were calculated. The net effect of probiotic products on lipid concentrations was calculated as the difference in the mean lipid concentration change between the intervention and control groups in parallel trials and the difference in the lipid concentration between the intervention and control periods in crossover trials. Unreported standard deviation values were imputed from standard errors or CIs using a standard formula or calculated by assuming a correlation coefficient of 0.8 between the variances at baseline and final lipid concentrations.

The homogeneity of the effect size was tested using the Q test at the $P < 0.05$ level of significance. To measure the percentage of total variation across studies by heterogeneity rather than by chance, the I^2 statistic was calculated. To calculate the combined effect size, we used either fixed or random effects models based on the results of the Q statistics. To explore the influence of other factors, a series of subgroup analyses was performed. Subgroups were selected based on baseline total cholesterol (high, >240 mg/dL; borderline high, 200–240 mg/dL; normal, <200 mg/dL), study location (Asian or Non-Asian region), intervention duration (<8 or ≥8 weeks), study design (parallel or crossover), blinding (single blind or double blind), probiotic strain (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, or *Enterococcus faecium*), probiotic delivery method (milk, yogurt/cheese, or capsule/drink), methodological quality (high vs. low), sponsorship by probiotics company, and number of participants in each trial. A sensitivity analysis was also conducted for study one at a time to examine the influence of a single study on the overall effect.

We assessed publication bias using Begg funnel plot and Egger test. If publication bias exists, the Begg funnel is asymmetric or the Egger test P-value is <0.05. STATA version 10.0 (StataCorp, College Station, TX) was used for all analyses. A P-value <0.05 was considered statistically significant.

RESULTS

Search Results

A flow chart depicting the literature search and selection is presented in Figure 1. Using the search terms mentioned above, 271 articles were retrieved. We screened these articles based on the title/abstract and excluded 212 articles. We then evaluated the full text of the remaining 59 articles, and 34 articles were excluded for the following reasons: not written in English (n = 2); outcome measure was not the lipid profile (n = 6); did not investigate only the effect of probiotics (n = 19); no usable data were reported (n = 6); or duplicated data (n = 1). There was agreement between the 2 reviewers for study screening (κ statistic = 0.93). Although this study analyzed 27 articles, 2 articles can be viewed as 2 or 3 separate trials. The study by Agerholm-Larsen et al[10] examined 3 different yogurts manufactured with 3 kinds of probiotic strains and was thus considered 3 trials. Another study by Ivey[11] used 2 different delivery methods (milk and yogurt). Three trials did not report the effect of probiotics on LDL cholesterol.[12–14] Therefore, 27 articles[10–36] are included in this meta-analysis: 30 randomized controlled trials for total cholesterol, HDL cholesterol, and triglycerides and 27 trials for LDL cholesterol.

Trial Characteristics

The characteristics of the studies included in this meta-analysis are shown in Table 1, comprising 30 clinical
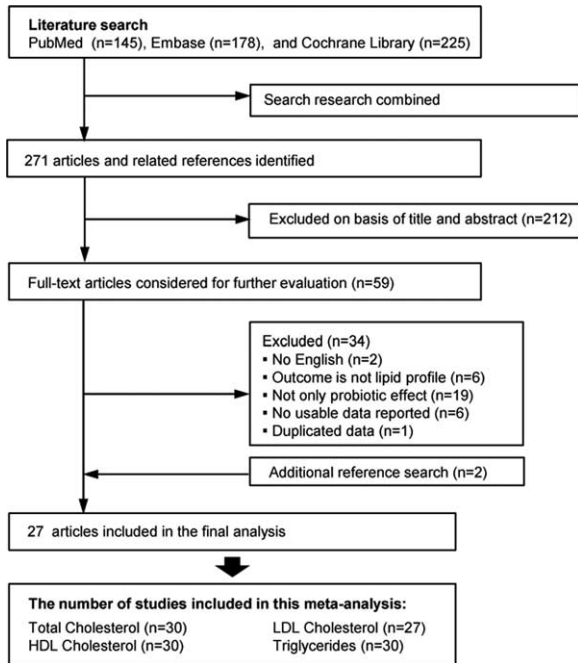
FIGURE 1. Flow diagram of study identification and selection.

randomized controlled trials. When divided by baseline total cholesterol level, 8 studies considered participants with high cholesterol, 12 studies considered participants with borderline cholesterol, and 10 studies considered participants with normal cholesterol. Eleven studies were performed in Asia and 19 in Western countries. Intervention duration ranged from 4 to 12 weeks and 17 studies lasted less than 8 weeks. Twenty-four studies had a parallel design, 5 used a crossover design, and 1 conducted trials within subjects. Ten studies were single blinded and 20 studies were double blinded. Eighteen studies had a sample size less than 50. When we evaluated study quality, 20 studies scored less than 4 because most did not describe the blinding or randomization processes appropriately. Several probiotic strains were used including *L. acidophilus* (11 studies), *B. lactis* (6 studies), *L. plantarum* (3 studies), *L. helveticus* (3 studies), and *E. faecium* (3 studies), and so on. For the probiotic delivery method, 8 studies used milk, 15 studies used yogurt or cheese, and 7 studies used a capsule or drink. Among the included studies, 24 reported being supported by probiotic companies: 15 studies were funded by probiotic companies and 9 studies were provided with intervention materials (probiotics).

Quality Assessment

To assess the methodological quality of the 27 included studies, we used both the modified Jadad scale and Cochrane risk of bias assessment tool (see Table S1, http://links.lww.com/MD/A461, Supplemental Content, http://links.lww.com/MD/A461, which illustrated the methodological quality of 27 included trials). Using the modified Jadad scale, 10 studies (33%) were rated as high-quality studies (modified Jadad scores ≥4). Most studies were reported as randomized and double blind, but did not describe the appropriate methods. Based on the Cochrane risk of bias assessment tool, 7 studies[13,14,17,18,28,30,36] had at least 1 domain rated as high risk of

bias: neither the participants nor the evaluators were blinded in 4 studies,[13,17,18,30] and the details of dropout were not reported in 3 studies.[14,28,36] Most studies did not describe the randomization sequence generation, allocation concealment, or blinding of outcome assessment; thus, they were rated as having an unclear risk of bias. Although most studies reported using a double-blind study design, blinding of outcome assessment was rated as unclear risk because the assessors might not be blinded to treatment allocation.

The Effect of Probiotics on Lipid Concentrations

The pooled mean net change in total cholesterol for those treated with probiotics compared to controls was −7.8 mg/dL (95% CI: −10.4, −5.2; *P* for heterogeneity <0.01, $I^2 = 64\%$) (Table 2, Figure 2). The pooled mean net change in LDL cholesterol for those treated with probiotics compared to controls was −7.3 mg/dL (95% CI: −10.1, −4.4; *P* for heterogeneity <0.01, $I^2 = 77\%$) (Table 2, Figure 3). In subgroup analyses, the effect of probiotics was slightly stronger in studies that used subjects with high baseline cholesterol, that used particular study designs (longer durations, larger sample sizes, double-blind), and that were supported by probiotics companies (Table 2). Among the strains included in more than 3 studies, *L. acidophilus*, a mixture of *L. acidophilus* and *B. lactis*, and *L. plantarum* were associated with significant reductions in total cholesterol and LDL cholesterol.

The pooled mean net changes in HDL cholesterol and triglycerides were not statistically significant (Table 2; see Figure S1, http://links.lww.com/MD/A461 and Figure S2, http://links.lww.com/MD/A461, Supplemental contents, http://links.lww.com/MD/A461, which illustrated the forest plots of estimates of mean difference in HDL cholesterol and triglycerides). In subgroup analyses, only studies with high-quality scores showed a significant decrease in triglycerides.

To investigate the robustness of these findings, sensitivity analyses were conducted by excluding studies of pregnant women[17] or type 2 diabetics,[21,28] studies including participants under 20 years old,[24,25] and low-quality studies (Table 3). We could not observe a strong difference in probiotics effect after excluding these studies.

Publication Bias

In this meta-analysis, publication bias was assessed by examining Begg funnel plots (see Figure S3, http://links.lww.com/MD/A461, Supplemental contents, http://links.lww.com/MD/A461, which illustrated publication bias) and conducting Egger linear regression tests, which suggested that the selection of publication was a likely source of bias. We then used the trim-and-fill method to adjust for funnel plot asymmetry, but the results showed no trimming performed and data unchanged (''no trimming performed, data unchanged''). However, we observed that the publication bias was reduced in the sensitivity analyses after excluding studies that were considered lower quality in this meta-analysis (Table 3).

DISCUSSION

This meta-analysis of 30 randomized controlled trials found that participants receiving probiotic bacteria supplementation had significantly lower concentrations of total cholesterol and LDL cholesterol compared to control subjects. However, the use of probiotics does not seem to change levels of HDL cholesterol or triglycerides. The present meta-analysis provides

TABLE 1. Characteristics of the Enrolled Randomized Controlled Studies in This Meta-Analysis

Study	Country	Probiotics	Delivery Method	Design	Duration (weeks)	No. of Subjects Randomized	Age, year (Mean Age)	Mean Baseline TC (mg/dL)
Agerbaek et al[15]	Denmark	*E. faecium*	Milk	P	6	57	44	231.3
Hata et al[12]	Japan	*L. helveticus; S. cerevisiae*	Milk	P	8	30	40−86	248.3
Anderson et al[16]	USA	*L. acidophilus*	Yogurt	X	4	40	(56.4)	248.3
Bertolami et al[19]	Brazil	*E. faecium*	Milk	X	8	32	36−65 (56)	249.0
de Roos et al[20]	Netherland	*L. acidophilus*	Yogurt	P	6	78	18−65 (40)	199.2
Agerholm-Larsen et al[10]	Denmark	*E. rhamnosus*	Yogurt	P	8	28	18−25 (37.9)	199.5
Agerholm-Larsen et al[10]	Denmark	*E. faecium*	Yogurt	P	8	34	18−25 (37.8)	195.7
Agerholm-Larsen et al[10]	Denmark	*L. acidophilus*	Yogurt	P	8	30	18−25 (38.6)	198.4
Kawase et al[13]	Japan	*L. casei*	Milk	P	8	20	30−51 (40.1)	223.9
Naruszewicz et al[29]	Sweden	*L. plantarum*	Drink	P	6	36	35−45 (42)	214.2
Xiao et al[36]	Japan	*B. longum*	Yogurt	P	4	32	(44)	214.2
Mizushima et al[14]	Japan	*L. helveticus; S. cerevisiae*	Milk	P	4	46	23−59	200.3
Lewis and Burmeister[27]	UK	*L. acidophilus*	Capsule	X	6	86	20−65	257.2
Fabian and Elmadfa[22]	Austria	*L. casei*	Yogurt	X	4	33	22−29	170.2
Simons et al[34]	Australia	*L. fermentum*	Capsule	P	10	44	30−75 (51.4)	241.3
Ataie-Jafari et al[18]	Iran	*L. acidophilus; B. lactis*	Yogurt	X	6	14	(50.5)	219.3
Sadrzadeh-Yeganeh et al[32]	Iran	*L. acidophilus; B. lactis*	Yogurt	P	6	59	19−49 (34)	180.2
Usinger et al[35]	Denmark	*L. helveticus*	Milk	P	8	94	54	197.2
Ejtahed et al[21]	Iran	*L. acidophilus; B. lactis*	Yogurt	P	6	60	30−60	191.4
Asemi et al[17]	Iran	*L. acidophilus; B. animalis*	Yogurt	−	9	70	18−30	248.3
Gobel et al[24]	Denmark	*L. salivarius*	NA	P	12	50	12−15	165.9
Jones et al[26]	Czech Republic	*L. reuteri*	Capsule	P	9	127		257.5
Fuentes et al[23]	Spain	*L. plantarum*	Capsule	P	12	60	18−65	250.6
Sharafedtinov et al[33]	Russia	*L. plantarum*	Cheese	P	3	40	30−60	213.9
Guardamagna et al[25]	Italy	*B. animalis; B. bifidum; B. longum*	Capsule	X	12	38	10.8 ± 2.1	223.1
Mohamadshahi et al[28]	Iran	*L. acidophilus; B. lactis*	Yogurt	P	8	42	(51)	220.4
Ogawa et al[30]	Japan	*L. gasseri*	Milk	WB	4	20		215.4
Rajkumar et al[31]	India	VSL#3	Capsule	P	6	30	40−60 (49)	181.8
Ivey et al[11]	Australia	*L. acidophilus; B. lactis*	Milk	P	6	79	>50 (65)	201.5
Ivey et al[11]	Australia	*L. acidophilus; B. lactis*	Yogurt	P	6	77	>50 (68)	210.4

B = bifidobacterium, E = enterococcus, L = lactobacillus, P = parallel, TC = total cholesterol, WB = within subject, X = crossover.

an updated and comprehensive report of the role of probiotics in lipid metabolism. Our findings are similar to those of previous meta-analyses of randomized controlled trials considering the association between probiotics and lipid profiles.[4,37] A previous meta-analysis of 13 clinical trials conducted by Guo et al[4] reported the hypocholesterolemic effects of probiotics on total cholesterol (6.6 mg/dL) and LDL cholesterol (5.0 mg/dL), which are similar to those observed in our meta-analysis. In a meta-analysis of 5 randomized controlled studies on the hypocholesterolemic effects of *E. faecium*, a significant reduction in serum total cholesterol (−8.5 mg/dL) and LDL cholesterol (−7.7 mg/dL) was observed after the intervention.[37]

However, these meta-analyses did not provide the information that might influence the differential effect of probiotics between trials. In this meta-analysis, we conducted subgroup analyses to consider possible explanations for heterogeneity[38] and found that the effect of probiotics may differ by factors such as probiotic strains, trial designs, and baseline lipid levels.

Most importantly, the bacterial strains, dosages, and delivery methods may alter the effect of probiotics on lipid concentrations. Several studies have implied strain-specific effects on blood cholesterol concentrations[11,16] that may be associated with differences in specific metabolite production among strains[33] or with survival ability in acid and bile

TABLE 2. Subgroup Analyses of Effect of Probiotics on Lipid Metabolism

Subgroup	No. Trials	Total Cholesterol		LDL Cholesterol		HDL Cholesterol		Triglycerides	
		Net Change (95% CI) mg/dL	P (I²%)*	Net Change (95% CI) mg/dL	P (I²%)	Net Change (95% CI) mg/dL	P (I²%)	Net Change (95% CI) mg/dL	P (I²%)
Total	30	-7.8 (-10.4, -5.2)	<0.01 (64)	-7.3 (-10.1, -4.4)	<0.01 (77)	-0.24 (-1.6, 1.1)	<0.01 (82)	-5.3 (-11.1, 0.4)	<0.01 (63)
Baseline TC†									
High	8	-12.6 (-20.4, -4.7)	<0.01 (83)	-9.4 (-17.5, -1.3)	<0.01 (80)	-2.1 (-5.0, 0.7)	<0.01 (68)	-15.4 (-34.0, 3.5)	<0.01 (83)
Borderline high	12	-7.8 (-11.1, -4.5)	0.32 (13)	-7.7 (-10.9, -4.5)	0.03 (52)	-0.9 (-3.1, 1.3)	<0.01 (72)	-6.4 (-19.2, 6.4)	<0.01 (65)
Normal	10	-5.0 (-8.3, -1.6)	<0.01 (63)	-4.8 (-8.7, -1.0)	<0.01 (83)	1.6 (-0.2, 3.4)	<0.01 (83)	-1.2 (-5.3, 2.9)	0.95 (0)
Location									
Asia	11	-8.3 (-12.8, -3.9)	<0.01 (61)	-9.1 (-14.2, -4.0)	0.01 (65)	-0.3 (-3.3, 2.7)	<0.01 (90)	-4.9 (-10.6, 0.8)	0.44 (1)
Non-–Asia	19	-7.5 (-10.9, -4.1)	<0.01 (67)	-6.5 (-10.1, -3.0)	<0.01 (80)	0.3 (-0.5, 1.0)	0.32 (11)	-4.0 (-11.8, 3.9)	<0.01 (73)
Duration of treatment									
<8 weeks	17	-6.5 (-8.6, -4.4)	0.37 (8)	-5.2 (-8.4, -2.1)	0.01 (53)	-0.5 (-2.6, 1.6)	<0.01 (84)	-1.4 (-5.3, 2.6)	0.05 (39)
≥8 weeks	13	-10.5 (-15.5, -5.5)	<0.01 (81)	-10.2 (-15.2, -5.1)	<0.01 (87)	0.1 (-1.6, 1.8)	<0.01 (73)	-11.0 (-22.2, 0.2)	<0.01 (77)
Study design									
Parallel	24	-7.9 (-10.9, -4.9)	<0.01 (69)	-7.8 (-11.0, -4.6)	<0.01 (79)	0.1 (-1.4, 1.6)	<0.01 (83)	-4.1 (-10.3, 2.1)	<0.01 (64)
Crossover	5	-7.6 (-11.9, -3.2)	0.18 (36)	-5.7 (-14.1, 2.7)	<0.01 (74)	-1.6 (-3.7, 0.6)	0.29 (20)	-10.9 (-27.7, 5.9)	0.03 (62)
Blinding									
Single-blind	9	-6.6 (-12.1, -1.2)	0.01 (59)	-6.6 (-13.3, 0.1)	0.03 (60)	-2.4 (-5.0, 0.2)	<0.01 (62)	-1.8 (-7.0, 3.3)	0.37 (8)
Double-blind	21	-8.3 (-11.3, -5.3)	<0.01 (63)	-7.5 (-10.7, -4.2)	<0.01 (80)	0.6 (-0.8, 2.0)	<0.01 (80)	-5.5 (-13.2, 2.2)	<0.01 (71)
Sample size									
<50	18	-6.9 (-9.8, -4.0)	<0.01 (56)	-7.5 (-11.1, -3.8)	<0.01 (78)	-0.7 (-2.7, 1.4)	<0.01 (88)	-5.0 (-10.6, 0.7)	0.08 (34)
≥50	12	-9.0 (-14.2, -3.7)	<0.01 (72)	-7.1 (-12.2, -2.0)	<0.01 (77)	0.5 (-0.5, 1.5)	0.65 (0)	-5.6 (-17.3, 6.0)	<0.01 (79)
Methodological quality									
Low (Jadad score <4)	20	-7.6 (-11.0, -4.3)	<0.01 (68)	-7.4 (-11.0, -3.8)	<0.01 (80)	-0.7 (-2.0, 0.6)	<0.01 (58)	-4.4 (-11.4, 2.7)	<0.01 (68)
High (Jadad score ≥4)	10	-8.4 (-12.5, -4.3)	0.05 (47)	-7.2 (-12.3, -2.2)	<0.01 (71)	1.0 (-1.4, 3.4)	<0.01 (86)	-8.0 (-14.9, -1.2)	0.08 (42)
Probiotic strain									
L. acidophilus	11	-6.1 (-10.4, -1.9)	0.05 (46)	-5.7 (-10.4, -0.9)	<0.01 (68)	0.2 (-1.7, 2.1)	<0.01 (65)	-0.7 (-6.5, 5.2)	0.88 (0)
L. acidophilus + B. lactis	6	-6.0 (-10.4, -1.6)	0.15 (39)	-8.2 (-14.9, -1.5)	0.01 (65)	0.7 (-2.3, 3.8)	<0.01 (79)	-2.1 (-9.5, 5.3)	0.66 (0)
L. plantarum	3	-17.5 (-24.6, -10.3)	0.14 (50)	-13.9 (-19.3, -8.5)	0.91 (0)	-1.7 (-7.1, 3.6)	0.01 (79)	-22.3 (-90.8, 46.1)	<0.01 (94)
L. helveticus	3	-2.0 (-5.6, 1.6)	0.36 (3)	-7.7 (-11.9, -3.5)	—	-4.9 (-10.6, 0.8)	0.01 (81)	4.0 (-4.2, 12.2)	0.89 (0)
E. faecium	3	-8.4 (-18.4, 1.6)	<0.01 (83)	-8.8 (-20.5, 3.0)	<0.01 (90)	1.0 (-0.7, 2.6)	0.32 (13)	4.9 (-2.8, 12.6)	0.21 (36)
Delivery method									
Milk	8	-6.8 (-11.7, -1.9)	0.04 (52)	-9.2 (-13.4, -4.9)	0.19 (35)	-2.1 (-4.5, 0.3)	<0.01 (66)	3.8 (-2.0, 9.5)	0.15 (36)
Yogurt/cheese	15	-8.3 (-12.4, -4.1)	<0.01 (71)	-7.6 (-11.5, -3.6)	<0.01 (82)	0.8 (-0.6, 2.1)	<0.01 (59)	-8.1 (-16.9, 0.74)	<0.01 (69)
Capsule/drink	7	-8.4 (-13.3, -3.5)	0.01 (56)	-5.8 (-12.2, 0.7)	<0.01 (76)	-0.1 (-3.5, 3.3)	<0.01 (86)	-7.9 (-20.7, 4.9)	0.02 (60)
Sponsorship by probiotics company									
Yes	24	-8.1 (-11.0, -5.2)	<0.01 (67)	-7.6 (-10.7, -4.5)	<0.01 (78)	-0.32 (-1.85, 1.20)	<0.01 (83)	-4.4 (-10.7, 1.8)	<0.01 (67)
Funding	15	-8.3 (-11.9, -4.6)	<0.01 (64)	-7.7 (-11.9, -3.5)	<0.01 (83)	0.06 (-0.77, 0.88)	0.16 (27)	-1.0 (-7.8, 5.7)	0.04 (42)
Probiotics	9	-8.0 (-13.2, -2.7)	<0.01 (74)	-7.4 (-11.6, -3.1)	0.02 (57)	0.52 (-2.47, 3.51)	<0.01 (89)	-9.5 (-21.2, 2.2)	<0.01 (82)
No	6	-6.5 (-13.0, -0.1)	0.09 (47)	-6.1 (-15.1, 3.0)	0.01 (72)	0.04 (-3.71, 3.79)	<0.01 (80)	-7.5 (-18.3, 3.4)	0.21 (31)

B = bifidobacterium, CI = confidence interval, E = enterococcus, HDL = high-density cholesterol, L = lactobacillus, LDL = low-density cholesterol, TC = total cholesterol.
* P for heterogeneity.
† Baseline total cholesterol: high, ≥240 mg/dL; borderline, 200–240 mg/dL; low, <200 mg/dL.
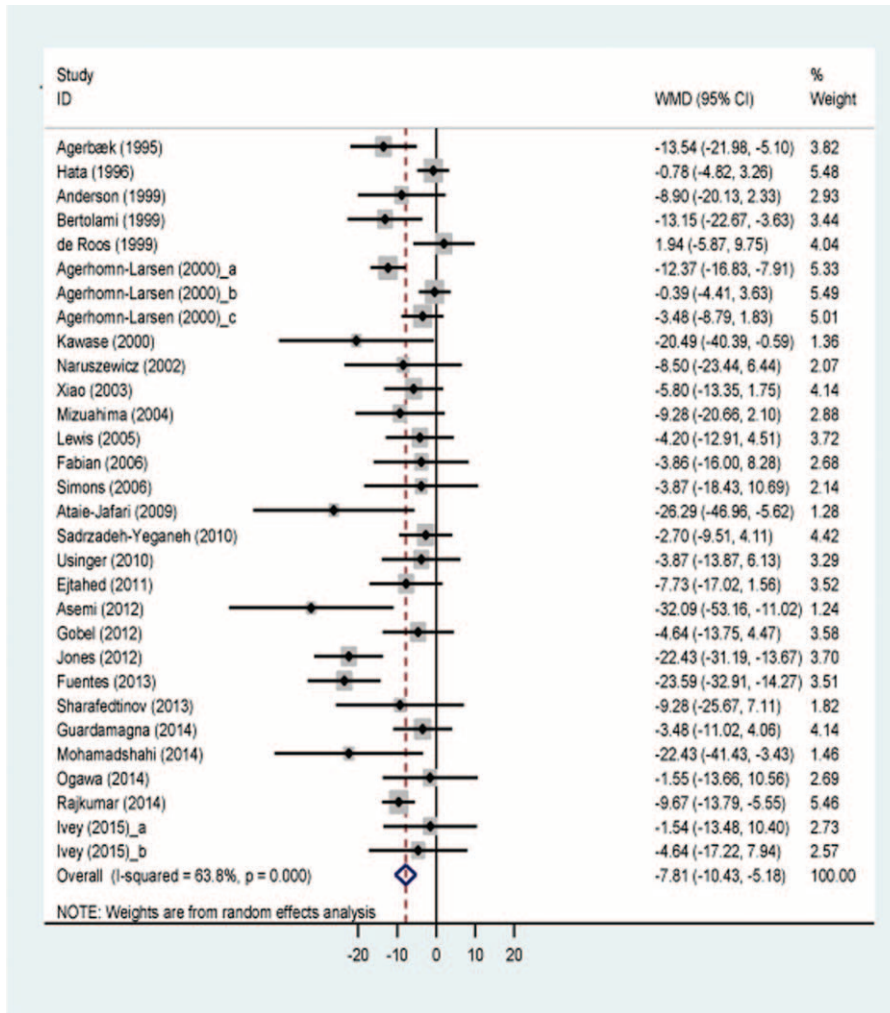
FIGURE 2. Forest plots of estimates of mean differences (95% CI) in total cholesterol using a random effects model.

environments.[27] In the present meta-analysis, we observed slightly different effects according to the probiotic strain. Subgroup analyses indicated that *L. acidophilus*, a mixture of *L. acidophilus* and *B. lactis* mixtures, and *L. plantarum* had significant beneficial effects, while *L. helveticus* and *E. faecium* did not. Various *Lactobacillus* spp. have demonstrated a variety of health-improving effects.[39] *L. plantarum* and *L. acidophilus* are able to survive in acid and bile environments and easily colonize the human intestinal tract.[27,40] Thus, these strains are candidates for therapeutic dietary interventions for hyperlipidemia. However, these effects may be altered by the probiotic dose and delivery method (eg, fermented dairy products, freeze-dried bacteria). Although dairy products could be a more effective mediums for administering probiotic bacteria,[32] the addition of large amounts of dairy products to the diet may increase fat consumption. Therefore, it is difficult to interpret the results of hypocholesterolemic effects of dairy products.[16] Determining the optimal strains that produce a hypocholesterolemic effect[27] and reinforce healthy bacteria without altering the balanced microbial ecosystem of healthy individuals is important.[41] Various processing advances, such as microencapsulation and bacterial coating, and the addition of prebiotic compounds used as growth factors by probiotic

organisms help optimize the delivery and survival of strains at the site of action.[39]

The clinical heterogeneity of participants, particularly of baseline lipid profiles, may affect the role of probiotics in lipid metabolism. In this meta-analysis, subgroup analyses using participants with high baseline cholesterol showed a much stronger improvement in total and LDL cholesterol. This finding suggests that the hypocholesterolemic benefits of probiotics may be stronger in populations with higher baseline total cholesterol levels,[11] and the lack of an effect reported in some studies may be the result of participants' relatively good baseline cholesterol levels.[22,32] In addition, participant weight change during an intervention could influence the observed effect. Sharafedtinov et al[33] reported that the consumption of probiotic cheese was associated with a more efficient reduction in BMI compared with ordinary cheese. Finally, studies conducted in Asia showed slightly greater beneficial effects, and these differences may be associated with dietary and lifestyle differences such as the use of antibiotics or the consumption of fruits and vegetables.

Study design may influence the probiotic effect. In this meta-analysis, subgroup analyses found that double-blind studies showed greater improvement in lipid metabolism than
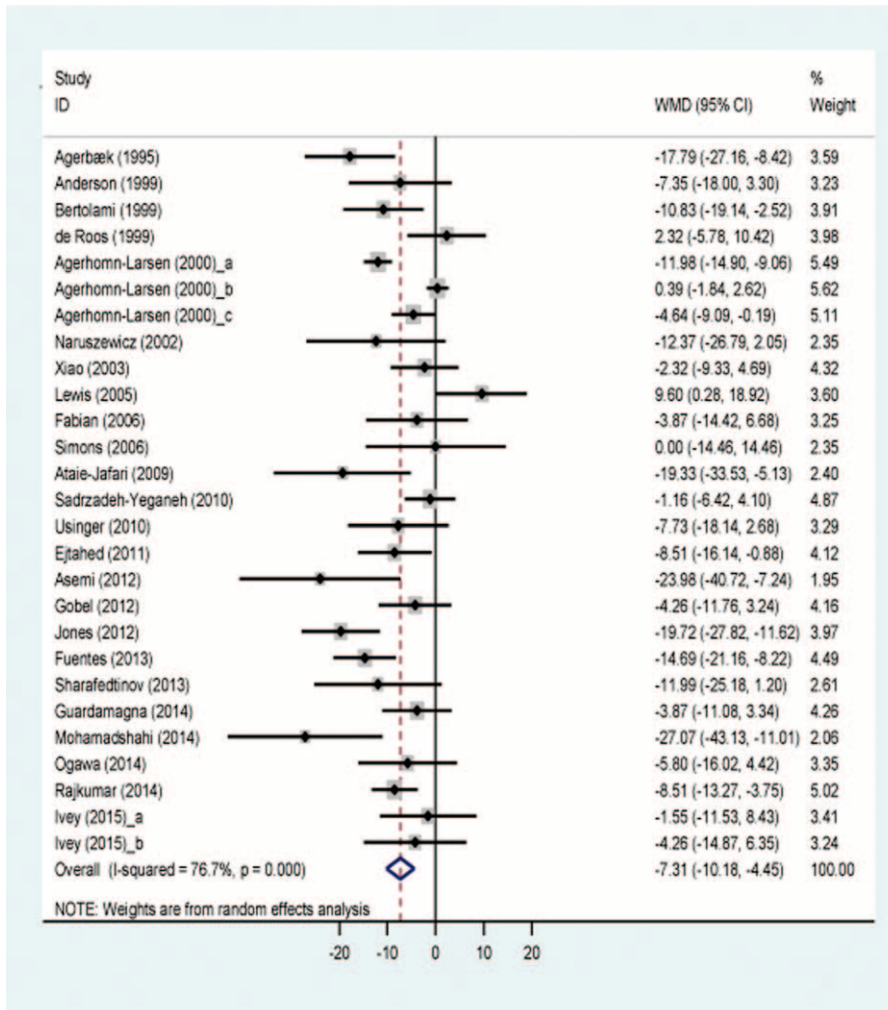
FIGURE 3. Forest plots of estimates of mean differences (95% CI) in LDL cholesterol using a random effects model.

those using single blinding. Parallel and crossover studies showed similar results. Although crossover studies have methodological advantages compared to parallel studies because participants act as their own controls, insufficient washout periods may induce additional biases.[4] The length of the treatment period also could affect the findings. A stronger association was observed when the study lasted more than 8 weeks, and a reduction in compliance over time could explain the decreased effects observed in some long-term studies. Findings from these subgroup analyses may help us interpret the hypocholesterolemic effects of probiotics reported in previous studies and provide information for improving the probiotic effect on lipid metabolism.

Although the underlying cholesterol-lowering mechanisms have not yet been sufficiently elucidated, several mechanisms have been proposed.[42] Probiotic bacteria can reduce the absorption of cholesterol in the intestine by binding and incorporating it into the cell membrane. Some of these bacteria can assimilate cholesterol directly from the gastrointestinal tract.[43] Additionally, probiotics may reduce the enterohepatic circulation of bile salts due to hydrolase activity, which catalyzes the hydrolysis of deconjugated bile salts into free bile acids and coprecipitates with cholesterol.[44–46] Consequently, the liver requires higher mobilization of systemic cholesterol for the de novo synthesis of bile salts, thus reducing plasma cholesterol levels. Furthermore, the short chain fatty acids produced by probiotic bacteria may also inhibit hepatic cholesterol synthesis and/or redistribution of cholesterol from the plasma to the liver.[47] The present meta-analysis implies a stronger effect of probiotics on total cholesterol and LDL cholesterol than on triglyceride and HDL cholesterol levels. It has been suggested that probiotics may alter the pathways of cholesterol esters and lipoprotein transporters[45] and may promote the excretion of cholesterol and bile acid rather than affecting hepatic cholesterol synthesis.[36] However, additional studies are required to further elucidate the underlying mechanisms.

The meta-analysis of clinical trials faces several important limitations. As mentioned above, the studies included in this meta-analysis showed significant heterogeneity as indicated by the Q statistics and I². Although we conducted stratified analyses to identify the sources of heterogeneity, such heterogeneity did not disappear in most subgroups, except for certain probiotic strains. Clinical heterogeneity between studies can lead to statistical heterogeneity in their results. In addition, this

TABLE 3. The Effect of Probiotics on Lipid Metabolism in Sensitivity Analysis

Meta-Analysis	No. Trials	Total Cholesterol Net Change (95% CI) mg/dL	Total Cholesterol P (I²%)*	LDL Cholesterol Net Change (95% CI) mg/dL	LDL Cholesterol P (I²%)*	HDL Cholesterol Net Change (95% CI) mg/dL	HDL Cholesterol P (I²%)*	Triglycerides Net Change (95% CI) mg/dL	Triglycerides P (I²%)*
A	30	−7.8 (−10.4, −5.2)	<0.01 (64)	−7.3 (−10.1, −4.4)	<0.01 (77)	−0.24 (−1.6, 1.1)	<0.01 (82)	−5.3 (−11.1, 0.4)	<0.01 (63)
P for bias		0.05		0.02		<0.01		0.09	
B	27	−7.2 (−9.9, −4.6)	<0.01 (64)	−6.5 (−9.4, −3.5)	<0.01 (77)	−0.6 (−2.1, 0.9)	<0.01 (83)	−4.7 (−10.7, 1.3)	<0.01 (66)
P for bias		0.16		0.28		<0.01		0.17	
C	28	−7.7 (−10.4, −5.0)	<0.01 (65)	−7.6 (−10.7, −4.6)	<0.01 (78)	−0.3 (−1.8, 1.1)	<0.01 (83)	−3.9 (−9.5, 1.6)	<0.01 (58)
P for bias		0.08		0.10		<0.01		0.09	
D	25	−7.7 (−10.4, −5.0)	<0.01 (65)	−6.7 (−9.9, −3.5)	<0.01 (79)	−0.8 (−2.3, 0.8)	<0.01 (84)	−3.2 (−9.0, 2.6)	<0.01 (61)
P for bias		0.08		0.27		<0.01		0.20	
E	18	−7.4 (−10.4, −4.4)	<0.01 (69)	−7.0 (−10.6, −3.4)	<0.01 (82)	−0.4 (−2.1, 1.4)	<0.01 (86)	−3.5 (−11.0, 3.9)	<0.01 (68)
P for bias		0.31		0.32		<0.01		0.19	

CI = confidence interval, HDL = high-density cholesterol, LDL = low-density cholesterol, A = included all studies, B = excluded studies with pregnant women or type 2 diabetics, C = excluded studies with participants aged under 20 years old, D = excluded studies with pregnant women, type 2 diabetes, or participants under 20 years old, E = excluded studies with pregnant women, type 2 diabetes, participants under 20 years old, or study quality below 3.
* P for heterogeneity.

meta-analysis indicated possible publication bias, possibly because we included only studies published in English. Asymmetrical funnel plots may indicate that studies with low precision tend to show a more beneficial effect of probiotics on total cholesterol.[38] Recent large meta-analyses published in major medical journals report publication bias because positive and significant findings are more likely to be published.[48] The low methodological quality of included studies is another important source of bias. All these biases are more likely to affect small studies rather than large ones because smaller studies require larger treatment effects to be published.[38] However, in subgroup analyses, we found stronger beneficial effects in high-quality studies and among larger sample sizes. Therefore, observed publication bias could be due to either heterogeneity or true treatment effects. One important source of potential bias is sponsorship because industry-sponsored research is more likely to favor the product developed by the company sponsoring the study compared to research funded by other sources.[49] In this meta-analysis, most studies reported receiving financial support or intervention products from probiotics companies. We also observed slightly stronger beneficial effects in trials supported by probiotics companies than in those not supported. Therefore, we cannot completely exclude the possibility that industry sponsorship might have influenced the designs, results, or interpretations of individual clinical trials.

The hypocholesterolemic effect of probiotics in this meta-analysis should be interpreted with caution. Because this is a meta-analysis of randomized controlled trials, the quality of this study is affected by the quality of data from the original publications. Although randomized trials are considered valid compared to other studies, true intervention effects could be biased by limited methodological quality.[49] In addition, the lipid-lowering effect of probiotics is not particularly strong compared to other nonmedical interventions[50] and might have been confounded by other factors (eg, dietary habits, physical activity) that the present study did not consider. Finally, the side effects and benefits of these probiotics should be investigated further.

In conclusion, this meta-analysis found that consumption of certain probiotic strains could improve lipid metabolism, particularly by reducing total cholesterol and LDL cholesterol. Although the overall effect of probiotics on total cholesterol and LDL cholesterol is significant, uncertainty remains regarding the extent of their effectiveness. Considering several limitations observed in this meta-analysis and previous clinical trials, clinical evidence is not sufficient to recommend probiotics as a nonpharmacologic alternative for improving lipid metabolism. Therefore, well-designed clinical trials with long follow-up periods should be conducted to confirm the efficacy and safety of probiotics for lowering cholesterol levels.

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