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SYSTEMATIC REVIEWS AND META-ANALYSES

The impact of probiotic yogurt consumption on lipid profiles in subjects with mild to moderate hypercholesterolemia: A systematic review and meta-analysis of randomized controlled trials

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Meta-analysis

Abstract *Background and aims:* Potential beneficial effect of probiotic yogurt on the lipid profile has raised much interest. However, the results are inconsistent in this regard. The aim of the study is to determine the effects of probiotic yogurt on serum lipid profile in individuals with mild to moderate hypercholesterolemia.
Methods and results: Online databases including PubMed, Scopus, ISI Web of Science, Cochrane Central Register of Controlled Trials, Science Direct, Google Scholar and Igaku Chuo Zasshi were searched until March 19th 2019. The effect sizes were expressed as the weighted mean difference (WMD) with 95% confidence interval (CI). Seven eligible trials with 274 participants were included in this systematic review. Pooling of 9 effect sizes from these seven articles revealed a significant reduction in total cholesterol and low density lipoprotein cholesterol levels following probiotic yogurt consumption (mean difference: −8.73 mg/dl, 95% CI: −15.98, −1.48, p-value = 0.018 and mean difference: −10.611 mg/dl, 95% CI: −16.529, −4.693, p-value = 0.000, respectively) without significant heterogeneity among the studies (I^2 = 40.6%, p-value = 0.1 and I^2 = 24.2%, p-value = 0.229, respectively). The results showed no significant changes in high density lipoprotein cholesterol and triglyceride levels. Also, none of the variables showed a significant change for sensitivity analysis.
Conclusion: Available evidence suggests that probiotic yogurt can significantly reduce total cholesterol and LDL-c in subjects with mild to moderate hypercholesterolemia without a significant effect on HDL-c and triglyceride levels.

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Introduction

Hypercholesterolemia is a major risk factor for lifestyle-related diseases such as atherosclerosis and cardiovascular diseases (CVDs), including coronary heart disease (CHD) and stroke [1]. In fact, the possibility of heart attack is three times higher in individuals with hypercholesterolemia than those with normal blood lipid profile [2]. Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability adjusted life years (DALYS), or 2.0% of total DALYS [3]. Each 1% reduction of the cholesterol level has been said to result in 2.3% reduction of the coronary related risks [4]. A 10% reduction in serum cholesterol in men aged 40 has been reported to result in a 50% reduction in heart diseases within 5 years [3]. Statins are commonly accepted as a treatment of choice for lowering low-density lipoprotein (LDL) cholesterol, reducing coronary heart disease morbidity and mortality [5]. However, many patients have potential indications for non-pharmacological treatment of dyslipidemia, including adults with medium-to-low Cardiovascular (CV) risk and mild dyslipidemia, dyslipidemic children-adolescents with a family history of premature CV disease, dyslipidemic adults intolerant to drugs because of the side effects, and adults with human immunodeficiency virus (HIV) infection and mild dyslipidemia [6]. As adherence to medications for treatment of a symptomless condition such as high LDL-c levels is important, factors leading to the risk for non-adherence to medications such as fear of drug-drug interactions or side effects of drugs, negative earlier experience with pharmacological therapies, presence of psychological problems/cognitive impairments, ignoring the importance of medication intake, medication acting as a reminder of the patients' condition or the compulsion to take medications, and making the patient feel old or bad about themselves should be taken into consideration. In the case of statins, factors such as age, gender, income, and race which are the causes of non-adherence should also be regarded [5,7]. Even in patients who are diagnosed with CVDs, adherence to statin therapy often remains poor. Therefore, it is advantageous to identify non-drug treatments and use them alone or in combination with drugs for the treatment of hypercholesterolemia. Probiotic bacteria are defined by the World Health Organization (WHO) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [8]. In a report from WHO (2001), no "acute negative effects" associated with the consumption of probiotics has been mentioned [9]. Previously, several experimental and clinical studies reported that probiotic bacteria such as Lactobacillus and Bifidobacterium had beneficial effects on serum lipid profile [10]. Although the findings on the lipid lowering effect of probiotics in several human clinical studies are controversial, some systematic reviews have supported their hypolipidemic role based on randomized controlled trials [10–16]. However, these reviews are often inconsistent due to missing the required sub-group analyses [11,12,15], missing sub-group analysis based on the health status of the subjects in terms of disease (diabetes mellitus, hypertension, non-alcoholic fatty liver, obesity and overweight) as well as pregnancy, smoking and other variables [10,13,14,16], absence of subgroup analysis in the context of severity of hypercholesterolemia (mild to moderate-severe) [10,14], and limitations of the quantity and quality of the studies reviewed [10,11,15]. A number of previous meta-analyses have conducted subgroup analysis for probiotic dairy products in general and probiotic capsules in particular [10,13,14,16]. However, milk, yogurt and kefir have different constituents and the analyses of their effects as a whole may create bias in detecting the real effects of probiotics on serum lipid profile. For example the contribution of probiotic bacteria in yogurt to the improvement of intestinal micro-flora has been widely recognized for their effectiveness, while these bacteria should overcome the adverse effects of the low pH of yogurt and antagonistic action of other fermenting flora [17]. Moreover, we found some clinical studies reporting the effects of probiotics, in the form of yogurt, on the lipid profiles which were missed in the previous meta-analyses [10,13,14,16]. Finally, because of intolerance to lactose, the consumption of probiotics in the form of milk may not be easily acceptable by everyone. Thus, it seems that daily intake of probiotic yogurt alone or in combination with drugs can be a better choice.

The purpose of this study was to evaluate the effects of probiotic yogurt compared with ordinary yogurt on lipid profiles in subjects with mild to moderate hypercholesterolemia.

Method

The current systematic review and meta-analysis followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [18] for every stage of processing, analyzing, and reporting the data. The study protocol is registered in the Prospective Register of Systematic Reviews (PROSPERO) [protocol code: CRD42018097134].

Search strategy

We searched for electronically available research studies in the PubMed/Medline, Scopus, ISI Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), Science Direct, Google Scholar and Igaku Chuo Zasshi databases to identify relevant studies published before March 19th 2019, without language, time or any other restrictions. In Igaku Chuo Zasshi database, the search was done both in Japanese and in English languages. The type of article was limited to randomized controlled trials (RCTs) or clinical trials. The following keywords were used: ((probiotic*) OR (Lactobacillus) OR (Bifidobacterium)) AND ((yogurt) OR (yoghurt) OR (milk) OR (Kefir) OR ("fermented dairy")) AND (("total cholesterol") OR ("LDL-c") OR ("LDL

cholesterol") OR (cholesterol) OR ("HDL-c") OR ("HDL cholesterol") OR ("lipid profile") OR ("lipid status") OR ("blood lipids") OR ("cardiovascular disease") OR ("heart disease") OR (lipid*) OR (triglyceride*)) AND ((hypercholesterolemia) OR ("mildly hypercholesterolemia") OR ("moderately hypercholesterolemia") OR (hypercholesterolemic) OR (elevated cholesterol)) AND NOT ((rat) OR (mouse) OR (animal) OR (rabbit) OR (hamster) OR ("in vitro ")).

Eligibility criteria

Screening the titles and abstracts, followed by the full texts assessment of the eligible articles were performed by two independent investigators (B.P & S.F). The inclusion criteria in our meta-analysis were:

1 Clinical trials in which participants took probiotic yogurt and lipid profiles (low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol and triglyceride) were then studied too.
2 Studies in which participants were people with hypercholesterolemia with no other illnesses and their Body Mass Index (BMI) were less than 30.
3 Studies in which the criteria for mild to moderate hypercholesterolemia were addressed (total cholesterol: 200−300 mg/dl or 5.2−7.76 mmol/l).

Age limit and specific time frame were not considered for search and all studies conducted on this topic by March 2019 were reviewed.

Exclusion criteria in this study were:

1 Animal and in vitro studies.
2 Studies investigating the effects of fermented yogurt, without mentioning the dose and type of probiotic bacteria.
3 Studies including pregnant or breast-feeding women or individuals with certain diseases or those who had previously undergone intestinal surgery.
4 Studies investigating the effect of probiotic supplementation and probiotic milk but not probiotic yogurt.
5 Studies in which subjects were hypercholesterolemic and normocholesterolemic, but the results for hypercholesterolemic patients were not separated from the results of normocholesterolemic subjects.
6 Studies using fermented milk as probiotic without mentioning the word yogurt.

Data extraction and quality assessment

The studies were selected by two independent researchers (B.P) and (S.F) on the basis of inclusion and exclusion criteria. Any disagreement between researchers was resolved by consulting the third researcher (F.SH). The following information was collected: author's name, study location, study design, study population, mean age, sex, sample size, intervention group, control group, probiotic dosage, probiotic strain, and period of intervention. This information is shown in Table 1.

Risk of bias assessment

The quality of the studies was independently evaluated by 2 researchers (B.P) and (S.F) according to the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0 [19] and by using the following criteria:

1 random sequence generation
2 allocation concealment
3 blinding of participants and personnel
4 blinding of outcome assessment
5 incomplete outcome data, and
6 selective outcome reporting.

There were six key domains according to which each study was assigned in terms of overall risk of bias, including low risk (low for all key domains), high risk (high for one or more key domains.), and unclear risk (unclear for one or more key domains).

Statistical analysis

For all outcomes, the effect sizes were measured by the mean difference between the intervention and the control group at the follow-up. Where the effect size was not reported, the difference in the mean values at the baseline and at the end of the study were used. We extracted the mean and Standard Deviation (SD) from the studies reviewed and where the data were reported in a different format. This method was used by Hozo et al. as follows: SD = square root [(SD pre-treatment) 2 + (SD post-treatment) 2 − (2R × SD pre-treatment × SD post-treatment)] [20]. The groups were combined by applying a weighted average when we had >1 control group, to enable a single pairwise comparison. In order to estimate effect sizes, the random effects model (DerSimonian and Laird method) was used and the results were provided across weighted mean difference (WMD) and 95% confidence interval (CI). We used Plot digitizer software when the results were only presented in the graphic form. Heterogeneity was calculated by the I^2 index [21]. We considered I^2 index of greater than 50% as the substantial heterogeneity among the trials. Subgroup analysis was performed to identify factors for high heterogeneity. We considered the values less or more than median as the cut off values for each aforementioned quantitative parameter of subgroups. The sensitivity analysis was done by using the leave-one-out method [22] to examine the impact of each study on the results. The funnel plot was used to determine publication bias, by either Beggs' rank correlation or Eggers' regression test. STATA version 11.0 was used for statistical analysis (Stata Corp, College Station, TX) and P values < 0.05 was considered statistically significant.

Table 1 Characteristics of the randomized clinical trials that were included in the systematic review.

Author (year) (reference)	Country	Clinical Trial Design	Population	Mean Age (year)	Sex	Sample Size Case/Placebo	Intervention group	Control group	Probiotic dose (cfu)	Probiotic strain	Period (week)
Xiao, J. Z et al. (2003) [28]	Japan	Parallel	Healthy H.C	43.8	Male	16/16	Probiotic yogurt (300 mg)	ordinary yogurt (300 mg)	$>10^8$	Bifidobacterium longum strain BL1	4
Kiessling, G et al. (2002) [17]	Jena	Cross-over	Healthy H.C	37	Female	14/14	Probiotic yogurt (300 mg)	ordinary yogurt (300 mg)	Lactobacillus. ($10^6 - 10^8$) B. longum ($>10^5$)	Lactobacillus acidophilus 145, Bifidobacterium longum 913	7
Jones, M.L et al. (2012) [8]	Canada	Parallel	Healthy H.C	51.8	Both	56/58	Probiotic yogurt (125 mg)	ordinary yogurt (125 mg)	5×10^9	Lactobacillus reuteri (NCIMB 30242)	6
Baroutkoub, A et al. (2010) [4]	Iran	Cross-over	Healthy H.C	43.5	Both	46/46	Probiotic yogurt (300 mg)	ordinary yogurt (300 mg)	10^6	L. acidophilus, Bifidobacteria	6
Anderson, J.W et al. (1999) [29]	Oklahoma	Cross-over	Healthy H.C	58	Both	19/19	Probiotic yogurt (200 mg)	ordinary yogurt (200 mg)	$>1 \times 10^7$	Lactobacillus Acidophilus L1	4
Ataie-Jafari. A et al. (2009) [31]	Iran	Cross-over	Healthy H.C	50.5	Both	14/14	Probiotic yogurt (300 mg)	ordinary yogurt (300 mg)	10^6	Lactobacillus acidophilus, Bifidobacterium lactis	6
Ataie-Jafari. A et al. (2007) [30]	Iran	Parallel	Healthy H.C	50.5	Both	8/6	Probiotic yogurt (300 mg)	ordinary yogurt (300 mg)	10^6	Lactobacillus acidophilus, Bifidobacterium lactis	6

Results

Study selection

A flow chart depicting the literature search and selection is presented in Fig. 1. Using the key terms of the study, we identified 125 articles through searching the databases and four additional articles through other sources. First, duplicate articles (n = 37) were removed and then another 80 articles, recognized to be irrelevant, were removed by reading their titles and abstracts. Next, we evaluated the full text of the remaining 12 articles from which five articles were excluded for the following reasons: 1- it was inaccessible article [23], 2- it did not include individuals with mild to moderate hypercholesterolemia [24–26], and 3- the outcome of interest was not reported [27]. The two reviewers (B.P) and (S.F) agreed on the study screening procedure. Although we finally analyzed seven articles, two of them can be viewed as separate trials. One of them was the study by Xiao et al. (2003), which separately reported the results for people with baseline total cholesterol > 200 mg/dl and those with baseline total cholesterol > 240 mg/dl [28]. The other study was performed by Anderson et al. applying two intervention times. These last two studies can be regarded as two independent studies [29]. Therefore, seven articles were included in this systematic review with the effect sizes of 9.

Study and participant characteristics

The characteristics of the studies included in the present systematic review and meta-analysis are shown in Table 1. Three studies were conducted in Iran [4,30,31] and the others were conducted in Japan [28], Germany

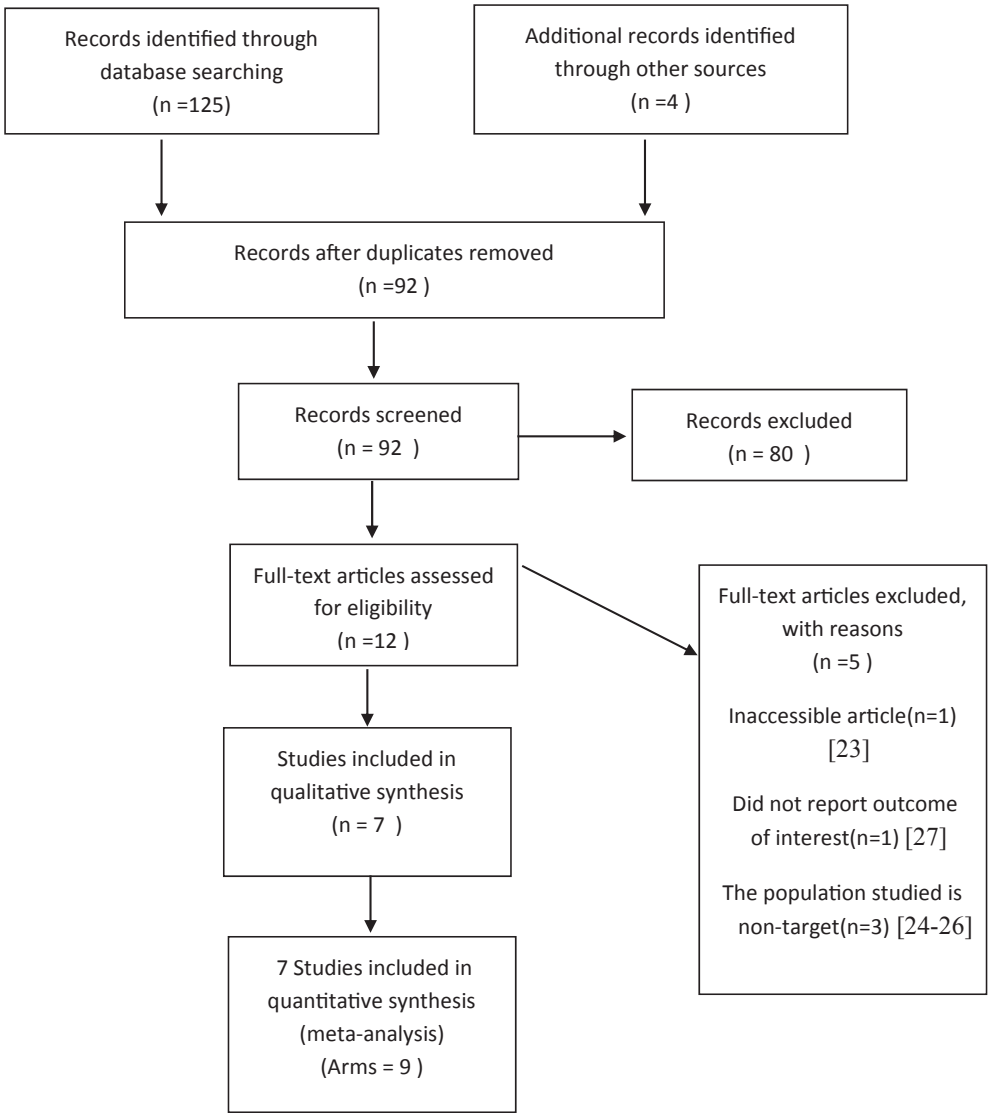

Figure 1 Flow chart of the study selection process.

[17], Canada [8] and the U.S.A [29]. From the total seven studies, four studies were crossover in design and consisted of 93 participants [4,17,29,31] The intervention period was six weeks in four studies [4,8,30,31], four weeks in two studies [28,29] and seven weeks in one study [17]. In the majority of trials, the daily consumption of probiotic yogurt in subjects was 300 mg/d and in one study it was 125 mg/d [8]. The probiotic dosage was 10^6 cfu in three of the studies [4,30,31] and it was $>10^7$cfu [29], $>10^8$cfu [28], $10^6 - 10^8$cfu [17], and $>10^9$cfu in the rest of the studies [8]. All the studies used ordinary yogurt as placebo which was similar in dose to probiotic yogurt. The majority of included studies consisted of both genders whereas one study was performed merely on male adults [28] and one study on female individuals [17]. The age range of the participants was 37–58 years old in these studies.

Risk of bias assessment

As shown in Table 2, except for one study that was assessed as high risk in random sequence generation [4], the other studies were randomized, but did not explicitly mentioned the random sequence generation methods; therefore, they were regarded as unclear risk of bias [8,17,28–31]. One study was assessed as high risk in allocation concealment [4], and six as unclear risk [8,17,28–31]. There were two studies which were double-blind randomized controlled trials (RCTs) and were thus considered as low risk of bias for blinding of participants and personnel [8,29]. There were also five studies with single blind trials which were therefore considered as unclear risk of bias [4,17,30,31,28]. None of the trials provided a clear description of blinding of outcome assessment and other issues. Two studies provided incomplete outcomes [8,28] and five articles were not clear on this matter [4,17,29–31]. One study was assessed as high risk in selective reporting [4], two as unclear on risk [8,30], and the other four studies as low risk of bias [17,28,29,31]. Except for one study that was assessed as high risk in quality [4], Since the other studies were considered as unclear risk of bias for at least one of the six key domains, we found the quality of these studies "unclear" [8,17,28–31].

Meta-analysis

Total cholesterol (TC)

There were seven studies with 352 participants (case = 179, and control = 173) which reported TC, LDL-c, HDL-c and triglyceride as an outcome measure. Combined results of the random-effects model showed a significant reduction in TC following the probiotic yogurt consumption (MD: −8.73 mg/dl, 95% CI: −15.98,-1.48, p = 0.018) without a significant heterogeneity among the studies (I^2 = 40.6%, p = 0.1) (Fig. 2). Subgroup analysis for duration of intervention showed that the reduction of TC was significantly higher in participants with intervention duration of >4 weeks (WMD = −14.210; 95% CI:-24.609, −3.812; P = 0.007) and in those receiving probiotic yogurt with the dose of ≥300 mg/d (WMD = −12.400, 95% CI, −21.116, −3.684; P = 0.005). However, no association was observed in participants with intervention duration of ≤4 weeks and in those receiving probiotic yogurt with the dose of <300 mg/d (see Table 3). Sensitivity analysis indicated that no study reported a significant impact on the overall effect sizes of TC (Supplemental Fig. 1). Assessment of publication bias by visual inspection of funnel plot did not show any evidence of publication bias in the meta-analysis of probiotic yogurt consumption on TC (p = 0.364) (Supplemental Fig. 2).

High Density Lipoprotein cholesterol (HDL-c)

Combined results of the random-effects model showed no significant increase in HDL-c following probiotic yogurt consumption (MD: 1.567 mg/dl, 95% CI: −1.586, 4.719, p = 0.330) and no significant heterogeneity among the studies (I^2 = 28.0%, p = 0.19) (Fig. 3). Sensitivity analysis revealed that no study had a significant impact on the overall effect sizes of HDL-c (Supplemental Fig. 1). Assessment of publication bias by visual inspection of funnel plot did not show any evidence of publication bias in the meta-analysis of probiotic yogurt consumption on HDL-c (p = 0.991) (Supplemental Fig. 2).

Low Density Lipoprotein cholesterol (LDL-c)

Combined results of the random-effects model showed a significant reduction in LDL-c following probiotic yogurt consumption (MD: −10.611 mg/dl, 95% CI: −16.529, −4.693, p = 0.000) without a significant heterogeneity

Table 2 Risk of bias assessment according to the Cochrane collaboration's risk of bias assessment tool.

Study, Year (reference)	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Overall assessment of risk of bias
Xiao, J. Z et al. (2003) [28]	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Kiessling, G et al. (2002) [17]	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Jones, M.L et al. (2012) [8]	Unclear	Unclear	Low	Unclear	Low	Unclear	Unclear
Baroutkoub, A et al. (2010) [4]	High	High	Unclear	Unclear	Unclear	High	High
Anderson, J. W et al. (1999) [29]	Unclear	Unclear	Low	Unclear	Unclear	Low	Unclear
Ataie-Jafari, A Et al. (2009) [31]	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Ataie-Jafari, A et al. (2007) [30]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear

Table 3 Subgroup analyses of a probiotic yogurt consumption on serum Total cholesterol, LDL-c, HDL-c and TG levels.

	No.	I^2(%)	p-heterogeneity intergroup	WMD (95% CI) (weighted mean difference)	Significance test (P_{value})
Total cholesterol	9	40.6	0.1	−8.734 (−15.986, −1.481)	0.018
Time(week)					
4≥	4	34.7	0.20	−4.165 (−13.216, 4.886)	0.36
>4	5	34.5	0.21	−14.210 (−24.609, −3.812)	0.007
Yogurt dose(mg/day)					
300>	3	51.8	0.126	−2.195 (−15.315, 10.924)	0.743
300≤	6	32.3	0.193	−12.400 (−21.116, −3.684)	0.005
Age(year)					
<50 years old	4	8	0.35	−7.957 (−16.355, 0.441)	0.063
≥50 years old	4	69.4	0.02	−9.039 (−25.709, 7.631)	0.28
NR	1	_	_	−12.480 (−24.037, −0.923)	0.034
HDL-c	9	28	0.196	1.567 (-1.586, 4.719)	0.330
Time(week)					
4≥	4	0	0.927	0.968 (-3.481, 5.417)	0.670
>4	5	62	0.032	2.033 (-3.394, 7.461)	0.463
Yogurt dose(mg/day)					
300>	3	0	0.997	2.146 (-1.891, 6.184)	0.297
300≤	6	54.4	0.052	1.219 (-3.940, 6.378)	0.643
Age(year)					
<50 years old	4	59	0.062	3.147 (-3.657, 9.951)	0.365
≥50 years old	4	_	0.483	−0.236 (−4.395, 3.924)	0.912
NR	1	0	_	2 (-3.482, 7.482)	0.475
LDL -c	9	24.2	0.229	−10.611 (-16.529, -4.693)	0.000
Time(week)					
4≥	4	0	0.968	−4.190 (−11.175, 2.795)	0.024
>4	5	0	0.467	−16.773 (−23.228, −10.317)	0.000
Yogurt dose(mg/day)					
300>	3	24.8	0.264	−9.981 (−16.599, −4.693)	0.016
300≤	6	35.5	0.163	−12.026 (−21.696, −2.356)	0.015
Age(year)					
<50 years old	4	50.4	0.109	−9.155 (−21.523, 3.214)	0.147
≥50 years old	4	0	0.398	−8.972 (−18.237, 0.294)	0.058
NR	1	_	_	−14.820 (−22.540, −7.100)	0.000
TG	9	5.5	0.390	4.524 (-10.101, 19.148)	0.544
Time(week)					
4≥	4	0	0.713	1.774 (−20.882, 24.429)	0.878
>4	5	42.5	0.138	−0.306 (−26.734, 26.121)	0.982
Yogurt dose(mg/day)					
300>	3	0	0.925	24.585 (3.16, 45.559)	0.022
300≤	6	0	0.795	−10.458 (−29.309, 8.393)	0.277
Age(year)					
<50 years old	4	0	0.500	−10.566 (−31.481, 10.349)	0.322
≥50 years old	4	0	0.805	6.891 (−20.966, 34.748)	0.628
NR	1	_	_	27.590 (1.878, 53.302)	0.035

among the studies (I^2 = 24.2%, p = 0.229) (Fig. 4). Sensitivity analysis indicated that no study had a significant impact on the overall effect sizes of LDL-c (Supplemental Fig. 1). Assessment of publication bias by visual inspection of funnel plot did not show any evidence of publication bias in the meta-analysis of probiotic yogurt consumption on LDL-c (p = 0.830) (Supplemental Fig. 2).

Triglyceride (TG)

Combined results of the random-effects model showed no significant reduction in TG following probiotic yogurt consumption (MD: 4.524 mg/dl, 95% CI: −10.101, 19.148, p = 0.544) and no significant heterogeneity among the studies (I^2 = 5.5%, p = 0.390) (Fig. 5). Sensitivity analysis indicated that no study reported a significant impact on the overall effect sizes of TG (Supplemental Fig. 1). Assessment of publication bias by visual inspection of funnel plot did not show any evidence of publication bias in the meta-analysis of the effect of probiotic yogurt consumption on TG (p = 0.068) (Supplemental Fig. 2).

Discussion

This meta-analysis systematically reviewed seven randomized controlled trials which had examined the effect of probiotic yogurt on serum lipid profile in mild to moderate hypercholesterolemic individuals. To our knowledge, this is the first meta-analysis investigating this topic. Results of our analysis showed that serum total cholesterol and LDL-c levels significantly decreased in the group receiving probiotic yogurt as compared with the control group. However, the effect of probiotics on HDL-c and triglyceride was not statistically significant.

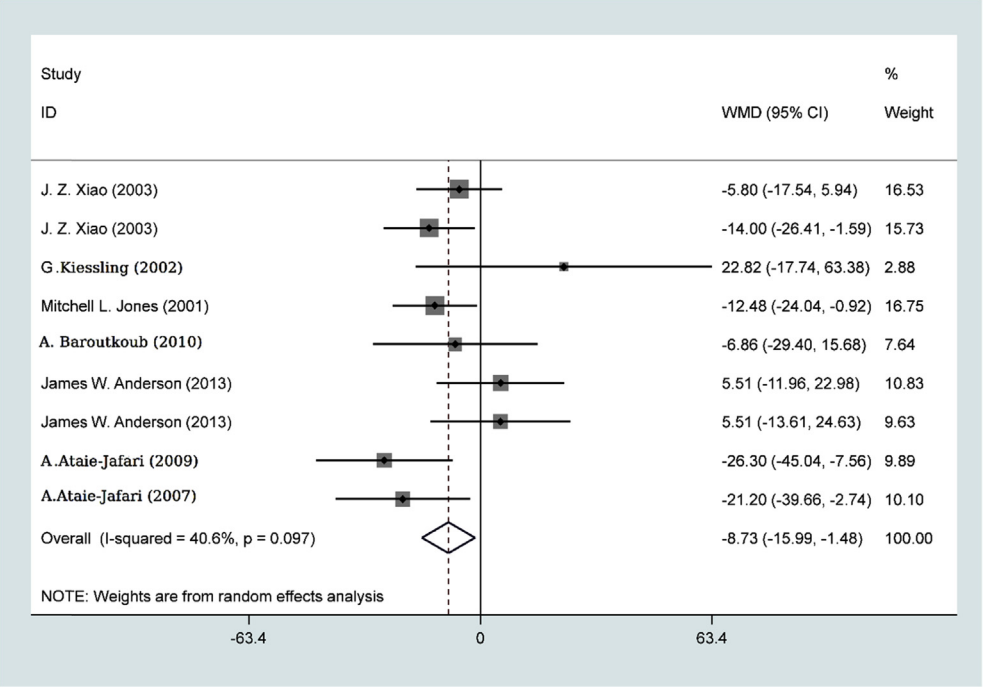

Figure 2 Forest plot of randomized controlled trials investigating the effects of probiotic yogurt on total cholesterol.

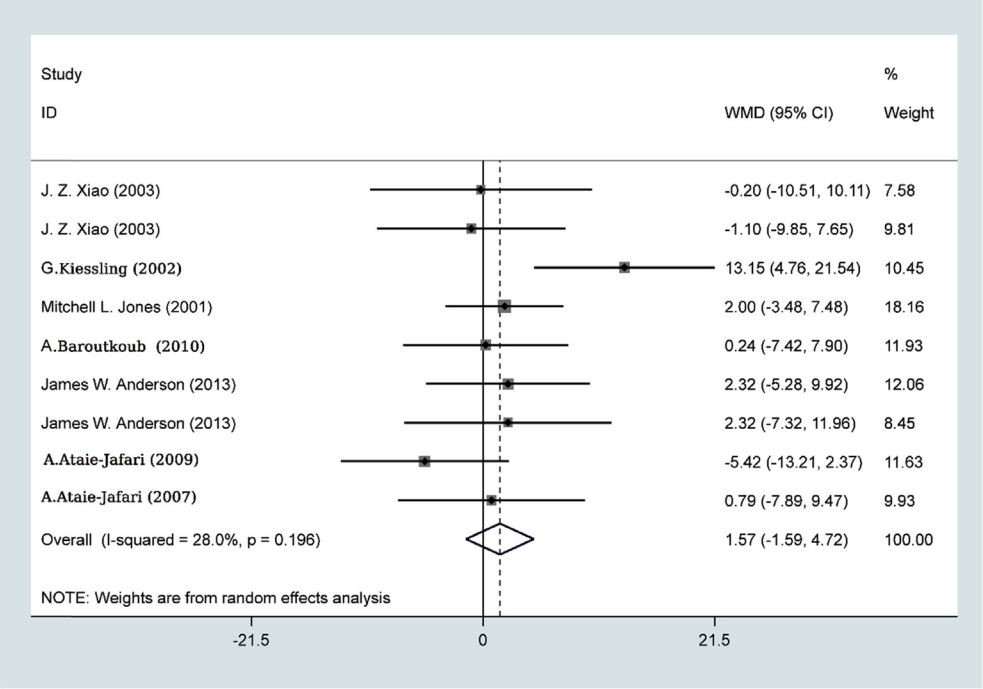

Figure 3 Forest plot of randomized controlled trials investigating the effects of probiotic yogurt on HDL-c.

Considering the association between probiotic yogurt and serum lipid profile, our findings are similar to those of previous meta-analyses of randomized controlled trials [10–16]. A previously conducted meta-analysis by Larsen et al. [11] has reported reductions in total cholesterol (−8.51 mg dl) and LDL-c (−7.74 mg dl) levels after consumption of probiotic yogurt. Another study performed by Guo et al. [12] has showed that subjects receiving probiotic yogurt had a significantly lower total cholesterol (−6.40 mg dl) and LDL-c (−4.90 mg dl) compared to controls. Moreover, Cho et al. [13] have remarked that the pooled mean net change in total

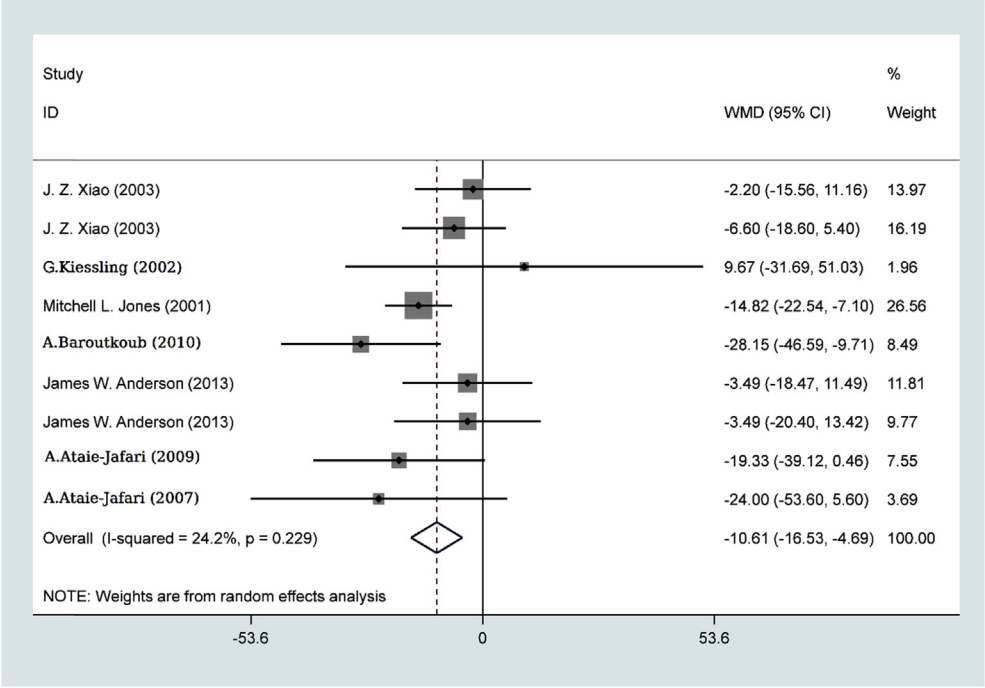

Figure 4 Forest plot of randomized controlled trials investigating the effects of probiotic yogurt on LDL-c.

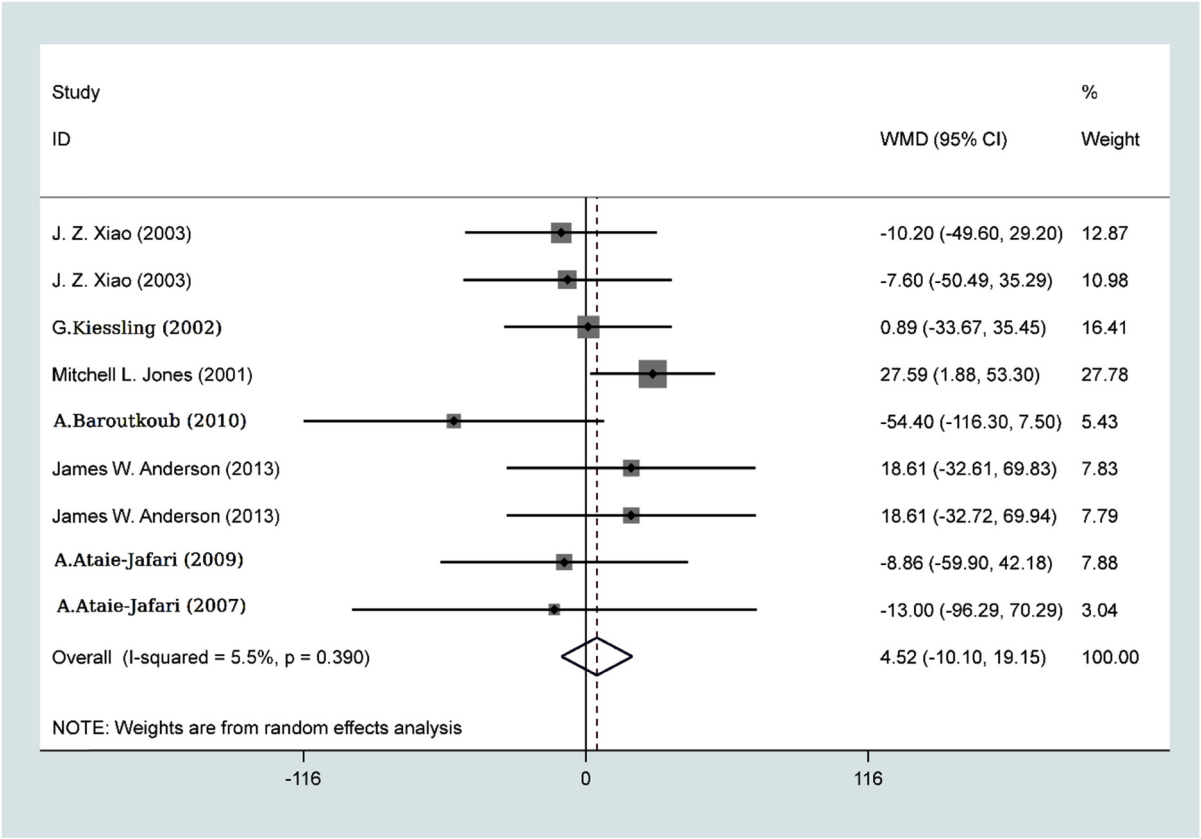

Figure 5 Forest plot of randomized controlled trials investigating the effects of probiotic yogurt on Triglyceride.

cholesterol and in LDL-c were −7.8 mg/dl and −7.3 mg/dl, respectively for those treated with probiotic yogurt. Shimizu et al. [10] have discussed that probiotic intervention causes significant changes in total cholesterol (MD = −0.17 mmol/l) and LDL-c (MD = −0.22 mmol/l). Similarly, Jing Suna et al. [14] have showed that the pooled effect of total cholesterol and LDL-c were - 0.27 mmol/l and - 0.23 mmol/l, respectively in the probiotic group compared with the control group. Finally, Wang et al. [16] have showed that the total cholesterol level significantly reduced in probiotics group (MD = −13.27).

Our results about HDL-c and triglyceride are similar to the findings of the above mentioned studies which have reported no significant difference in experimental groups compared with controls. These findings may be due to the greater potential of the probiotics to reduce total cholesterol and LDL-c than the levels of HDL-c and triglyceride.

Treatment strategies to achieve optimal plasma cholesterol levels include both lifestyle and pharmacological interventions [32]. Statins, owing to their undisputed effectiveness in reducing plasma LDL-c levels, are the cornerstone of pharmacotherapy for hypercholesterolemia [33]. But the use of statins can be associated with muscle-related side effects, cognitive and memory problems, and new-onset diabetes in a dose-related manner [5]. Also, non-adherence and discontinuation of statin therapy often leads to inadequate control of plasma cholesterol levels and an increased cardiovascular risk. On the other hand, the initial therapeutic approach to hypercholesterolemia should always include non-pharmacological measures such as: Low dietary intake of cholesterol, saturated and “trans” fats and increased intake of dietary fiber, as well as exercise programs suited to the patients’ physical capabilities [32]. Hence, there is a critical need to identity additional effective hypolipidemic agents that can be used in combination with statins and non-pharmacological interventions. Over the past two decades, there has been a surge of interest to use natural products for the management of hypercholesterolemia [33]. Probiotic products are widely available in the markets and are promoted as useful dietary supplements for creating homeostasis of the ecosystem of microorganisms in the colon which is the most metabolically active organ in the body [10,34].

It has been suggested that probiotics may reduce blood cholesterol in the form of cholesterol ester via the interrelated pathways of lipid transporters [35], and promote the excretion of the cholesterol and bile acid rather than affecting hepatic cholesterol synthesis [28]. In addition, another proposed mechanism of action associated with bile salt hydrolase (BSH) activity involves the inhibition of Niemann-Pick C1 Like 1 which is responsible for the bulk movement of cholesterol into the enterocytes; the genetic inactivation often results in a significant reduction of cholesterol absorption. Beside, a study has demonstrated that an increased circulating bile acid is correlated with a reduction in serum LDL-c and apoB-100 which is a primary structural protein of the atherogenic lipoproteins, and its decrease results in reducing LDL-c serum levels [8].

Subgroup analysis in the present study for duration of intervention showed that the reduction of total cholesterol was significantly higher in participants with intervention duration >4 weeks. In line with our study, there are several studies reporting significantly higher decrease in total cholesterol in participants with intervention duration >4weeks [10,14,16]. In another subgroup analysis performed, we noticed that the reduction of total cholesterol was significantly higher in participants who received probiotic yogurt with the dose of ≥300 mg/day. Therefore, the effect of probiotic yogurt appears to be more on lipid profiles if longer consumption period and higher intake are considered.

By and large, the cholesterol-lowering mechanisms of probiotics have not yet been sufficiently elucidated. Further studies on the large intestine which is the main site where probiotics of fermented milk products can function are suggested. Intestinal lactobacilli may reduce serum cholesterol levels through the incorporation of cholesterol into the cellular membrane to inhibit the formation of intestinal cholesterol micelles, assimilation of cholesterol by growing cells, production of BSH which catalyzes the hydrolysis of conjugated bile salt into free bile acids [12], and fermentation of indigestible carbohydrates and production of short chain fatty acids (SCFAs) [36].

The present study has several strengths. First, there was no time and language limitations for the inclusion of the studies investigating the effect of probiotic yogurt on serum lipid profiles. Second, participants of the studies reviewed had a very low heterogeneity and were similar in levels of primary cholesterol and health status; because the difference in total cholesterol levels and health condition at the baseline may have a significant impact on the study results. Third, the amount of yogurt (maximum 300 mg/d) and probiotic dosage (maximum 10^9 cfu) was tolerable for daily consumption and could be used on the regular daily basis in all the included studies. Fourth, species in the Lactobacillus and Bifidobacterium genera are the most commonly used probiotics and all studies included in this meta-analysis used Lactobacillus acidophilus and Bifidobacterium as probiotics. Fifth, participants had not used cholesterol-lowering drugs in the studies reviewed, except for two studies in which this issue was not mentioned [28,30]. Sixth, all crossover studies had run in period, whether the run in period was long enough needs further study. However, there are some limitations to this meta-analysis. First, we analyzed both crossover and parallel studies which were different in terms of methods and biases. Second, there were some deficiencies in the quality of literature which could affect the final outcomes. In the studies we reviewed, except for one study [8], small sample-size and short term intervention time were used. This suggests that further randomized clinical studies with larger sample-size and longer intervention period to reduce possible biases are needed. In addition, the lipid-lowering effect of probiotic may be affected by participants’ diet and physical activities. In the studies we reviewed the participants were

asked not to change their regular diet and physical activity prior to the beginning of the study. Moreover, all the studies used the 24-h food recall questionnaire at the first and the end of the intervention period to estimate the individual nutrients and energy intake, except for two studies that did not mention this issue [28,30]. In most studies, there was no mention of physical activity adjustment.

Finally, in most clinical studies, probiotic and conventional yogurt were prepared as low-fat [4,8,28,29] and only in two studies [17,31], they were prepared in the form of moderate fat yogurt and full fat yogurt, respectively while in another study, there was no explanation for the amount of fat in yogurt [30]. Due to the few number of studies in each group, we were unable to perform a subgroup analysis on the amount of fat in yogurt. The United States Department of Agriculture (USDA) currently recommends that Americans consume fat-free and low-fat dairy instead of high-fat alternatives, and mention that "increasing the proportion of fat-free milk consumed to meet dairy group recommendations would decrease the amounts of sodium, cholesterol, and saturated fatty acids". Current evidence of high-fat versus low-fat dairy is not conclusive, and further studies are needed in this regard [37].

Conclusion

The results of our meta-analysis show that probiotic yogurt can significantly reduce total cholesterol and LDL-c levels in mild to moderate hypercholesterolemic individuals. The effects of probiotic yogurt on lowering total cholesterol were significantly higher in participants with intervention duration >4 weeks and in participants who received probiotic yogurt with the dose of ≥300 mg/day. However, the effect of probiotic on HDL-c and triglyceride was not statistically significant.

Authors' contributions

The authors' responsibilities were as follows: F.SH, BP and S.F conceived the study and designed the search strategy; B.P and S.F conducted the study selection; B.P and S.F conducted data extraction; B.P and S.F evaluated the risk of bias of included studies; S.F and H.K conducted the data analysis and interpretation of results; S.F and B.P wrote the first draft of the manuscript; F.SH and A.D revised the manuscript; and all authors read and approved the final version of the manuscript.

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Declaration of Competing Interest

All the authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.10.001.

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