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**Title: Effects of probiotics consumption on lowering lipids and
CVD risk factors: A systematic review and meta-analysis of
randomised controlled trials**

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Effects of probiotics consumption on lowering CVD risk factors: A systematic review and meta-analysis of randomised controlled trials

Abstract

This meta-analysis examined the effect of probiotics on the reduction of lipid components and co-existing risk factors associated with cardiovascular disease. All randomised controlled trials published in English on PubMed and Scopus from 2000 to 2014 were systematically searched. Using the PEDro scale to assess the quality of studies, a total of 15 studies with 788 subjects were selected for inclusion in the analysis. The mean difference and effect size with a 95% confidence interval (CI) were extracted from individual studies. Statistically significant pooled effects of probiotics were found on reduction of total cholesterol, low-density lipoprotein (LDL), body mass index (BMI), waist circumference and inflammatory markers. Subgroup analysis revealed statistically significant effects of probiotics on total cholesterol and LDL when the medium was fermented milk or yogurt ($P<0.001$) compared to capsule form, consumption was at least eight weeks in duration ($P<0.001$), and the probiotics consisted of multiple strains ($P<0.001$) rather than a single strain. A significant reduction was found in LDL in trials which contained *L.acidophilus* strain ($P<0.001$) compared to other types of strains. Our

findings suggest that probiotic supplementation use is effective in lowering the lipid level and co-existing factors associated with cardiovascular disease.

Key words: probiotics, cholesterol, low density lipoprotein, body mass index, weight, inflammatory markers

Key messages:

1. Probiotics supplementation use is effective in reducing total cholesterol, LDL, weight, waist, and inflammatory markers.
2. Probiotics use on total cholesterol and LDL is more effective when the medium was fermented milk or yogurt compared to capsule form, consumption was at least eight weeks in duration, and the probiotics consisted of multiple strains rather than a single strain.
3. A significant reduction was found in LDL in trials which contained *L.acidophilus* strain compared to other types of strains.
4. Probiotic supplementation use is effective in lowering the lipid level and co-existing factors associated with cardiovascular disease.

Effects of probiotics consumption on lowering CVD risk factors: A systematic review and meta-analysis of randomised controlled trials

1.1 Introduction

Cardiovascular disease (CVD) is a leading cause of mortality worldwide, with an increasing morbidity rate in both developing and developed countries.(1) A number of modifiable and major risk factors, such as elevated low-density lipoprotein cholesterol (LDL-C), increased triglyceride-rich lipoproteins, and low levels of high-density lipoprotein cholesterol (HDL-C),(1) are significant contributors to CVD. Additional co-existing risk factors include overweight and obesity,(2) inflammatory markers such as high-sensitivity C-reactive protein (CRP) and tumour necrosis factor alpha (TNF Alpha) (3). If not adequately addressed, these risk factors contribute to high cardiovascular morbidity and mortality rates.(4, 5)

Adopting healthy lifestyle habits is an important part of managing CVD risk factors and reducing costs associated with the disease.(1) In this context, probiotics have gained recent attention as products for managing CVD. Probiotics have been defined by the World Health Organization (WHO) as ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’.(6) It has been demonstrated that probiotics can modulate gut health (7, 8) and strengthen the immune system by regulating gut microbiota.(9).

Although a number of clinical studies have shown that probiotics can lower CVD risk factors, this research has significant limitations, (10, 11) there is conflicting and inconclusive evidence about the effects of probiotics on improving lipid components (11), and which forms, consumption duration, and dosage limits per strain are required to optimise risk reduction.

A meta-analysis conducted by Agerholm-Larsen and colleagues, which found that probiotic dairy product consumption resulted in the reductions in total and LDL cholesterol, focussed on a small number of pre-2000 trials with short term (4-8 weeks) interventions. (10) However, to date, no systematic review or meta-analysis has been conducted on findings from recent clinical trials investigating the effect of probiotics consumption on CVD risk factors. This study addresses this issue by using a meta-analytic approach to review the evidence from randomised controlled trials (RCTs) that examined the effects of probiotics and consumption characteristics on reducing cholesterol and other coexisting CVD risk factors.

1.2 Methods

1.2.1 Literature search

The review protocol was registered at the Prospero International Prospective Register of Systematic Reviews (Registration ID = PROSPERO 2014:CRD42014010591; website: http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42014010591). Relevant studies published between 2000 and 2014 in the following databases were systematically identified by the two authors independently for inclusion in the meta-analysis: PubMed, Scopus, Evidence-Based Medicine (EBM) Guidelines and the Cochrane Library. A combination of the following keywords was employed in this process: Probiotics AND cardiovascular disease risk factors OR lipid profiles OR inflammatory factors. Further identification occurred through the use of keywords related to specific CVD risk factors and types of probiotics including: *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, *Bifidobacterium*, *L. plantarum*, and *L. fermentum*. The title and abstract were initially screened for relevance. The full text of the relevant articles was then retrieved for further reading and assessment. The literature search and

presentation of results were undertaken according to Systematic Reviews and Meta-analysis (PRISMA) guidelines. Identified articles were imported into the EndNote reference management software to remove any duplicates. The reviewers then independently extracted data on patients' characteristics and outcome measures. Where there was unreported data, the research team contacted the authors of the studies in question to obtain the data. This occurred with two included studies. (12, 13)

1.2.2 Inclusion criteria

The research team chose the following criteria to determine the inclusion of studies using the PICO structure: Patient problem or population (P), intervention (I), comparison (C) and outcome(s) (O): (14, 15)

1. Participants were 18 years or older;
2. Clinical trials involved participants with CVD risk factors, including hypertension, abnormal lipid profiles, obesity, diabetes or metabolic syndromes;
3. Study design was a randomised control trial in which participants were randomly allocated into intervention (probiotic consumption) and control (placebo) groups, from which similar baseline data on key outcome variables was collected;
4. Outcome variables included total cholesterol, HDL, LDL, triglycerides, BMI, waist circumference, hs-CRP and TNF Alpha;
5. Only those papers with the largest sample and longest intervention duration were included where there were multiple publications and companion papers from the same population.

1.2.3 Data abstraction and quality assessment

Abstraction of data and assessment of studies according to the criteria was independently conducted by two reviewers. A summary of the review is presented in a PRISMA

flowchart (see Figure 1). A pre-piloted data form was used to extract data from the selected randomised controlled studies. Data on the effects of probiotics at-risk groups was extracted. At-risk groups were defined as participants who had hypertension, abnormal lipid profiles, obesity or metabolic abnormality. Outcomes variables that were examined included cholesterol, LDL, HDL, triglyceride, BMI and waist circumference, as well as inflammatory markers such as hs-CRP and TNF Alpha. Other variables examined included duration of probiotics intake, dosage of probiotics use, probiotics in fermented milk/yoghurt or in capsule forms, types of strains of probiotics, single-strain versus multiple strains of probiotics intake. The cut-off scores of 5.5 mmol/L and 3.5 mmol/L respectively for total cholesterol and LDL were taken to divide total cholesterol and LDL as elevated and normal levels, in accordance with guideline provided by Gould and colleagues.(16, 17)

The PEDro tool was used to assess the quality of the abstracted articles (see Table 1).(18) This tool categorises the quality of the evidence into three levels—high quality (8 or more points), moderate quality (4–7 points), low quality (3 points or less)—based on 10 factors. Studies that were deemed to be low quality were excluded.(18) The 10 factors were: (1) Random allocation of subjects into groups; (2) Concealed randomisation; (3) Similarity of baseline information between groups; (4) Blinding in relation to subjects and assessors; (5) Blinding in relation to researchers; (6) Blinding in relation to assessors; (7) Low attrition rate; (8) Use of ‘intention to treat’ analysis; (9) Use of variability measures such as standard deviation and/or standard error and; (10) Between-group comparison results. One point was allocated for each factor present in the study. Factors that were used to downgrade the quality of evidence in a study included the potential for bias such as non-randomisation, single blinding or no blinding, lack of baseline data, no between-group comparison results, no use of an ‘intention to treat’ measure and no use of

variability measures. There were no obvious discrepancies in ratings between the two assessors relating to the quality of the selected studies (Table S1).

1.3 Statistical Analysis

A mean difference with a 95% confidence interval (CI) was used. Random-effect models were used to take into account between-study variation. The heterogeneity level was estimated based on the I^2 index for all studies. Studies with values of less than 30% were considered to have low heterogeneity, 30-50% was considered moderate heterogeneity and values of greater than 50% substantial heterogeneity. Random-effect models were used to take into account between-study variation. The detailed effect of probiotics was further explored using subgroup analysis to assess whether there are possible sources of heterogeneity. These analyses were conducted in relation to the effects of: (1) Duration of the probiotics intake of less than eight weeks compared with eight weeks or longer; (2) Dosage level involving 10^9 colony-forming units (CFU) or more versus 10^8 CFU or less and fermented dairy products (10^9 colony-forming units (CFU) as cut off); (3) Medium type (i.e., milk-based versus capsule-based); (4) Single strain versus multiple strain; (5) *Acidophilus* versus non-*acidophilus* strains and; (6) People with hypercholesterolemia and other CVD risk factors. Publication bias was assessed by use of a funnel plot.(19) Sensitivity analysis was conducted to assess whether the inferences overly depended on a particular study.

1.4 Results

In the initial search, 1,734 articles were identified from the key databases (1,051 from PubMed, 667 from Scopus and 16 from Cochrane Library) (see Figure 1) and imported into EndNote. After removing duplicate studies, the remaining 1,011 studies were

screened for relevance through their title and abstract, and then assessed according to the eligibility criteria previously stated. This step resulted in 65 articles. A further review that was conducted using PEDro excluded 40 papers (22 had irrelevant outcome data, 10 lacked placebo groups, four used a language other than English, four were low quality, and 10 trials with healthy participants). The remaining 15 papers were included in the final quantitative analysis; 14 were of high quality (8 points or more) and one was of moderate quality (5–7 points). Table 1 shows the final list of included studies and their summary characteristics.

[Insert Figure 1]

1.4.1 Description of the included studies

Fifteen randomised and placebo controlled studies examining the effects of probiotics on the risk factors of CVD were included in the analysis (see Table 1), representing a total sample of 788 subjects. Among these studies, all were blinded to assessors, 11 were double blinded and four were single blinded. All studies had placebo controlled groups as comparison groups. Placebo groups used placebo capsules that were identical to probiotics capsules in appearance except that the bacterial strains were absent. (20-24) Studies that used milk or yogurt as the medium had placebo products that were prepared in the same way as the probiotic milk or yogurt except the bacterial strains were absent. (11-13, 20, 25-30) Countries in which the studies were conducted included the Canada, Australia, Brazil, Denmark, Sweden, Spain, Iran, Japan and Korea. Subject characteristics included hypercholesterolemia, (11, 21, 24, 26, 27), hypertension, (28, 29) diabetes, (20, 25), overweight and obesity, (13, 22, 23, 31), metabolic abnormality, (12) and heavy smoking. (30) All studies measured one or more of the following outcome variables: cholesterol, HDL, LDL, triglyceride, waist circumference, BMI and

inflammatory biomarkers. The summary description of the study characteristics is presented in Table 1.

[Insert Table 1]

1.4.2 Effects on cholesterol and lipid profiles

Ten studies reported the effects of probiotics on total cholesterol, 10 studies on LDL, 11 studies on HDL and 12 studies on triglyceride. There were 464 participants in studies (see Figure 3) examining total cholesterol, 533 participants for LDL, 579 participants for HDL and 614 participants for triglyceride. Of the 12 studies on triglyceride, four reported results involving 225 hypercholesterolemia participants, and eight studies involving 389 participants reported results in other health conditions including hypertension, diabetes and metabolic conditions.

Of the 10 studies on cholesterol eight reported a reduction in this factor after consuming probiotics. The mean reduction at baseline was 0.50 mmol/L (ranging from 0.13 to -1.43 mmol/L), compared with 0.19 mmol/L (ranging from 0.36 to -0.56 mmol/L) reduction in the control group. The pooled effect of the total cholesterol was -0.27 mmol/L (95CI: -0.38 to -0.16, $P<0.00001$) in the probiotics group compared with the control group. The forest plot of the effect is presented in Figure 2.A. The heterogeneity was not significant ($I^2=35.5\%$, $P=0.12$).

Nine of ten studies on LDL presented changes in this factor after consuming probiotics. The mean reduction of LDL was 0.49 mmol/L (ranging 0.16 to -1.89 mmol/L). The pooled effect of the LDL was -0.23 mmol/L (95% CI: -0.33 to -0.13 mmol/L, $P<0.00001$) in the mean difference in the probiotics group compared with the control group (see Figures 2.B), with significant heterogeneity ($I^2=56.58\%$, $P=0.01$).

Five of 11 studies on HDL showed an increase in this factor, while six studies showed a decrease in HDL after consuming probiotics. There was an average decrease of

0.03 mmol/L (ranging from 0.13 to -0.26 mmol/L). The pooled effect of the HLD was 0.01 (95% CI: -0.01 to 0.03 mmol/L, $P=0.39$), with non-significant heterogeneity ($I^2=0\%$, $P=0.57$) (See Figure 2.C).

Five of 12 studies on triglycerides showed a decrease in this factor, six studies showed an increase and one study showed no change after consuming probiotics. The lowest decrease in triglycerides was 0.01 mmol/L and the highest decrease was 0.29 mmol/L. The meta-analysis result showed non-statistical decrease of 0.01 mmol/L (95% CI: -0.20 to 0.22 mmol/L, $P=0.92$), with non-significant heterogeneity ($I^2=0\%$, $P=0.57$) (See Figure 2.D).

[Insert Figure 2]

1.4.3 Effects on BMI and waist circumference

Four studies (13, 22, 23, 25) reported BMI results among 234 subjects, and four studies (13, 22, 23, 32) reported waist circumference results among 304 subjects (see Figure 3). Three of the four studies showed a decrease in BMI after consuming probiotics. The mean reduction of BMI following probiotics consumption was 0.3 kg/m^2 (-0.12 to 0.50 kg/m^2). The pooled effect of the BMI was -0.52 kg/m^2 (95% CI: -0.80 to -0.24 kg/m^2 , $P<0.001$), with unmeasurable heterogeneity ($I^2=0\%$, $P=0.59$, see Figure 3.A). All four studies showed a decrease in waist circumference. The average reduction of waist circumference was 1.82cm, and the range of decrease in waist circumference was 2.0 cm to 1.56 cm. The pooled effect of the waist circumference as -2.11 cm (95% CI: -3.54 to -0.68 cm) with unmeasurable heterogeneity ($I^2=0\%$, $P=0.63$) (see Figure 3.B).

[Insert Figure 3]

1.4.4 Effects on inflammatory markers

The effects of probiotics on inflammatory markers including hr-CRP and TNF Alpha were measured. Two studies (20, 33) on hr-CRP and TNF results among 69 subjects

were reported. Probiotics caused an average hr-CRP reduction of 1.67 mg/l (range 0.20 mg/l to 2.26 mg/l reduction) in probiotics group. The pooled effect was -2.95 (95% CI: -0.99 to -4.91, $P=0.002$, see Figure 3C). TNF Alpha had an average reduction of 3.25 pg/ml (range 0 pg/ml to 9.0 pg/ml reduction) in probiotics group, with the pooled effect of the TNF Alpha was -2.34 (95% CI: -0.53 to -4.14 pg/ml, $P=0.486$, see Figure 3D), with no heterogeneity for both hrs-CRP ($I^2=0\%$, $P=0.002$) and TNF Alpha ($I^2=0\%$, $P=0.49$).

[Insert Figure 4]

1.4.5 Subgroup and sensitivity analysis

The subgroup analysis found that the effects of the probiotics on cholesterol were statistically significant when it was in milk/yoghurt form ($P<0.001$) compared to those using probiotics in capsule form ($P>0.05$). Probiotics with multiple strains ($P<0.001$), and the trials that were eight weeks or longer in duration ($P<0.001$) had significant effects on total cholesterol and LDL compared to those trials with single strain and using probiotics for less than eight weeks. A greater reduction with clinical significance was found when trials had baseline total cholesterol of more than 5.5 mmol/L ($P<0.001$) compared with trials with total cholesterol of less than 5.5 mmol/L ($P<0.05$) (see Table 2). The reduction in cholesterol in trials was significant across both trials with acidophilus strain and those without acidophilus strains.

The subgroup analysis found that the effect of the probiotics on LDL was statistically significant when it was in milk/yoghurt form ($P<0.001$) compared to those trials using probiotics in capsule form, when the probiotics consisted of multiple strains ($P<0.001$) rather than a single strain, and the trials contained acidophilus strain compared to trials that did not include the acidophilus strains. A greater reduction with

clinical significance was also found in trials with baseline LDL of 3.5 mmol/L or more ($P<0.001$) compared with trials with less than 3.5 mmol/L ($P<0.05$) (see Table 2).

The subgroup analysis found that the effects of the probiotics diet on HDL and triglyceride were not related to the form of probiotics, dosage, number of strains, duration of administration of the probiotics consumption and health status of the participants (see Table 3).

[Insert Table 2]

[Insert Table 3]

Sensitivity analyses revealed that no particular study significantly affected the summary effects for total cholesterol, LDL, BMI, waist circumference.

1.4.6 Publication bias

The results for all factors showed minimal asymmetry, suggesting minimal publication bias. A visual inspection of the funnel plots showed no clear evidence of publication bias with regard to effects on total cholesterol, LDL, HDL, triglyceride, BMI and waist circumference (see Figure 5). Findings from the Egger's regression test (total cholesterol: $P=0.56$, LDL: $P=0.74$; HDL: $P=0.97$; triglyceride: $P=0.93$; BMI: $P=0.94$; waist circumference: $P=0.35$) (See Table 4) supported a finding that there was no publication bias. Trim and fill did not change the overall effects for all variables. These tests did not reveal any statistically significant evidence of publication bias for any assessed outcomes.

[Insert Figure 5]

[Table 4]

1.5 Discussion

This meta-analysis systematically reviewed 15 randomised controlled trials that examined the effects of probiotics on reducing cholesterol and other co-existing risk

factors associated with CVD. To our knowledge, this is the first meta-analysis of high quality randomised controlled trials investigating this topic. The pooled effects based on the meta-analysis revealed that probiotics reduced CVD risk factors, including high blood pressure and lipid components, as well as improving BMI, waist circumference and CRP. However, the effects of probiotics on HDL, triglycerides and TNF were not statistically significant. The reduction in total cholesterol and LDL was similar to that reported in another meta-analysis of the effect of statin doses on reducing total cholesterol and LDL. (34)

Probiotics were found to provide multiple benefits—not only reducing cholesterol and LDL, but also improving co-existing risk factors such as BMI, waist circumference and inflammatory biomarkers. Worldwide, these major risk and coexisting risk factors are often ignored in treatment regimes, yet they need to be addressed as reduction in lipid components alone does not significantly reduce the mortality and morbidity associated with CVD. Our results are consistent with previous published reviews regarding the effects of probiotics on the risk factors of CVD, including cholesterol (35, 36) and LDL.(35, 36) is still high.

1.5.1 Effects of probiotics on lipid profiles and CVD risk factors

Sub-group analyses revealed that the significant effects of probiotics on the reduction of total cholesterol and LDL may be due to the effects of probiotic forms (i.e., milk/yoghurt vs capsules), duration of intake, and number of strains in the products. Most of the trials (11 out of 15) used a fermented dairy product, rather than a capsule. Probiotics in the fermented dairy form had significant pooled effects on the total cholesterol and LDL, and probiotics in the capsule form had no significant reduction of total cholesterol and LDL. These findings are consistent with two clinical trials that found that probiotics using a

fermented dairy product was effective in reducing total cholesterol and LDL to 10%. (37, 38)

It was found that trials using multiple probiotic strains showed a statistically significant reduction of total cholesterol, whereas trials with a single strain did not have a significant reduction in total cholesterol. This result is similar to the finding that trials with multiple strains had better effects compared with those with a single strain on blood pressure.(39) Due to a limited number of trials with a single strain, further studies are warranted to confirm this finding. In the studies we reviewed, a number of genera of bacteria were used in probiotics, including *L. acidophilus*, *L.plantarum*, *Bifidobacterium*, *Enterococcus*, *S.thermophilus*, *L.bulgaricus*, *Streptococcus*, and *Thermophiles*. We found that *L.acidophilus* has the strongest effect on LDL reduction. This may be because *L. acidophilus* can pass through the digestive tract intact and alive compared to other strains, thereby optimising its activity level in the large intestine(40).

The mechanism through which probiotics in fermented milk acts might be through the production of an enzyme called bile salt hydrolase, (41). This enzyme reduces the absorption of cholesterol in the bloodstream and instead, it becomes trapped in the gut, and later excreted in fecal matter. The conversion of cholesterol to bile salts in the liver and subsequent secretion and fecal excretion provides the major route (~ 90%) for the elimination of elevated or excess cholesterol. Another mechanism by which the lipid lowering effects occurs is through probiotics stimulating the production of short-chain fatty acids in the gut, which modulate blood lipid levels.(42, 43) This mechanism acts by removing cholesterol through the incorporation of cholesterol into cellular membranes in the intestines, thereby reducing serum cholesterol in humans.(44)

The impact of probiotics on cholesterol and LDL maybe also explained by its effects on the reduction of co-existing risk factors, including BMI, waist circumference and inflammatory markers such as CRP. The reduction of these factors is important because of their cumulative effect on CVD. (1, 45) Outcomes of a number of clinical trials indicate the weight-lowering and anti-oxidative properties of probiotics as manifested by the improvement in the BMI and waist circumference. (46). This effect may be due to the calcium in dairy products, which can reduce body fat and body-mass. (46) In addition, probiotics can attenuate acute-phase inflammation by changing the intestinal inflammatory barrier, and impact the inflammatory process via the effect of intestinal microflora on bile and metabolism. The reduction of these risk factors through probiotics consumption has significant implications for reducing the mortality and morbidity attributable to CVD when major risk factors such as cholesterol and LDL are also controlled.(1)

No studies in this meta-analysis reported any adverse side effects related to the mediums used (fermented milk and capsules), indicating that they were safe and well tolerated. In addition, 14 of the 15 studies had less than a 15% attrition rate over the course of the intervention, suggesting that the subjects had a high level of satisfaction with the consumption of probiotics. Fourteen of the 15 included trials did not use medication. When the study using herbal extracts in both probiotics and placebo groups were excluded from the analysis, (23) the results remained significant. This suggests that probiotics consumption provides health benefits equivalent to medication use and has achieved significant intervention effects at both the statistical and clinical level without medication intake or controlled medication use.

1.5.2 Strengths, limitations and implications

A strength of this study is that all studies published since 2000 have been included in the meta-analysis, and where there was unreported data, the authors were contacted to provide it. Therefore, all available data were included, thereby increasing the power of the study.(39) All included studies in this review used placebos as the control group; thus, the effect of intervention due to probiotics consumption could be examined. The results of these analyses should therefore be viewed as accurate and precise. This is the first meta-analysis study on RCTs relating to the effect of probiotics on lipid profiles. This meta-analysis suggests an avenue for future intervention programs to investigate the effects of probiotics consumption on improving lipid profiles.

A number of limitations of this study should be acknowledged. The studies did not systematically report treatment effects in clinically sensible subgroups, such as risk strata defined by the Framingham Risk Score.(47) A previous randomised control study suggested that the treatment effects of dietary-based interventions vary across these strata, with benefits confined to high-risk individuals who have at least two to three clinical risk factors.(48) Further exploration of such subgroup differences within the context of RCTs will entail an individual patient data meta-analysis. There was inconsistent use of strain types among some of the studies, with some using one strain and others multiple strains, making it difficult to assess which strain or strains contributed to the effect of probiotics. Further RCTs involving long-term interventions that consistently compare single versus multiple strains of probiotics supplement regimens are required to further clarify these findings. It is also acknowledged that we did not include studies published in languages other than English so it is unknown whether results from these studies would have modified our findings.

The efficacy of the probiotics in reducing the risk of complications, reoccurrence of major cardiovascular events, and mortality in patients with more severe heart

conditions was not examined in our study. Future research is required using a large sample size and long-term intervention RCT to confirm that alternative nutrition regimens are effective with regard to the reduction of not only the major risk factors of CVD, but also the coexisting inflammatory factors. This will enhance our understanding of the application of probiotics in CVD prevention and treatment. Further research is also needed into whether probiotics has a superior effect to pharmaceutical drug administration in order to determine whether it should be included as a treatment option for CVD. At the very least, the field of translational cardiovascular medicine should explore probiotics as an important dietary supplement for reducing total cholesterol and LDL, as well as other coexisting risk factors of CVD.

1.6 Conclusions

The findings of this study suggest that probiotics supplementation use is effective in reducing total cholesterol, LDL and the coexisting risk factors of CVD. Probiotics are more effective when they are taken as a fermented milk or yogurt product, consumed for at least eight weeks and include multiple strains. By managing multiple risk factors and coexisting risk factors associated with CVD, probiotics may provide a powerful avenue to reduce current high rates of cardiovascular morbidity and mortality.

Table 1

Characteristics of included clinical trials

Studies	Participant s (No. of P/C)	Design; Location	Age Mean (SD) P/C	Sex (M/F)	Probiotic (wk)	Probiotics strains	Dose (CFU/ g and/o r quantity/d)	Placebo group	Measured Outcomes
1. Agerholm-Larsen et al 2000	OW (56/14)	DB, PC, P; Denmark	ND/ 39.4 (2.1)	20/50	Yogurt (8)	Streptococcus (S.) thermophilus and Enterococcus (E.) faecium	540 ml/d	Placebo yogurt with delta acidlactone	TC, LDL, HDL cholesterol, TG
2. Andreasen et al 2010	T2DM (21/24)	DB, PC, P; Denmark	55 (ND)/ 60 (ND)	45/0	Capsule (4)	L. acidophilus	10 ¹⁰	Placebo capsule	CRP; TNF alpha
3. Asemi et al 2013	Healthy (37/33)	SB, PC, P; Iran	24.2 (3.3)/ 25.7 (3.1)	0/70	Yogurt (9)	S. thermophilus, L. bulgaricus, L. acidophilus and Bifidobacterium (B.) animalis	10 ⁷	Placebo yogurt	TC, LDL, HDL cholesterol, TG
4. Ataie-Jafari et al 2008	HC (7/7)	SB, PC, Cr; Iran	ND/ ND	4/10	Yogurt (16)	L. acidophilus and B. lactis	10 ⁶	Placebo yogurt	TC, LDL, HDL cholesterol, TG
5. Barreto et al 2014	Metabolic syndrome (12/12)	SB, PC, P; Brazil	62 (ND)/ 63 (ND)	20/4	Fermented Milk ()	L. plantarum	1.25x 10 ⁷ CFU/g 80 ml/d	Non-fermented milk	TC, LDL, HDL cholesterol, TG
6. Ejtahed et al 2011	T2DM (30/30)	DB, PC, P; Iran	50.87 (1.40)/ 51.00 (1.34)	23/37	Yogurt (6)	L. acidophilus and B. lactis	10 ⁶ for each strain	Conventional yogurt	TC, LDL, HDL cholesterol, TG

7. Fuentes et al 2012	HC (30/30)	DB, PC, P; Spain	ND/ ND	ND	Capsule (12)	<i>L. plantarum</i>	1.2×10^9	Placebo capsule	TC, LDL, HDL cholesterol, TG
8. Inoue et al 2003	HTN (18/17)	SB, PC, P; Japan	56.2 (2.1)/ 53.9 (3.3)	20/15	Fermented Milk (12)	<i>L. casei</i> and <i>L. lactococcus</i>	10 – 12 mg/100 ml per day	Lactic acid placebo with the same taste	TC; TG
9. Jones et al 2012	HC (56/58)	DB, PC, P; Canada	51-89 (ND)/ 48-79 (ND)	41/73	Yogurt (6)	<i>L. reuteri</i>	5×10^9 CFU, twice daily	Placebo yogurt	TC, LDL, HDL cholesterol, TG
10. June et al 2013	OB (28/29)	DB, PC, P; Korea	ND/ ND	22/35	Capsule (12)	<i>L. gasseri</i>	10^{10}	Placebo capsules	TC, LDL, HDL cholesterol, TG
11. Kadooka et al 2010	OB (43/44)	DB, PC, P; Japan	48.3 (9.3)/ 49.2 (9.1)	59/28	Fermented Milk (12)	<i>L. gasseri</i>	5×10^{10} CFU	Placebo yogurt	BMI; WC
12. Lee et al 2014	OB (25/25)	DB, PC, P; Korea	ND/ ND	ND	Capsules (8)	<i>S. thermophiles</i> , <i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. lactis</i> , <i>B. longum</i> , and <i>B. breve</i> .	5×10^9 CFU, two capsules per day	Placebo capsules	BMI; WC
13. Mizushima et al 2004	HTN (23/23)	DB, PC, P; Japan	44.3 (10.3)/ 48.5 (8.9)	46/0	Sour Milk (4)	<i>L. Helveticus</i> and <i>Saccharomyces cerevisiae</i>	160 g/d	Placebo acidified milk	HDL-C; TC; TG
14. Naruszewicz et al 2002	Smokers (18/18)	DB, PC, P; Sweden	42.3 (2.8)/ 41.8 (2.8)	18/18	Sour Milk (6)	<i>L. plantarum</i>	5×10^7 CFU	Placebo milk manufactured in the same company	TC, LDL, HDL cholesterol, TG
15. Simons et al 2006	HC (23/21)	DB, PC, P; Australia	50 (12)/ 53 (11)	16/28	Capsule (10)	<i>L. fermentum</i>	8×10^9 CFU	Placebo capsule	TC, LDL, HDL cholesterol, TG

A: Control or Probiotic group (cross-over); B: Probiotic or Control group (cross-over); BMI: Body Mass Index; CFU: Colony-forming Unit; Cr.: Crossover; CRP: C-reactive Protein; TB: Triple Blinded; DB: Double Blinded; T2DM: Diabetic patients; HC: Hypercholesterolemic patients; IBD: Inflammatory Bowel Disease; HDL-C: High Density Lipoprotein Cholesterol; HTN: Hypertensive patients; IL-6: Interleukin 6; LDL-C: Low Density Lipoprotein Cholesterol; ND: Not Determined; OB: Obese subjects; OW: Overweight subjects; P: Parallel; PC: Placebo Controlled; P/C: Probiotic group/Control group; RA: Rheumatoid Arthritis patients; SB: Single Blinded; SD: Standard Deviation; TC: Total Cholesterol; TG: Triglyceride; TNF: Tumor Necrosis Factor; WC: Waist Circumference.

Table 2

The subgroup analyses of the effect of probiotics on total cholesterol and LDL-C by probiotics administration criteria

Subgroups	Total Cholesterol					LDL-C				
	No of studies	I ²	Q-test	Mean difference (95% CI)	P between	No of studies	I ²	Q-test	Mean difference (95% CI)	P between
<i>Form of probiotics</i>					0.67					0.65
Capsule	3	76.2%	8.4	-0.21 [-0.70, 0.28]		4	79.9%	9.9	-0.30 [-1.01, 0.41]	
Milk or yoghurt	10	4.8%	6.3	-0.31 [-0.42, -0.20]***		11	0%	1.8	-0.47 [-0.70, -0.25]***	
<i>Probiotics dosage</i>					0.40					0.79
Large dose ($\geq 10^9$ CFU)	4	71.3%	36	-0.30 [-0.50, -0.10] ***		5	72.1%	10.8	-0.37 [-0.64, -0.16]***	
Small dosage ($<10^9$ CFU or milk)	6	0%	21	- 0.32 [-0.41, -0.24]		5	0%	1.6	-0.44 [-0.72, -0.16]***	
<i>Number of strains of probiotics</i>					0.69					0.47
Single Strain of probiotics	4	64.3%	8.4	-0.22 [-0.65, 0.21]		6	55.1%	11.2	-0.34 [-0.68, -0.00]**	
Multiple strains of probiotics	6	20.6%	6.3	-0.31 [-0.47, -0.16]***		4	0%	0.8	-0.52 [-0.85, -0.18]***	
<i>Duration of probiotics administration</i>					0.93					0.81
Probiotic use for < 8 weeks	2	0%	0.1	-0.22 [-0.50, -0.05]		4	0%	0.4	-0.45[-0.72, -0.17]***	
Probiotic use for ≥ 8 weeks	8	50.5%	14.1	-0.32 [-0.53, -0.11]***		6	58.1%	11.9	-0.24 [-0.78, -0.01]**	
<i>Health status of study sample</i>					0.68					0.30
Hypercholesterolemic patients	7	33.6%	9.0	-0.30 [-0.38, -0.21]***		5	7.6%	4.3	-0.29 [-0.56, -0.03]***	
Hypertension	3	28.1%	2.8	-0.51 [-0.73, -0.28]***		4	51.6%	6.2	-0.55 [-0.97, -0.14]***	
<i>By specific probiotic strains</i>					0.63					0.64
Acidophilus strain	3	50.9%	4.1	-0.50 [-0.95, -0.05]**		3	0%	0.8	-0.52 [-0.85, -0.18]***	
Non-Acidophilus strains	7	52.4%	10.5	-0.26 [-0.46, -0.06]**		5	62.8%	10.8	-0.26 [-0.75, 0.02]	
<i>By baseline level</i>					0.02*					0.08
<5.5 mmol/L for TC or <3.5mmol for LDL	5	34.5%	18	-0.20 (-0.38, -0.02)*		3	10%	2.2	-0.18 (-0.33, -0.03)*	
≥ 5.5 mmol/L for TC or ≥ 3.5 for LDL	5	0%	7	-0.54 (-0.75, -0.33)***		6	0%	3.4	-0.38 (-0.38, -0.16)***	

*effect size significant at $p < 0.05$; ** effect size significant at $p < 0.01$; *** effect size significant at $p < 0.001$; LDL-C: Low Density Lipoprotein Cholesterol

Table 3 Con't

The subgroup analyses of the effect of probiotics on HDL-C and triglycerides by probiotics administration criteria

Subgroups	HDL-C					Triglyceride				
	No	I ²	Q-test	Mean difference (95% CI)	P between	No	I ²	Q-test	Mean difference (95% CI)	P between
Form of probiotics					0.83					0.28
Capsule	3	0%	1.7	0.05 [-0.030, 0.12]		3	0%	1.9	-0.12 [-0.36, 0.12]	
Milk or yoghurt	7	0%	1.7	0.02 [-0.01, 0.05]		9	75.7%	32.9	-0.02 [-0.18, 0.33]	
Probiotics dosage					0.89					0.47
Large dose ($\geq 10^9$ CFU)	4	0%	1.9	0.04 [-0.03, 0.11]		5	80.3%	20.3	0.12 [-0.29, 0.54]	
Small dosage ($<10^9$ CF/ milk form)	5	43%	1.7	0.02 [-0.01, 0.05]		7	0%	5.1	-0.03 [-0.11, 0.04]	
Number of strains of probiotics					0.75					0.43
Single Strain of probiotics	5	0%	2.2	0.05 [-0.02, 0.12]		6	76.1%	20.9	0.11 [-0.25, 0.47]	
Multiple strains of probiotics	4	0%	1.0	0.02 [-0.01, 0.05]		6	0%	4.3	-0.09 [-0.11, 0.04]	
Duration of probiotics administration					0.73					0.14
Probiotic use for < 8 weeks	3	0%	0.5	0.06 [-0.04, 0.16]		4	72.7%	11.0	0.25 [-0.14, 0.63]	
Probiotic use for ≥ 8 weeks	6	0%	2.7	0.02 [-0.01, 0.05]		8	0%	6.4	0.00 [-0.12, 0.69]	
Health status of study sample					0.99					0.76
Hypercholesterolemic patients	5	0%	1.9	0.02 [0.0, 0.05]		4	0%	5.9	-0.02 [-0.10, 0.05]	
Hypertension	4	0%	1.9	0.03 [-0.05, 0.10]		12	85.6%	20.9	-0.06 [-0.47, 0.59]	
By specific probiotic strains					0.29					0.07
Acidophilus strain	3	0%	1.0	0.02 [-0.08, 0.12]		3	0%	1.14	-0.23 [-0.47, 0.02]	
Non-Acidophilus strains	6	0%	2.8	0.03 [0.0, 0.05]		8	77.0%	30.45	0.11 [-0.13, 0.36]	

HDL-C: High Density Lipoprotein Cholesterol

Table 4. Egger test results on lipids, BMI and waist circumference

Egger test	t (95% CI)	P
Cholesterol	0.56 (-0.74 to 1.31)	0.56
LDL	0.35 (-3.39 to 4.58)	0.74
HDL	0.04 (-0.69 to 0.71)	0.97
TG	0.09 (-1.66 to 1.81)	0.93
BMI	0.08 (-4.88 to 5.07)	0.94
Waist circumference	1.20 (-4.35 to 7.71)	0.35

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