Efficacy of Probiotics in Patients of Cardiovascular Disease Risk: A Systematic Review and Meta-Analysis

Asher Dixon,^{1,2,4} Kai Robertson,³ Amanda Yung,² Michael Que,² Hayden Randall,^{2,4}
Don Wellalagodage,^{2,4} Tynan Cox,¹ Dylan Robertson,¹ Cheng Chi,¹ and Jing Sun,¹

¹School of Medicine, Griffith University, Gold Coast, Queensland, Australia; ²Sydney Medical School, University of Sydney, Camperdown NSW; ³School of Medical Science, Griffith University ⁴St Paul's College Graduate House, Sydney, Australia

Correspondence: Associate Professor Jing Sun, School of Medicine, Griffith University, Gold Coast campus. Q4222 Email: j.sun@griffith.edu.au

Abstract:

Purpose of Review: This meta-analysis evaluated the effect of probiotics on blood pressure, blood glucose, body mass index (BMI), and lipid profile of patients with cardiovascular disease risk factors.

Recent Findings: Randomised controlled trials published on PubMed, Scopus, Embase, Grey Literature and the Cochrane Central Register of Controlled Trials (CENTRAL) from 1990 to 2020 were systematically searched. The PEDro scale was used to assess the quality of studies. A total of 34 studies with 2,177 adults were selected for inclusion in the analysis. The mean difference and effect size with a 95% confidence interval (CI) were analysed for the pooled results. Statistically significant pooled effects of probiotics were found in the reduction of SBP by -1.31 mmHg (95% CI: -2.23, -0.39, $P = 0.005$), DBP by -1.87 mmHg (95% CI: -2.41, -1.33, $P < 0.001$), total cholesterol by -6.05 mg/dL (95% CI: -8.49, -3.61, $P < 0.001$), LDL-C by -8.77 mg/dL (95% CI: -11.86, -5.69, $P < 0.001$), serum glucose by -4.92 mg/dL (95% CI: -6.15, -3.69, $P < 0.001$), HbA1C by -0.18% (95% CI: -0.31, -0.06, $P = 0.005$), and BMI by -0.31 kg/m² (95% CI: -0.41, -0.21, $P < 0.001$). No significant changes were observed in the outcome of triglycerides and HDL-C.

Summary: Probiotic supplementation had beneficial effects on blood pressure, blood glucose, BMI, and lipid profile levels in patients with cardiovascular disease risk factors. These effects were associated with longer treatment duration, higher dosage, use of alternate formulations, diabetic patients, and female populations.

Keywords: Probiotics, Cardiovascular Disease, Hypertension, Diabetes, Microbiome

Introduction:

Cardiovascular disease (CVD) is the leading cause of death worldwide. In 2016, CVDs were responsible for approximately 17.9 million deaths, or 31% of all deaths globally [1]. By 2025, over 5 million men and 2.8 million women are projected to die prematurely from CVD [2]. The most prominent physiological risk factors for CVDs are a cluster of medical conditions known as

metabolic syndrome. This “deadly quartet” of metabolic abnormalities include obesity, glucose intolerance/insulin resistance, dyslipidaemia, and hypertension [3].

The presence of cardiovascular-risk factors, or any one of its components, confers a greatly increased risk of developing CVD, including ischaemic heart disease and stroke [4]. While there are variable definitions of cardiovascular disease risk such as metabolic syndrome, it is estimated to be prevalent in approximately one quarter of the world population, putting a significant proportion of humanity at risk of developing CVD [5]. Thus, CVDs and its associated comorbidities represent a significant burden to world health.

Currently, CVD risk reduction is commonly managed by medications such as statins and antihypertensives [6]. Control of risk factors for CVD can also be achieved through lifestyle modification, especially through diet and exercise. For instance, lowering salt intake has been shown to reduce blood pressure substantially [7]. However, due to the immense and ever-growing burden of CVDs, alternative approaches are required in the coming years to reduce morbidity and mortality associated with CVDs.

The past decade has seen an upsurge of interest in the contribution of the human microbiome to health and disease. The human gastrointestinal microbiota is incredibly diverse [8] and plays key roles in maintaining the gastrointestinal epithelium, modulation of the immune system, digestion, and nutrient metabolism [9]. Alteration of the microbiota in a pathological fashion, known as dysbiosis, may be involved in the pathogenesis of many diseases, including inflammatory bowel disease [10], cancer [11], obesity [12], and diabetes mellitus [13]. Dysbiosis itself can be caused by medical and lifestyle factors such as infection, host genetics, antibiotic use, and a high-fat, low-fibre diet [14]. Crucially, there have also been studies linking dysbiosis to hypertension, insulin resistance, dyslipidaemia, and increased adiposity [15] [16], thus raising the possibility of managing CVD risk factors by treating dysbiosis.

One method of treating dysbiosis is by probiotics. Probiotics, formally defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [17], have shown efficacy in helping prevent or alleviate gut-related disorders such as irritable bowel syndrome [18], diarrhoea [19][20], colorectal cancer [21], and lactose intolerance [22]. The most common strains used in probiotics are lactic acid bacteria belonging to genera *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* [23]. Possible mechanisms by which probiotics exert their effect include restoring microbiota diversity, decreasing cholesterol absorption, reducing the production of pro-inflammatory molecules, and the synthesis of various metabolites [24][25]. Probiotics are most frequently consumed in the form of food or drink, such as yogurts, or as dietary supplement pills [26]. Probiotic studies have also shown promising results in treating hypertension, dyslipidaemia, insulin resistance, and obesity, with potential to have protective and therapeutic effects against heart failure and myocardial infarction [27*][28][29].

Patients with CVD risk factors, such as metabolic syndrome, face a greatly increased risk of cardiovascular events and may therefore have the greatest potential for benefit from probiotic therapy. Thus, our systematic review and meta-analysis aims to elucidate whether probiotic therapy is effective in lowering the physiological and biomedical risk factors of CVD (namely obesity, blood pressure, blood glucose, and lipids) in patients who are afflicted by cardiovascular comorbidities such as hypertension, Type II diabetes, obesity, and dyslipidaemia.

Methods:

Search Methods: The review protocol was registered with the Prospero International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42014010591); registration number CRD2014010591 [29]. The Population, Intervention, Control, Outcome (PICO) principle was used to search the literature [30]. The population was hypertensive adults > 18 years of age with cardiovascular disease risk, metabolic syndrome, type II diabetes mellitus, hypertension, obesity, hypercholesterolaemia. The intervention was probiotic supplementation treatment within the intervention group; the control included patients receiving no treatment, placebo or alternate cardiovascular medications; the primary outcomes used were blood pressure, cholesterol, triglycerides, fasting blood glucose, HbA1C, LDL-C, HDL-C and the secondary outcome was BMI. The keywords used to identify relevant studies through advanced searches were as follows: Hypertension AND (Cardiovascular Disease OR Metabolic Syndrome OR Diabetes) AND Probiotic Agent AND Randomised Controlled Trial. For cases where data was not adequately reported, authors were contacted in order to retrieve the necessary information.

Inclusion Criteria: Inclusion of studies within the meta-analysis followed strict inclusion criteria as follows: (1) Published in a peer-reviewed journal in the past 30 years or Grey Literature (February 1990 until February 2020); (2) Study design was an RCT; (3) Participants were adult patients with hypertension, obesity, cardiovascular disease, metabolic syndrome, type II diabetes or hypercholesterolaemia; (4) probiotics were used as an intervention approach; (5) primary outcome variables included blood pressure, Total cholesterol, HbA1C, serum glucose, LDL-C, HDL-C with BMI as an additional outcome; (6) If multiple studies were published on the same population only the most recent study is to be included.

Data Collection: Studies that reported insufficient or incomplete data had their corresponding authors contacted, seeking acquisition of necessary additional data. No authors provided information on outcomes for studies and their values were excluded in the analysis.

Data Extraction: Two authors completed the initial search with review of each search strategy, three authors resolved disagreements. AD completed the first search with AY completing a paralleled search. DR participated in discussion when disagreements were raised. All citations and abstracts were downloaded to EndNote X9 for review. Duplicates were deleted and the titles and abstracts of the remaining articles were screened for inclusion and checked by a second examiner (Figure 1. outlines this process). Several databases were used to identify articles for inclusion such as PubMed, Scopus, Embase, Grey Literature and the Cochrane Central Register of Controlled Trials (CENTRAL). The following data were extracted from each of the included articles: study setting, design location, study blinding, participant demographics, attrition rate, comorbidities, participant age & gender, probiotic treatment duration & route, strain and form of probiotic used for intervention, dosage of intervention treatment, control used, measured outcomes, information for assessment of the risk of bias and reported major findings.

Quality Assessment: The Physiotherapy Evidence Database tool (PEDro scale) was used to rapidly assess the internal validity (criteria 2-9), external validity (criteria 1) and sufficiency of statistical information (criteria 10-11) of randomised clinical trials. The Pedro tool categorises the quality of studies into three levels: high quality (≥ 8 points), moderate quality

(4-7 points), and low-quality (≤ 3 points). Criteria 1-11 are as follows: eligibility criteria specified, subject randomisation, allocation concealment, the similarity of baseline prognosis between groups, blinding of subjects, therapists and assessors, a primary outcome measurement on $\geq 85\%$ of initial subjects, use of intention-to-treat analysis, use of variability measures and use of between-group comparison methods. Each paper was assessed independently by two reviewers and any discrepancies were resolved via discussion (Table 1).

Statistical Analysis: The effect of probiotics on CV health outcome variables was described with the use of a pooled effect sized method. Random – effect meta-analyses were performed to describe the overall effect size. Mean difference was used to present the effect size for blood pressure, total cholesterol, LDL-C, HDL-C, triglycerides, serum glucose, HbA1c and BMI. CIs and the weighted mean difference were calculated for each outcome variable. Analysis of each study to assess for inconsistency included; visual assessment of CI for overlap and I^2 statistics of heterogeneity. Subgroup analyses were performed including gender ratio (Female > Male, Male > Female), dosage of probiotics ($< 1.0 \times 10^9$ CFU compared with $> 1.0 \times 10^9$ CFU), attrition ($> 15\%$ compared with $< 15\%$), form of probiotic (Capsule, Milk, Yogurt, Other), study blinding (SB, DB and NB) and duration of study (< 1.5 months compared with > 1.5 months). It can be noted, study numbers within the subgroup tables (3-7) represent the total number of studies included within each paper. As such individual papers may have included more than one study. Publication bias was assessed using Egger's Test (Table 8).

Figure 1. PRISMA flow chart

Results:

The initial database search yielded 901 potential articles and 15 additional articles were found through the reference lists of published articles. Duplicate articles (28) were removed. A total of 888 studies remained for screening through abstract and title for relevant articles to the study. Application of the Physiotherapy Evidence Database process confirmed the remaining selection of 34 studies to be included in the final Meta-Analysis (Table 1).

Participants: An outline of included studies are provided in Table 2. Outcomes were reported on 2,177 adults receiving probiotic intervention (1176) or control (1001) with removal of duplicate controls. Probiotics treatment was used to assess different cardiovascular outcomes, with ten studies measuring blood pressure (38, 43, 45, 51, 52, 55, 57, 59, 60, 64); twenty-four studies measuring total cholesterol (31, 33, 34, 35, 38, 39, 41, 42, 43, 44, 45, 49, 50, 51, 52, 55, 56, 57, 59, 60, 61, 62, 63); twenty-one studies measuring LDL-C (31, 32, 33, 34, 35, 39, 41, 43, 44, 45, 49, 50, 52, 55, 56, 57, 58, 59, 60, 61, 63); twenty-three studies measuring HDL-C (31, 32, 33, 34, 35, 39, 40, 41, 43, 44, 45, 49, 50, 51, 52, 55, 56, 57, 58, 59, 60, 61, 63); twenty five studies measuring triglycerides (31, 33, 34, 35, 37, 38, 39, 41, 42, 43, 44, 45, 49, 50, 51, 52, 55, 56, 57, 59, 60, 61, 62, 63); twenty studies measuring serum glucose (33, 35, 36, 37, 38, 41, 42, 45, 47, 49, 50, 52, 53, 55, 56, 57, 58, 59, 61, 62, 63); sixteen studies measuring HbA1C (33, 35, 36, 38, 39, 41, 45, 47, 49, 50, 52, 53, 54, 55, 56, 59); and nineteen studies measuring BMI (33, 35, 38, 39, 41, 45, 46, 48, 49, 50, 52, 53, 54, 57, 58, 60, 61, 63, 64). These studies were conducted in 11 different locations, including Australia (43), Brazil (34, 39), Czech Republic (44), Denmark (32), Estonia (60), Iran (31, 33, 35, 36, 37, 47, 49, 50, 53, 54, 56, 57, 58, 59, 61, 63, 64), Japan (40, 41, 42, 46, 51, 55), Korea (45, 58), Malaysia (38), Poland (62) and Sweden (52).

Effect on Blood Pressure: Ten studies with a total sample of 755 adults measured the mean difference of blood pressure between the intervention and control groups. The meta-analysis showed there was a statistically significant difference in the mean systolic and diastolic blood pressure, with an overall difference of -1.31 mmHg (95% CI: -2.23, -0.39) in SBP ($P = 0.005$) and -1.87 mmHg (95% CI: -2.41, -1.33) in DBP ($P < 0.001$) in favour of probiotic intervention. The heterogeneity was not statistically significant ($I^2 = 0\%$, $P = 0.48$). The forest plot of the effect is presented in Figure 2A and Figure 2B. A subgroup analysis (Table 3) found the effect of probiotics on SBP and DBP was more statistically significant in patients with Diabetes Mellitus demonstrating a 5.82 mmHg reduction in SBP ($P = 0.02$) and a 3.81 mmHg reduction in DBP ($P < 0.001$). Probiotics were more effective when in yoghurt form, reducing SBP and DBP by 3.56 mmHg ($P = 0.07$) and 3.54 mmHg ($P = 0.001$) respectively. SBP reductions of 2.38 mmHg ($P = 0.04$) were observed in patients treated for > 1.5 months. Probiotics appeared more effective on SBP and DBP in male populations demonstrating reductions of 2.36 mmHg ($P = 0.05$) and 0.81 mmHg ($P = 0.007$) respectively. A reduction in DBP was observed in both long and short duration patient groups, with DBP reductions of 1.98 mmHg ($P = 0.01$) and 2.14 mmHg ($P = 0.001$) respectively. Subgroup comparisons between dosage, blinding, attrition and hypertensive or hypercholesterolaemic patients could not be calculated due to lack of data.

Effect on Total Cholesterol: Twenty-four studies with a total sample of 1,727 adults measured the mean difference in cholesterol between the intervention and control. The meta-analysis showed there was a statistically significant difference in the mean cholesterol, with an overall difference of -6.05 mg/dL (95% CI: -8.49, -3.61) in Total cholesterol ($P < 0.001$) in favour of probiotic intervention. The heterogeneity was not statistically significant ($I^2 =$

0%, $P = 0.80$). The forest plot of the effect is presented in Figure 2C. A subgroup analysis (Table 4) detected a cholesterol reduction of 16.54 mg/dL ($P < 0.001$) in hypercholesterolaemic patients. A significant reduction in serum cholesterol was recorded in patients treated for < 1.5 months, -11.61 mg/dL ($P < 0.001$), and > 1.5 months, -4.84 mg/dL ($P < 0.001$). Patients that received probiotics in yoghurt, milk and other forms (e.g. kefir and powder) had significant reductions in serum cholesterol: -9.48 mg/dL ($P = 0.006$), -8.74 mg/dL ($P = 0.004$), and -5.22 mg/dL ($P = 0.003$) respectively. Probiotics were more effective in reducing cholesterol in predominantly male patient groups than predominantly female patient groups with reductions of 6.44 mg/dL ($P = 0.01$) and 5.59 mg/dL ($P = 0.002$) respectively. Double blinded studies reported a statistically significant reduction in cholesterol of 6.43 mg/dL ($P < 0.001$). A reduction in cholesterol was apparent in patients receiving higher dosages ($> 1.0 \times 10^9$ CFU) of probiotics, -6.38 mg/dL ($P < 0.001$). Studies with lower attrition rates ($< 15\%$) reported a more significant reduction in serum cholesterol, -6.01 mg/dL ($P < 0.001$). Subgroup comparisons in Non-blinded studies could not be calculated due to a lack of data.

Effect on LDL-C: Twenty-one studies with a total sample of 1,526 adults measured the mean difference in LDL between the intervention and control. The meta-analysis showed there was a statistically significant difference in LDL, with an overall difference of -8.77 mg/dL (95% CI: -11.86, -5.69), ($P < 0.001$) in favour of probiotic treatment. There is evidence of moderate heterogeneity ($I^2 = 78\%$, $P < 0.001$). The forest plot of the effect is presented in Figure 2H. A subgroup analysis (Table 5) found the effect of probiotics on LDL-C was more statistically significant in patients with Hypercholesterolaemia demonstrating a -20.67 mg/dL reduction ($P = 0.001$). Patients with diabetes and obesity were close to follow with reductions in LDL-C of -8.09 mg/dL ($P = 0.03$) and -7.27 mg/dL ($P = 0.001$) respectively. When administered as yoghurt, probiotics demonstrated a significant decline in LDL-C of -18.06 mg/dL ($P = 0.001$). Milk and capsule form similarly showed significant reductions in LDL-C of -9.82 mg/dL ($P = 0.007$) and -4.35 mg/dL ($P = 0.001$). Shorter durations of treatment (< 1.5 months) proved to have significant reductions in LDL-C -14.01 mg/dL ($P = 0.001$). Studies with treatment duration > 1.5 months similarly proved to reduce LDL-C (-7.08 mg/dL, ($P = 0.001$)). A greater reduction in LDL-C was apparent in patients receiving higher dosages ($> 1.0 \times 10^9$ CFU) of probiotics, -8.38 mg/dL ($P = 0.001$). Probiotics were more effective in lowering LDL-C in patient groups that were predominantly female, demonstrating a -9.87 mg/dL ($P = 0.001$). Subgroup comparisons on the blinding of studies and attrition could not be calculated due to a lack of data.

Effect on HDL-C: Twenty-three studies with a total sample of 1,602 adults measured the mean difference in HDL-C between the intervention and control. The meta-analysis showed there was no statistically significant difference in HDL-C, with an overall difference of 1.05 mg/dL (95% CI: -0.33, 2.43), ($P = 0.14$). There is evidence of severe heterogeneity ($I^2 = 90\%$, $P < 0.001$). The forest plot of the effect is presented in Figure 2I. A subgroup analysis (Table 5) found that probiotics were most effective in Hypercholesterolaemic and Diabetic patients with an increase in HDL-C of 6.36 mg/dL ($P = 0.04$) and 2.03 mg/dL ($P = 0.01$) respectively. It can be noted that obese patients sustained a reduction in HDL-C of -1.21 mg/dL ($P = 0.001$). Similarly, patient groups that were predominately female indicated significant reductions of HDL-C -1.02 mg/dL ($P = 0.02$). The remaining data revealed that probiotic treatment did not elicit a significant difference in HDL-C between subgroups: Dosage, formulation, study duration. Subgroup comparisons on the blinding of studies and attrition could not be calculated due to a lack of data.

Effect on Triglycerides: Twenty-five studies with a total sample of 1,590 adults measured the mean difference in cholesterol between the intervention and control. The meta-analysis showed there was no statistically significant difference in the mean serum triglycerides in patients taking probiotics with an overall difference of 3.24 mg/dL (95% CI: -0.07, 6.54), ($P = 0.06$). There is evidence of moderate heterogeneity ($I^2 = 61\%$, $P < 0.001$) within the study sample. The forest plot of the effect is presented in Figure 2D. A subgroup analysis (Table 4) detected a triglyceride reduction of 14.17 mg/dL ($P = 0.02$) in Diabetes Mellitus patients treated with probiotics. The remaining data revealed that probiotic treatment did not elicit a significant difference in serum triglycerides between subgroups: sex, dosage, attrition, formulation, blinding or study duration. Subgroup comparisons in Non-blinded studies could not be calculated due to a lack of data.

Effect on Fasting Glucoses: Twenty studies with a total sample of 1,481 adults measured the mean difference in serum fasting glucose between the intervention and control. The meta-analysis showed there was a statistically significant difference in serum glucose, with an overall difference of -4.92 mg/dL (95% CI: -6.15, -3.69), ($P < 0.001$) in favour of probiotic treatment. There is evidence of substantial heterogeneity ($I^2 = 83\%$, $P < 0.001$) within the study sample. The forest plot of the effect is presented in Figure 2E. A subgroup analysis (Table 6) detected a serum fasting glucose reduction of 16.15 mg/dL ($P = 0.001$) in short term treatment models where patients were treated for < 1.5 months with probiotics. Patients that received probiotics in capsule, yoghurt and other forms (e.g. kefir and powder) had significant reductions in serum fasting glucose: -13.47 mg/dL ($P = 0.03$), -12.88 mg/dL ($P = 0.03$) and 4.41 mg/dL ($P = 0.001$) respectively. It was also observed that probiotics reduced serum fasting glucose by 12.94 mg/dL ($P < 0.001$) in Diabetes Mellitus patients treated with probiotics. Subgroup analysis revealed a greater reduction of serum fasting glucose in predominantly male patient groups, -5.69 mg/dL ($P = 0.001$). A greater reduction in serum glucose was apparent in patients receiving higher dosages ($> 1.0 \times 10^9$ CFU) of probiotics, -5.29 mg/dL ($P = 0.001$). Double blinded studies reported a serum fasting glucose reduction of 5.27 mg/dL ($P = 0.001$). No significant changes in fasting glucose could be identified between groups of attrition, with a -5.63 mg/dL ($P = 0.001$) reduction in $> 15\%$ attrition and a -5.29 mg/dL ($P = 0.001$) reduction in $< 15\%$ attrition. Subgroup comparisons in non-blinded studies and patients with hypertension or hypercholesterolaemia could not be calculated due to a lack of data.

Effect on HbA1C: Sixteen studies with a total sample of 1,122 adults measured the mean difference in HbA1C between the intervention and control. The meta-analysis showed there was a statistically significant difference in HbA1C, with an overall difference of -0.18% (95% CI: -0.31, -0.06), ($P = 0.005$) in favour of probiotic treatment. There is evidence of substantial heterogeneity ($I^2 = 92\%$, $P < 0.001$) within the study sample. The forest plot of the effect is presented in Figure 2F. A subgroup analysis (Table 6) demonstrated that yoghurt probiotic formulations reduced HbA1C by 55% ($P = 0.03$), statistically significant reductions were not seen in other formulations. It was also found probiotics were more effective in reducing HbA1C in patients treated for < 1.5 months, -50% ($P = 0.001$). Furthermore, serum HbA1C was reduced by 32% ($P = 0.04$) in patients within the 'other' health group (e.g. multiple comorbidities, such as metabolic syndrome). Studies with higher attrition rates ($> 15\%$) demonstrated a slightly larger reduction in HbA1C, -20% ($P = 0.01$), when compared with lower attrition rate studies ($< 15\%$), -19% ($P = 0.001$). Probiotics were more effective at reducing HbA1C in patient groups that were predominantly female, with a reduction of 18% ($P < 0.001$). Subgroup analysis revealed there was a more promising trend in the reduction of HbA1C in higher doses ($> 1 \times 10^9$ CFU) of probiotics, -12% ($P = 0.06$), than lower doses.

Subgroup comparisons of study blinding and patients with hypertension or hypercholesterolaemia could not be calculated due to a lack of data.

Effect on BMI: Nineteen studies with a total sample of 1,205 adults measured the mean difference in BMI between the intervention and control. The meta-analysis showed there was a statistically significant difference in BMI, with an overall difference of -0.31 kg/m^2 (95% CI: $-0.41, -0.21$), ($P < 0.001$) in favour of probiotic treatment. The heterogeneity was not statistically significant ($I^2 = 0\%$, $P = 0.51$). The forest plot of the effect is presented in Figure 2G. A subgroup analysis (Table 7) found that probiotics were able to reduce BMI by 0.50 kg/m^2 ($P = 0.001$) when administered in other forms (e.g. kefir and powder). Probiotics were also able to reduce BMI by 0.48 kg/m^2 ($P = 0.001$) in patients with obesity. A greater reduction in BMI was apparent in patients receiving higher dosages ($> 1.0 \times 10^9 \text{ CFU}$) of probiotics, -0.41 kg/m^2 ($P = 0.001$). Probiotics were more effective in lowering BMI in patient groups that were predominantly female, demonstrating a 0.35 kg/m^2 ($P = 0.001$) reduction in BMI in these groups. It was also found probiotics were more effective in lowering BMI in patients treated for > 1.5 months, -0.31 kg/m^2 ($P = 0.001$). Studies with lower attrition rates ($< 15\%$) reported a significant reduction in BMI, -0.29 kg/m^2 ($P = 0.001$). Subgroup comparisons on the blinding of studies could not be calculated due to a lack of data.

Publication Bias: Findings from the Egger's test (table 8) (SBP: $P = 0.205$; DBP: $P = 0.611$; Total Cholesterol: $P = 0.637$; Triglycerides: $P = .533$; Glucose: $P = 0.001$; HbA1C: $P = 0.403$; and BMI: 0.117 ; LDL-C: $P = 0.066$; HDL-C: $P = 0.215$) supported that there was no publication bias, except within Glucose. The sensitivity analysis for glucose was conducted removing any study did not change the overall results.

Conclusions:

The Pooled effects based on the meta-analysis found that probiotics improved the health outcomes in patients at risk of cardiovascular disease. This included an overall statistically significant reduction in systolic and diastolic blood pressure, serum cholesterol, serum triglycerides, serum glucose, HbA1C, LDL-C and BMI. It can also be noted there was an overall increase in HDL-C although it is not statistically significant. All outcomes demonstrated a statistically significant reduction in cardiovascular risk factors except HDL-C. These findings are highly relevant in patients at risk of cardiovascular disease, with polypharmacy becoming an increasing issue in global health [65]. The capacity to reduce cardiovascular risk factors in conjunction of prescription of pharmaceuticals indicates a significant advance in the field of the human microbiome. Our results are consistent with previously published reviews regarding the effects of probiotics in reducing blood pressure [66], cholesterol [67], serum triglycerides [68][69], serum glucose [70], HbA1C [71] and BMI [72]. Despite correlations these previous studies focused on mono-morbid patient groups rather than a whole society at risk of cardiovascular disease with comorbidities or measured only one outcome variable. None of these studies provided subgroup analysis on our key variables. Our studies included RCT's focusing on adults at risk of cardiovascular disease with comorbidities (diabetes, dyslipidaemia, metabolic syndrome, hypercholesterolaemia, hypertension).

Effects of Probiotics on BP, TC, LDL-C, HDL-C TAG, Glucose, HbA1C and BMI:

Subgroup analyses revealed the beneficial effects of probiotics may be related to patient morbidity. Diabetic patients displayed the greatest reduction in blood pressure, fasting glucose and triglycerides out of all of the health groups. With a 5.82 mmHg reduction in SBP ($P = 0.02$) and a 3.81 mmHg reduction in DBP ($P < 0.001$). Similarly fasting glucose displayed the most significant reduction in diabetics with 12.94 mg/dL ($P < 0.001$). Triglycerides and HDL-C also demonstrated the most beneficial effects in diabetic patients with a 14.17 mg/dL ($P = 0.02$) reduction and 2.03 mg/dL ($P = 0.01$) mean difference between the intervention and control group.

Outcome variables further indicated analyses may be related to morbidity with hypercholesterolaemic patients displaying the greatest reduction in total cholesterol (16.54 mg/dL, $P < 0.001$) and LDL-C (-20.67 mg/dL, $P = 0.001$), as did obese patients in BMI (0.48 kg/m², $P = 0.001$). These results may be explained by the inherent association between these morbidities and the outcome variable, given each patient subgroup showed the most significant reduction in the variable that was most related to their condition. This is a potential form of selection bias [73], whereby it can be expected that each of these subgroups would have a correlation with a given outcome measure.

Probiotics in Yogurt form demonstrated the most significant reductions in the cardiovascular risk factors of total cholesterol (-9.48 mg/dL), fasting glucose (-12.88mg/dL), HbA1C (-0.55%) and LDL-C (-18.06 mg/dL); reductions in blood pressure were also observed. Probiotics within the 'other forms' category (Kefir and powder) demonstrated a significant reduction in BMI (-0.5 kg/m²). One mechanism for explaining this characteristic, is the greater diversity of probiotic species found in kefir and their capacity to stimulate gastric emptying [74]. As such studies have shown a correlation between probiotic strain diversity and gastric motility [75], hence reducing the time for absorption and digestion.

Studies with greater proportions of female patients indicated more significant reductions in outcome measures, specifically; total cholesterol (-5.59 mg/dL, $P = 0.002$), fasting glucose (-5.63 mg/dL, $P = 0.001$), HbA1C (-0.18%, $P = 0.007$), LDL-C (-9.87 mg/dL, $P = 0.001$), and BMI (-0.35 kg/m², $P = 0.001$). One explanation for this is the relationship between the oestrogen-gut microbiome axis, whereby women are at greater risk of microbiota decline [78], thus providing a physiological template for microbiota correction and reduction in CVD risk factors.

Longer treatment duration (> 1.5 months) was favoured holistically within the outcome of blood pressure, with reductions of -2.38 mmHg in SBP ($P = 0.04$) and -1.98 mmHg in DBP ($P = 0.01$). Reduction over extended period of time could be expected, as resting blood pressure have been shown to require long-term lifestyle intervention [76].

Shorter treatment duration (< 1.5 months) was statistically favoured for reduction in total cholesterol (-11.61 mg/dL, $P < 0.001$), LDL-C (-14.01 mg/dL, $P = 0.001$), fasting glucose (-16.15 mg/dL, $P = 0.003$) and HbA1C (-0.5%, $P = 0.009$)
One mechanism for this can be explained by initial increase in gastric motility providing the majority of the effect in total cholesterol reduction [77] by a reduced absorption time, this may have residual effects with metabolism.

Subgroup analyses revealed higher treatment doses ($> 1 \times 10^9$ CFU) were more effective in reducing BMI (0.41kg/m², $P = 0.001$), total cholesterol (6.38 mg/dL, $P < 0.001$), HbA1C (12%, $P = 0.06$), and fasting glucose (16.15 mg/dL, $P = 0.001$) respectively. It can also be

said, results were trending towards higher treatment doses in triglycerides outcome groups for greater effect.

Strengths, Limitations and Implications: Our review used a thorough and comprehensive search strategy in identifying and assessing each article's quality for inclusion within the meta-analysis. Reviewer bias was minimised through individual and independent screening of potential articles before collaboration for final inclusion. One strength of this meta-analysis was the use of broad search terms to allow for manual inclusion of all relevant articles, maximising the power of the study. All articles included were RCT's and from the last 30 years, providing a relevant and up-to date review. Any missing data from studies prompted contacting of authors, and thus all possible data was included in this meta-analysis at the time of publication. All studies included adults at risk of cardiovascular disease and therefore the effect of probiotics could be assessed. Subgroup analysis provided statistical insight into formulations, dosages and patient groups most susceptible to treatment. This meta-analysis strengthens prior published findings. For example, a meta-analysis [27], which included 15 studies (788 adults with CVD risk) reported statistically significant reduction in cholesterol and BMI, whereas our study had 34 RCT's (n=2,177 adults with CVD risk) which found probiotics significantly reduce the risk of cardiovascular disease in high risk patient groups. A limitation of this study is the inconsistent use of strain types among studies, disallowing for accurate assessment of individual strain efficacy. However, this allowed for cross analysis of the relationship between strain number and therapeutic efficacy. Another limitation of this study is the small population samples within each study, these was primarily mitigated through inclusion of all available studies, however, a larger sample size could improve the statistical power of the study. These limitations are primarily due to a lack of literature available. Further RCT's observing the effects of single and multiple strain use on patients with cardiovascular risk factors are required to further clarify findings.

Acknowledgments:

Authorship: The authors' responsibilities were as follows – AD: Conceptualised and designed the study, developed search strategy, collected and extracted the data, interpreted the data, all table formulation, referencing, PEDro table development, article drafting, edited and submitted manuscript, critically revised the article – KR: data extraction – AY: data collection, data extraction, formulated table 2 – MQ: data extraction, referencing, table formulation, edited manuscript – HR: data extraction, formulated table 2 – DW: PEDro table development – TC: data extraction, referencing, subgroup table formatting, edited. DR: data extraction – CC: statistical analysis and subgroup calculations – JS: Designed the study, statistical analysis and subgroup calculations.

Compliance with Ethics Guidelines:

Conflict of Interest: The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent: This article does not contain any studies with human or animal subjects performed by any of the authors.

Figure 2. Forest plots of the effects of probiotics on Systolic Blood Pressure (A), Diastolic blood Pressure (B), Cholesterol (C), Triglycerides (D), Serum Glucose (E), HbA1C (F), BMI (G), LDL-C (H), HDL-C (I).

D

Effect of Probiotics on Triglycerides

E

Effect of Probiotics on Glucose

F Effect of Probiotics on HbA1C

G Effect of Probiotics on BMI

H

Effect of Probiotics on LDL

I

Effect of Probiotics on HDL

Table 1. PEDro Analysis of remaining studies.

Trial	Specified Eligibility Criteria	Random Allocation	Allocation Concealment	Blinding of Subjects	Blinding of Assessors	Blinding of Researchers	Similar at baseline	Key outcome measurement 85%	Intention to treat	Between group comparison	Measure of variability	Total score
Abbasi et al. (2018) [31] **	X	X	X	X	X	X	X	X	-	X	X	10/11
Agerholm-Larsen et al (2000) [32]	X	X	-	X	X	-	-	X	-	X	X	7/11
Asemi et al. (2013) [33]	X	X	-	X	X	-	X	X	-	X	X	8/11
Cavallini et al. (2016) [34]	X	X	X	X	X	X	X	X	-	X	X	10/11
Ebrahimi et al. (2017) [35]	X	X	X	X	X	X	X	X	-	X	X	10/11
Ejtahed et al. (2012) [36]	X	X	X	X	X	X	X	X	-	X	X	10/11
Feizollahzadeh et al. (2017) [37] *	X	X	-	X	X	-	X	-	-	X	X	7/11
Firouzi et al. (2017) [38] **	X	X	X	X	X	X	X	-	X	X	X	10/11
Gomes et al. (2017) [39]	X	X	X	X	X	X	X	-	X	X	X	10/11
Hata et al. (1996) [40]	X	X	-	X	-	-	X	-	-	X	X	6/11
Higashikawa et al. (2016) [41]	X	X	X	X	X	X	X	X	X	X	X	11/11
Inoue et al. (2003) [42]	X	X	-	X	-	-	X	X	-	X	X	7/11
Ivey et al. (2015) [43]	X	X	-	-	-	-	X	X	-	X	X	6/11
Jones et al. (2012) [44]	X	X	-	X	X	-	X	X	X	X	X	9/11
Jung et al. (2013) [45]	X	X	X	X	X	-	X	-	X	X	X	9/11
Kadooka et al. (2010) [46]	X	X	-	X	X	-	X	X	-	X	X	8/11
Kassaian et al. (2018) [47] *	X	X	X	X	X	X	X	-	-	X	X	9/11
Kim et al. (2017) [48] **	X	X	X	X	X	-	X	X	-	X	X	9/11
Madjd et al. (2016) [49]	X	X	X	X	-	-	X	X	X	X	X	9/11
Mafi et al. (2018) [50] *	X	X	X	X	X	-	X	X	X	X	X	10/11
Mizushima et al. (2004) [51]	X	X	-	X	X	-	X	X	-	X	X	8/11
Mobini et al. (2017) [52] *	X	X	-	X	X	X	X	X	-	X	X	9/11
Mohamadshahi et al. (2018) [53]	X	X	-	X	X	-	X	X	-	X	X	8/11
Mohammadi-Sartang et al. (2014) [54]	X	X	-	X	X	-	X	X	-	X	X	8/11
Naito et al. (2018) [55]	X	X	X	X	X	X	X	X	-	X	X	10/11
Ostadrahimi et al. (2015) [56]	X	X	X	X	X	-	X	X	-	X	X	9/11
Raygan et al. (2018) [57]	X	X	X	X	X	X	X	X	X	X	X	11/11
Razmpoosh et al. (2019) [58] *	X	X	X	X	X	X	-	X	-	X	X	9/11
Rezaei et al. (2017) [59] **	X	X	X	X	X	X	-	X	-	X	X	9/11
Sharafedinov et al. (2013) [60]	X	X	X	X	X	-	-	-	-	X	X	7/11
Shakeri et al. (2014) [61]	X	X	-	X	X	-	X	X	X	X	X	9/11
Szulinska et al. (2018) [62] *	X	X	X	X	X	X	-	X	-	X	X	9/11
Tajabadi-Ebrahimi et al. (2017) [63] **	X	X	X	X	X	X	X	X	X	X	X	11/11
Zarrati et al. (2014) [64]	X	X	X	X	X	-	X	X	-	X	X	9/11

(X) = Characteristic within study, (-) = Characteristic absent within study.

Table 2. Study Characteristics

Study (year) (ref.)	Design; location	Participants at beginning <i>n</i> (P/C)	Attrition rate <i>n</i> (%)	Comorbidities	Age, P/C, multiple P groups labelled as (1), (2)	Sex, <i>n</i> (P, C), (M/F)	Control	Probiotic strain(s)	Dose, CFU/g	Measured outcomes	Key results
Abbasi et al. (2018)	DB; Iran	44	4 (9.0)	OW	56.9/53.6	Not mentioned	Soymilk	<i>Lactobacillus Plantarum</i> A7	2x10 ⁷	Chol, TAG	Consumption of probiotic soymilk improved LDL and serum TAG significantly more than conventional soymilk ($P = 0.01$ and $P = 0.007$ respectively).
Agerholm-Larsen et al. (2000)	DB; Denmark	36	0 (.0)	OW, OB	38.6/38.3	4/12, 3/7	Calcium lactate placebo	<i>Streptococcus thermophilus</i> , <i>Lactobacillus acidophilus</i>	4.5x10 ¹¹	SBP, DBP, Chol, TG	Cholesterol, SBP and DBP pressure were significantly decreased ($P < 0.05$) by probiotic yoghurt products
Asemi et al. (2013)	DB; Iran	54	0 (0)	T2D	50.5/52.6	9/18, 9/18	Placebo capsule	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , <i>L. bulgaricus</i> , <i>B. breve</i> , <i>B. longum</i> , <i>Streptococcus thermophilus</i>	9.52x10 ¹⁰	HbA1c, TC, TAG, BMI	Consumption of probiotic supplements prevented a rise in plasma glucose ($P = 0.01$).
Cavallini et al. (2016)	DB; Brazil	49	0(0)	HCL	(1) 48.1/45.4, (2) 45.1/45.4	(1) 17/0, (2) 17/0, 15/0	Placebo unfermented soy product	(1) <i>E. faecium</i> , <i>L. helveticus</i> , without Isoflavin, (2) <i>E. faecium</i> , <i>L. helveticus</i> , with Isoflavin (genistin, genistein, daidzin, daidzein)	(1) 2x10 ¹⁰ (2) 2x10 ¹⁰	TC, TAG	The probiotic with Isoflavin group experienced significantly reduced total cholesterol and LDL ($P < 0.05$ and $P < 0.05$ respectively), but not for the group without Isoflavin.
Ebrahimi et al. (2017)	DB; Iran	70	0 (0)	T2D	58.7/58.6	23/12, 19/26	Placebo capsule	<i>Lactobacillus</i> family, <i>Bifidobacterium</i> family, <i>Streptococcus thermophilus</i>	10 ⁹	FBG, Hb1c, BMI, TC, TAG	Probiotic supplementation significantly decreased plasma glucose and HbA1c levels ($P = 0.05$ and $P < 0.01$ respectively).
Ejtahed et al. (2012)	DB; Iran	64	4(6.25)	T2D	50.9/51.0	11/19, 12/18	Conventional yogurt	<i>B. lactis</i> , <i>L. acidophilus</i>	2.03x10 ¹⁰	FBG, HbA1c	Consumption of probiotic yogurt significantly decreased plasma glucose and HbA1c levels ($P < 0.01$ and $P < 0.05$ respectively).
Feizollahzadeh et al. (2017)	DB; Iran	48	8(16.7)	T2D	56.9/53.6	9/11, 10/10	Soymilk	<i>L. plantarum</i> A7)	2x10 ⁷	FBG, TG	In the probiotic group, there was a significant decrease in LDL ($P = 0.023$) but no significant changes in plasma glucose.
Firouzi et al. (2017)	DB; Malaysia	136	35 (25.74)	T2DM	52.9/54.2	31/17, 34/19	Placebo sachet w/water	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. lactis</i> , <i>B. bifidum</i> , <i>B. longum</i> , <i>B. infantis</i>	3x10 ¹⁰	All Outcomes	No difference between probiotics and control group in all variables
Gomes et al. (2017)	DB; Brazil	60	17 (28.3)	OW/OB	20-59/20-59	0/21, 0/22	Placebo sachet	<i>L. acidophilus</i> LA-14, <i>L. casei</i> LC-11, <i>L. lactis</i> LL-23, <i>B. bifidum</i> BB-06, <i>B. lactis</i> BL-4	1x10 ⁹	BMI, HbA1C, TC, TAG	The probiotic group showed a greater decrease in BMI ($P < 0.001$). No significant differences were observed in LDL or triglyceride levels.
Hata et al. (1996)	SB; Japan	35	6 (16.0)	OW, OB	76.5/73	4/13, 4/9	Placebo	<i>L. helveticus</i> . <i>S. cerevisiae</i>	7.025x10 ¹⁰	SBP, DBP, Chol, TG	Daily consumption of the commercial probiotic drink 'Calpis' significantly reduced SBP by 14mmHg and DBP by 7mmHg from baseline, whereas placebo had no significant effect. However, there was no difference between probiotic and placebo.
Higashikawa et al. (2016)	DB; Japan	62	4 (6.45)	OW	(1) 52.5/52.8, (2) 55.5/52.8	(1) 8/13, 7/13 (2) 8/13, 7/13	Placebo powder	(1) <i>Pediococcus pentosaceus</i> LP28, (2) heat killed <i>Pediococcus pentosaceus</i> LP28	(1) 1x10 ¹¹ (2) 1x10 ¹¹	BMI, FPG, HbA1c, TC, TG	For the heat-killed probiotic group only, there was a significant decrease in BMI compared to placebo ($P = 0.035$). There were no changes in plasma glucose, HbA1c or serum lipids.
Inoue et al. (2003)	SB; Japan	39	4 (10.26)	HTN	56.2/53.9	10/8, 10/7	Placebo milk	<i>L. casei</i> strain Shirota, <i>L. lactis</i> YIT 2027	< 10x10 ¹⁰	TC, TAG, FBG, SBP, DBP	The probiotic group experienced a significant decrease in SBP ($P < 0.05$).
Ivey et al. (2015)	DB; Australia	116	0 (.0)	OW, OB	(1) 68.0/68.0, (2) 65.0/65.0	(1) 25/15, 23/17 (2) 23/13, 23/17	Control milk with placebo capsule	(1) (<i>L. acidophilus</i> La5 and <i>B. animalis</i> subsp. <i>lactis</i> Bb12 dose Yoghurt) + (<i>L. acidophilus</i> La5 and <i>B. animalis</i> subsp. <i>lactis</i> Bb12 dose capsule), (2) <i>L. acidophilus</i> La5 and <i>B. animalis</i> subsp. <i>lactis</i> Bb12 dose + control milk	(1) 1.2x10 ¹⁰ (2) 6x10 ⁹	SBP, DBP, Chol, BMI	No difference between probiotics and control group in all variables
Jones et al. (20120)	DB; Czech Republic	120	11 (9.2)	HCL	51.9/48.7	21/34, 20/38	Placebo yoghurt	<i>L. reuteri</i>	5x10 ⁹	Chol, TAG	The probiotic group experienced significant decreases in LDL and total cholesterol ($P = 0.016$ and $P = 0.031$ respectively).
Jung et al. (2013)	DB; Korea	62	8 (12.9)	OW/OB	19-60/19-60	14/14, 9/20	Placebo capsule	<i>L. gasseri</i> BNR17	6x10 ¹⁰	All Outcomes	No difference between probiotics and control group in all variables
Kadooka et al. (2010)	DB; Japan	87	0 (0)	OB	48.3/49.2	19/14, 30/14	Placebo fermented milk	<i>L. gasseri</i> SBT2055 (LG2055)	5x10 ¹⁰	BMI	Consumption of probiotics significantly decreased BMI ($P < 0.001$).
Kassaian et al. (2018)	DB; Iran	91	35 (29.2)	Pre-T2DM	(1) 53.0/53.0, (2) 53.0/53.0	(1) 19/14, 12/16 (2) 13/17, 12/16	Placebo maltodextrin powder	(1) Probiotic (<i>L. acidophilus</i> , <i>B. lactis</i> ,	(1) 1x10 ⁹ (2) 1x10 ⁹	HbA1c, FPG	Probiotic supplementation resulted in a significant decrease in HbA1c ($P = 0.005$). Synbiotic supplementation resulted in a significantly greater decrease in plasma glucose and HbA1c ($P =$

								<i>B. bifidum</i> , <i>B. longum</i> (2) <i>Synbiotic (same bacteria)</i>			0.01 and <i>P</i> = 0.008 respectively).
Kim et al. (2017)	DB; Korea	120	54 (45)	OW	Not mentioned	Not mentioned	Placebo powder	<i>L. curvatus</i> HY7601, <i>L. plantarum</i> KY1032	2.5x10 ⁹	BMI	Probiotics resulted in a significant reduction in BMI (<i>P</i> = 0.080).
Madjd et al. (2016)	SB; Iran	89	0 (0)	OB	32.2/31.8	0/44, 0/45	Low-fat yoghurt, <i>S. thermophilus</i> and <i>L. bulgaricus</i> (starter cultures), hypo-energetic diet	<i>S. thermophilus</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> <i>LA5</i> , <i>B. lactis</i> <i>BB12</i>	1x10 ⁷	TC, HBA1C, FPG, TAG, BMI	The probiotic group had significantly greater decrease in total cholesterol and LDL (<i>P</i> = 0.024 and <i>P</i> = 0.018 respectively). There was no significant difference in plasma glucose.
Mafi et al. (2018)	DB; Iran	60	6 (10)	T2D	58.9/60.9	Not mentioned	Placebo capsule	<i>L. acidophilus</i> strain <i>ZT-L1</i> , <i>B.</i> <i>bifidum</i> strain <i>ZT-B1</i> , <i>L. reuteri</i> strain <i>ZT-Lre</i> , <i>L. fermentum</i> strain <i>ZT-L3</i>	8x10 ⁹	BMI, FPG, HBA1C, TAG, TC	Probiotic supplementation resulted in a significant decrease in plasma glucose and total triglycerides (<i>P</i> = 0.01 and <i>P</i> = 0.001 respectively).
Mizushima et al. (2004)	DB; Japan	46	4 (8.7)	HTN	44.3/10.3	23/0, 23/0	Placebo milk (acidified)	<i>L. helveticus</i> , <i>S. cerevisiae</i>	7x10 ¹¹ , 2.5x10 ⁹	SBP, DBP, TC, TAG	The probiotic group had a significant change in DBP (<i>P</i> = 0.039).
Mobini et al. (2017)	DB; Sweden	44	0 (0)	T2D, OB	(1) 66.0/65.0, (2) 64.0/65.0	(1) 12/3, 11/4 (2) 11/3, 11/4	Placebo powder	(1) <i>Lactobacillus reuteri</i> <i>DSM</i> <i>17938</i> , (2) <i>Lactobacillus reuteri</i> <i>DSM 17938</i>	(1) 1x10 ⁸ (2) 1x10 ¹⁰	All Outcomes	No difference between probiotics and control group in all variables
Mohamadshahi et al. (2018)	DB; Iran	44	2 (4.55)	T2M, OW or OB	53.0/49.0	Not mentioned	Placebo conventional yoghurt (<i>Lactobacillus</i> <i>delbrueckii</i> subsp. <i>bulgaricus</i> and <i>Streptococcus</i> <i>thermophilus</i>)	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> <i>Bb12</i> (<i>DSM 10140</i>) and <i>Lactobacillus acidophilus</i> strain <i>La5</i>	3.7×10 ⁶	FBG, BMI, HBA1C	Consumption of probiotic yogurt significantly decreased HbA1c levels (p = 0.032).
Mohammadi- Sartang et al. (2018)	DB; Iran	44	2 (4.7)	T2D, OW, OB	53.0/49.0	5/17, 5/17	Placebo yogurt	<i>B. animal lactis</i> <i>Bb12</i> + <i>L. acidophilus</i> <i>La4</i>	7.4x10 ⁶	HBA1C, BMI	Consumption of probiotic yogurt resulted in a significant decrease in triglyceride levels (<i>P</i> = 0.003).
Naito et al. (2018)	DB; Japan	100	2 (2.0)	OB, pre-T2D	48.6/47.4	48/0, 50.0	Milk placebo	<i>Milk fermented with LcS YIT</i> <i>9029</i>	1x10 ¹¹	All Outcomes	Daily administration of a fermented milk product significantly reduced plasma glucose, glycoalbumin and HbA1c at 8 weeks compared with baseline (<i>P</i> < 0.05). However, this improvement was only significantly better than placebo for glycoalbumin. Subgroup analysis revealed improved benefits for patients with severe glucose intolerance. LDL was significantly lowered in the probiotic group compared with control.
Ostadrahimi et al. (2015)	DB; Iran	60	0 (0)	T2D	35-65/5-65	Not mentioned	Placebo conventional fermented milk	<i>L. acidophilus</i> , <i>L. casei</i> , <i>B. lactis</i>	25x10 ⁶ , 15x10 ⁶ , 8x10 ⁶	TC, TAG, FPG, HBA1C	The probiotic group had a significant decrease in blood glucose and HbA1c (<i>P</i> = 0.01 and <i>P</i> = 0.02 respectively).
Raygan et al. (2018)	DB; Iran	60	0 (0)	T2D, CHD	60.7/61.8	Not mentioned	Placebo capsules	<i>B. bifidum</i> , <i>L. casei</i> , <i>L. acidophilus</i>	2x10 ⁹	SBP, DBP, BMI, FPG, TAG, TC	Probiotic supplementation significantly decreased plasma glucose (<i>P</i> = 0.005).
Razmpoosh et al. (2019)	DB; Iran	68	8 (11.8)	T2D	58.6/61.3	17/13, 16/14	Placebo capsule	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. rhamnosus</i> , <i>L. bulgaricus</i> , <i>B.</i> <i>breve</i> , <i>B. longum</i> , <i>S. thermophilus</i>	1.9x10 ¹¹	FPG, BMI, TC, TAG	There was a significant decrease in plasma glucose for the probiotic group (<i>P</i> = 0.001).
Rezaei et al. (2017)	DB; Iran	90	0 (0)	T2D	50.5/50.1	23/22, 21/24	Placebo yoghurt, starter cultures <i>S.</i> <i>thermophilus</i> , <i>L.</i> <i>bulgaricus</i>	<i>L. acidophilus</i> <i>La5</i> , <i>B. lactis</i> <i>Bb12</i>	2.22 x10 ⁹	SBP, DBP, FBG, TC, TAG, HBA1C	The probiotic group experienced significant decreases in plasma glucose, LDL, triglycerides, DBP and HbA1c levels (<i>P</i> < 0.05 for all).
Shakeri et al. (2014)	DB; Iran	98	6 (8.33)	T2D	(1) 52.3/53.1, (2) 52.3/53.1	Not mentioned	Control bread	(1) <i>L. sporogenes</i> , <i>inulin</i> (<i>HPX</i>), (2) <i>L. sporogenes</i>	(1) 1.2x10 ¹⁰ (2) 1.2x10 ¹⁰	BMI, FPG, TAG, TC,	Consumption of probiotic group with insulin resulted in a significant decrease in triglycerides (op = 0.005).
Sharafedtinov et al. (2013)	DB; Estonia	40	4 (10)	MetS	10.9/12.1	9/16, 4/11	Conventional cheese	<i>L. plantarum</i> <i>TENSIA</i>	2.55x10 ¹⁰	SBP, DBP, BMI, Chol, TG	Probiotics significantly lowered BMI (p = 0.03), as well as DBP (p = 0.02); SBP was lowered, though not significant
Szulinska et al. (2018)	SB; Poland	71	5 (12.3)	OB, (postmenopausal)	(1) 55.2/58.7, (2) 56.4/58.7	(1) 0/23, 0/24 (2) 0/24, 0/24	Placebo powder	(1) <i>B. bifidum</i> , <i>B. lactis</i> <i>W51</i> , <i>B. lactis</i> <i>W52</i> , <i>L.</i> <i>acidophilus</i> , <i>L. brevis</i> , <i>L. casei</i> , <i>L. salivarius</i> , <i>L. lactis</i> <i>W19</i> , <i>L. lactis</i> <i>W58</i>), (2) <i>Same as previous</i>	(1) 1x10 ¹⁰ (2) 2.5x10 ⁹	TC, BMI, TAG, FPG	Both high and low doses of probiotics significantly decreased SBP (p = 0.0498 and p = 0.0359 respectively).

Tajabadi-Ebrahimi et al. (2017)	DB; Iran	60	5 (8.3)	OW, T2D, CHD	64.2/64.0	Not mentioned	Placebo capsule	<i>L. acidophilus</i> , <i>L. casei</i> , <i>B. bifidum</i>	6x10 ⁹	BMI, FPG, TC, TAG	Consumption of probiotics significantly decreased plasma glucose (p = 0.03).
Zarrati et al. (2014)	DB; Iran	75	0 (0)	OW/OB	36.0/36.0	Not mentioned	Placebo yoghurt (starter cultures of <i>S. thermophilus</i> and <i>L. bulgaricus</i> .)	<i>S. thermophilus</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> LA5, <i>L. casei</i> DN001, <i>B. lactis</i> BB12	1x10 ⁷	BMI, SBP, DBP	Probiotic yogurt resulted in a significant reduction in BMI (p < 0.01).

Table 3. Subgroup Analysis of SBP and DBP

Subgroups	SBP							DBP						
	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>
Health														
DM	2	191	28	0.24	-5.82	-10.52, -1.12	0.02	2	191	28	0.45	-3.81	-5.68, -1.93	0.0001
Obesity	9	408	0	0.75	-2.38	-0.59, -0.18	0.03	9	408	0	0.38	-2.07	-3.75, -0.38	0.01
HTN														
Hypercholesterolemia														
Other	4	144	0	0.92	-0.78	-1.85, 0.29	0.15	4	144	0	0.75	-1.64	-2.24, -1.03	0.0001
Sex														
F>M	7	273	68	0.04	-2.64	-7.09, 2.81	0.25	7	273	68	0.06	-2.29	-4.30, -0.27	0.03
F<M	7	406	0	0.82	-2.36	-4.74, 0.03	0.05	7	406	0	0.81	-0.91	-3.30, -0.53	0.007
Dosage														
< 1.0×10 ⁹ CFU														
> 1.0×10 ⁹ CFU														
Attrition														
> 15%														
< 15%														
Form														
Capsule	5	173	0	0.92	-0.31	-4.99, 0.36	0.90	5	173	0	0.80	-0.25	-3.37, 2.88	0.88
Milk	4	214	0	0.42	-2.68	-5.81, 0.44	0.09	4	214	0	0.34	-1.97	-4.52, 0.58	0.13
Yoghurt	3	217	48	0.15	-3.56	-7.43, 0.31	0.07	3	217	48	0.60	-3.54	-5.13, -1.96	0.001
Other	4	185	0	0.71	-0.88	-0.94, 0.17	0.10	4	185	0	0.77	-1.68	-2.28, -1.08	0.001
Blinding														
SB														
DB														
NB														
Duration														
< 1.5 months	5	293	41	0.15	-2.00	-4.37, 0.37	0.10	5	293	41	0.21	-2.14	-3.39, -0.89	0.001
> 1.5 months	11	496	0	0.84	-2.38	-4.60, -0.16	0.04	11	496	0	0.64	-1.98	-3.57, -0.38	0.01

Table 4. Subgroup Analysis of TC and TAG

Subgroups	Total Cholesterol							Triglycerides						
	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>
Health														
DM	8	507	0	0.46	-7.53	-13.59, -1.47	0.01	9	547	22	0.26	-14.17	-26.26, -2.08	0.02
Obesity	15	683	0	0.88	-4.19	-7.28, -1.11	0.008	15	663	74	0.01	7.51	-2.36, 17.38	0.14
HTN	2	81	10	0.29	-6.14	-20.05, 7.77	0.39	2	81	0	0.90	16.10	-41.50, 73.71	0.58
Hypercholesterolemia	3	163	0	0.48	-16.54	-25.46, -7.62	0.0003	3	163	0	0.61	-33.69	-91.36, 23.98	0.25
Other	6	264	0	0.96	-7.31	-14.94, 0.32	0.06	6	264	37	0.16	-7.19	-27.64, 13.27	0.49
Sex														
F>M	15	726	9	0.36	-5.59	-9.08, -2.10	0.002	16	746	74	0.001	7.26	-5.65, 20.18	0.27
F<M	12	620	0	0.61	-6.44	-11.32, -1.57	0.01	12	620	2	0.42	2.32	-3.06, 7.70	0.40
Dosage														
< 1.0×10 ⁹ CFU	4	194	0	0.83	-3.67	-10.73, 3.39	0.31	5	234	0	0.97	1.04	-9.12, 11.19	0.84
> 1.0×10 ⁹ CFU	30	1504	0	0.68	-6.38	-8.98, -3.78	0.0001	30	1484	68	0.001	-2.70	-11.49, 6.09	0.55
Attrition														
> 15%	3	174	0	0.35	-6.90	-18.43, 4.62	0.24	4	214	0	0.85	-9.95	-23.51, 3.61	0.15
< 15%	31	1524	0	0.75	-6.01	-8.51, -3.52	0.0001	31	1504	63	0.001	-0.86	-9.01, 7.30	0.84
Form														
Capsule	10	477	0	0.72	-3.00	-9.08, 3.07	0.33	10	477	23	0.26	-7.18	-22.15, 7.79	0.35
Milk	9	398	0	0.58	-8.74	-14.62, -2.86	0.004	10	438	0	00.88	-6.99	-26.34, 12.36	0.48
Yoghurt	5	393	0	0.31	-9.48	-16.17, -2.48	0.006	5	393	40	0.15	1.61	-6.50, 9.71	0.70
Other	10	430	0	0.85	-5.22	-8.63, -1.82	0.003	10	410	84	0.001	-1.36	-20.86, 18.14	0.89
Blinding														
SB	3	154	0	0.63	-1.42	-10.29, 7.46	0.75	3	154	0	0.62	0.38	-10.27, 11.03	0.94
DB	31	1544	0	0.78	-6.43	-8.97, -3.89	0.0001	32	1564	63	0.001	-2.11	-10.49, 6.27	0.62
NB														
Duration														
< 1.5 months	9	516	0	0.44	-11.61	-17.37, -5.85	0.0001	9	516	12	0.34	2.84	-3.78, 9.45	0.40
> 1.5 months	25	1182	0	0.96	-4.84	-7.53, -2.14	0.0004	26	1202	65	0.001	-2.65	-12.42, 7.12	0.60

Table 5. Subgroup Analysis of LDL-C and HDL-C

Subgroups	LDL							HDL						
	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>
Health														
DM	7	406	90	0.0001	-8.09	-15.52, -0.65	0.03	7	406	85	0.001	2.03	0.39, 3.66	0.01
Obesity	12	562	60	0.01	-7.27	-10.55, -3.99	0.001	13	592	21	0.26	-1.21	-1.93, -0.50	0.001
HTN														
Hypercholesterolemia	3	163	49	0.14	-20.67	-31.00, -10.34	0.001	3	163	41	0.18	6.36	0.15, 12.57	0.04
Other	6	264	0	0.58	-5.61	-11.37, 0.15	0.06	6	264	76	0.001	1.26	-2.23, 4.76	0.48
Sex														
F>M	12	605	79	0.001	-9.87	-13.66, -6.08	0.001	13	635	41	0.09	-1.02	-1.88, -0.17	0.02
F<M	9	438	53	0.03	-6.35	-13.47, 0.77	0.08	10	484	30	0.17	1.22	-0.99, 3.43	0.28
Dosage														
< 1.0×10 ⁹ CFU	3	159	86	0.001	-10.73	-25.58, 4.13	0.16	2	159	0	0.99	0.25	-1.20, 1.70	0.74
> 1.0×10 ⁹ CFU	25	1236	78	0.001	-8.38	-11.62, -5.13	0.001	28	1312	91	0.001	1.16	-0.36, 2.68	0.13
Attrition														
> 15%														
< 15%														
Form														
Capsule	10	477	0	0.83	-4.35	-6.87, -1.84	0.007	10	477	85	0.001	1.95	-0.73, 4.62	0.15
Milk	6	287	64	0.02	-9.82	-18.28, -1.36	0.02	8	363	52	0.05	0.86	-2.07, 3.80	0.56
Yoghurt	4	370	52	0.10	-18.06	-24.88, -11.24	0.001	4	370	0	0.99	0.25	-1.82, 2.32	0.81
Other	8	261	88	0.001	-7.67	-13.16, -2.19	0.006	8	261	97	0.001	0.32	-2.41, 3.06	0.82
Blinding														
SB														
DB														
NB														
Duration														
< 1.5 months	8	470	53	0.04	-14.01	-20.67, -7.35	0.001	9	516	49	0.05	0.61	-2.21, 3.44	0.67
> 1.5 months	20	925	81	0.001	-7.08	-10.50, -3.66	0.001	21	955	94	0.001	1.08	-0.56, 2.72	0.20

Table 6. Subgroup Analysis of Fasting Glucose and HbA1C

Subgroups	Fasting Glucose							HbA1C						
	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>
Health														
DM	10	607	48	0.04	-12.94	-20.75, -5.13	0.001	6	435	94	0.001	-0.35	-1.09, 0.39	0.36
Obesity	7	377	63	0.01	-1.60	-3.61, 0.41	0.12	6	349	0	0.64	-0.02	-0.05, 0.02	0.31
HTN														
Hypercholesterolemia														
Other	8	351	55	0.03	-5.69	-6.30, -5.09	0.001	7	275	91	0.001	-0.32	-0.62, -0.01	0.04
Sex														
F>M	12	680	81	0.001	-4.17	-5.12, -3.23	0.001	11	584	92	0.001	-0.18	-0.32, -0.05	0.007
F<M	7	408	55	0.03	-5.69	-6.30, -5.09	0.001	5	313	76	0.002	0.13	-0.17, 0.43	0.40
Dosage														
< 1.0×10 ⁹ CFU	5	236	0	0.88	-1.16	-4.24, 1.92	0.46	4	205	93	0.001	-0.43	-1.01, 0.15	0.15
> 1.0×10 ⁹ CFU	21	1134	85	0.001	-5.29	-6.57, -4.01	0.001	15	854	90	0.001	-0.12	-0.24, 0.01	0.06
Attrition														
> 15%	4	226	0	0.97	-5.63	-5.82, -5.43	0.001	4	229	0	0.51	-0.19	-0.25, -0.14	0.001
< 15%	22	1144	85	0.001	-5.29	-6.57, -4.01	0.001	15	830	92	0.001	-0.20	-0.36, -0.04	0.01
Form														
Capsule	7	421	67	0.006	-13.47	-25.70, -1.24	0.03	4	241	83	0.001	0.14	-0.15, 0.44	0.34
Milk	4	233	95	0.001	-7.54	-23.84, 8.76	0.36	2	158	98	0.001	-0.61	-0.81, 0.58	0.31
Yoghurt	5	304	62	0.01	-12.88	-24.54, -1.22	0.03	5	325	90	0.001	-0.55	-1.03, -0.06	0.03
Other	10	412	80	0.001	-4.41	-5.32, -3.51	0.001	8	335	77	0.001	-0.08	-0.17, 0.02	0.12
Blinding														
SB	2	124	32	0.23	-3.15	-11.48, 5.17	0.46							
DB	24	1246	83	0.001	-5.27	-6.54, -4.00	0.001							
NB														
Duration														
< 1.5 months	3	210	0	0.84	-16.15	-26.88, -5.42	0.003	2	150	0	0.64	-0.50	-0.87, -0.13	0.009
> 1.5 months	23	1160	85	0.001	-4.77	-6.01, -3.54	0.001	17	909	92	0.001	-0.16	-0.29, -0.03	0.02

Table 7. Subgroup Analysis of BMI

Subgroups	BMI						
	Studies, <i>n</i>	Participants, <i>n</i>	<i>I</i> ²	Q-test	Mean Difference	95% CI	<i>P</i>
Health							
DM	6	357	0	0.99	-0.33	-1.28, 0.62	0.49
Obesity	11	623	0	0.84	-0.48	-0.62, -0.35	0.001
HTN							
Hypercholesterolemia							
Other	8	350	0	0.99	-0.09	-0.22, 0.05	0.21
Sex							
F>M	11	526	47	0.04	-0.35	-0.55, -0.15	0.001
F<M	7	460	0	0.98	-0.45	-1.03, 0.14	0.14
Dosage							
< 1.0×10 ⁹ CFU	4	205	0	0.99	-0.08	-0.23, 0.07	0.31
> 1.0×10 ⁹ CFU	21	1125	0	0.91	-0.41	-0.53, -0.29	0.001
Attrition							
> 15%	3	210	0	0.72	-0.40	-1.01, 0.21	0.20
< 15%	22	1120	7	0.37	-0.29	-0.41, -0.18	0.001
Form							
Capsule	7	421	0	0.99	-0.11	-0.37, 0.15	0.42
Milk							
Yoghurt	5	248	0	0.96	-0.09	-0.24, 0.06	0.24
Other	11	476	0	0.95	-0.50	-0.64, -0.36	0.001
Blinding							
SB							
DB							
NB							
Duration							
< 1.5 months	2	100	0	0.91	-0.28	-1.92, 1.36	0.74
> 1.5 months	23	1230	6	0.38	-0.30	-0.41, -0.18	0.001

Table 8. Egger's Test

Variable	Studies, <i>n</i>	<i>t</i>	95% CI	<i>P</i>
SBP	13	1.36	-1.03, 4.22	0.205
DBP	13	-0.53	-6.21, 3.84	0.611
Total Cholesterol	28	-0.48	-2.15, 1.34	0.637
Triglycerides	29	0.63	-2.06, 3.89	0.533
Glucose	22	-5.96	-12.54, -6.09	0.001
HbA1C	16	-0.86	-8.61, 3.67	0.403
BMI	21	-1.63	-5.45, 0.65	0.117
LDL	21	-1.93	-8.53, 0.28	0.066
HDL	23	1.27	-1.80, 7.61	0.215

References:

Papers of particular interest, published recently, have been highlighted as:

* Of importance

** Of major importance

1. Who.int. (2020). *Cardiovascular diseases (CVDs)*. [online] Available at: [https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-\(cvds\)](https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)) [Accessed 2 Feb. 2020].
2. Sacco RL, Roth GA, Reddy KS, Arnett DK, Bonita R, Gaziano TA, Heidenreich PA, Huffman MD, Mayosi BM, Mendis S, Murray CJ. The heart of 25 by 25: achieving the goal of reducing global and regional premature deaths from cardiovascular diseases and stroke: a modeling study from the American Heart Association and World Heart Federation. *Circulation*. 2016 Jun 7;133(23): e674-90.
3. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *The lancet*. 2005 Apr 16;365(9468):1415-28.
4. Zhang WW, Liu CY, Wang YJ, Xu ZQ, Chen Y, Zhou HD. Metabolic syndrome increases the risk of stroke: a 5-year follow-up study in a Chinese population. *Journal of neurology*. 2009 Sep 1;256(9):1493-9.
5. Saklayen MG. The global epidemic of the metabolic syndrome. *Current hypertension reports*. 2018 Feb 1;20(2):12.
6. Mancia G, Laurent S, Agabiti-Rosei E, Ambrosioni E, Burnier M, Caulfield MJ, Cifkova R, Clément D, Coca A, Dominiczak A, Erdine S. Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. *Blood pressure*. 2009 Jan 1;18(6):308-47.
7. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, Obarzanek E, Conlin PR, Miller ER, Simons-Morton DG, Karanja N. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *New England journal of medicine*. 2001 Jan 4;344(1):3-10.
8. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *science*. 2005 Jun 10;308(5728):1635-8
9. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. *World journal of gastroenterology: WJG*. 2015 Aug 7;21(29):8787.
10. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut*. 2004 Jan 1;53(1):1-4.
11. Sheflin AM, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. *Current oncology reports*. 2014 Oct 1;16(10):406.
12. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaïss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012 Feb;482(7384):179-85.
13. ** Li X, Watanabe K, Kimura I. Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. *Frontiers in immunology*. 2017 Dec 20; 8:1882. **This study revealed onset of diabetes is linked with microbiome dysbiosis, establishing a conceptual framework for treatment with probiotics.**

14. * Levy M, Kolodziejczyk AA, Thaïss CA, Elinav E. Dysbiosis and the immune system. *Nature Reviews Immunology*. 2017 Apr;17(4):219. **This paper outlined the polymorphic relationship between genetic, lifestyle and a high-cholesterol diet with microbiome dysbiosis.**
15. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, Sahay B. Gut dysbiosis is linked to hypertension. *Hypertension*. 2015 Jun;65(6):1331-40.
16. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013 Aug;500(7464):541-6.
17. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature reviews Gastroenterology & hepatology*. 2014 Aug;11(8):506-14.
18. Moayyedi P, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, Quigley EM. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut*. 2010 Mar 1;59(3):325-32.
19. D'souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *Bmj*. 2002 Jun 8;324(7350):1361.
20. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *The Lancet infectious diseases*. 2006 Jun 1;6(6):374-82.
21. Ambalam P, Raman M, Purama RK, Doble M. Probiotics, prebiotics and colorectal cancer prevention. *Best practice & research Clinical gastroenterology*. 2016 Feb 1;30(1):119-31.
22. Oak SJ, Jha R. The effects of probiotics in lactose intolerance: a systematic review. *Critical reviews in food science and nutrition*. 2019 Jun 17;59(11):1675-83.
23. Klein G, Pack A, Bonaparte C, Reuter G. Taxonomy and physiology of probiotic lactic acid bacteria. *International journal of food microbiology*. 1998 May 26;41(2):103-25.
24. Lye HS, Kuan CY, Ewe JA, Fung WY, Liong MT. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *International journal of molecular sciences*. 2009 Sep;10(9):3755-75.
25. Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanisms of action of probiotics: recent advances. *Inflammatory bowel diseases*. 2009 Feb 1;15(2):300-10.
26. McFarland LV. From yaks to yogurt: the history, development, and current use of probiotics. *Clinical Infectious Diseases*. 2015 May 15;60(suppl_2): S85-90.
27. Sun J, Buys N. Effects of probiotics consumption on lowering lipids and CVD risk factors: a systematic review and meta-analysis of randomized controlled trials. *Annals of medicine*. 2015 Aug 18;47(6):430-40.
28. ** Gomes AC, Bueno AA, de Souza RG, Mota JF. Gut microbiota, probiotics and diabetes. *Nutrition journal*. 2014 Dec;13(1):60. **This systematic review and meta-analysis demonstrated significant reductions in TC and BMI following probiotic treatment. Subgroup analyses further revealed that longer treatment duration and the use of multiple species were more efficacious in reducing TC than shorter treatment duration and the use of a single species.**
29. Ettinger G, MacDonald K, Reid G, Burton JP. The influence of the human microbiome and probiotics on cardiovascular health. *Gut microbes*. 2014 Nov 2;5(6):719-28.

30. Huang X, Lin J, Demner-Fushman D. Evaluation of PICO as a knowledge representation for clinical questions. In AMIA annual symposium proceedings 2006 (Vol. 2006, p. 359). American Medical Informatics Association.
31. ** Abbasi B, Mirlohi M, Daniali M, Ghiasvand R. Effects of probiotic soymilk on lipid panel in type 2 diabetic patients with nephropathy: A double-blind randomized clinical trial. *Prog. Nutr.* 2018 Jan 1; 20:70-8. **This RCT demonstrated consumption of probiotic soymilk significantly reduced LDL-C and TAG in overweight patients, with greater effect than conventional soymilk.**
32. Agerholm-Larsen L, Raben A, Haulrik N, Hansen AS, Manders M, Astrup A. Effect of 8-week intake of probiotic milk products on risk factors for cardiovascular diseases. *European journal of clinical nutrition.* 2000 Apr;54(4):288-97.
33. Asemi Z, Zare Z, Shakeri H, Sabihi SS, Esmailzadeh A. Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. *Annals of nutrition and metabolism.* 2013;63(1-2):1-9.
34. Cardoso Umbelino Cavallini D, Manzoni J, Simone M, Bedani R, Roselino MN, Celiberto LS, Vendramini RC, De Valdez GF, Saes Parra Abdalla D, Aparecida Pinto R, Rosetto D. Probiotic soy product supplemented with isoflavones improves the lipid profile of moderately hypercholesterolemic men: a randomized controlled trial. *Nutrients.* 2016 Jan;8(1):52.
35. sadat Ebrahimi Z, Nasli-Esfahani E, Nadjarzade A, Mozaffari-khosravi H. Effect of symbiotic supplementation on glycemic control, lipid profiles and microalbuminuria in patients with non-obese type 2 diabetes: a randomized, double-blind, clinical trial. *Journal of Diabetes & Metabolic Disorders.* 2017 Dec;16(1):23.
36. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition.* 2012 May 1;28(5):539-43.
37. * Feizollahzadeh S, Ghiasvand R, Rezaei A, Khanahmad H, Hariri M. Effect of probiotic soymilk on serum levels of adiponectin, inflammatory mediators, lipid profile, and fasting blood glucose among patients with type ii diabetes mellitus. Probiotics and antimicrobial proteins. 2017 Mar 1;9(1):41-7. **This RCT demonstrated that consumption of probiotic soymilk resulted in no significant changes in triglyceride levels in participants with T2D.**
38. ** Firouzi S, Majid HA, Ismail A, Kamaruddin NA, Barakatun-Nisak MY. Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized controlled trial. *European journal of nutrition.* 2017 Jun 1;56(4):1535-50. **This RCT involving individuals with T2D showed significant improvements in HbA1C without any significant decrease in FBG following consumption of probiotic powder. This study also demonstrated non-significant reductions in TG and TC levels, along with a significant reduction in SBP from baseline within the probiotics group.**
39. Gomes AC, de Sousa RG, Botelho PB, Gomes TL, Prada PO, Mota JF. The additional effects of a probiotic mix on abdominal adiposity and antioxidant Status: a double-blind, randomized trial. *Obesity.* 2017 Jan;25(1):30-8.
40. Hata Y, Yamamoto M, Ohni M, Nakajima K, Nakamura Y, Takano T. A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. *The American journal of clinical nutrition.* 1996 Nov 1;64(5):767-71.
41. Higashikawa F, Noda M, Awaya T, Danshiitsoodol N, Matoba Y, Kumagai T, Sugiyama M. Antiobesity effect of *Pediococcus pentosaceus* LP28 on overweight subjects: a randomized, double-blind, placebo-controlled clinical trial. *European journal of clinical nutrition.* 2016 May;70(5):582-7.

42. Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M, Sansawa H. Blood-pressure-lowering effect of a novel fermented milk containing γ -aminobutyric acid (GABA) in mild hypertensives. *European journal of clinical nutrition*. 2003 Mar;57(3):490-5.
43. Ivey KL, Hodgson JM, Kerr DA, Thompson PL, Stojceski B, Prince RL. The effect of yoghurt and its probiotics on blood pressure and serum lipid profile; a randomised controlled trial. *Nutrition, Metabolism and Cardiovascular Diseases*. 2015 Jan 1;25(1):46-51.
44. Jones ML, Martoni CJ, Parent M, Prakash S. Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. *British Journal of Nutrition*. 2012 May;107(10):1505-13.
45. Jung SP, Lee KM, Kang JH, Yun SI, Park HO, Moon Y, Kim JY. Effect of *Lactobacillus gasseri* BNR17 on overweight and obese adults: a randomized, double-blind clinical trial. *Korean journal of family medicine*. 2013 Mar;34(2):80.
46. Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T. Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. *European journal of clinical nutrition*. 2010 Jun;64(6):636-43.
47. * Kassaian N, Feizi A, Aminorroaya A, Jafari P, Ebrahimi MT, Amini M. The effects of probiotics and synbiotic supplementation on glucose and insulin metabolism in adults with prediabetes: a double-blind randomized clinical trial. *Acta diabetologica*. 2018 Oct 1;55(10):1019-28. **This RCT observed the effects of probiotic/synbiotic supplementation on pre-diabetic participants. Intervention resulted in significant improvements in FBG and HbA1c levels.**
48. ** Kim M, Kim M, Kang M, Yoo HJ, Kim MS, Ahn YT, Sim JH, Jee SH, Lee JH. Effects of weight loss using supplementation with *Lactobacillus* strains on body fat and medium chain acylcarnitines in overweight individuals. *Food & function*. 2017;8(1):250-61. **This RCT showed a significant reduction in BMI in overweight participants who consumed probiotic supplement.**
49. Madjd A, Taylor MA, Mousavi N, Delavari A, Malekzadeh R, Macdonald IA, Farshchi HR. Comparison of the effect of daily consumption of probiotic compared with low-fat conventional yogurt on weight loss in healthy obese women following an energy-restricted diet: a randomized controlled trial. *The American journal of clinical nutrition*. 2016 Feb 1;103(2):323-9.
50. * Mafi A, Namazi G, Soleimani A, Bahmani F, Aghadavod E, Asemi Z. Metabolic and genetic response to probiotics supplementation in patients with diabetic nephropathy: a randomized, double-blind, placebo-controlled trial. *Food & function*. 2018;9(9):4763-70. **This RCT enrolled individuals with T2D. When compared to placebo, probiotic supplementation resulted in significant reductions in FBG and TG, along with non-significant reductions in HbA1c and TC.**
51. Mizushima S, Ohshige K, Watanabe J, Kimura M, Kadowaki T, Nakamura Y, Tochikubo O, Ueshima H. Randomized controlled trial of sour milk on blood pressure in borderline hypertensive men. *American journal of hypertension*. 2004 Aug 1;17(8):701-6.
52. * Mobini R, Tremaroli V, Ståhlman M, Karlsson F, Levin M, Ljungberg M, Sohlin M, Bertéus Forslund H, Perkins R, Bäckhed F, Jansson PA. Metabolic effects of *Lactobacillus reuteri* DSM 17938 in people with type 2 diabetes: A randomized controlled trial. *Diabetes, Obesity and Metabolism*. 2017 Apr;19(4):579-89. **This study enrolled patients with T2D but demonstrated no significant differences between placebo and probiotic groups in all variables.**

53. Mohamadshahi M, Veissi M, Haidari F, Shahbazian H, Kaydani GA, Mohammadi F. Effects of probiotic yogurt consumption on inflammatory biomarkers in patients with type 2 diabetes. *BioImpacts: BI*. 2014;4(2):83.
54. Mohammadi-Sartang M, Bellissimo N, de Zepetnek JT, Brett NR, Mazloomi SM, Fararouie M, Bedeltavana A, Famouri M, Mazloom Z. The effect of daily fortified yogurt consumption on weight loss in adults with metabolic syndrome: A 10-week randomized controlled trial. *Nutrition, Metabolism and Cardiovascular Diseases*. 2018 Jun 1;28(6):565-74.
55. Naito E, Yoshida Y, Kunihiro S, Makino K, Kasahara K, Kounoshi Y, Aida M, Hoshi R, Watanabe O, Igarashi T, Miyazaki K. Effect of *Lactobacillus casei* strain Shirota-fermented milk on metabolic abnormalities in obese prediabetic Japanese men: a randomised, double-blind, placebo-controlled trial. *Bioscience of microbiota, food and health*. 2017:17-012.
56. Ostadrahimi A, Taghizadeh A, Mobasser M, Farrin N, Payahoo L, Gheshlaghi ZB, Vahedjabbari M. Effect of probiotic fermented milk (kefir) on glycemic control and lipid profile in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Iranian journal of public health*. 2015 Feb;44(2):228.
57. Raygan F, Rezavandi Z, Bahmani F, Ostadmohammadi V, Mansournia MA, Tajabadi-Ebrahimi M, Borzabadi S, Asemi Z. The effects of probiotic supplementation on metabolic status in type 2 diabetic patients with coronary heart disease. *Diabetology & metabolic syndrome*. 2018 Dec 1;10(1):51.
58. * Razmpoosh E, Javadi A, Ejtahed HS, Mirmiran P, Javadi M, Yousefinejad A. The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo-controlled trial. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2019 Jan 1;13(1):175-82. **This RCT revealed a significant decrease in FPG and a significant increase in HDL-C following multi-strain probiotic supplementation in patients with Type 2 diabetes.**
59. ** Rezaei M, Sanagoo A, Jouybari L, Behnampoo N, Kavosi A. The effect of probiotic yogurt on blood glucose and cardiovascular biomarkers in patients with type II diabetes: a randomized controlled trial. *Evidence Based Care*. 2017;6(4):26-35. **Significant reductions in FBG, TG, DBP, and HbA1c were revealed in this RCT following consumption of probiotic yogurt in patients with T2D.**
60. Sharafedinov KK, Plotnikova OA, Alexeeva RI, Sentsova TB, Songisepp E, Stsepetova J, Smidt I, Mikelsaar M. Hypocaloric diet supplemented with probiotic cheese improves body mass index and blood pressure indices of obese hypertensive patients—a randomized double-blind placebo-controlled pilot study. *Nutrition journal*. 2013 Dec 1;12(1):138.
61. Shakeri H, Hadaegh H, Abedi F, Tajabadi-Ebrahimi M, Mazroei N, Ghandi Y, Asemi Z. Consumption of synbiotic bread decreases triacylglycerol and VLDL levels while increasing HDL levels in serum from patients with type-2 diabetes. *Lipids*. 2014 Jul;49(7):695-701.
62. * Szulińska M, Łoniewski I, Skrypnik K, Sobieska M, Korybalska K, Suliburska J, Bogdański P. Multispecies probiotic supplementation favorably affects vascular function and reduces arterial stiffness in obese postmenopausal women—A 12-week placebo-controlled and randomized clinical study. *Nutrients*. 2018 Nov;10(11): 1672. **This RCT revealed a significant reduction in SBP following probiotic supplementation in obese postmenopausal women.**
63. ** Tajabadi-Ebrahimi M, Sharifi N, Farrokhian A, Raygan F, Karamali F, Razzaghi R, Taheri S, Asemi Z. A randomized controlled clinical trial investigating the effect of synbiotic administration on markers of insulin metabolism and lipid profiles in overweight type 2 diabetic patients with coronary heart disease. *Experimental and*

Clinical Endocrinology & Diabetes. 2017 Jan;125(01):21-7. **This RCT showed a significant decrease in FPG and a significant increase in HDL-C levels following synbiotic supplementation in overweight, type 2 diabetic patients with coronary heart disease.**

64. Zarrati M, Salehi E, Nourijelyani K, Mofid V, Zadeh MJ, Najafi F, Ghaflati Z, Bidad K, Chamari M, Karimi M, Shidfar F. Effects of probiotic yogurt on fat distribution and gene expression of proinflammatory factors in peripheral blood mononuclear cells in overweight and obese people with or without weight-loss diet. *Journal of the American College of Nutrition*. 2014 Nov 2;33(6):417-25.
65. Kantor ED, Rehm CD, Haas JS, Chan AT, Giovannucci EL. Trends in prescription drug use among adults in the United States from 1999-2012. *Jama*. 2015 Nov 3;314(17):1818-30.
66. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension*. 2014 Oct;64(4):897-903.
67. Wang L, Guo MJ, Gao Q, Yang JF, Yang L, Pang XL, Jiang XJ. The effects of probiotics on total cholesterol: A meta-analysis of randomized controlled trials. *Medicine*. 2018 Feb;97(5).
68. Ahn HY, Kim M, Ahn YT, Sim JH, Choi ID, Lee SH, Lee JH. The triglyceride-lowering effect of supplementation with dual probiotic strains, *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032: Reduction of fasting plasma lysophosphatidylcholines in nondiabetic and hypertriglyceridemic subjects. *Nutrition, Metabolism and Cardiovascular Diseases*. 2015 Aug 1;25(8):724-33.
69. Pereira DI, Gibson GR. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical reviews in biochemistry and molecular biology*. 2002 Jan 1;37(4):259-81.
70. Yao K, Zeng L, He Q, Wang W, Lei J, Zou X. Effect of probiotics on glucose and lipid metabolism in type 2 diabetes mellitus: a meta-analysis of 12 randomized controlled trials. *Medical science monitor: international medical journal of experimental and clinical research*. 2017; 23:3044.
71. Zhang Q, Wu Y, Fei X. Effect of probiotics on glucose metabolism in patients with type 2 diabetes mellitus: A meta-analysis of randomized controlled trials. *Medicina*. 2016 Feb;52(1):28-34.
72. John GK, Wang L, Nanavati J, Singh R, Mullin G. Mo1963-Lactobacillus Probiotics Reduce Body Mass Index, Body Weight, and Fat Mass in Overweight and Obese Subjects: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Gastroenterology*. 2018 May 1;154(6):S-865.
73. Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. *Epidemiology*. 2004 Sep 1;615-25.
74. Zubillaga M, Weill R, Postaire E, Goldman C, Caro R, Boccio J. Effect of probiotics and functional foods and their use in different diseases. *Nutrition Research*. 2001 Mar 1;21(3):569-79.
75. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, Pusani C, Pinelli G, Privitera G, Cesari I, Faierman SA, Caccialanza R. Probiotics and prebiotic fiber for constipation associated with Parkinson disease: an RCT. *Neurology*. 2016 Sep 20;87(12):1274-80.
76. Gremeaux V, Drigny J, Nigam A, Juneau M, Guilbeault V, Latour E, Gayda M. Long-term lifestyle intervention with optimized high-intensity interval training improves body composition, cardiometabolic risk, and exercise parameters in patients with abdominal obesity. *American Journal of Physical Medicine & Rehabilitation*. 2012 Nov 1;91(11):941-50.

77. Dimidi E, Christodoulides S, Scott SM, Whelan K. Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. *Advances in Nutrition*. 2017 May;8(3):484-94.
78. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen–gut microbiome axis: physiological and clinical implications. *Maturitas*. 2017 Sep 1; 103:45-53.