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Effects of Probiotics, Prebiotics and Synbiotics on Gut Microbiota and Lipid Profiles in Patients with Dyslipidaemia: A Systematic Review

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ABSTRACT

Purpose: This systematic review appraised previous findings to uncover the potential of pro-/ pre-/ synbiotics on regulation of gut microbiota and lipid profiles of patients with dyslipidaemia or cardiovascular diseases (CVD).
Method: The literature search was performed using PubMed, Scopus, ScienceDirect and the Cochrane Library. Studies were shortlisted based on the inclusion and exclusion criteria and evaluated for risk of bias using the "RoB 2" tool and the NIH quality assessment tool for before-after (Pre-Post) studies with no control group. Due to heterogeneity, a narrative synthesis was performed.
Results: 21 publications were shortlisted, including parallel, crossover and pre-post designs. Most studies were high risk of bias (n=17). Synbiotic interventions (low risk studies from a single trial) demonstrated significant changes in gut microbiota, including increased Bacteroidetes and Bifidobacterium spp., alongside an increase in high-density lipoprotein cholesterol (HDL-C). Pro-/ and prebiotic interventions were also associated with increases in beneficial taxa (Bifidobacterium spp., Faecalibacterium spp., Lactobacillus spp.) and improvements in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). However, with gut microbiota diversity and composition as the primary outcome, findings were inconclusive with most studies presenting high risk of bias due to considerable heterogeneity in study designs, interventions, and microbiota assessment methods.
Conclusion: Evidence from this review remains inconclusive regarding the potential of microbiota-targeted therapies (pro-/ pre-/ and synbiotic) towards dyslipidaemia or CVD due to considerable heterogeneity in microbiota assessment, interventions, study designs and predominance of high risk of bias throughout the studies. This highlights the importance of well-designed and standardised clinical trials.

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Introduction

Cardiovascular diseases (CVD) are the leading cause of death globally.[1] In 2022, there was an estimated 19.8 million deaths due to CVD, accounting for approximately 32% of all global deaths.[2] It is widely recognised that dyslipidaemia is a significant risk factor for CVD,[3] hence a good predictor of many cardiovascular and cerebrovascular events. As such, prevention and management of dyslipidaemia are deemed important in reducing the incidence of CVD.[4]

At present, the hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, remain the mainstay of lipid-modifying drugs primary outcome at play a crucial role in prevention of CVD in patients with dyslipidaemia.[5,6] Despite being the cornerstone of dyslipidaemia management, the effective use of statins is often compromised by poor adherence which could be attributed to factors such as affordability, running out of medication, adverse media reporting and side effects.[7] Statins are known to be linked with statin-associated muscle symptoms (SAMS), new-onset diabetes and elevated liver enzymes levels.[8]

The limitations of the current pharmacological approach against dyslipidaemia raise the need for non-pharmacological alternatives. In this regard, dietary modification is acknowledged as an effective primary strategy for both prevention and management of dyslipidaemia and CVD.[9] Pro-/ pre-/ and synbiotics are some of the alternative dietary supplements that have been widely reviewed for their potential beneficial effects on improving lipid profiles. Probiotics are live microorganisms that, when consumed in suitable proportions, provide health benefits to the host,[10] whilst prebiotics are substrates that are selectively utilised by host microorganisms, such as beneficial gut bacteria, to confer health benefits.[11] Synbiotics are mixtures comprising of pro- and prebiotics. They can be either complementary, whereby pro-/ and prebiotics work independently, or synergistically, with substrates specifically supporting the co-administered microorganisms to enhance health.[12]

Previous reviews have consistently highlighted that pro-/ and synbiotic supplementations could potentially improve the lipid profiles of individuals with dyslipidaemia or CVD, as evidenced by significant reductions in LDL-C and TC following the said interventions.[13-16] Elsewhere, a review by Pan et al[17] demonstrated that pro-/ pre-/ and synbiotic interventions significantly improved lipid profiles in non-alcoholic fatty liver disease patients by reducing triglycerides (TG), TC and LDL-C. None of the previous reviews had specifically examined the relationship between lipid lowering interventions, lipid profiles and gut microbiota of participants with dyslipidaemia or CVD. A balanced gut microbiota is important as it improves an individual's health. Dysbiosis, or imbalanced microbial diversity and composition, on the other hand, has been linked to the progression of CVD through mechanisms that involve pro-inflammatory metabolites and altered metabolism characterised by increased levels of pro-inflammatory trimethylamine-N-oxide (TMAO), disruptions in bile acid synthesis, imbalances in short-chain fatty acid (SCFA) production (energy metabolism), and changes in lipid metabolism.[18,19] Particularly, high levels of TMAO could cause endothelial damage, inhibit reverse cholesterol transport, increase the conversion of macrophages into foam cells and platelet activation which causes thrombosis.[20] Reduced or imbalanced SCFA, on the other hand, could impair gut barrier integrity and promote systematic inflammation, leading to increased endotoxin translocation and metabolic dysregulation, which collectively drive the development and progression of CVD.[21] Recently, emerging evidence suggests that pro-/ pre-/ and synbiotic interventions may modulate the gut microbiota in ways that could potentially improve lipid homeostasis and cardiovascular health.[22,23] As such, this study aimed at systematically reviewing the benefits of these interventions on lipid profiles and gut microbiota in individuals with dyslipidaemia and CVD, hence reducing the risk of developing dyslipidaemia or CVD. The decision to include populations with both dyslipidaemia and established CVD was guided by their shared biological basis and their position along a

continuous disease spectrum. Studying them together captures both risk factor (dyslipidaemia) and clinical manifestation (CVD).

Methods

Literature search strategy

The literature search was performed using four online databases, namely PubMed, Scopus, ScienceDirect and the Cochrane Library. The search strategy involved the integration of pre-defined keywords, MeSH terms, free text and Boolean operators (refer Table S1 for full search strategies). The search terms included "CVD", "cardiovascular disease patients", "coronary atherosclerosis", "vascular diseases", "arterial occlusive disease", "arteriosclerosis", "atherosclerosis", "coronary artery disease", "coronary disease", "coronary occlusion", "myocardial ischemia", "coronary stenosis", "cerebrovascular disorder", "peripheral vascular disease", "cardiovascular diseases", "hypocholesterol*", "dyslipid*", "HDL-C", "high density lipoprotein cholesterol", "LDL-C", "Low density lipoprotein cholesterol", "TG", "triglyceride", "TC", "total cholesterol", "non-HDL-C", "non-high density lipoprotein cholesterol", "lipoprotein", "lipids", "cholesterol", "hyperlipid*", "microbiome", "gut microbiome", "gut heart axis", "gut-heart axis", "gastrointestinal microbiome", "intestinal microbiome", "gut microbiota", "gastrointestinal microbiome", "Lactic acid bacteria", "LAB", "lactobacillus", "bifidobacterium", "probiotic yogurt", "probiotic yoghurt", "postbiotic", "prebiotic", "synbiotic", "dietary supplements", and "probiotics". The final search was performed on March 2026 without any restrictions on the publication date. This systematic review was registered under the International Prospective Register of Systematic Reviews (PROSPERO: CRD42024571796).

Study selection and data extraction

The present systematic review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[24] Briefly, all duplicated studies from the literature search were first eliminated. The remaining studies were screened for their relevance to the present systematic review based on their titles, abstracts and keywords. The eligibility of each potential study was then evaluated independently by four investigators (MAIA, SML, CFN and KR) based on the inclusion and exclusion criteria. Discrepancies in the study selection were resolved through discussion and consensus. The inclusion criteria were as follows: (i) published clinical trials, (ii) sample population of patients with CVD or dyslipidaemia (including hyperlipidaemia) that fulfils the diagnosis criteria, (iii) use of pro-/ post-/ pre-/ synbiotics or yogurt as the only active intervention, and (iv) evaluation of gut microbiota and lipid profiles. The exclusion criteria were as follows: (i) review articles, (ii) non-human studies, (iii) observational studies, (iv) randomised controlled trials (RCT) involving children, pregnant or lactating women; subjects with gastrointestinal disorders; the use of intragastric balloon and/ or gastrointestinal surgery, including bariatric surgery; patients using drugs that may affect body weight and body composition; patients with diabetes, hypertension, renal or hepatic dysfunction, non-alcoholic fatty liver disease, cancer, critical illness, autoimmune or immunosuppressive diseases, thyroid disease, polycystic ovary syndrome or metabolic syndrome, (v) abstracts or conference proceedings, (vii) non-English literature and (vi) pilot studies and (vii) studies with insufficient data. The primary outcome of this review included gut microbiota diversity and composition, while the secondary outcomes were lipid profiles, FPG and anthropometric parameters. Relevant data (i.e., country, sample size, sex, body mass index (BMI), gut microbiota, lipid profiles and anthropometric measurements) from the shortlisted studies was extracted independently by four investigators

(MAIA, SML, CFN and KR) using a standardised electronic extraction form. Discrepancies in data extraction were resolved through discussion and consensus.

Risk of bias assessment

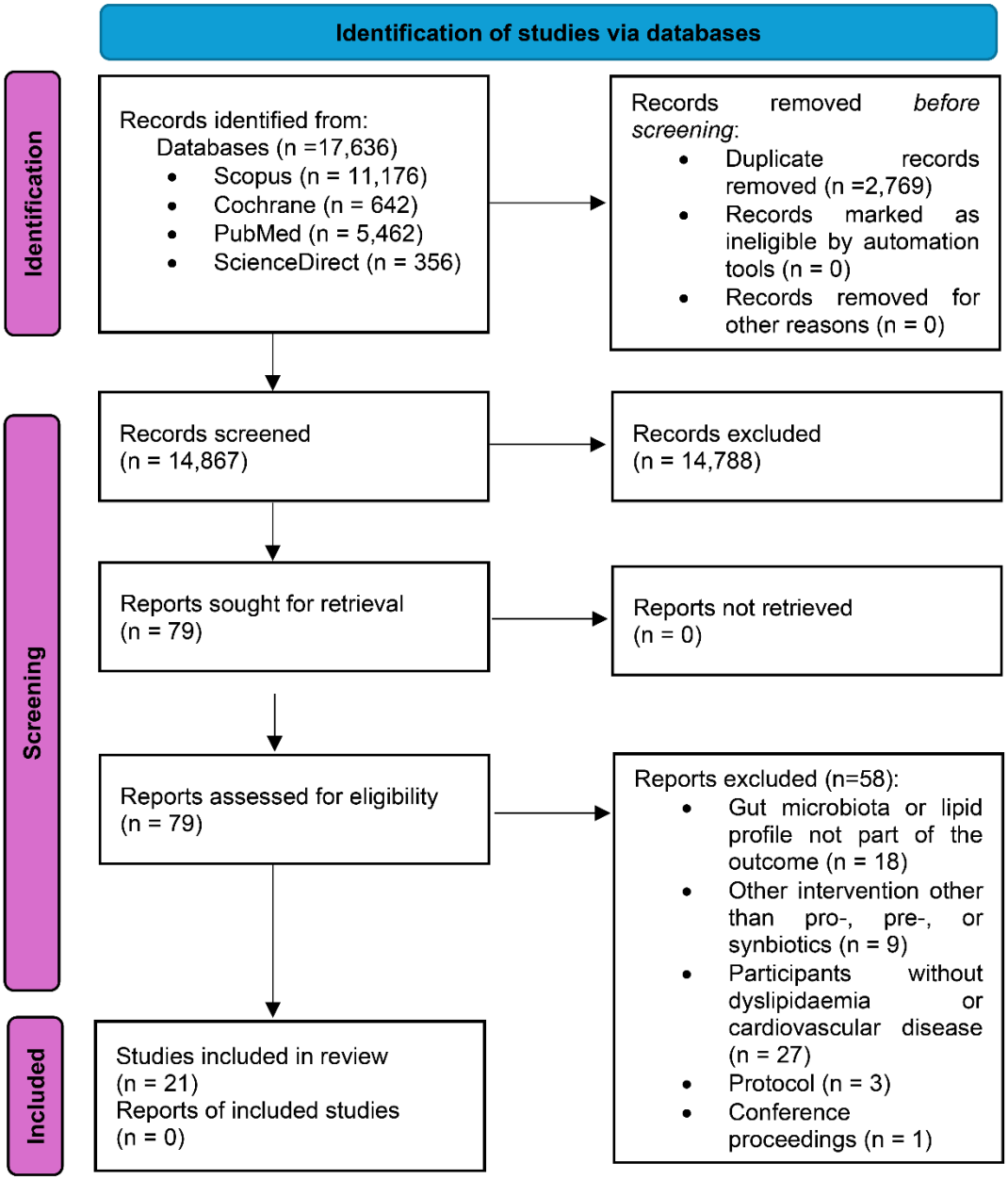
The risk of bias assessment of the shortlisted studies was performed independently by two reviewers (MAIA, KR). Discrepancies were resolved through discussion with input from two additional reviewers acting as adjudicators (SML, CFN). Inter-rater agreement was evaluated based on percent absolute agreement.[25] The shortlisted parallel intervention studies were assessed based on the "RoB 2" tool.[26] The tool comprises a set of "signalling questions" in fixed domains that direct the evaluation of bias sources, such as handling of missing data, randomisation and deviations from the intended intervention, amongst others. The tool uses an algorithm-based approach to categorise bias as "low", "some concerns", or "high". As for the crossover trials, the "RoB 2" tool adapted for crossover trials was used, whereby it has an additional domain (i.e., DS: risk of bias arising from period and carryover effects) that assess bias arising from period and carryover effects, which captures potential issues related to washout periods and treatment carryover effects in the study. On the other hand, the shortlisted pre-post studies were assessed based on the "NIH Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group".[27] It is a checklist designed to evaluate the internal validity of studies that measure outcomes before and after an intervention without a control group. It assesses factors like study objectives, participant selection, sample size, outcome measurements, and statistical analysis to determine the reliability and potential bias in the study results. In this regard, the shortlisted studies were rated based on the overall quality scores: ≥ 9 (good), $5 - 8$ (fair), ≤ 4 (poor).

Results

Description of the shortlisted studies

Figure 1 shows the PRISMA flowchart of the present systematic review. A total of 17,636 publications were initially identified from the literature search through four online databases, of which 2,769 publications were identified as duplicates and were thus excluded. Subsequent screening of the remaining studies based on their title, abstracts and keywords found 14,788 of them to be irrelevant and were excluded. Of the remaining 79 publications, 58 were excluded due to the following reasons: (ⅰ) gut microbiota or lipid profiles outcomes were not reported or not quantitatively assessed (e.g. no data on microbial taxa abundance/ diversity indices or lipid parameters) (n = 18), (ⅱ) involved other interventions apart from pro-/ pre-/ or synbiotics (n = 9), (ⅲ) participants without dyslipidaemia or CVD (n = 27), (ⅳ) protocol (n = 3), (v) conference proceeding (n = 1) while the rest of the 21 publications (2 linked publications were of the same trial) that met the inclusion criteria were shortlisted for the present systematic review, comprising a total of 1,186 participants.

Figure 1. PRISMA flowchart of the study selection



Study characteristics

Table 1 summarises the characteristics of the shortlisted publications which comprised 16 parallel, four crossover and one pre-post study design. For the shortlisted publications of parallel studies (n = 1,024 participants), six publications involved the use of probiotics in their interventions whilst eight studies used prebiotics, and the remaining two linked publications (single trial) utilised synbiotics in their intervention. For the shortlisted crossover studies (n = 111 participants), one study involved the use of probiotics whilst three studies applied prebiotics as interventions. The only shortlisted pre-post study (n = 47 participants) tested probiotics as the intervention. Whist majority of the shortlisted studies involved participants with dyslipidaemia, there were only two studies with CVD participants that fulfilled the predefined inclusion criteria.

Table 1 Characteristics of the shortlisted clinical trials

Ref.	Design	Country	Age [placebo (p)/ intervention (i)]	Prescribed lipid-lowering drugs	Health status	BMI (kg/m²) [placebo (p)/ intervention (i)]	Sample size (n)	Study duration	Intervention	DNA extraction kit	Gut microbiome assessment
a) Parallel studies (n = 16)											
Probiotics											
Chiu et al[28] 2021	Randomised, double-blind, placebo-controlled	Taiwan	18-60	NI	Mild hypercholesterolaemia	25.50 ± 3.15 (p)/ 23.40 ± 3.38 (i)	40	10 weeks then 2 weeks follow-up period	Probiotic milk formula: *L. acidophilus* (La5) *L. casei* (TMC) *B. lactis* (Bb12)	NA	Enumeration
Jiang et al[29] 2022	Randomised, single-blind, placebo-controlled	China	56.3 ± 5.3 (p)/ 55.9 ± 5.4 (i)	No	Dyslipidaemia	25.7 ± 3.5 (p)/ 25.3 ± 3.3 (i)	109	12 weeks	*L. paracasei* N1115	PSP® Spin Stool DNA Plus Kit	V4 region of bacterial 16S rRNA genes
Chu et al[30] 2023	Randomised, double-blind, placebo-controlled	China	58.27 ± 8.39 (p)/ 55.76 ± 8.87(i)	NI	Hyperlipidaemia	24.35 ± 2.67 (p)/ 26.02 ± 3.09 (i)	62	6 weeks	*B. longum* CCFM1077	FastDNA ® Spin Kit	V3–V4 region of the 16S rRNA gene
Kerlikowsky et al[31] 2025	Randomised, double-blinded, placebo-controlled	Germany	30-75	No	Mild hypercholesterolaemia	26.2 ± 3.9 (p)/ 26.4 ± 4.2 (i)	86	12 weeks	*L. plantarum* CECT7527(KABP011) *L. plantarum* CECT7528 (KABP012) *L. plantarum* CECT7529 (KABP013)	ZymoBIOMICS 96 MagBead DNA Kit	16S rRNA gene amplification of the V4 region
Wang et al[32] 2023	Randomised, double-blind, placebo-controlled	China	48.67 ± 11.79 (p)/ 51.17 ± 10.14 (i)	NI	Hyperlipidaemia	27.09 ± 5.79 (p)/ 26.44 ± 4.16 (i)	56	3 months	Probiotic-X: *L. casei* Zhang *B. lactis* V9 *B. lactis* Probio M-8 *L. plantarum* P-8	QIAamp DNA Stool minikit	Shotgun metagenomic sequencing
Spasova et al[33] 2024	Randomised, double-blind, placebo-controlled	Bulgaria	58.86 ± 9.38 (p)/ 58.14 ± 7.09 (i)	Yes	Atherosclerotic cardiovascular disease	NA	30	12 weeks	*L. plantarum* GLP3	NA	Targeted microbiome sequencing analysis

Table 1 continue overleaf

Ref.	Design	Country	Age [placebo (p)/ intervention (i)]	Prescribed lipid-lowering drugs	Health status	BMI (kg/m²) [placebo (p)/ intervention (i)]	Sample size (n)	Study duration	Intervention	DNA extraction kit	Gut microbiome assessment
Prebiotics											
Chiu et al[34] 2016	Randomised, double-blind, placebo-controlled	Taiwan	30 - 60	No	Mild hypercholesterolaemia	25.81 ± 2.83 (p)/ 24.62 ± 3.84 (i)	44	8 weeks	Fermented plant extract	NA	Enumeration
Chiu et al[35] 2017	Randomised, double-blind, placebo-controlled	Taiwan	18-53	No	Mild hypercholesterolaemia	23.59 ± 4.04 (p)/ 21.57 ± 2.45 (i,1)/ 22.82 ± 2.98 (i,2)	60	4 weeks then 2 weeks follow-up period	Prune essence concentrate	NA	Enumeration
Xu et al[36] 2021	Randomised, single-blind, controlled	China	49.08 ± 11.09 (p)/ 48.74 ± 10.90 (i)	No	Mild hypercholesterolaemia	23.22 ± 2.44 (p)/ 23.38 ± 2.41 (i)	187	45 days	Oat	QIAamp, Powerfecal Pro DNA Kit	16S rDNA gene targeted qPCR
Xu et al[37] 2022	Randomised, single-blind, placebo-controlled	China	44.03 ± 10.23 (p)/ 45.35 ± 12.50 (i)	No	Mild hypercholesterolaemia	22.71 ± 2.90 (p)/ 23.06 ± 2.64 (i)	62	45 days	Oat porridge	FastDNA SPIN Kit	V3–V4 region of the 16S rRNA gene
Contento et al[38] 2019	Randomised, double-blind, placebo-controlled	Italy	30-65	No	Hypercholesterolaemia	24 ± 3.0 (p)/ 24 ± 3.4 (i)	62	8 weeks	Olive oil pomace-enriched biscuit	FastDNA SPIN Kit	V3–V4 region of the 16S rRNA gene
Khandouzi et al[39] 2022	Randomised, controlled	Iran	56.22 ± 9.02 (p)/ 56.67 ±7.44 (i)	Yes	Coronary artery disease	26.75 ± 2.37 (p)/ 26.55 ± 2.58 (i)	50	12 weeks	Flaxseed	QIAamp DNA Stool Mini Kit	16S rDNA gene targeted qPCR
Ren et al[40] 2024	Randomised	China	25 - 65	NI	Hyperlipidaemia	NI	56	3 months	Autoclaving-treated germinated brown rice	NA	V3-V4 region of the 16S rDNA gene
Granado-Serrano et al[41] 2022	Randomised, three-arm, double blind	Spain	37-68	No	Hypercholesterolaemia	27.1 ± 3.3	68	2 months	Arm 1: Insoluble fibre cookies (wheat bran) consisted of fibre-rich cookies Arm 2: 5 g of psyllium plantago Arm 3: Onion-based antioxidant fibre	QIAamp DNA Stool Mini Kit	V3–V4 region of the 16S rRNA gene

Table 1 continue overleaf

Ref.	Design	Country	Age [placebo (p)/ intervention (i)]	Prescribed lipid-lowering drugs	Health status	BMI (kg/m²) [placebo (p)/ intervention (i)]	Sample size (n)	Study duration	Intervention	DNA extraction kit	Gut microbiome assessment
Synbiotics											
Salamat, Jahan-Mihan et al[42] 2025* Salamat, Tabandeh[19] 2024*	Randomised, double-blind, placebo-controlled	Iran	42.4	No	Dyslipidaemia	28.7 ± 4.1 (p)/ 30.1 ± 4.9 (i)	56	12 weeks	*L. acidophilus* ATCC4356 *L. fermentum* DSM14241 *L. plantarum* ATCC14917 *B. longum* BAA-999 *B. lactis* ATCC27536 *Saccharomyces boulardii* CNCM I745 Inulin Fructooligosaccharide	Stool DNA isolation kit	Hydrolysis probe targeting the 16S rRNA gene
b) Crossover studies (n = 4)											
Probiotics											
Redondo et al[43] 2019	Randomised	Spain	43.43 ± 13.35	No	Borderline-high hypercholesterolaemia	24.90 ± 3.40 (i,1)/ 25.30 ± 3.33 (i,2)/ 25.22 ± 3.11 (i,3)	30	5 weeks periods 4 weeks washouts	Arm 1: Cow's whole milk yogurt: *L. bulgaricus Streptococcus thermophilus* Arm 2: Ewe's semi-skimmed milk yogurt: *L. bulgaricuss S. thermophilus* Arm 3: Ewe's whole-milk yogurt: *L. bulgaricuss S. thermophilus*	QIAamp DNA Stool Mini Kit	16S rDNA gene targeted qPCR
Prebiotics											
Martín-Peláez et al[44] 2017	Randomised, controlled, double-blind	Spain	35-80	NI	Hypercholesterolaemia	27.85 ± 4.71	33	3 weeks period 2 weeks washout	Arm 1 virgin olive oil Arm 2 PC-enriched virgin olive oil Arm 3 PC-enriched virgin olive oil thyme	NA	Enumeration
Ranaivo et al[45] 2022	Randomised, controlled, double-blind	France	44 ± 10	NI	Cardiometabolic risk (with dyslipidaemia)	28 ± 2	16	3 weeks period 4-6 weeks washout	Chitin-glucan	QIAamp DNA Stool Mini Kit	V5–V6 region of the 16S rRNA gene
Connolly et al[46] 2016	Randomised, controlled, double-blind	United Kingdom	23-64	No	Mild to moderate hypercholesterolaemia	26.4 ± 5.7	32	6 weeks period 4 weeks washouts	Arm 1 Whole-grain Oat-based Granola breakfast cereal Arm 2 NWG breakfast cereal	NA	Enumeration

Table 1 continue overleaf

Ref.	Design	Country	Age [placebo (p)/ intervention (i)]	Prescribed lipid-lowering drugs	Health status	BMI (kg/m²) [placebo (p)/ intervention (i)]	Sample size (n)	Study duration	Intervention	DNA extraction kit	Gut microbiome assessment
c) Pre-post studies (n = 1)											
Probiotics											
Wang et al[47] 2018	1 arm intervention study	China	64.02 ± 6.43	NI	Mild dyslipidaemia and hyperglycaemia	24.61 ± 2.06	47	3 weeks	*Bifidobacterium bifidum* TMC3115	TIANamp Stool DNA Kit	V4 region of the 16S rRNA gene

Abbreviation: BMI, body mass index; NI, no information; NWG, non-whole grain; PC, phenolic compound;
* Salamat, Jahan-Mihan et al[42] 2025 and Salamat, Tabandeh et al[19] 2024 are 2 shortlisted publication from the same trial

Risk of bias

Table 2 summarises the risk of bias assessment outcomes of the shortlisted studies. The inter-rater agreements for shortlisted publications of parallel randomised trials (n=16) assessed using the "RoB 2" tool, crossover trials (n=4) assessed using an adapted crossover "RoB 2" tool, and pre–post studies (n=1) assessed using the NIH quality assessment tool were 81.25%, 83.33% and 91.67%, respectively, which were deemed good or acceptable.[25] Of the 16 shortlisted publications of parallel studies (Table 2a), the two linked publications of the same trial were of low risk and one study presented with some concerns of bias. The remaining studies were of high risk. As for the shortlisted crossover studies (Table 2b), all four studies were presented with high risk of bias. The only shortlisted pre-post study (Table 2c), fulfilled a good overall score according to the "NIH Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group" (overall quality scores ≥ 9). The high risk of bias identified in both the shortlisted parallel and crossover studies was attributed mainly to deviation from intended interventions [Domain 2 (D2)], followed by the measurement of the outcome [Domain 4 (D4)] and missing outcome data [Domain 3 (D3)]. Collectively, 17 of the shortlisted studies were considered as high risk of bias, out of which 11 (i.e., all) were prebiotic studies. As such, findings from these studies with high risk of bias should be interpreted with caution.

Table 2. Risk of bias assessment outcomes of the shortlisted studies using the "Cochrane Collaboration's Risk of Bias Tool 2" and the "NIH Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group"

a) Parallel studies	Domain					Overall
	D1	D2	D3	D4	D5	
Chiu et al[34] 2016	Some concerns	High	High	High	Some concerns	High
Chiu et al[35] 2017	Some concerns	Some concerns	Some concerns	High	Some concerns	High
Conterno[38] et al 2019	Low	High	High	Low	Some concerns	High
Chiu et al[28] 2021	Some concerns	Low	Low	High	Some concerns	High
Xu et al[36] 2021	Some concerns	High	Some concerns	High	Some concerns	High
Jiang et al[29] 2022	Some concerns	Some concerns	Low	Low	Low	Some concerns
Khandouzi et al[39] 2022	Some concerns	High	Some concerns	High	Some concerns	High
Xu et al[37] 2022	Some concerns	High	Some concerns	Low	Some concerns	High
Chu et al[30] 2023	Low	High	High	Low	Low	High
Granado-Serrano et al[41] 2022	Some concerns	High	Some concerns	Low	Some concerns	High
Kerlikowsky et al[31] 2025	Some concerns	High	High	Low	Some concerns	High
Wang et al[32] 2023	Some concerns	High	High	Low	Some concerns	High
Salamat, Jahan-Mihan et al[42] 2025*	Low	Low	Low	Low	Low	Low
Salamat, Tabandeh et al[19] 2024*	Low	Low	Low	Low	Low	Low
Ren et al[40] 2024	Some concerns	High	High	Low	Some concerns	High
Spasova et al[33] 2024	Low	Low	Low	High	Some concerns	High

NOTE: D1, Risk of bias arising from the randomisation process; D2, Risk of bias due to (effect of assignment to intervention); D3, Risk of bias due to missing outcome data; D4, Risk of bias in measurement of outcome; D5, Risk of bias in selection of the reported result;
High: high risk of bias; Low: low risk of bias; Some concerns: some concerns of bias
* Salamat, Jahan-Mihan et al[42] 2025 and Salamat, Tabandeh et al[19] 2024 are 2 shortlisted publication from the same trial

b) Crossover studies	Domain						Overall
	D1	DS	D2	D3	D4	D5	
Connolly et al[46] 2016	Some concerns	Low	High	Low	High	Low	High
Martín-Peláez et al[44] 2017	Some concerns	Low	High	Low	High	Some concerns	High
Redondo et al[43] 2019	Some concerns	Low	Some concerns	Low	High	Some concerns	High
Ranaivo et al[45] 2022	Some concerns	Low	High	Some concerns	Low	Low	High

NOTE: D1, Risk of bias arising from the randomisation process; DS, Risk of bias arising from period and carryover effects; D2, Risk of bias due to deviations from intended interventions (effect of assignment to intervention); D3, Risk of bias due to missing outcome data; D4, Risk of bias in measurement of outcome; D5, Risk of bias in selection of the reported result.
High: high risk of bias; Low: low risk of bias; Some concerns: some concerns of bias

c) Pre-post studies	Questions												Quality
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	
Wang et al[47] 2018	Yes	Yes	Yes	Yes	NR	Yes	Yes	NR	Yes	Yes	No	Yes	Good

NOTE: NR not reported; No, high risk of bias; Yes, low risk of bias. Q1, was the study question or objective clearly stated?; Q2: Were eligibility/ selection criteria for the study population prespecified and clearly described?; Q3: Were the participants in the study representative of those who would be eligible for the test/ service/ intervention in the general or clinical population

of interest?; Q4: Were all eligible participants that met the prespecified entry criteria enrolled?; Q5: Was the sample size sufficiently large to provide confidence in the findings?; Q6: Was the test/ service/ intervention clearly described and delivered consistently across the study population?; Q7: Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?; Q8: Were the people assessing the outcomes blinded to the participants' exposures/ interventions?; Q9: Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?; Q10: Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p-values for the pre-to-post changes?; Q11: Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?; Q12: If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.), did the statistical analysis take into account the use of individual-level data to determine effects at the group level?

Qualitative appraisal

Given the substantial heterogeneity in the interventions, study designs, microbiome assessment methods and risk of bias across the shortlisted studies, the present qualitative appraisal described only the significant changes ($p < 0.05$) that fulfilled either one of the following criteria; (ⅰ) significant findings from a single study must be of low risk of bias, (ii) significant findings of the same parameter or taxa from more than one shortlisted studies (n > 1) with either low risk of bias some concerns and/ or high risk of bias within the same intervention category (pro-/ pre-/ or synbiotics) must be at least the predominant trend.

Primary outcome: effects of pro-/ pre-/ and synbiotics on gut microbiota

Given the substantial heterogeneity in interventions, study designs and microbiota assessment methods across the shortlisted studies, the gut microbiota findings were synthesised descriptively based on the direction of significant change in the reported taxa. This is in line with established guidelines for synthesis without meta-analysis.[48] Table S2 summarises the shortlisted studies with their findings of the effects of pro-/ pre-/ or synbiotic supplementation on gut microbiota diversity and composition. There were no shortlisted studies of either postbiotic or yogurt interventions given that none from the present literature search met the predefined inclusion criteria.

The shortlisted single study or studies with low risk of bias that reported consistent significant changes is shown in Table S2a. Wang et al[47] reported a significant increase in the genus, *Dorea* and the species, *Bifidobacterium bifidum* post probiotic *Bifidobacterium bifidum* TMC3115 intervention. However, it was designed as a pre-post study without a control group (Table 1), and the gut microbiota species was measured through a real-time quantitative PCR (qPCR) with species-specific primers, indicating a targeted measurement approach. This may introduce selection bias and should therefore be interpreted as a targeted change rather than a comprehensive microbiome shift. In terms of synbiotic intervention (*L. acidophilus* ATCC4356 + *L. fermentum* DSM14241 + *L. plantarum* ATCC14917 + *B. longum* BAA-999 + *B. lactis* ATCC27536 + *Saccharomyces boulardii* CNCM I745 + inulin + fructooligosaccharide), Salamat, Jahan-Mihan, et al[42] and Salamat, Tabandeh et al[19] reported significant increase of the phylum Bacteroidetes. Salamat, Tabandeh et al[19] also reported a significant increase of the genus *Bifidobacterium* spp. post synbiotic intervention.

For the other shortlisted studies of low, some concerns and/ or high risk of bias that reported consistent or predominant trend of significant changes (Table S2b), probiotic interventions (5/5 shortlisted studies), either single- or multi-strain (mainly bifidobacteria and lactobacilli), resulted in a significant increase of the genus *Lactobacillus* spp.. Although the trend was consistent across the shortlisted studies, the findings should be interpreted with caution as 3/5 shortlisted studies[28,30,33] are of high risk of bias (Table 2), heterogenous in their respective study designs and microbiota assessment methods (enumeration vs 16S sequencing). In terms of

prebiotic interventions, the genera *Bifidobacterium* spp. (5/7 shortlisted studies),[34,35,40,44,46] *Dialister* spp. (3/3 shortlisted studies),[36,37,40] and *Faecalibacterium* (2/2 shortlisted studies)[37,40] were significantly increased post intervention. At the species level, fermented plant extract[34] and prune essence[35] significantly reduced pathogens such as *C. perfringens* and *E. coli*. Nevertheless, these findings should be interpreted cautiously given that all shortlisted prebiotic studies are of high risk of bias as well as heterogenous in their respective study designs and microbiota assessment methods (four shortlisted studies used enumeration whilst one of them is a crossover study).[34,35,44,46]

For the remaining shortlisted studies (Table S2c), their findings were either derived from a single study of some concerns or high risk of bias or characterised by no significant changes or mixed outcomes of the same parameter or taxa.

Secondary outcomes: effects of pro-/ pre-/ and synbiotics on lipid profile, FPG and anthropometric measurements

Table S3 summarises the shortlisted studies with their findings of the effects of pro-/, pre-/ or synbiotic supplementations on lipid profile.

For shortlisted single study or studies with low risk of bias that reported consistent significant changes (Table S3a), synbiotic intervention[19,42] significantly increased HDL-C (+5%) but has no effects on LDL-C, TC and TG.

For other shortlisted studies of low, some concerns and/ or high risk of bias (Table S3b) that reported consistent or predominant trend of significant changes, 5/8 shortlisted probiotic studies[28,30-32,47] reported significant decrease of LDL-C (-3% to -14%) whilst 6/8 shortlisted probiotic studies [28-32,47] reported significant TC reduction (-2% to -14%) post intervention. These findings should, however, be interpreted cautiously given that the majority of the studies are of high risk of bias (Table 2) and heterogenous in their study designs. On another note, probiotic intervention yielded no consistent pattern of effects on HDL-C, TG and non-HDL-C.

As for prebiotic intervention, 6/11 shortlisted studies[34-37,40,46] reported a decrease of LDL-C (-6% to -13%) and TC (-7% to -10%), respectively. Moreover, 2/2 shortlisted studies[36,37] exhibited a significant reduction of non-high-density lipoprotein cholesterol (non-HDL-C), which is the "TC minus HDL".[49] Although prebiotic exhibited promising results of LDL-C, TC and non-HDL-C reduction, it should be interpreted cautiously given all shortlisted prebiotic studies are of high risk of bias (Table 2) and heterogenous in their study design (Table S3). On another note, prebiotics yielded no consistent pattern of effects on HDL-C and TG.

In terms of other secondary outcomes, pro-/ pre-/ and synbiotics yielded no consistent pattern of effects on FPG and anthropometric parameters.

Potential relationship between gut microbiota and lipid profile.

Table 3 shows the potential relationship between gut microbiota changes and lipid profile. For single studies or studies with low risk of bias that reported consistent significant changes (Table 3a), evidence from the pre-post probiotic intervention [47] reported an increase in the genus *Dorea* spp. and *Bifidobacterium bifidum* with a decrease in LDL-C and TC. As for the two linked synbiotic publications (from the same trial),[19,42]a significant increase in the phylum Bacteroidetes and genus *Bifidobacterium* spp. alongside an increase in HDL-C following intervention was reported. These findings from the probiotic and synbiotic intervention may suggest a potential association microbiota changes and lipid profile.

Table 3 Significant gut microbiota changes post pro-/ pre-/ synbiotic intervention in relation to significant lipid changes.

Pro-/ pre-/ synbiotics	Intervention	Gut microbiota	Microbiota assessment method	Lipid changes	RoB	Study design	Reference
a) Findings from shortlisted single study or studies with low risk of bias that reported consistent and/ or significant changes							
Probiotic	*B. bifidum* TMC3115	↑ *Dorea* (Genus) ↑ *Bifidobacterium bifidum* (Species)	16S+qPCR	↓ LDL-C ↓ TC	Low	Pre-post	Wang et al[47] 2018
Synbiotic	*L. acidophilus* ATCC4356 *L. fermentum* DSM14241 *L. plantarum* ATCC14917 *B. longum* BAA-999 *B. lactis* ATCC27536 *Saccharomyces boulardii* CNCM I745. Inulin & fructooligosaccharide	↑ Bacteroidetes (Phyla)	16S	↑ HDL-C	Low	Parallel	Salamat, Jahan-Mihan et al[42] 2025* Salamat, Tabandeh et al[19] 2024*
Synbiotic	*L. acidophilus* ATCC4356 *L. fermentum* DSM14241 *L. plantarum* ATCC14917 *B. longum* BAA-999 *B. lactis* ATCC27536 *Saccharomyces boulardii* CNCM I745. Inulin & fructooligosaccharide	↑ *Bifidobacterium* spp. (Genus)	16S	↑ HDL-C	Low	Parallel	Salamat, Tabandeh et al[19] 2024*
b) Findings from shortlisted studies with low, some concerns and/ or high risk of bias that reported consistent or predominant trend of significant changes							
Probiotic	*L. acidophilus* (La5) *L. casei* (TMC) *B. lactis* (Bb12)	↑ *Lactobacillus* spp. (Genus)	16S	↓ LDL-C ↓ TC ↑ HDL-C	High	Parallel	Chiu et al[28] 2021
Probiotic	*B. longum* CCFM1077		16S	↓ LDL-C ↓ TC ↑ HDL-C	High	Parallel	Chu et al[30] 2023
Probiotic	*L. paracasei* N1115		16S	↓ TC	Some concerns	Parallel	Jiang et al[29] 2022
Probiotic	*L. plantarum* GLP3		16S	No significant changes	High	Parallel	Spasova et al[33] 2024
Probiotic	*B. bifidum* TMC3115		16S	↓ LDL-C ↓ TC	Low	Parallel	Wang et al[47] 2018
Prebiotic	Fermented plant extract	↑ *Bifidobacterium* spp. (Genus)	Enumeration	↓ LDL-C ↓ TC	High	Parallel	Chiu et al[34] 2016
Prebiotic	Prune essence concentrate		Enumeration	↓ LDL-C ↓ TC	High	Parallel	Chiu et al[35] 2017
Prebiotic	Whole-grain oat-based granola breakfast cereal		Enumeration	↓ LDL-C ↓ TC	High	Crossover	Connolly et al[46] 2016
Prebiotic	Phenolic compound enriched virgin olive oil		Enumeration	No significant changes	High	Crossover	Martín-Peláez et al[44] 2017

Pro-/ pre-/ synbiotics	Intervention	Gut microbiota	Microbiota assessment method	Lipid changes	RoB	Study design	Reference
	Autoclaving-treated germinated brown rice		16S	↓ LDL-C ↓ TC ↑ HDL-C ↓ TG	High	Parallel	Ren et al[40] 2024
	Flaxseed	↔ *Bifidobacterium* spp. (Genus)	16S	No significant changes	High	Parallel	Khandouzi et al[39] 2022
	Oat		16S	↓ LDL-C ↓ TC ↓ Non-HDL-C	High	Parallel	Xu et al[36] 2021
	Autoclaving-treated germinated brown rice	↑ *Dialister* spp. (Genus)	16S	↓ LDL-C ↓ TC ↑ HDL-C ↓ TG	High	Parallel	Ren et al[40] 2024
	Oat		16S	↓ LDL-C ↓ TC ↓ Non-HDL-C	High	Parallel	Xu et al[36] 2021
	Oat porridge		16S	↓ LDL-C ↓ TC ↓ Non-HDL-C	High	Parallel	Xu et al[37] 2022
	Autoclaving-treated germinated brown rice	↑ *Faecalibacterium* (Genus)	16S	↓ LDL-C ↓ TC ↑ HDL-C ↓ TG	High	Parallel	Ren et al[40] 2024
	Oat porridge		16S	↓ LDL-C ↓ TC ↓ Non-HDL-C	High	Parallel	Xu et al[37] 2022
	Fermented plant extract	↓ *Clostridium perfringens* (Species)	Enumeration	↓ LDL-C ↓ TC	High	Parallel	Chiu et al[34] 2016
	Prune essence concentrate		Enumeration	↓ LDL-C ↓ TC	High	Parallel	Chiu et al[35] 2017
	Oat	↔ *Clostridium perfringens* (Species)	qPCR	↓ LDL-C ↓ TC ↓ Non-HDL-C	High	Parallel	Xu et al[36] 2021
Prebiotic	Fermented plant extract	↓ *Escherichia coli* (Species)	Enumeration	↓ LDL-C ↓ TC	High	Parallel	Chiu et al[34] 2016
	Prune essence concentrate		Enumeration	↓ LDL-C ↓ TC	High	Parallel	Chiu et al[35] 2017

↑: Significantly increase, ↓: Significantly decrease, ↔: No significant changes
Abbreviations: FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.
a) Findings from shortlisted single study or studies with low risk of bias that reported consistent and/ or significant changes;
b) Findings from shortlisted studies with low, some concerns and/ or high risk of bias that reported consistent or predominant trend of significant changes;
* Salamat, Jahan-Mihan et al[42] 2025 and Salamat, Tabandeh et al[19] 2024 are 2 shortlisted publication from the same trial

The studies with low, some concerns and/ or high risk of bias (Table 3b) that reported consistent or predominant trend of significant changes is shown in Table 3b. All 5/5[28-30,33,47] studies with probiotic intervention reported significant increase of *Lactobacillus* spp. with mixed lipid modification (3/5: significant reduction in LDL-C, 4/5: significant reduction in TC, 2/5 significant increase in HDL-C). As for the prebiotic interventions, 5/7 studies reported a significant increase of *Bifidobacterium* spp. post intervention of which 4/5[34,35,40,46] studies showed reduction in LDL-C and TC. Prebiotic intervention increased the genera *Dialister* spp. (3/3 studies)[36,37,40] and *Faecalibacterium* (2/2 studies)[37,40] with significant reduction in LDL-C, TC and non-HDL-C. For species, two studies[34,35] showed significant reduction of pathogenic *C. perfringens* and *E. coli* post prebiotic intervention, with reduction in both LDL-C and TC.

Discussion

Gut microbiota influence lipid changes

There is growing evidence that support the close interaction of gut microbiota with the host and its influence on the host's health and disease status, including dyslipidaemia and CVD. Alterations in the intestinal environment often inhibit the growth of beneficial bacteria, including SCFA-producing species, hence disrupting gut homeostasis and promoting dyslipidaemia. This would in turn exacerbate gut dysbiosis, leading to a cycle of metabolic imbalance.[50] As part of the efforts in uncovering effective strategies for both prevention and management of dyslipidaemia and CVD, the present systematic review revealed the beneficial effects of pro-/ pre-/ and synbiotics against these metabolic diseases through regulation of the gut microbiota.

In terms of probiotic intervention (Table 3a), it remains unclear as to whether the significant increase of *Dorea* influenced the lipid profile given that Wang et al[47] found no association between this genus and the reduced LDL-C and TC post intervention. Consistent with this, findings in a recent human cohort study found that higher abundance of the genus *Dorea* is associated with lower levels of LDL-C after supplementation with green tea with rhubarb root, however, no causality can be inferred from these association.[51] another randomised study using human genetic data found that higher genetically predicted abundance of *Dorea* was associated with lower TG levels, whereas evidence for LDL-C reduction was weaker and less consistent, suggesting a modest potential role in lipid metabolism.[52] There is no strong evidence that *Dorea* directly influences the lipid metabolism in a clinically meaningful manner.

Increased *Bifidobacterium bifidum* with probiotic intervention (Table 3a) was observed with the improved lipid profile even though its causal effect has yet to be established. While preclinical studies on high-fat diet rat model suggest possible lipid-lowering mechanisms,[53] their relevance to humans is uncertain. However, *Bifidobacterium bifidum* has been shown to produce SCFA[54] that could in turn activate the adenosine monophosphate-activated protein kinase (AMPK) pathway, thereby inhibiting HMG-CoA reductase[42,55] and decreasing the synthesis of cholesterol.

In addition, probiotic interventions were also found to increase *Lactobacillus* spp. (Table 3b) that may enhance bile salt hydrolase (BSH) activity,[30] which may explain the reduction of LDL-C and TC post intervention. BSH activity could promote the deconjugation of bile acids in the liver and the release of free bile salts, which would eventually be excreted through the faeces. To compensate for the bile acid loss, the liver would then draw cholesterol from the circulation to synthesise more bile acids. This compensatory response would ultimately lower blood cholesterol levels.[30,31] Besides, *Lactobacillus* spp. could also produce beneficial SCFA which improves lipid metabolism.[55] That being said, the findings from the shortlisted studies still need to be interpreted cautiously due to the heterogeneity of their respective study designs and microbiota assessment methods as well high risk of bias (3/5 shortlisted studies).[28,30,33] One of the shortlisted probiotic studies,[30] for example, did not perform the intention-to-treat (ITT) analysis despite having 11% of participant dropouts. This had caused the shortlisted study to lose the benefit of randomisation,[56] giving rise to increased bias. The remaining two shortlisted studies, which performed enumeration[28] and targeted microbiome analysis[33] of the *Lactobacillus* spp., exhibited bias in terms of the use of assessment methods that were unsuitable to reflect the status of the entire gut microbiota.

In terms of prebiotic interventions, the majority of the shortlisted prebiotic studies[34,35,40,44,46] (Table 3b) reported an increase of the SCFA-producing genus *Bifidobacterium* spp., which may explain, at least in part, the significant reduction of LDL-C and TC post intervention. However, these findings should be interpreted with caution given the absence of ITT analysis in these shortlisted studies which may have introduced potential bias

due to incomplete data that may have been excluded from the final analysis. This may in turn influence the observed association between the gut microbiota modulation and lipid changes. Additionally, two shortlisted prebiotic studies[37,40] reported about the significant increase of the genus *Faecalibacterium*, which is also closely related to SCFA production.[37] This may explain, at least in part, the significant reduction of LDL-C and TC post intervention.

Besides, the shortlisted studies[36,37,40] also reported a significant increase of the genus *Dialister* spp. post prebiotic intervention. The roles of *Dialister* spp. in lipid metabolism remain to be fully understood as previous studies reported mixed outcomes. It was previously found that *Dialister* spp. was less abundant in individuals with dyslipidaemia[57] but more abundant in patients with cerebral infarction and ischaemia.[58] Whilst some *Dialister* spp. were positively correlated with HDL-C, *Dialister succinatiphilus*, in particular, was positively correlated with LDL-C.[59] Elsewhere, *Dialister invisus* was found to be associated with type 2 diabetes mellitus and metabolic syndrome, which are often characterised by altered lipid profiles.[60] As such, further research is needed to define the roles of *Dialister* spp. in the context of lipid metabolism.

Importantly, the shortlisted prebiotic studies reported reduced pathogens post intervention. More specifically, fermented plant extract and prune essence concentrate significantly lowered the abundance of *C. perfringens* and *E. coli*.[34,35] However, the shortlisted studies measured *C. perfringens* and *E. coli* through enumeration, which may contribute to a bias in microbiota assessment. It is noteworthy that the overall findings from the shortlisted prebiotic studies must be interpreted cautiously as they were of high risk of bias either due to deviation from intended intervention, microbiota assessment methods or missing data.

In terms of synbiotic intervention (Table 3a), the increase of the phylum Bacteroidetes and the genus *Bifidobacterium* spp.[19,42] may explain the increase of HDL-C. Bacteroidetes is one of the primary bacterial phyla in the gut which is strongly associated with the synthesis of beneficial SCFA such as butyrate and propionate.[19] Likewise, *Bifidobacterium* spp. could also produce beneficial SCFA. SCFA was found to upregulate Apolipoprotein A-1 (ApoA-1) and ATP-binding cassette subfamily A member 1 (ABCA1), both of which play crucial roles in lipid metabolism.[61] ApoA-1 is the key to synthesis of HDL particles as it is able to remove excess cholesterol and phospholipid from cell and tissues, turning them into nascent HDL. These nascent disc-shaped HDL are subsequently converted into mature spherical HDL through the action of lecithin:cholesterol acyltransferase (LCAT), which esterifies free cholesterol. This would prompt the migration of cholesteryl esters into the HDL particle core, giving its round mature shape that transport low-density lipoprotein (LDL).[62] The transporter ABCA1, on the other hand, could mediate the cholesterol and phospholipid efflux, which together could boosts the levels of HDL-C in the blood.[63] The overall findings from the synbiotic intervention appear to be less controversial based on their low risk of bias.

These previous evidence shows that gut microbiota modulation may represent one of the potential mechanisms of action for lipid modification.[19,28-30,35-37,42,46] However, further well-designed clinical studies on pro-/ pre-/ and synbiotics, are needed to clarify the role of the gut microbiome in mediating lipid-lowering effects. Previous systematic reviews have reported reductions in TC and LDL-C following these interventions,[13-17] and similar trend was observed in the present review. However, due to the generally high risk of bias of the previous shortlisted studies, the certainty of the current evidence remains limited.

Risk of bias and research gap

The risk of bias assessment of the shortlisted studies identified some biases in their methods. The risk of bias assessment using the "RoB 2" tool identified D2 as the most concerning source of high risk of bias. D2, which measures the risk of bias towards deviation from intended interventions, was most overlooked in the present shortlisted studies. Most of these studies failed to apply appropriate analysis to estimate the effect of assignment to intervention. The most appropriate analysis that should be used is the ITT analysis which is crucial to avoid overestimation of the efficacy of an intervention and for maintaining the integrity of randomisation. The ITT analysis typically considers all participants in the groups to which they were originally assigned regardless of whether they completed the intervention or adhered to the protocol, thus preserving the benefits of randomisation and reducing bias.[56] These shortlisted studies also deviated from the intended interventions, which were likely to significantly influence the trial outcomes. These deviations primarily involved cases of non-adherence to the assigned interventions, one of the reasons is due to a dropout rate exceeding 5% of the originally randomised participants. As such, these studies should employ the ITT analysis to evaluate the effect of assignment to intervention as one cannot control the dropout rate of the participants in the clinical trials.

The second most concerning source of high risk of bias of the present shortlisted studies was D4, which measures the risk of bias in the measurement of the outcome. The outcome of the present systematic review was the gut microbiota and the accepted methods of unbiased measurement of gut microbiota included shotgun metagenomic sequencing and quantitative real time polymerase chain reaction (qPCR), targeting the V3-V6 region of the 16s rRNA gene. The present review also considered the objectives of the shortlisted studies when determining the potential methodological risks of bias. The present shortlisted studies with high risk of bias, used enumeration technique of selected bacteria,[28,34,35,44,46] or adopted the qPCR method that targeted only specific bacteria,[36,39,43] which certainly did not reflect the entire gut microbiota as stated in their objectives.

The third most concerning source of high risk of bias of the present shortlisted studies was D3, which measures and evaluates the extent and impact of missing data on the results. Based on the risk of bias assessment tool, the number of participants for the final analysis needs to be more than 95% of the original number of randomised participants. Many of the shortlisted studies reported dropout rates exceeding 5% but did not clearly specify the reasons for participant dropout. The shortlisted studies must clearly explain the reasons for participant dropout, ensuring that both the number and causes of dropouts were reported for each intervention group and should remain relatively balanced across the groups. Transparent reporting of the number of dropouts is crucial as it can impact the potential bias and overall validity of the study.[64]

The other limitations from the shortlisted studies include variations in sample size, which may influence the accuracy and significance of study results. The highest sample size from the present eligible studies was 187[36] whereas the lowest being 16.[45] From the shortlisted studies, seven of them includes fewer than 50 participants, while 9 studies recruit participants between 50 and 100 and the remaining two have a sample size of more than 100 participants (Table 1). Smaller sample sizes are generally considered more prone to bias, often leading to exaggerated findings compared to larger studies. Future clinical trials should conduct rigorous sample size calculations to ensure that trials are neither underpowered nor excessively large, thereby maximising their impact.[65]

Limitations and future perspectives

The present systematic review acknowledges several limitations of the shortlisted studies. Firstly, the heterogeneous study population which included individuals with dyslipidaemia and CVD. This systematic review had included CVD studies in addition to the dyslipidaemic studies to uncover the potentials of pro-/ pre-/ synbiotic intervention as an adjunct therapy to lipid-modifying drugs in treating both the underlying risk factor (dyslipidaemia) and its clinical manifestation (CVD). Notably, the two shortlisted CVD studies are randomised placebo-controlled trials which minimise baseline differences between groups and reduce confounding from both known and unknown factors.[66] This would then improve comparability between intervention and control groups within each shortlisted study. In other words, the internal validity of these studies remains preserved despite the differences in participants characteristics. Both the population of dyslipidaemia and CVD remain different in terms of their baseline metabolic status and background therapies (lipid-lowering medications), which may have influenced lipid outcomes independently of microbiota modulation.[67] The two shortlisted CVD studies which included participants who were taking lipid modifying drugs[33,39] however reported no significant changes in lipid profile post pro-/ prebiotic interventions. As such, the potential influence of population heterogeneity on lipid outcomes appears to be limited in the context of this systematic review whereby the studied population was predominantly individuals with dyslipidaemia who were not prescribed lipid modifying drugs.

This present systematic review had shortlisted studies of multiple trial designs which included parallel, crossover and pre-post designs. In terms of the parallel studies, between-group comparisons were the primary estimate of effect and these designs minimise confounding variable, hence providing the most robust evidence among the shortlisted studies.[66] In terms of the crossover studies, they may be susceptible to carryover effects if washout periods between intervention phases were insufficient, which may influence treatment estimates.[68,69] However, from the present assessment using the "RoB 2" tool adapted for crossover studies, all the shortlisted crossover studies demonstrated low risk on the carryover effects. The four crossover studies employed washout period of 2-4 weeks. This is relevant as meaningful alterations in the gut microbiota occur within two weeks.[70] This validates the between period comparisons in these studies as the washout periods were likely sufficient for microbiota recovery between intervention phase, minimising residual treatment effects. In terms of the single pre-post study, the "NIH Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group" tool was used to capture design vulnerabilities (lack of a concurrent control group and confounding by time).[27] Given the well-recognised limitations of single group pre-post designs (susceptibility to regression to the mean, history, and maturation effects), the findings from this study were presented as suggestive rather than causal, ensuring it does not drive the overall conclusions relative to the randomised evidence.[71]

The gut microbiota assessment methods of the shortlisted studies were also heterogenous. The gut microbiota was assessed primarily using either 16S rRNA gene sequencing (sometimes complimented by qPCR), enumeration or shotgun metagenomics, each with distinct strengths and limitations. The 16S rRNA gene, sequences mainly bacteria but offers poor taxonomic and functional resolution and typically cannot resolve taxa below genus level.[72] Relative abundance data from sequencing may be influenced by compositional bias and differences in total microbial load, whereas qPCR-based normalisation can provide estimates of absolute bacterial counts. In contrast, shotgun metagenomics sequencing analyses total microbial DNA present in a sample, enabling the detection of a broad range of microorganisms (bacteria, archaea, viruses, fungi) and enables higher taxonomic resolution (until species level identification) and detection of a broader range of taxa (low-abundance organisms).[73,74] These methodological differences may influence the observed microbial profiles and limit direct

comparability across studies. Taxa identified as "unchanged" using 16s rRNA sequencing may reflect limited sensitivity rather than true absence of effect, especially for low-abundance genera[72] compared to shotgun metagenomics methods. As for enumeration, culture-based methods capture only a subset of the microbiota and may underestimate overall diversity[75] compared to the other approaches. Future studies should standardise the use of microbiota assessment method to enhance the comparability of findings across studies.

Additionally, other variations in terms of intervention duration, diets, dosages and delivery methods amongst the shortlisted studies could also further hinder the comparability of findings, making it challenging to establish definitive conclusions. Due to the usage of different stool sample collection methods and DNA extraction kits, the metagenomic data of every study varied to a particular extent. These discrepancies could hinder direct comparisons between studies that employ different methodologies. Therefore, standardised guidelines for stool sample collection, DNA extraction, and gut microbiota analysis are needed to improve consistency and facilitate inter-study comparisons.[76] The heterogeneity in study design, intervention protocols and method of measuring outcomes limits the ability to draw definitive conclusions. Future studies should focus on standardising methodologies with appropriate intervention periods to offer a more solid and clinically relevant results.[66] Diet should also be considered when interpreting effects of interventions on gut microbiota modulation, as habitual diet can independently influence microbial composition irrespective of intervention.[77] Future studies should incorporate standardised dietary monitoring, validated dietary assessments such as 3-day food diary or Food Frequency Questionnaire (FFQ) and controlled diet protocols to improve isolation of the true effects of the interventions.

Conclusion

Altogether, the current evidence from this qualitative appraisal of previous pro-/ pre-/ and synbiotic studies remains inconclusive regarding the role of these microbiota-targeted interventions towards dyslipidaemia or CVD due to considerable heterogeneity and bias. The least controversial evidence from low risk of bias studies in this review suggest that synbiotics intervention caused an increase in the phyla Bacteroidetes and genus *Bifidobacterium* spp., which may influence the improvement of HDL-C. As for the other studies, mostly reported an increase in beneficial taxa such as *Bifidobacterium* spp., *Faecalibacterium*, *Lactobacillus* spp. and improvements in TC and LDL-C following pro-/ or prebiotics intervention. However, interpretation for these findings was limited due to heterogeneity in interventions, microbiota assessment, study designs and predominance of high risk of bias throughout the studies. This highlights the importance of well-designed and standardised clinical trials to clarify the clinical relevance of these microbiota-targeted interventions.

Declaration of Competing Interest

None.

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Data availability

Data will be made available on request**.**

Clinical trial number

Not applicable

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