Birjand University of Medical Sciences

Potential Benefits of Probiotics and Prebiotics in Cardiovascular Disease

Presenter

Farshad Mirzavi

Assistant Professor of Clinical Biochemistry

Cardiovascular Diseases Research Center

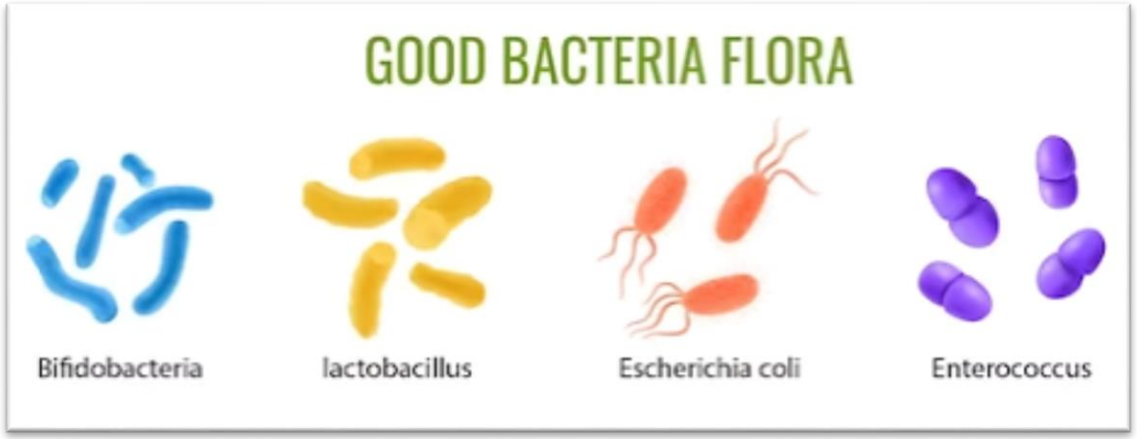
Birjand University of Medical Sciences

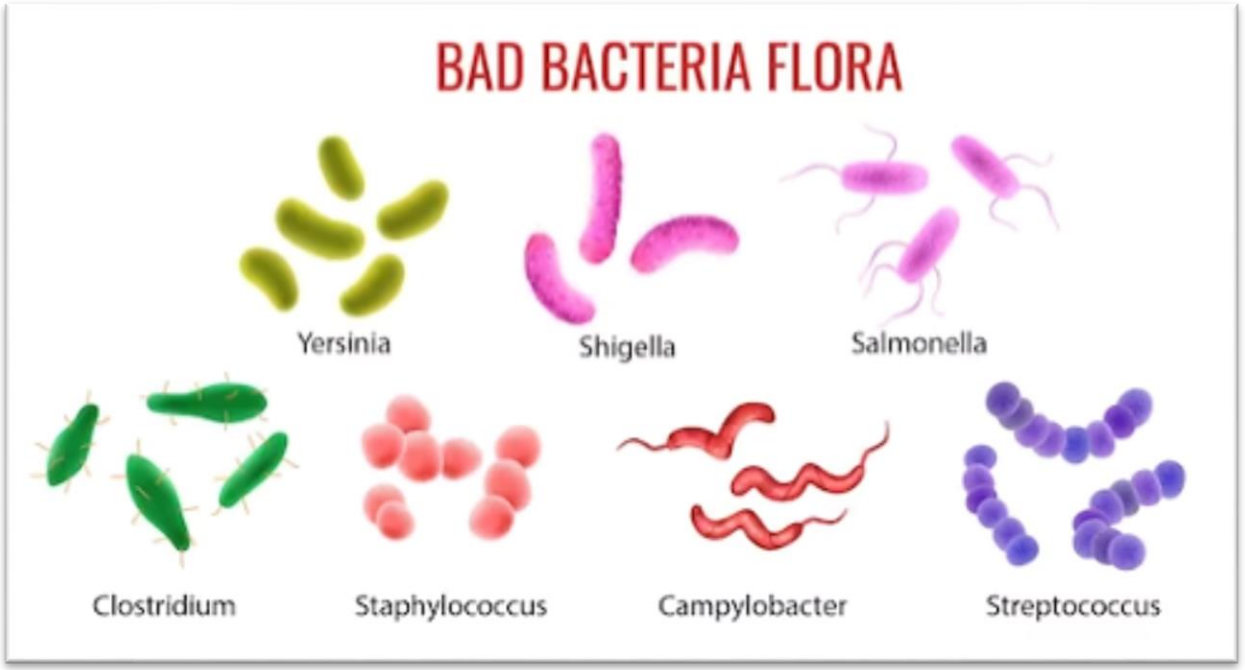
Probiotics and Prebiotics: General Information

- **Probiotic:** live microorganisms which, when administered in adequate doses, confer a health benefit on the host (FAO 2001).
- **Prebiotic:** a prebiotic is a non-viable food component that confers a health benefit by modulation of the gut microbiota (FAO 2007).
- **Synbiotic:** a product that contains both probiotics and prebiotics
- **Gut microbiota, gut microbiome, or gut flora:** are the microorganisms, including bacteria, fungi, and viruses, that live in the digestive tracts.
- **Dysbiosis:** the imbalance of gut microbiota associated with an unhealthy outcome.
- **Colony-forming unit (CFU):** the number of viable microorganisms in a probiotic supplement.

Probiotics and Prebiotics: General Information…

- According to the current state of knowledge, probiotics encompass both **bacteria** (Lactobacillus, Leuconostoc, Pediococcus, Propionibacterium, Bifidobacterium, Bacillus, some Streptococcus, Enterococcus, Escherichia coli) and **yeast** (Saccharomyces) genera.





Probiotic supplements might not be universally-effective and safe

- ✓The FAO/WHO guidelines on probiotic evaluation from 2002 reported that probiotics may theoretically be linked to specific types of side effects in patients with underlying medical conditions.
- ✓The at-risk population groups are broadly characterized by weakened immune systems, gut dysbiosis, and/or impaired intestinal barriers, therefore, it is important to carefully assess the safety associated with deliberate administration of probiotics.

Table 2
Risks associated with rampant dietary intake of probiotics.

Category	Microorganisms	Population	Mechanisms	References
Systemic infection	*Lactobacillus rhamnosus* GG	Pre-term infant with short gut syndrome	*Lactobacillus* bacteremia	[112]
	L. rhamnosus GG	Critically ill children with antibiotic related diarrhea	Sepsis	[113]
	L. rhamnosus GG	11-month old infant with short gut syndrome	*Lactobacillus* bacteremia	[114]
	L. rhamnosus GG	17-year-old boy with ulcerative colitis	*Lactobacillus* bacteremia	[115]
	Lactobacillus sp.	58-year-old immunocompetent with mechanical ventilation	*Lactobacillus* bacteremia and sepsis	[116]
	Three strains of *L. rhamnosus*	24-year-old female cardiosurgical patient	Probiotic sepsis	[23]
	Bifidobacterium longum	74-year-old man with polymetastatic prostatic adenocarcinoma	*Bifidobacterium* bacteremia	[117]
	B. longum subspecies infantis	Pre-term infants	*Bifidobacterium* bacteremia	[118]
	B. longum	Low birth-weight infants	*Bifidobacterium* bacteremia	[119]
	B. breve	2-year-old boy with Philadelphia chromosome-positive acute B-cell lymphoblastic leukemia	*Bifidobacterium* sepsis	[120]
	E. coli NISSLE strain 1917	Pre-term infants	Severe sepsis	[41]
	Saccharomyces cerevisiae	48-year-old diabetic with multiple co-morbidities	Multiple organ failure and septic shock in association with toxic megacolon and probiotic fungemia	[121]
	S. cerevisiae var. *boulardii*	Immunocompromised 73-year-old patient on chemotherapy	Fungemia	[25]
	S. cerevisiae var. *boulardii*	8-year-old boy with respiratory distress (Intensive care unit patient)	Fungemia	[122]
	S. cerevisiae var. *boulardii*	Premature neonate receiving nutrition enterally	Fungal septicemia	[123]
	S. cerevisiae var. *boulardii*	Critically ill patients	Fungemia	[82]
	L. plantarum	30-year-old male with rheumatic valve disease	Endocarditis	[124]
	L. casei	53-year-old immunocompetent patient	Endocarditis	[125]
	L. jensenii	47-year-old immunocompetent patient	Endocarditis	[126]
	L. paracasei	77-year-old male patient with prostate cancer	Endocarditis	[127]
	L. acidophilus	48-year-old male with heart disease and dental manipulations	Endocarditis	[45]
	L. rhamnosus	> 65-year-old patient with hemorrhagic telangiectasia (HHT)	Endocarditis	[128]
Localized infection	*L. casei*	60-year-old with renal transplant patient	Intra-abdominal abscess	[129]
	L. paracasei	65-year-old diabetic patient	Bacteremia and liver abscess	[47]
	L. rhamnosus	11-month-old female with trisomy 21 with respiratory viral infection	Probiotic associated pneumonia	[48]

Kothari, Damini, Seema Patel, and Soo-Ki Kim. "Probiotic supplements might not be universally-effective and safe: A review." *Biomedicine & Pharmacotherapy* 111 (2019): 537-547.

Probiotic supplements in clinical research

❑There are several criteria for evaluating whether probiotics could be used in food in clinical research:

(1) proper identification, characterization, and maintenance of probiotic strains

(2) keeping the studied probiotics in live condition

(3) ensuring they are alive at the site of action in the studies.

❖**According to the provisions of the WHO, the number of living cells in probiotic foods at the time of human consumption may not be lower than 10^6 cells per 1 mL or 1 g of product. Furthermore, the therapeutic dose is 10^8–10^9 cells in 1 mL or 1 g of product.**

Gut Microbiota, Its Diet-Derived Products, and Cardiovascular Diseases

- Evidence from research suggests that there is a strong correlation between the gut microbiome and the development of cardiovascular disease.
- In particular, a correlation has been found between the gut microbiome and the production of N-trimethylamine oxide, derived from dietary components such as choline and carnitine.
- Certain bacteria that are found in the gut are capable of converting choline and carnitine (components that are found in red meat and other animal foods) into **N-trimethylamine oxide, potentially increasing risk factors for heart disease.**

Gut Microbiota, Its Diet-Derived Products, and Cardiovascular Diseases

- Elevated blood levels of N-trimethylamine oxide have been directly linked to adverse outcomes in patients with such conditions, such as coronary artery disease and heart failure.
- Over time, the microbiome begins to produce toxic molecules, including N-trimethylamine oxide, which enter the bloodstream, **causing inflammation**.
- The age-related microbial imbalance of the gut microbiome contributes to the development of oxidative stress and inflammation that underlie arterial dysfunction. Such findings, therefore, support that good gut microbiota helps prevent cardiovascular disease.

Table 1. Studies concerning gut dysbiosis in CVD [6].

Study Groups	Microbiota Results
Non ischemic heart failure with reduced ejection fraction; $n = 28$ (vs. 19 controls)	↑ *Streptococcus, Veillonella, Eggerthela* ↓ *Prevotella, SMB53 (Clostridiaceae)*
Patients with ischemic or dilated cardiomyopathy; $n = 84$ (vs. 266 controls)	↑ *Prevotella, Hungatella (Lacnospiraceae), Succiniclasticum* ↓ *Blautia, Anaerostipes, Faecalibacterium, Lachnospiraceae, Bifidobacterium, Eubacterium, Coprococcus*
Stable systolic heart failure; $n = 20$ (vs. 20 controls)	↑ *Escherichia-Shigella* ↓ *Blautia, Collinsella, Ruminococcaceae, Erysipelotrichaceaem Faecalibacterium*
Patients with ischemic or dilated cardiomyopathy; $n = 53$ (vs. 40 controls)	↑ *Ruminococcus, Acinetobacter, Veillonella* ↓ *Faecalibacterium, Alistipes, Oscilibacter*
Patients with hypertension (≥140/90 mmHg); $n = 60$ (vs. 60 controls)	↑ *Klebsiella, Salmonella, Streptococcus, Clostridium, Parabacteroides, Eggerthella* ↓ *Faecalibacterium, Roseburia, Synergistetes*
Patients with hypertension (≥140/90 mmHg) and pre-hypertensive patients (125/80–139/90 mmHg); $n = 155$ (vs. 41 controls)	↑ *Prevotella, Klebsiella, Porphyromonas* ↓ *Faecalibacterium, Roseburia, Bifidobacterium, Oscillibacter, Coprococcus, Butyrivibrio*
Patients with coronary artery disease; $n = 70$ (vs. 98 controls)	↑ *Escherichia-Shigella, Lactobacillus, Enterococcus, Streptococcus* ↓ *Faecalibacterium, Roseburia, Eubacterium, Subdoligranulum*

Oniszczuk, Anna, et al. "Role of gut microbiota, probiotics and prebiotics in the cardiovascular diseases." *Molecules* 26.4 (2021): 1172.

The Influence of Probiotics and Prebiotics on the Factors Causing CVD

- The underlying mechanisms of probiotics on CVD are relatively complicated and are yet to be elucidated. Sánchez et al. proposed 3 different mechanisms contributing to the beneficial effects of probiotics on human health:

(1) amelioration of the epithelial barrier function

(2) competing against pathogens

(3) immunomodulation

Probiotics and CVD

- ✓ Reduces trimethylamine N-oxide (TMAO) levels
- ✓ Anti inflammatory activity
- ✓ Anti oxidative activity
- ✓ Decrease cholesterol absorption

Product Facts
Serving Size 1 Capsule 500 mg
Serving Per Container: 30

15 Billion Live Cultures

Lactobacillus Acidophilus	$> 7.5 \times 10^9$ CFU
Bifidobacterium Bifidum	$> 6.0 \times 10^9$ CFU
Bifidobacterium Longum	$> 1.5 \times 10^9$ CFU

Daily Value not established

Other ingredients
Maltodextrin and gelatin capsules

The cholesterol-lowering mechanism

- The cholesterol-lowering mechanism requires the synergistic performance of prebiotics and probiotics. The proposed mechanism was as follows:

- ✓ ***Bile salt deconjugation by BSH.*** The bile salt deconjugation process is carried out by probiotic bacteria's bile salt hydrolase (BSH) enzyme. The deconjugation process will reduce cholesterol. Generally, conjugated bile salts are circulated through the enterohepatic tract. In contrast, deconjugated bile salts are more soluble in water and can be excreted in the feces. Bile salts that are excreted in the feces must be replaced by new bile salts synthesized from blood cholesterol. Therefore, the more bile salts that come out through the feces, the more cholesterol is taken from the blood, lowering cholesterol levels in the blood.
- ✓ ***Coprecipitation of cholesterol with deconjugated bile.*** Prebiotic fermentation by probiotic bacteria produces SCFA, which results in a decrease in pH. Cholesterol will coprecipitate with deconjugated bile salts at a pH lower than 5.5.
- ✓ ***Cholesterol use by cellular membranes.*** Cholesterol is absorbed by the cellular membrane of probiotic bacteria and used for the growth of these bacteria. *Lactobacillus* carries out this mechanism. This mechanism causes a decrease in cholesterol absorption by the blood in the intestines, thereby reducing cholesterol levels in the blood.

Table 1. Beneficial effects of probiotics on CHD and stroke.

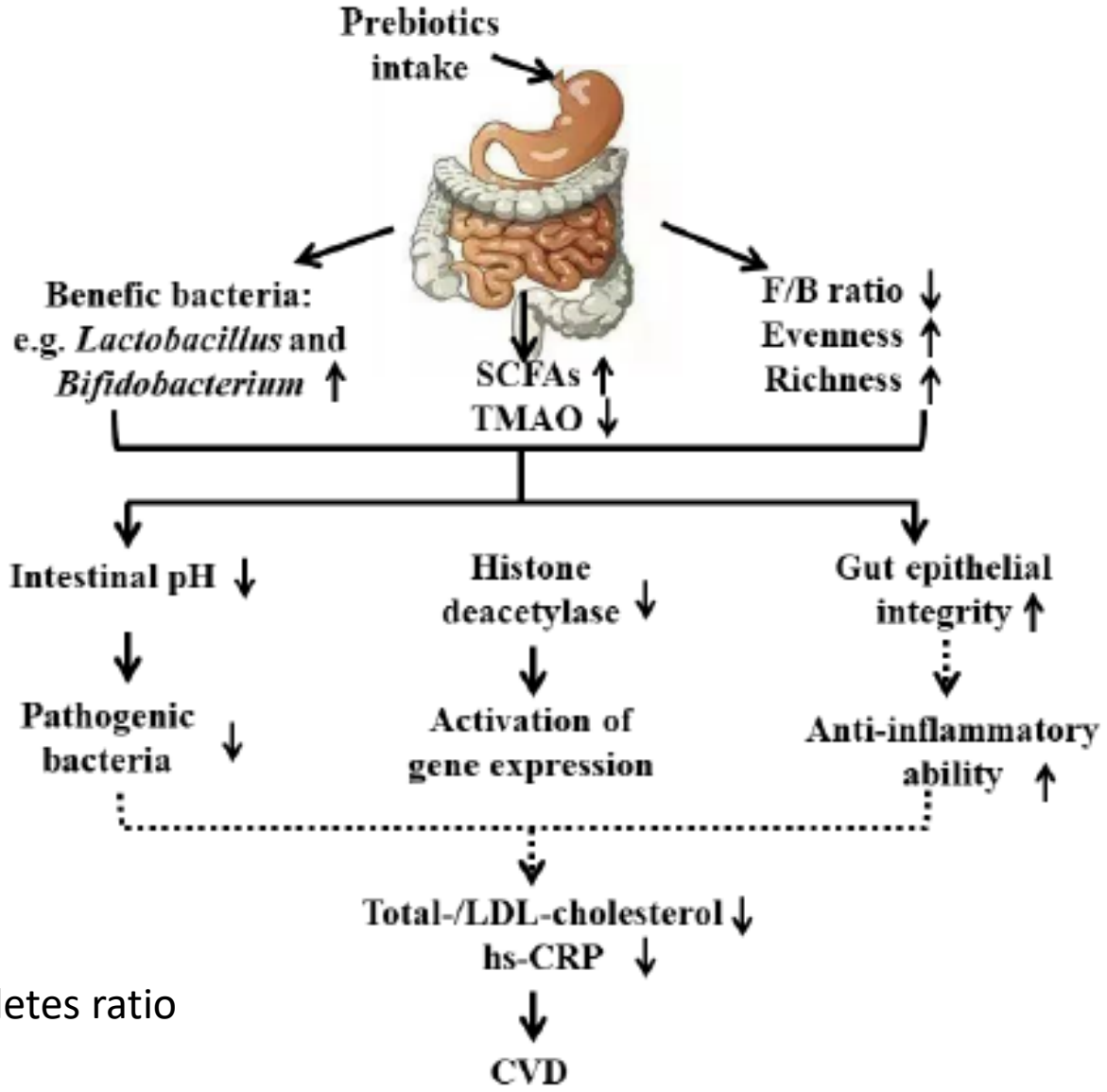
Disease	Product	Probiotic Strains	Subject	Dose	Duration	Outcomes	Reference
Type 2 diabetic patients with CHD	Probiotic	*B. bifidum*, *L. casei*, *L. acidophilus*	Human	2×10^9 CFU/day	12 weeks	Fasting plasma glucose, insulin, insulin resistane and total/HDL cholesterol ratio ↓ Insulin sensitivity and HDL cholesterol levels ↑, hs-CRP ↓ Antioxidant capacity and total glutathione levels ↑	[67]
Diabetic people with CHD	Vitamin D and probiotics	*L. zisttakhmir*	Human	8×10^9 CFU/g	12 weeks	Serum insulin levels ↓ Serum 25-OH-vitamin D ↑ Serum HDL cholesterol levels ↑ Serum hs-CRP, plasma NO, and plasma TCA ↑	[68]
Type 2 diabetic patients with CHD	Probiotic and selenium	*L. acidophilus*, *L. reuteri*, *L.fermentum*, *B. bifidum*	Human	2×10^9 CFU/g	12 weeks	Fasting plasma glucose, serum insulin levels, insulin resistance ↓ Triglycerides, VLDL and total cholesterol, and hs CRP ↓ NO ↑	[69]
Type 2 diabetic patients with CHD	Synbiotic	*L. acidophilus*, *L. casei*, *B. bifidum*	Human	2×10^9 CFU/g	12 weeks	Fasting plasma glucose, serum insulin concentrations ↓ HLDL-cholesterol levels ↑	[70]
Overweight, diabetes, and CHD	Synbiotic	*L. acidophilus* strain T16 (IBRC-M10785), *L. casei* strain T2 (IBRC-M10783) *B. bifidum* strain T1	Human	2×10^9 CFU/g	12 weeks	Serum hs-CRP and plasma MDA ↓ Plasma NO ↑	[71]
Men with stable CAD	Probiotic	*L. plantarum* 299v (Lp299v)	Human	2×10^{10} CFU/d	6 weeks	NO ↑ IL-8, IL-12, and leptin levels↓	[72]
CAD patients	Probiotic	*L. rhamnosus* GG (LGG)	Human	1.6×10^9 CFU/d	12 weeks	IL1-Beta ↓ LPS ↓	[73]
CAD patients	Synbiotic	*L. rhamnosus* GG (LGG)	Human	1.9×10^9 CFU/d	8 weeks	hs-CRP ↓ LPS ↓ TNF-α ↓	[74]

Wu, Haicui, and Jiachi Chiou. "Potential benefits of probiotics and prebiotics for coronary heart disease and stroke." *Nutrients* 13.8 (2021): 2878.

Prebiotics and CVD

- Prebiotics contain only substances which stimulate microorganism growth; there are no bacteria in their composition.
- These substances can be obtained from various sources, including soybeans, raw oats and honey. However, the most popular prebiotics are **plant oligosaccharides**.
- Nondigestible carbohydrates, including polysaccharides (resistant starch, pectin, and dextrin) and oligosaccharides, such as fructo oligosaccharides, galacto oligosaccharide, raffinose oligosaccharides, lactulose, and inulin, possess prebiotic properties.
- Prebiotics are fermented by the gut bacteria and **produce short-chain fatty acids**, e.g., propionate, butyrate, and acetate.
- The production of short-chain fatty acids has positive effects, including improvement of intestinal membrane integrity and absorption of minerals, lowering both glycemic levels and body weight, improved immunity, and modulation of metabolic, cardiovascular, and inflammatory biomarkers.

Mechanism of the beneficial role of prebiotics on CVD



F/B ratio: Firmicutes to Bacteroidetes ratio
TMAO: trimethylamine N-oxide.

Wu, Haicui, and Jiachi Chiou. "Potential benefits of probiotics and prebiotics for coronary heart disease and stroke." *Nutrients* 13.8 (2021): 2878.

Table 2. Beneficial effects of prebiotics on various CVDs.

Disease	Product	Prebiotics	Subject	Dose	Duration	Outcomes	Reference
Type 2 diabetic patients with CHD	Synbiotic	Inulin	Human	800 mg/day	12 weeks	Fasting plasma glucose, serum insulin concentrations ↓ HLDL cholesterol levels ↑	[70]
Overweight, diabetes, and CHD	Synbiotic	Inulin	Human	800 mg/day	12 weeks	Serum hs-CRP and plasma MDA ↓ Plasma NO ↑	[71]
CAD patient	Synbiotic	Inulin	Human	15 g/day	2 months	hs-CRP, LPS, TNF-α ↓	[74]
Chronic kidney disease patients	Prebiotic	Inulin	Human	19 g/day	6 months	Serum insulin and fasting glucose levels, HOMA-IR, total cholesterol, Triglycerides, CRP and homocysteine ↓ HDL cholesterol ↑	[122]
Women with type 2 diabetes	Prebiotic	Inulin and oligofructose	Human	10 g/day	8 weeks	Total antioxidant capacity ↑ Fasting plasma glucose, HbA1c, total cholesterol, LDL cholesterol, TC/HDL-c ratio, LDL-c/HDL-c ratio and malondialdehyde ↓	[123]

Clinical Nutrition 40 (2021) 4915–4931

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Meta-analyses

Effects of probiotics on body adiposity and cardiovascular risk markers in individuals with overweight and obesity: A systematic review and meta-analysis of randomized controlled trials

Karine Scanci da Silva Pontes [a], Marcella Rodrigues Guedes [a], Michelle Rabello da Cunha [b], Samanta de Souza Mattos [b], Maria Inês Barreto Silva [c, d], Mario Fritsch Neves [b], Bianca Cristina Antunes Alves Marques [b, e], Márcia Regina Simas Torres Klein [c, *]

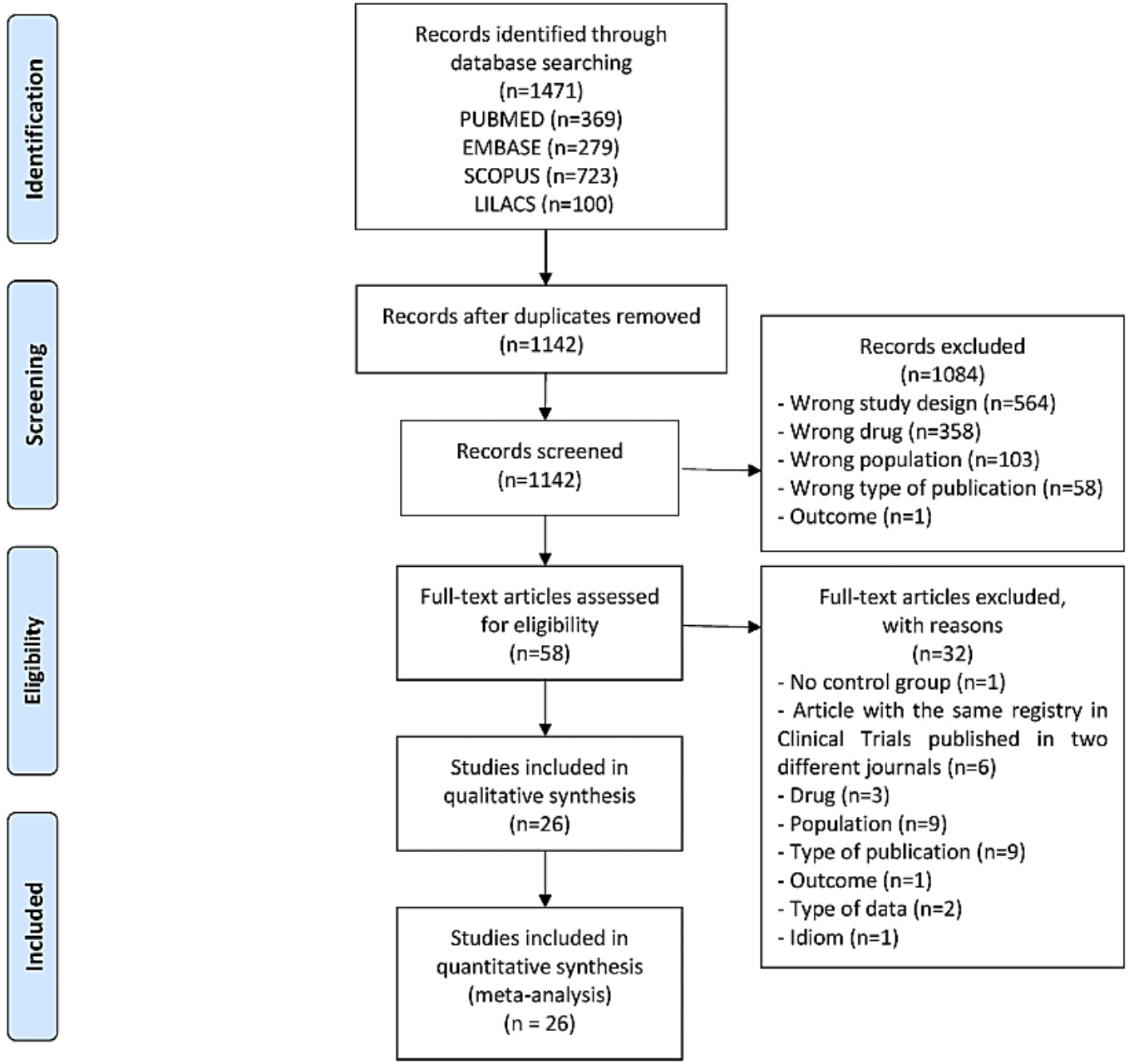


Fig. 1. PRISMA flow diagram of study.

Table 1
Characteristics of the randomized controlled trials included in systematic review.

Author Year, Location	Design	Population	Baseline characteristics (Probiotic/control)			Sample size (PB/C)	Probiotic			Duration (weeks)	Control group	Dietary intervention	Main outcomes
			BMI (kg/m^2)	Age (years)	Sex % F		Vehicle	Strains	Dose (CFU)				
Agerholm-Larsen 2000 [19], Denmark	DB, PC	Healthy overweight and obese adults	30.0/30.0	38.6/39.4	75/64	16/14	Yogurt	*S. thermophilus* *L. acidophilus*	~9 × 10^9	8	Yogurt fermented with delta-acid-lactone	No	BW, BMI, WHR, BF, LIP, BP
			30.2/30.0	37.9/39.4	72/64	14/14		*S. thermophilus* *L. rhamnosus*	~9 × 10^{10}				
			30.1/30.0	37.8/39.4	75/64	16/14		*E. faecium* *S. thermophilus*	~27 × 10^9				
Banach 2020 [35], Poland	SB, PC	Obese	35.6/34.2	34.9/34.2	63/67	27/27	Yogurt	*L. acidophilus* LA5 and *B. lactis* BB12	ND	12	Only diet	Yes	BW, BMI, BF
Brahe 2015(36), Denmark	SB, PC	Obese women (Post-M)	34.2/34.3	31.4/58.5	53/47	18/16	Sachet	*L. paracasei* F19	9.4 × 10^{10}	6	Maltodextrin	No	LIP, GLU, INS, HOMA-IR, LPS, IL-6, TNF-α, CRP
Culpepper 2019 [37], USA	DB, PC, CO	Health obese adults	36.2/36.1	56.0/53.6	100/73	18/60	Capsule	*B. subtilis* R0179	2.5 × 10^9	6	Potato starch and stearate	No	LIP, GLU, INS, HOMA-IR
			36.1/36.1	53.4/53.6	67/73	24/60		*L. plantarum* HA119	5 × 10^9				
			35.9/36.1	51.3/53.6	56/73	18/60		*B. lactis* B94	5 × 10^9				
Fathi 2016 [38] and 2017 [20], Iran	DB, PC	Healthy overweight or obese women (Pre-M)	29.5/28.9	35.2/37.0	47/53	18/20	Kefir	ND	ND	8	Low-fat dairy products	Yes	BW, BMI, WC, LIP
Hajipoor et al. 2021 [39], Iran	DB, PC	Obese	36.3/34.6	40.9/35.37	71/81	28/31	Yogurt	*L. acidophilus* LAB5 and *B. lactis* BB12	8 × 10^9	10	Low-fat yogurt	Yes	BF, LIP
Hibberd 2019 [21], Finland	DB, PC	Healthy overweight or obese adults	30.9/31.0	49.1/48.3	72/72	25/36	Sachet	*B.animalislactis* 420	1 × 10^{10}	24	Microcrystalline cellulose	No	BW, BMI, WHR, BF, LIP, GLU, HbA1C, INS, CRP
Higashikawa 2016 [40], Japan	DB, PC	Healthy overweight	26.8/27.4	52.5/52.8	62/65	21/20	Powder	*P.pentosaceus* LP28	1 × 10^{11}	12	Dextrina	No	BMI, WC, BF, LIP, GLU, HbA1C, INS, HOMA-IR, LEP, ADIP
Ivey 2014(41) and 2015(42), Australia	DB, PC	Overweight	30.8/30.8	64.7/65.4	41/43	39/40	Yogurt and capsule	*L. acidophilus* LA5 and *B. animalislactis* BB12	3 × 10^9	6	Milk and capsule	No	LIP, GLU, HbA1C, INS, HOMA-IR, BP
Kadooka 2010 [22], Japan	DB, PC	Healthy overweight	27.5/27.2	48.3/49	33/32	43/44	Fermented milk	*L.gasseri* SBT2055 LG2055	1 × 10^{11}	12	Fermented milk	No	BW, BMI, WC, WHR, BF, AF, ADIP
Kim 2017 [43], South Korea	DB, PC	Overweight	26.6/27.1	ND	ND	32/34	Powder	*L. curvatus* HY7601 and *L. plantarum* KY1032	1 × 10^{10}	12	Crystalline cellulose, lactose, and blue berry flavouring agent.	No	BW, BMI, BF, AF
Kim 2018 [44], South Korea	DB, PC	Overweight and obese	28.2/28.6	37.9/38.1	77/53	30/30	Capsule	*L. gasseri* BNR17	1 × 10^{10}	12	Maltodextrin, crystalline cellulose, and magnesium stearate.	Yes	BW, BMI, WC, WHR, BF, AF, LIP, GLU, HbA1C, INS, HOMA-IR, TNF-α, CRP, LEP, ADIP
Krumbeck 2018 [45], USA	DB, PC	Obese	34.6/36.4	44.7/43.9	64/77	14/17	Sachet	*B. adolescentis* IVS1	1 × 10^9	3	Lactose	No	BW, BMI, WC, LP, GLU, BP
			37.5/36.4	43.9/43.9	86/77			*B. lactis* BB12	1 × 10^9				
Lim 2020 [46], Korea	DB, PC	Overweight and obese	28.0/28.5	46.4/47.2	75/70	47/48	Powder	*L. sakei* CJLS03	1 × 10^{10}	12	Powder	Yes	BW, BMI, WC, BF, AF, HbA1C, INS, HOMA-IR, BP
Madjd 2016 [47], Iran	SB, PC	Healthy overweight and obese women (Pre-M)	32.1/32.1	32.2/31.8	100/100	44/45	Yogurt	*L. acidophilus* LA5 and *B. lactis* BB12	2 × 10^7	12	Yogurt	Yes	BW, BMI, WC, LIP, GLU, HbA1C, INS, HOMA-IR
Majewska 2020 [48], Poland	DB, PC	Obese women	36.6/36.1	55.2/58.7	100/100	25/25	Sachet	*B .bifidum* W23, *B. lactis* W51, *B. lactis* W52, *L .acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lc. lactis* W19 and *Lc. lactis* W58.	1 × 10^{10}	12	ND	ND	LIP, TNF-α

Results: Twenty-six RCTs (n = 1720) were included. Data pooling showed a significant effect of probiotics in reducing body weight (MD:-0.70 kg; 95%CI:-1.04,-0.35 kg; $P < 0.0001$), body mass index (BMI) (MD:-0.24 kg/m^2; 95%CI:-0.35,-0.12 kg/m^2; $P = 0.0001$), waist circumference (WC) (MD:-1.13 cm; 95%CI:-1.54,-0.73 cm; $P < 0.0001$), fat mass (MD:-0.71 kg; 95%CI:-1.10,-0.32 kg; $P = 0.0004$), tumor necrosis factor-α (MD:-0.16 pg/ml; 95%CI:-0.24,-0.08 pg/ml; $P = 0.0001$), insulin (MD:-0.85mcU/ml; 95%CI:-1.50,-0.21mcU/ml; $P = 0.010$), total cholesterol (MD:-0.16 mmol/l; 95%CI:-0.26,-0.05 mmol/l; $P = 0.003$) and LDL (MD:-0.09 mmol/l; 95%CI:-0.16,-0.03 mmol/l; $P = 0.006$) compared with control groups. There was a significant decrease in body weight, BMI and WC in studies using both single and multi-bacterial species. Decreases in body adiposity parameters were only observed in studies using a probiotic dose of $\geq 10^{10}$ CFU and for $\geq$8 weeks duration.

Conclusions: The present meta-analysis suggests that probiotics consumption may be helpful for improving body weight, body adiposity and some CVD risk markers in individuals with overweight and obesity.

Thank you for your attention