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SMALL ANIMAL MODELS OF CARDIOVASCULAR DISEASE FOR EVALUATING THE CHOLESTEROL-LOWERING ACTIVITY OF PROBIOTIC STRAINS

Cardiovascular diseases (CVDs) are major contributors to global morbidity and mortality, causing approximately 17 million deaths annually worldwide. The incidence of CVDs is rising among economically productive age groups, exacerbated by increasing rates of diabetes, obesity, and smoking-related conditions. Despite significant advances in managing low-density lipoprotein (LDL) cholesterol, the residual risk of atherosclerotic cardiovascular disease (ASCVD) persists, partly attributed to remnant cholesterol in triglyceride-rich lipoproteins. This review aims to evaluate suitable animal models of CVD for demonstrating the therapeutic efficacy of probiotic strains with cholesterol-lowering activity. Animal models that closely mimic human CVD conditions are essential for elucidating underlying disease mechanisms. Probiotics have shown promising preventive effects on CVD through the restoration of gut microbiota dysbiosis and anti-inflammatory responses. Mechanisms include reduction of oxidative stress, lowering of hypercholesterolemia, and modulation of bile acid metabolism. The advantages and limitations of animal models in CVD research are discussed, highlighting the strength of rodent models such as mice, which are cost-effective, genetically manipulable, and replicate key aspects of human CVD pathophysiology. Various contemporary mouse models are reviewed for their suitability in studying atherosclerosis, myocardial infarction, and other CVDs. Each model offers unique insights into disease mechanisms and responses to therapeutic interventions. Thus, selecting appropriate animal models is crucial for advancing our understanding of probiotic-mediated therapies in CVD. By leveraging these models, researchers can explore novel strategies to mitigate CVD risk factors and enhance therapeutic outcomes.

Keywords: *cardiovascular diseases, probiotics, animal models, atherosclerosis, cholesterol metabolism, therapeutic efficacy.*

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Cardiovascular diseases (CVDs) including heart attack, stroke, and heart failure (HF) are a leading cause of morbidity and mortality, contributing to an estimated 17 million deaths annually around the world (Wargocka-Matuszewska et al., 2023; Golforoush, et al., 2020; Savojia et al., 2019). The phenomenon is most often observed in the older age group, however, there is an increasing tendency in the economically most productive groups. Over the next few years, the incidence of CVD may multiply due to the aging of the population. The more and more common occurrence of diseases such as diabetes and obesity, as well as the conditions associated with smoking, intensifies the frequency of CVD (Aparicio et al., 2021; Amini et al., 2021; Kondo et al., 2019; Ray et al., 2022; Hansen et al., 2024). There are many underlying pathologies that lead to CVDs:

- *Atherosclerosis*. Defined by cholesterol deposition in large- and medium-sized arteries.
- *Myocardial infarction*. Unstable atherosclerotic plaque erosion leads to acute myocardial infarction, where oxygen supply to myocytes becomes restricted.
- *Arrhythmia*. Improper function of cardiac ion channels or cell junctions, or physical obstacles (e.g., infarcted tissue) disturbing electrical wave propagation.
- *Cardiomyopathy*. A general term encompassing a variety of symptoms, including heart muscle enlargement, thickening, and rigidity.
- *Cardiac fibrosis*. The scarring process characterized by cardiac fibroblast over-proliferation, myofibroblast activation, and increased deposition of fibrous extracellular matrix proteins.

For many years, low-density lipoprotein (LDL) cholesterol has been the primary lipid target for preventing cardiovascular disease, supported by extensive evidence from observational, genetic, and randomized controlled trials. Nevertheless, even after LDL cholesterol is reduced to the recommended levels, there remains a residual risk of atherosclerotic cardiovascular disease (ASCVD). This residual risk may be partly attributed to remnant cholesterol in triglyceride-rich lipoproteins. These lipoproteins are linked to an increased risk of ASCVD through both observational and genetic studies. Remnant cholesterol is calculated by subtracting LDL cholesterol and high-density lipoprotein (HDL) cholesterol from the total cholesterol. It encompasses cholesterol in very low-density lipoproteins, intermediate-density lipoproteins, and chylomicron remnants in the non-fasting state (Langsted et al., 2020; Andreadou et al., 2020).

Therefore, the **aim** of the study was to select the most correct and useable animal model of CVD to demonstrate the positive therapeutic effects of probiotic strains with cholesterol-lowering activity on these pathologies. The use of animal models recapitulating human condition is essential to understand the mechanisms underlying cardiac diseases (Savojia et al., 2019).

Current state of cardiovascular disease treatment. Disturbed cholesterol homeostasis is not only a pathological basis of cardiovascular and cerebrovascular diseases but also contributes to the progression of other conditions, including neurodegenerative diseases and cancers. Maintaining cholesterol homeostasis is crucial physiologically. Normally, cholesterol homeostasis is maintained by a dynamic balance among intake, biosynthesis, transport, cellular uptake and efflux, and esterification (Duan et al., 2022).

Currently, several treatments are recognized for their ability to lower triglyceride-rich lipoproteins:

- Statins reduce both LDL cholesterol and triglyceride-rich lipoproteins, though the percentage reduction is smaller for triglycerides compared to LDL cholesterol (Morofuji et al., 2022)
- Fibrates lower triglycerides and, in subgroup analyses of individuals with elevated triglycerides, appear to reduce the risk of atherosclerotic cardiovascular disease (ASCVD) (Duan et al., 2022)
- High doses of omega-3 fatty acids lower triglycerides (Nicholls et al., 2018)
- Inhibition of angiopoietin-related protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) represents potential new targets. Antisense oligo-

nucleotides and antibodies have been developed to lower the plasma levels of apoC-III or ANGPTL3, thus significantly reducing plasma triglycerides (Nordestgaard et al., 2018). These drugs are expected to enter phase III trials in the near future

▪ PROMINENT trial evaluates whether reducing triglycerides and remnant cholesterol with a novel selective peroxisome proliferator-activated receptor alpha (PPARα) modulator will lower major adverse cardiovascular events (MACE) in high-risk individuals with diabetes and high triglycerides (Pradhan et al., 2018)

▪ Ezetimibe is a cholesterol absorption inhibitor used to lower total cholesterol, LDL-C, apolipoprotein B (Apo-B), and non-HDL-C in primary hyperlipidemia and familial cholesterolemia. It is a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption. (Khan et al., 2020)

▪ Consumption of prebiotics offers protective benefits against CVD. Among the various prebiotics, inulin and inulin-containing prebiotics have been most extensively studied for their beneficial effects on different CVDs, including coronary heart disease (CHD), diabetes associated with CHD, coronary artery disease, chronic kidney disease, atherosclerosis, and hypercholesterolemia, in both human patients and animal models (Moludi et al., 2021). Supplementation with inulin (or in combination with other components) has been shown to reduce the levels of cholesterol, including total and LDL cholesterol, C-reactive protein**,** and various inflammatory cytokines, while also improving antioxidant parameters and gut microbiota dysbiosis. In addition to inulin, other prebiotics and prebiotic complexes have also demonstrated beneficial roles in CVD. For instance, a prebiotic complex derived from fermented wheat bran was found to correct intestinal dysbiosis and endotoxemia in female rats with experimentally induced heart failure (Hoving et al, 2018). Soluble fiber supplementation (such as Minolest) has shown positive effects on the lipid profile of individuals with mild hypercholesterolemia and a low risk of coronary artery disease (Wu & Chiou, 2021). Moreover, larch arabinogalactan, a component of pectin, was observed to reduce myocardial injury by inhibiting apoptotic pathways in a rat model of ischemia-reperfusion. Chitosan oligosaccharides also demonstrated protective effects in CHD by enhancing antioxidant capacities and improving lipid profiles, likely by promoting the growth of beneficial probiotic species in the gut (Jiang et al, 2019). Overall, prebiotics may alleviate CVD symptoms through various mechanisms, including reducing inflammation, enhancing antioxidant capacity, and restoring balance to the dysbiotic gut microbiota.

▪ Consumption of probiotics offers protective benefits against CVD. Four mechanisms have been proposed to explain the beneficial effects of probiotics on CVD: 1) amelioration of the epithelial barrier function; 2) competition with pathogens for nutrients and adhesion sites; 3) effects on other tissues via the immune system and neurotransmitter production; and 4) immunomodulation (Sánchez et al., 2017). The preventive effects of probiotics on CVD are thought to occur through the restoration of the gut microbiota dysbiosis and anti-inflammatory responses. These mechanisms likely include reducing oxidative stress, lowering hypercholesterolemia, and decreasing high blood pressure (Oniszczuk et al., 2021). Changes in gut microbiota associated with the mediation of cholesterol metabolism, uric acid metabolism, oxidative stress, and inflammatory reactions through various metabolites could be involved in the development of atherosclerosis, a major risk factor for coronary heart disease and stroke. (Vasquez et al., 2019; Brandsma et al., 2019). In comparison with pharmaceutical agents, nutraceuticals generated from food after fermentation by probiotic bacteria have evoked greater interest in atherosclerosis prevention (O'Morain & Ramji, 2020; Jiang et al., 2020; Hassan, 2020). Probiotics play a potential role in preventing atherosclerosis through the reduction of trimethylamine N-oxide (TMAO) levels (Din, 2019; Qiu,

2018). CVDs, such as dyslipidemia and diabetes, could be caused by disorders in the metabolism of bile acids (BAs). *Lactobacilli* play an important role in BA biotransformation by promoting the activity of microbial bile salt hydrolase, regenerating primary free bile acids, and facilitating the microbial formation of secondary BAs as well as a range of intermediates (Prete et al., 2020). Obligate microflora provides hypolipidemic and hypotensive effects by deconjugating bile acids and reducing their resorption through the synthesis of specialized hydrolases; incorporating cholesterol into the lipid layer of the cell membrane; transforming cholesterol into coprostanol and removing it from the body via feces; and inhibiting cholesterol synthesis in the liver (Starovoitova et al., 2024; Hassan et al., 2019; Redinbo, 2020; Bhat et al., 2019; Daliri et al., 2022; Alaqil et al., 2020; O'Morain & Ramji, 2020; Jia et al., 2023; Asan-Ozusaglam & Gunyakti, 2019; Park et al., 2018; Bendali et al., 2017; Singhal et al., 2019; Zhang et al., 2017; Saikia et al., 2018; Bidura et al., 2019; Majeed et al., 2019; Huang et al., 2021; Haldar & Gandhi, 2019; Tom et al., 2021; Pimenta et al., 2018; Palaniyandi et al., 2020; Yusuf et al., 2020; Fernandez-Calderon et al., 2022; Dixon et al., 2020; Aswani et al., 2021; Neverovskyi et al., 2021; Pushpass et al., 2022; Frappier et al., 2022; Romero & Duarte, 2023; Taslim et al., 2023).

Thus, probiotics containing cholesterol-assimilating strains, especially in the encapsulated form (which increases the survival of probiotic microorganisms as they pass through the upper gastrointestinal tract, thereby indirectly enhancing the therapeutic effect of such treatments and products) (Starovoitova et al., 2022), can effectively complement the complex therapy of patients with cardiovascular diseases, cancer, and other conditions. These treatments are free from negative side effects associated with statins, such as hepatotoxicity; they are not addictive and do not require lifelong use. Additionally, functional foods enriched with probiotic microorganisms exhibiting hypocholesterolemic activity can be used not only in therapy but also in the prevention of diseases associated with high serum cholesterol levels (Starovoitova et al., 2024).

Pros and cons of animal models of CVD. The main advantages and limitations of animal models used in the study of cardiovascular disease are as follows:

Advantages:

- direct information;
- availability of control;
- controlled modification of variables;
- accurate techniques;
- possibility to create new models;
- availability of different models;
- greater analytical potential;
- access to structures.

Limitations:

- need to extrapolate the results;
- differences in genetic regulation;
- anatomical differences;
- various pathophysiological mechanisms;
- differences between species;
- variations induced by the techniques used;
- *in vivo* models versus *in vitro*;
- different responses to drugs.

Large animal models, such as canine and primate models, have a long history of use in CVD research. Although they effectively simulate the pathological characteristics of human patients, there are inevitable limitations, including high feeding costs and difficulties in genetic modification. Rodents, however, are the most common models for CVD research and are indispensable tools for studying the pathological features, clinical symptoms, and drug development of human diseases. Rodent models not only effectively simulate the characteristics and indicators of human CVD but also offer the advantages of strong reproductive ability and ease of detection, providing great convenience for scientific research (Table 1). Some existing rodent models for CVD are not yet fully mature or effective. More effort is needed to develop more suitable experimental rodent models for human CVDs.

Table 1. **Rodent Models and Methods for Common Cardiovascular Diseases**

Cardiovascular diseases	Modeling methods	Pros and cons
	Special diet models	
Coronary atherosclerotic heart disease	Feed apolipoprotein E deficient (Apoe−/−) mice with a high-fat diet (Gomez et al., 2018; Seijkens et al., 2018) Feed lipoprotein receptor-deficient (LDLR−/−) mice with a high-fat diet (Pan et al., 2018)	Low cost of food, simple operation Large individual differences, long experiment period
Ejection fraction-preserving heart failure	Feed Dahl salt-sensitive (DSS) rats with a high-salt diet (Cho et al., 2017)	
Coronary medial calcification	Feed dilute brown non-agouti (DBA/2) mice with a high-phosphate diet (Lau, 2013)	
Coronary intimal calcification	Feed fetuin-A/apolipoprotein E-deficient (Ahsg−/−), Apoe−/− mice with a high-phosphate diet (Jia et al., 2023)	
	Drug or immunogen-mediated models	
Low renin hypertension	Aldosterone/salt therapy (ALDOST) (Jia et al., 2023)	Significant effect, simple operation Large individual differences, risk of infecting operators
Advanced heart failure	Chronic isoproterenol stimulation (Wang, 2016)	
Rheumatic heart disease	Group A β-hemolytic streptococci (Jia et al., 2023)	
Pulmonary heart disease	Injection of monocrotaline combined with chronic hypoxic environment (Jia et al., 2023)	
Viral myocarditis	Coxsackie virus B3 (CVB3) (Remels et al., 2018; Althof et al., 2018)	
Experimental autoimmune myocarditis	α-Myosin heavy chain (α-MHC) (Miyawaki et al., 2017)	
Coronary medial calcification	Adenine (Zhou et al., 2019)	
Kawasaki disease	Water-soluble extract of *Candid albicans* (CAWS) (Miyabe et al., 2019), *Lactobacillus casei* cell wall extract (LCWE) (Jia et al., 2023)	
	Surgically induced models	
Myocardial infarction	Left anterior descending coronary artery ligation (Aghajanian et al., 2019; Park et la., 2019)	Significant effect Difficult operation, high technical requirements
Heart failure	Transverse aortic constriction (TAC) surgery (Greco et al., 2016)	
	Genetic models	
Coronary atherosclerotic heart disease	Apoe-/- mice (Jia et al., 2023)	Uniform phenotype, good controllability Long breeding time, high cost
Essential hypertension	Spontaneously hypertensive rats (SHRs) (Huc et al., 2018)	
Congenital heart disease	Nkx2-5 defect mice (mutations in the *Nkx2-5* gene are the main cause of congenital heart disease) (Furtado et al., 2017), GATA4 defect mice (the transcription factor GATA4 is a critical regulator of cardiac gene expression where it controls embryonic development, cardiomyocyte differentiation, and stress responsiveness of the adult heart. Traditional deletion of Gata4 causes embryonic lethality associated with endoderm defects and cardiac malformations, precluding an analysis of the role of GATA4 in the adult myocardium.) (Oka et al., 2006)	
Coronary artery calcification	Osteoprotegerin knockout (OPG-/-) mice (Liu et al., 2017)	
Arterial intimal calcification	Low-density lipoprotein receptor-deficient (LDLR−/−): runt-related transcription factor-2 Runx2$^{\Delta SM}$mice (Lin et al., 2016)	

Additionally, the advantages and disadvantages of small and large animal models can be discussed (Table 2) (Sosnovik & Scherrer-Crosbie, 2022; Poli et al., 2023; Liao et al., 2017; Wargocka-Matuszewska et al, 2023; von Scheidt, 2017; Golforoush, et al., 2020; Zhou et al., 2022; Hussein et al., 2023).

Since small animal models of CVD offer more advantages over large animal models, we will next examine existing small animal models for evaluating the cholesterol-lowering activity of probiotic strains and their positive effects on these diseases *in vivo*.

Contemporary small animal models of CVD. In this review, we will discuss murine models, as the mouse has become the preferred model for cardiovascular research for several reasons, including ease of handling, low procedural costs, and the ability to manipulate the mouse genome.

Mice are naturally resistant to atherosclerosis, likely because pro-atherogenic LDL-C is rapidly degraded from plasma, and the athero-protective HDL-C levels are much higher than the LDL-C levels.

Since the creation of hypercholesterolemic apolipoprotein E (apoE) gene knockout (KO) and LDL receptor (LDL-R) KO mice, which exhibit both spontaneous and diet-accelerated atherosclerosis (AS), mice have become the most widely used animal models in cardiovascular research. However, atherosclerotic plaques in mice are usually restricted to the aorta and aortic sinus, with their coronary arteries often remaining lesion-free (Sosnovik & Scherrer-Crosbie, 2022; Poli et al., 2023; Liao et al., 2017; Wargocka-Matuszewska et al., 2023; von Scheidt, 2017; Golforoush, et al., 2020; Zhou et al., 2022; Hussein et al., 2023).

LDLR–/– mice model. The LDLR–/– mouse serves as a model for familial hypercholesterolemia due to mutations affecting the LDL receptor (LDLR), with a plasma lipoprotein profile similar to that of humans. These genetically LDLR-deficient mice exhibit delayed clearance of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) from plasma. Consequently, LDLR–/– mice show a moderate increase in plasma cholesterol levels and slowly develop atherosclerosis on a normal chow diet.

Interestingly, the severity of hypercholesterolemia and atherosclerotic lesions in LDLR–/– mice can be accelerated by feeding a high-fat and

Table 2. **Advantages and disadvantages of animal models of cardiovascular diseases**

Aspects	Small animal models (rodents)	Large animal models (swine)
Advantages	Ease breading and handling	Closer to human anatomy, better tissue availability, and more accurate minimally invasive measurements
	Short reproductive cycle	Closer to human lipoprotein profile except for human HDL subclasses
	Relatively cheap	Moderately sensitive atherosclerosis on normal diet
	Well-defined genome	Vascular lesion distribution similar to that in humans
	Ease of genetic manipulation	Rare thrombosis due to plaque rupture
	Large litter number	Suitable for translational research
Disadvantages	Resistance to atherosclerosis development in Wild type (need for transgenic model)	Costly and difficult maintenance and handling
	Different gross anatomy compared to humans	No genetic modifications
	Different lipoproteins profile to humans / high level of lipid	Limited genetic models available
	Compromised lesion formation	Rare thrombosis due to plaque rupture
	Absence of plaque rupture and thrombosis	Ethical concerns

high-cholesterol diet, by mutating the apoB gene to an uneditable version, and by crossing with either leptin-deficient mice or apoB100 transgenic mice. Under these conditions, lesions in the aorta can progress beyond the foam-cell fatty streak stage to the fibroproliferative intermediate stage. The main features of the model are milder lipoprotein profile alteration compared to ApoE—/— mice and the development of atherosclerotic lesions in a time-dependent manner (Khan et al., 2018; Pan et al., 2018; Chen et al., 2024).

Apolipoprotein E knock-out mice (ApoE–/– mice) model. In 1992, two separate research groups simultaneously generated the apoE–/– mouse model by homologous recombination in embryonic stem cells. Homozygous deficiency in the apoE gene results in a marked increase in the plasma levels of LDL and VLDL due to impaired clearance through the LDLR and LDLR-related proteins. The apoE–/– mouse exhibits the entire spectrum of atherogenesis-associated lesions and is the first mouse model described to develop lesions similar to those in humans. Under normal dietary conditions, apoE–/– mice have dramatically elevated plasma cholesterol levels and develop extensive atherosclerotic lesions throughout the aorta. This process can be exacerbated by a high-fat diet, with female mice being more susceptible than males.

Chronological analysis of atherosclerotic lesions in apoE–/– mice has shown that the sequence of lesion formation is strikingly similar to that in larger animal models and humans.

Currently, apoE–/– mice are the most widely used animal model for studying atherosclerosis. Researchers have examined the effects of many genes on the development of atherosclerosis by crossing apoE–/– mice with other genetically manipulated animals. Additionally, the apoE–/– mouse serves as a valuable tool for: 1) identifying atherosclerosis susceptibility-modifying genes using a candidate-gene and gene-mapping methods; 2) deciphering molecular mechanisms and cell types involved in atherogenesis; 3) investigating the effects of drugs on atherosclerosis; 4) assessing novel therapies that prevent lesion progression. The main features of the model are spontaneously developing atherosclerosis on a normal diet; lesion progression, cell types present in the atherosclerotic plaque, and the presence of oxidized LDL, all reflecting the situation observed in humans. Notably, the apoE–/– mouse model has been used to test the additional therapeutic effects of statins beyond their cholesterol-lowering capabilities (Liao et al., 2017; Liao et al., 2021; Liu et al., 2022; Gomez et al., 2018; Seijkens et al., 2018; Chen et al., 2024).

***The LDLR and apoE double-deficient mouse* (LDLR–/–apoE–/–) model**, which develops severe hyperlipidemia and atherosclerosis even on a regular chow diet, has been proposed as a suitable model for studying the antiatherosclerotic effects of compounds without the need for an atherogenic diet. However, the response of both LDLR–/– and LDLR–/–apoE–/– mice to treatment with hypolipidemic drugs varies, ranging from lowering plasma cholesterol without reducing atherosclerosis to a weak reduction in lesions with or without decreased plasma cholesterol. In contrast, these mice respond effectively to agonists of the peroxisome proliferator-activated receptor (PPAR) or liver X receptor. This variability suggests that LDLR–/– mice may not be well-suited for analyzing the cholesterol-lowering and antiatherogenic effects of drugs (Torikai et al., 2023; Chen et al., 2024).

PCSK9 adeno-associated virus mice model. This model was developed without using germline genetic engineering. Creating this murine model required only a single injection of a recombinant adeno-associated virus (AAV) containing gain-of-function mutant forms of PCSK9, specifically human PCSK9D374Y or mouse PCSK9D377Y (AAVmPCSK9). These genes, in combination with a high-fat diet, were sufficient to reduce LDLR expression, increase plasma LDL cholesterol, and induce atherosclerosis in mice. Aortic root lesions developed in PCSK9 adeno-associated vi-

rus mice after atherosclerosis induction by a high-fat diet. An important feature of this model is its diet dependence, which also allows for the study of atherosclerosis regression. The main features of the model include the development of atherosclerosis on a fat-rich diet and the ability to study plaque calcification (Peled et al., 2017).

Mouse models of diabetes-accelerated atherosclerosis. Diabetes significantly increases the risk of CVD. Its cardiovascular complications primarily include ischemic heart disease, driven by accelerated atherosclerosis, and diabetic cardiomyopathy. To study atherosclerosis and cardiomyopathy in the context of diabetes, several mouse models are used, notably apoE−/− and LDLR−/− mice, where type 1 diabetes is induced through streptozotocin or viral injection. In these models, the induction of diabetes does not significantly alter the plasma lipid levels, effectively replicating the accelerated atherosclerosis observed in patients with type 1 diabetes (Liao et al., 2017; Liao et al., 2021; Liu et al., 2022; Gomez et al., 2018; Seijkens et al., 2018; Chen et al., 2024).

Calcium-chloride-induced abdominal aortic aneurysm* (AAA) *model. In this method, calcium chloride is applied periaortically between the renal arteries and the iliac bifurcation. Within 14 days, significant aortic dilation is observed, which becomes more severe when calcium chloride is used in conjunction with thioglycolate or the animals are fed a high-cholesterol diet. Unlike other models, this method induces AAA without requiring mechanical intervention (Krishna et al., 2020).

Elastase-induced AAA model. The elastase-induced model for AAAs in mice involves elastase perfusion in the mouse aorta, which initially causes mild-to-moderate dilation. Within 14 days, this dilation progresses to more than a 100% increase in aortic diameter. Elastase-induced injury elevates the expression of matrix metalloproteinases (MMPs), cathepsins, and other proteases, notably localizing MMP-9 to aneurysm-infiltrating macrophages. This model effectively mimics many characteristics of human AAAs, making it a valuable tool for systematically assessing the roles of individual gene products in aneurysmal degeneration. Compared to the calcium-chloride-induced AAA model, the elastase-induced method requires mechanical stress to replicate medial elastic degradation. However, it closely parallels the sequence of events seen in human AAA development (Xue et al., 2022; Wen et al., 2020).

Angiotensin II-Induced AAA model. This procedure was originally developed to determine whether elevated plasma concentrations of Angiotensin II (Ang II) directly influence the atherogenic process in aged hyperlipidemic apoE−/− mice. Ang II also induced the formation of large suprarenal AAAs in these animals. The severity of AAAs is significantly higher in hyperlipidemic apoE−/− or LDLR−/− male mice (affecting approximately 60% of mice) compared to normolipidemic mice. Here, neither hyperlipidemia nor atherosclerosis alone is considered a major determinant of aneurysm formation. This model has provided critical insights into the role of the renin-angiotensin system (RAS) in aneurysmal disease. However, two main limitations should be noted: the aneurysms in this model are located suprarenally, unlike the infrarenal location typical in humans, and the clinical relevance of RAS inhibition remains uncertain, as studies linking RAS to human AAAs have yielded controversial results (Sawada et al., 2022; Ren et al., 2022).

Spontaneous mouse mutants. The blotchy mouse strain carries a spontaneous mutation on the X chromosome, resulting in abnormal intestinal copper absorption. This defect leads to weak elastic tissue due to impaired crosslinking of elastin and collagen, causing the development of aortic aneurysms, primarily in the aortic arch and thoracic aorta, and occasionally in the abdominal aorta. However, interpreting results from this model is challenging because the mutation causes several severe additional effects beyond the aortic aneurysm formation (Liao et al., 2017; Liao et al., 2021).

Gene-targeted disruption of the muscle LIM protein (MLP) in mice serves as a model for dilated cardiomyopathy and heart failure. MLP is a regulator of myogenic differentiation. Mice homozygous for the MLP knockout develop dilated cardiomyopathy associated with myocardial hypertrophy, interstitial cell proliferation, and fibrosis. Adult mice exhibit clinical and hemodynamic signs of heart failure similar to those in humans. Due to these similarities, it has been suggested that molecular mechanisms resulting in MLP dysfunction may be involved in the development of human dilated cardiomyopathy and congestive heart failure (CHF) (Liao et al., 2017; Liao et al., 2021).

Doxorubicin cardiomyopathy. Doxorubicin exhibits both acute and chronic cardiotoxicity and has been used to induce heart failure in various animal species. Several mechanisms involved in the pathophysiology of doxorubicin-induced heart failure have been suggested, including free radical generation and lipid peroxidation, reactive sulfhydryl group interactions, binding to channel regulatory sites, and inhibition of mRNA and protein synthesis (Linders et al., 2024). It has also been shown that the suggested model (an optimal dose of doxorubicin for the simulation of congestive heart failure of 2.5 mg/animal, with a cumulative dose of 12.45 mg/kg in 4 injections every 3 days) can be used for research purposes (Spivak et al., 2013a). Moreover, gold nanoparticles of 30 nm and their AuNPs-Simdax conjugate have demonstrated positive results in biosafety and biocompatibility both *in vitro* and *in vivo*. AuNPs-Simdax and AuNPs show similar significant cardioprotective effects in rats with doxorubicin-induced heart failure, surpassing those of Simdax. Intrapleural (local) delivery is preferred over intravenous (systemic) delivery according to all tested parameters. Furthermore, sonoporation has been shown to enhance gold nanoparticle delivery to myocardial cells *in vivo* (Spivak et al., 2013b).

Murine models with Scavenger receptor class B type I (SR-BI) deficiency. SR-BI is an 85 kDa membrane glycoprotein, which contains a large extracellular domain, two transmembrane domains, a short cytoplasmic N-terminal domain, and a PDZK1-binding motif-containing C-terminal domain. Known as the primary HDL receptor with high binding affinity, SR-BI not only mediates the efflux of unesterified cholesterol (UC) from peripheral cells to circulating HDLs but also promotes the selective uptake of cholesterol esters from HDLs for biliary secretion or glucocorticoid synthesis. Modulation of its expression through the deficiency of the homologous gene or disruption of its adaptor protein PDZK1 significantly affects lipid metabolism, especially HDL metabolism. The accumulation of UC in HDLs from SR-BI-deficient mice diminishes the normal anti-atherogenic functions of HDLs. In hypercholesterolemic conditions, more UC accumulates, leading to the formation of pro-atherogenic (toxic) HDLs. Consequently, SR-BI deficiency aggravates atherosclerosis (AS). Moreover, it can even lead to occlusive coronary AS followed by spontaneous myocardial infarction in apoE KO mice on a regular chow diet (Liao et al., 2017).

SR-BI knockout and ApoE-hypomorphic mice (SR-BI KO/ApoeR61h/h mice). This model was generated by breeding two mouse strains, namely SR-BI-deficient (SR-BI KO) mice and hypomorphic apoE (ApoeR61h/h) mice, resulting in SR-BI KO/ApoeR61h/h mice. The most significant feature of the SR-BI KO/ApoeR61h/h mouse is the development of atherosclerosis and coronary heart disease in response to an atherogenic diet rich in fat, cholesterol, and cholate. This model allows investigators to control the disease onset and the severity of symptoms. It is of particular interest that due to the lack of small animal models that closely resemble the severe symptoms of atherosclerosis (formation of advanced plaques), severe coronary heart disease and even premature death are seen in humans. The main features of this model are the development of atherosclerosis and coronary heart disease on a diet rich in fat, cholesterol, and cholate,

the formation of advanced plaques, and the presence of severe coronary heart disease and premature death similar to those seen in humans. (Gonzalez et al., 2018; Burke & Huff, 2018).

Transgenic mice. Transgenic technologies have produced a range of valuable mouse models for studying hyperlipidemia and atherosclerosis. Notably, mice expressing mutant forms of apoE, such as apoE3Leiden (E3L) and apoE (Arg112→Cys142), are among the most extensively studied. These mice exhibit a lipoprotein profile similar to that of patients with dysbetalipoproteinemia, where plasma total cholesterol and triglycerides are predominantly confined to VLDL and LDL. E3L transgenic mice develop atherosclerotic lesions with characteristics akin to human vasculopathy, ranging from fatty streaks to mild, moderate, and severe plaques. Moreover, E3L transgenic mice, as well as the more recently developed E3L/Cholesteryl Ester Transfer Protein (CETP) transgenic mice, have demonstrated greater sensitivity to a variety of hypolipidemic drugs and peroxisome proliferator-activated receptor (PPAR) agonists compared to apoE−/− and LDLR−/− mice (Paalvast et al., 2017; Paalvast et al., 2022; Curry et al., 2024). For example:

▪ *apoE3Leiden.CETP mice*. The developed apoE3-Leiden.CETP (E3L.CETP) mouse model of atherosclerosis closely replicates the features of human disease. Among the similarities are the ability to form atherosclerotic lesions of all stages (type I to V) in a diet-induced manner and the response of diseased animals to treatments with drugs such as statins, fibrates, and ezetimibe. The model was created by combining the apoE3-Leiden transgene, which provides reduced clearance of triglyceride-rich lipoproteins, and the CETP transgene, which humanizes the cholesterol profile. The main features of the model are the formation of all stages of atherosclerotic lesions in a diet-induced manner and a human-like response to treatment with drugs such as statins, fibrates, and ezetimibe (Paalvast et al., 2017; Paalvast et al., 2022; Curry et al., 2024).

▪ *Apolipoprotein E-deficient fibrillin-1 mutant mice (ApoE—/—Fbn1C1039G+/— mice)*. These mice are characterized by impaired production of fibrillin-1, which leads to the fragmentation of elastic fibers observed in aortic stiffening. This condition is a potential cause of plaque rupture. The model also shares common features with atherosclerotic (apoE—/—) mice and can therefore be used to study human unstable plaques. ApoE—/—Fbn1C1039G+/— mice develop atherosclerosis in response to a high-fat diet, and this process is accelerated compared to regular apoE—/— mice. The main features of the model are the resemblance to plaque rupture and human-like complications (Curry et al., 2024).

Murine models with nitric oxide synthase (NOS) deficiency. The endothelium plays a critical role in cardiovascular health by regulating vascular tone, growth, thrombosis, and thrombolysis and inhibiting inflammation and smooth muscle cell proliferation. These functions are largely mediated by nitric oxide (NO) produced by endogenous NOS, which includes neuronal, inducible, and endothelial isoforms (nNOS, iNOS, and eNOS, respectively). eNOS is particularly important, as its dysfunction and the resulting decrease in NO production are key factors in the onset and progression of atherosclerosis.

In mice, eNOS knockout leads to elevated blood pressure variability, ejaculatory abnormalities, and impaired wound healing and angiogenesis. When eNOS knockout mice are bred with apoE knockout mice, the resulting eNOS/apoE double knockout mice develop coronary arteriosclerosis, myocardial ischemia/infarction, heart failure, and aortic aneurysms and dissections on a western diet. Despite the loss of eNOS, other NOS isoforms are upregulated, suggesting compensatory interactions within the NOS family.

To study the effects of the entire NOS system, triple knockout (tKO) mice were generated. These mice exhibited severe cardiovascular abnormalities, including hypertension, dysfunctional vascular relaxation and constriction, myo-

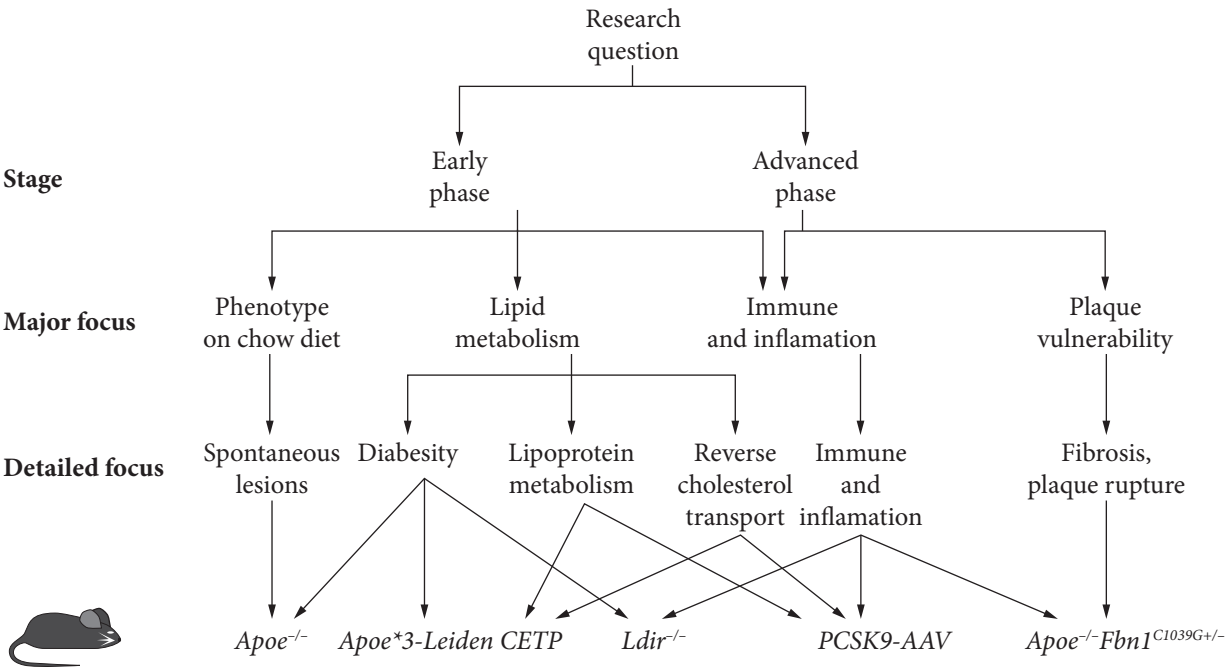

Fig. 1. Roadmap to facilitate the choice of an atherosclerotic mouse model (Oppi, 2019)

cardial infarction, left ventricular hypertrophy, and early death. While dyslipidemia is present, the coronary arteriosclerosis observed in them resembles that in eNOS/apoE double knockout mice. Additionally, significant mast cell infiltration in the coronary artery adventitia suggests that coronary spasm, potentially caused by mast cell-derived histamine release, may contribute to these conditions (Liao et al., 2021; Liao et al., 2017; Tenopoulou et al., 2018; Liu et al., 2022).

Murine models with fibrillin deficiency. Elastic fibers, composed of a cross-linked elastin core and fibrillin-rich microfibrils, are crucial for arterial elasticity and resilience. Disturbance of these fibers due to factors like aging, metabolic syndrome, and genetic defects can lead to vessel stiffness and weakness, causing conditions such as hypertension and aneurysms. Fibrillin-1, a key microfibril component, binds and sequesters growth factors and releases proteases that degrade elastin fibers. Deficiency in fibrillin-1 causes Marfan syndrome, characterized by aneurysms and skeletal defects. In apoE KO mice on a western diet, a fibrillin-1 mutation led to elastin fragmentation, accelerating atherosclerosis, intraplaque hemorrhage, and neovascularization, resulting in spontaneous plaque rupture. This caused myocardial and cerebral ischemia/infarction and death. Such mice are valuable for studying vulnerable plaque progression and therapeutic interventions (Liao et al., 2017).

Other murine models. Apart from the above strains, three other models also exhibited **cor**onary heart disease when fed atherogenic diets. These models include apoE/LDL-R dKO mice, apoE KO mice with macrophage-targeted overexpression of urokinase, and apoE KO mice with Akt1 deficiency.

There is even a roadmap to facilitate the choice of an atherosclerotic mouse model. This scheme should help researchers choose the most appropriate atherosclerotic mouse model based on their specific research questions (Oppi, 2019) (Fig.1.).

Conclusions. This review has demonstrated that probiotics containing cholesterol-assim-

ilating strains can effectively complement the complex therapy of patients with cardiovascular diseases and other conditions associated with high serum cholesterol levels. These treatments are free from the negative side effects associated with statins, such as hepatotoxicity, and are neither addictive, nor require lifelong use.

Also discussed are the advantages and disadvantages of using small and large animals as models for CVD. Small animals, particularly mice, are commonly used in basic research on the molecular mechanisms of atherosclerosis and related cardiovascular disorders. The establishment of diet-induced CVD models has provided researchers with more options for manipulating the disease onset and progression compared to spontaneous CVD models. Despite their limitations, these animal models are invaluable tools for translational research.

Rodents are the most common models for CVD research and are indispensable for studying the pathological features, clinical symptoms, and drug development for human diseases. They effectively simulate the characteristics and indicators of human CVD and offer advantages such as strong reproductive ability and ease of detection, which provide great convenience for scientific research.

In summary, small animal models of CVD can be used to demonstrate the positive therapeutic effects of probiotic strains with cholesterol-lowering activity against pathologies *in vivo*. The use of animal models that recapitulate human conditions is essential for understanding the mechanisms underlying cardiac diseases.

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МОДЕЛІ СЕРЦЕВО-СУДИННИХ ЗАХВОРЮВАНЬ НА ДРІБНИХ ТВАРИНАХ ДЛЯ ОЦІНКИ ХОЛЕСТЕРИНОЗНИЖУВАЛЬНОЇ АКТИВНОСТІ ПРОБІОТИЧНИХ ШТАМІВ

Серцево-судинні захворювання є основними причинами глобальної захворюваності та смертності, спричиняючи приблизно 17 мільйонів смертей щорічно в усьому світі. Частота цих захворювань зростає серед економічно активних вікових груп, що посилюється збільшенням рівня діабету, ожиріння та захворювань, пов'язаних з курінням. Незважаючи на значний прогрес у контролі рівня холестерину ліпопротеїнів низької щільності, залишковий ризик атеросклеротичної серцево-судинної хвороби зберігається, частково через залишковий холестерин у ліпопротеїнах, багатих на тригліцериди. Цей огляд має на меті оцінити відповідні тваринні моделі серцево-судинних захворювань для демонстрації терапевтичної ефективності пробіотичних штамів, що мають здатність знижувати рівень холестерину. Тваринні моделі, які якісно імітують умови серцево-судинних захворювань у людей, є необхідними для з'ясування основних механізмів захворювання. Пробіотики показали перспективні профілактичні ефекти на серцево-судинні захворювання шляхом відновлення дисбіозу кишкової мікробіоти та протизапальних реакцій. Механізми включають зниження оксидативного стресу, зниження гіперхолестеринемії та моделювання метаболізму жовчних кислот. Обговорюються переваги та обмеження тваринних моделей у дослідженнях серцево-судинних захворювань, підкреслюючи сильні сторони гризунів, таких як миші, які є економічно вигідними, генетично маніпульованими і відтворюють ключові аспекти патофізіології серцево-судинних захворювань у людей. Розглядаються різні сучасні мишачі моделі щодо їх придатності для вивчення атеросклерозу, інфаркту міокарда та інших серцево-судинних захворювань. Кожна модель пропонує унікальне розуміння механізмів захворювання та реакцій на терапевтичні втручання. Таким чином, вибір відповідних тваринних моделей є важливим для покращення нашого розуміння терапій, опосередкованих пробіотиками, при серцево-судинних захворюваннях. Використовуючи ці моделі, дослідники можуть вивчати нові стратегії для зменшення факторів ризику серцево-судинних захворювань та підвищення терапевтичних результатів.

Ключові слова: *серцево-судинні захворювання, пробіотики, тваринні моделі, атеросклероз, метаболізм холестерину, терапевтична ефективність.*