Biomedical Research and Therapy 2026, 13(03):8410-8425

Open Access Full Text Article

Review

Integrative Insights into Animal Models for Antihyperlipidemic Drug Screening: Bridging Preclinical and Translational Gaps in Research

Mithilesh Kesari[1], Javed Akhtar Ansari[1,*], Misbahul Hasan Syed[1], Farogh Ahsan[1], Juber Akhtar[1], Sana Ahmad[1], Najish Parveen[1]

ABSTRACT

Hyperlipidemia is a principal modifiable risk factor for atherosclerosis and cardiovascular disease, underscoring the critical need for predictive preclinical models to evaluate lipid-lowering therapies. Animal models are indispensable for elucidating lipid metabolism, validating pharmacological targets, and screening novel antihyperlipidemic agents prior to clinical translation. This integrative review presents an updated, critical synthesis of the animal models utilized in antihyperlipidemic drug screening, encompassing diet-induced, chemically induced, genetically modified, microbiota-modulated, and large-animal systems. Each model is evaluated based on its physiological relevance, methodological characteristics, and translational reliability. Small animals—such as mice, rats, and hamsters—provide accessible, cost-effective, and genetically versatile platforms for mechanistic investigations and early-stage screening. Conversely, larger species, including rabbits, pigs, and non-human primates, more accurately replicate human lipid profiles, atherosclerotic pathology, and cardiovascular physiology. Recent advancements in microbiota modulation, CRISPR-based genetic engineering, and multi-omics technologies have significantly enhanced mechanistic understanding and improved predictive accuracy in these models. By integrating classical and next-generation methodologies, this review proposes a translational framework designed to support ethical, reproducible, and clinically relevant preclinical research in lipid pharmacology.

Key words: Hyperlipidemia, animal models, lipid metabolism, translational pharmacology, antihyperlipidemic drug screening, preclinical evaluation

[1]*Faculty of Pharmacy, Integral University, Kursi Road, Lucknow, India*

Correspondence

Javed Akhtar Ansari, Faculty of Pharmacy, Integral University, Kursi Road, Lucknow, India

Email: javeda@iul.ac.in

History

- Received: Oct 05, 2025
- Accepted: Mar 23, 2026
- Published Online: Mar 31, 2026

DOI : 10.15419/9gdf9182

INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, accounting for approximately 17.9 million deaths annually—representing nearly 32% of all global deaths [1]. Among the modifiable risk factors contributing to cardiovascular morbidity and mortality, hyperlipidemia occupies a central role. It is characterized by elevated plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), alongside reduced levels of high-density lipoprotein cholesterol (HDL-C). These lipid derangements significantly accelerate atherogenesis and its associated clinical complications [2,3]. Hyperlipidemia drives endothelial dysfunction, arterial lipid deposition, and chronic vascular inflammation. Consequently, it remains a primary therapeutic target in the prevention and management of atherosclerotic cardiovascular disease.

Clinical Burden and Therapeutic Limitations

Hyperlipidemia is a core component of metabolic syndrome and frequently coexists with obesity, insulin resistance, and hypertension. Its global prevalence continues to rise, driven by sedentary lifestyles, high-fat dietary patterns, and an aging population [4]. While statins remain the cornerstone of pharmacological therapy, their clinical utility is occasionally constrained by adverse effects, residual cardiovascular risk, interindividual response variability, and long-term adherence challenges [5]. These limitations have stimulated the development of alternative lipid-lowering strategies, such as PCSK9 inhibitors, cholesterol absorption inhibitors, CETP inhibitors, and nutraceutical-based interventions. Concurrently, the advent of precision medicine has amplified the demand for preclinical models that more accurately recapitulate human lipid metabolism and disease heterogeneity [6]. Historically, traditional drug discovery pipelines have been hindered by poor translation from animal stud-

Cite this article : Kesari M, Ansari JA, Syed MH, Ahsan F, Akhtar J, Ahmad S, Parveen N. **Integrative Insights into Animal Models for Antihyperlipidemic Drug Screening: Bridging Preclinical and Translational Gaps in Research**. *Biomed. Res. Ther.* 2026; 13(03):8410-8425.

ies to clinical outcomes. This translational gap primarily stems from species-specific divergences in lipoprotein metabolism, inflammatory responses, and plaque biology [7,8].

Role of Animal Models in Hyperlipidemia Research

Animal models provide a critical foundation for investigating lipid metabolism, identifying therapeutic targets, and evaluating the safety and efficacy of candidate antihyperlipidemic agents prior to human trials [8]. These models are broadly classified into three categories. First, induced models utilize dietary, chemical, or hormonal interventions to generate hyperlipidemia. Second, genetically modified models—encompassing knockout, knock-in, or transgenic animals—are designed to mimic inherited dyslipidemias, such as familial hypercholesterolemia. Third, spontaneous models, exemplified by the Watanabe heritable hyperlipidemic (WHHL) rabbit, naturally develop hyperlipidemia and atherosclerosis over time. Each model presents distinct advantages for mechanistic and translational research. However, species-specific limitations persist, notably differences in cholesteryl ester transfer protein (CETP) activity, apolipoprotein expression, and LDL receptor regulation. These factors demand careful consideration when interpreting experimental outcomes and extrapolating preclinical findings to human physiology [8–10].

Importance of Model Selection and Standardization

Appropriate model selection is paramount for the meaningful preclinical evaluation of antihyperlipidemic therapies. A mismatch between a compound's pharmacodynamic properties and the metabolic background of the selected model can yield misleading efficacy or safety conclusions. For instance, wild-type mice lack CETP activity, inherently limiting their suitability for evaluating HDL-targeted therapies. In contrast, rabbits and non-human primates express CETP, thereby more closely mirroring human lipoprotein dynamics [9–11]. Beyond model selection, inadequate standardization of induction protocols, dietary compositions, outcome measures, and reporting practices continues to be a major source of irreproducibility in preclinical research. International guidelines and regulatory frameworks—including those established by the OECD, ICH, and the ARRIVE consortium—strongly emphasize the necessity of ethical conduct, methodological rigor, and transparent reporting in animal studies [12].

Emerging Trends in Model Development

Recent technological advancements have significantly expanded the scope and sophistication of animal models utilized in lipid research. CRISPR/Cas9-mediated genome editing has facilitated the generation of humanized models that express key human apolipoproteins, LDL receptors, or PCSK9 variants. These innovations have markedly enhanced the biological relevance of preclinical models for investigating inherited and complex lipid disorders [13]. Furthermore, multi-omics approaches—encompassing transcriptomics, proteomics, metabolomics, and lipidomics—are increasingly being integrated into animal studies to deliver systems-level insights into lipid regulation and pharmacological responses. Non-invasive imaging modalities, advanced biomarker profiling, and computational modeling further elevate the precision of disease monitoring and therapeutic evaluation [14]. The present review aims to provide a comprehensive and critical overview of the animal models employed in antihyperlipidemic drug screening. Emphasis is placed on their biological relevance, mechanistic utility, and translational potential, whilst also identifying persistent gaps and opportunities for refining the predictive validity of preclinical lipid research.

Methods: Literature Search and Study Selection

Search Strategy

A comprehensive and systematic literature search was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines to identify studies evaluating animal models utilized for antihyperlipidemic drug screening. Four electronic databases—PubMed/MEDLINE, Web of Science (Core Collection), Scopus, and Google Scholar—were queried to ensure a broad capture of experimental, mechanistic, and translational literature.

In PubMed/MEDLINE, the search strategy integrated Medical Subject Headings (MeSH) and free-text terms using Boolean operators, structured as follows: ("Hyperlipidemia"[MeSH] OR "Dyslipidemias"[MeSH] OR hyperlipidemia OR dyslipidemia OR hypercholesterolemia OR hypertriglyceridemia) AND ("Animal Models"[MeSH] OR "Disease Models, Animal"[MeSH] OR animal model* OR rodent* OR mouse OR mice OR rat OR rabbit OR hamster OR zebrafish OR primate*) AND

("Drug Screening" OR "Pharmacological Screening" OR "Preclinical Evaluation" OR antihyperlipidemic*). Detailed, database-specific search strategies are outlined in **Supplementary Table S1**. Boolean operators, truncation (*), and controlled vocabularies (e.g., MeSH) were tailored to meet the specific requirements of each database. For Google Scholar, simplified keyword combinations were applied, and the first 300 relevance-ranked records were screened to mitigate redundancy and exclude low-quality retrievals.

Eligibility Criteria

Across all databases, the search was restricted to English-language publications released between January 2000 and March 2025. Eligible studies comprised original research articles, as well as narrative and systematic reviews, that delineated animal models of hyperlipidemia, dyslipidemia, or atherosclerosis within the context of pharmacological screening or preclinical evaluation of antihyperlipidemic agents.

Studies were excluded if they met any of the following criteria:

- Consisted exclusively of *in vitro* or non-animal methodologies.
- Focused on disease models extraneous to lipid metabolism or atherosclerosis.
- Lacked a pharmacological intervention or drug-screening component.
- Were formatted as editorials, conference abstracts, commentaries, or opinion pieces.

"Non-relevant disease models" were defined *a priori* as those devoid of altered lipid metabolism or not explicitly intended for antihyperlipidemic drug evaluation.

Study Selection and Data Extraction

The initial database search yielded 2,453 records. Following the removal of 658 duplicate records utilizing reference management software (Zotero), 1,795 unique records advanced to title and abstract screening. During this phase, 1,303 records were excluded, categorized as *in vitro* or non-animal studies (n = 543), conference abstracts or editorials (n = 236), and non-relevant disease models (n = 524). Subsequently, 492 full-text articles underwent rigorous eligibility assessment.

At the full-text evaluation stage, an additional 205 articles were excluded, predominantly due to inadequate methodological detail, an absence of pertinent lipid-related outcomes, or publication in a language other than English (n = 72). Ultimately, 287 studies fulfilled all inclusion criteria and were incorporated into the qualitative synthesis.

Data extraction systematically captured the animal species and strain, model induction methodology (e.g., dietary, chemical, genetic, microbiota-modulated, or large-animal), pharmacological intervention, evaluated lipid parameters, and translational relevance. The selected studies were qualitatively synthesized by categorizing the models into diet-induced, chemically induced, genetic, microbiota-modulated, and large-animal paradigms. Any discrepancies arising during the screening or data extraction processes were resolved via consensus among the authors. The comprehensive study selection workflow is visually detailed in Figure 1 (PRISMA Flow Diagram).

LIPOPROTEIN FUNCTION AND CLASSIFICATION

Overview of Lipid Transport

Lipids—encompassing triglycerides (TG), cholesterol, phospholipids, and cholesteryl esters—are hydrophobic molecules essential for cellular architecture, membrane integrity, and energy metabolism. Due to their restricted solubility in aqueous environments, lipids necessitate specialized transport mechanisms to circulate within the bloodstream. This critical physiological function is fulfilled by lipoproteins [13,14]. Lipoproteins are spherical macromolecular complexes comprising a hydrophobic lipid core—predominantly containing triglycerides and cholesteryl esters—enveloped by an amphipathic shell of phospholipids, unesterified cholesterol, and apolipoproteins [14–16]. Beyond lipid transport, lipoproteins govern various physiological processes, including cellular lipid uptake, reverse cholesterol transport, steroidogenesis, and the maintenance of cellular membrane fluidity [16–18].

Structure and Components of Lipoproteins

Although lipoproteins exhibit heterogeneity in size and composition, they share a conserved structural architecture. The hydrophobic core houses neutral lipids, specifically triglycerides and cholesteryl esters, whereas the surface monolayer is enriched with phospholipids, unesterified cholesterol, and apolipoproteins. Apolipoproteins are integral to maintaining structural stability, facilitating receptor-mediated interactions, and modulat-

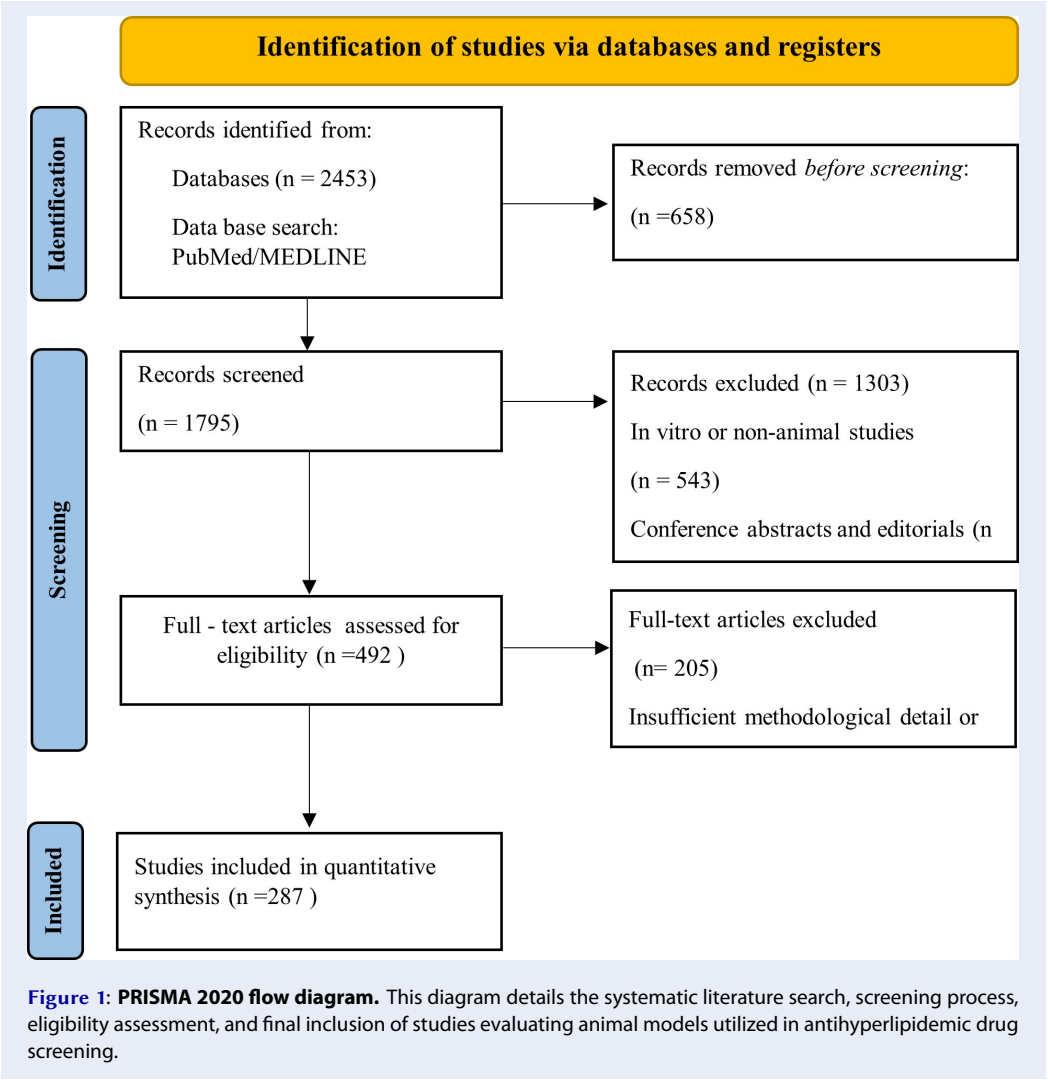

Figure 1: **PRISMA 2020 flow diagram.** This diagram details the systematic literature search, screening process, eligibility assessment, and final inclusion of studies evaluating animal models utilized in antihyperlipidemic drug screening.

ing the activity of key metabolic enzymes. For instance, apolipoprotein B-100 (ApoB-100) is obligatory for the assembly and secretion of very-low-density lipoprotein (VLDL) and its subsequent binding to the LDL receptor. Apolipoprotein A-I (ApoA-I) serves as the primary structural protein of high-density lipoprotein (HDL) and acts as a vital activator for lecithin-cholesterol acyltransferase (LCAT). Furthermore, apolipoprotein C-II activates lipoprotein lipase (LPL), whereas apolipoprotein E (ApoE) mediates the hepatic clearance of remnant lipoproteins [16,18].

Classification of Lipoproteins

Lipoproteins are systematically classified based on their hydrated density, particle diameter, lipid-to-protein ratio, electrophoretic mobility, and specific apolipoprotein composition. According to these physicochemical properties, lipoproteins are categorized into chylomicrons, VLDL, intermediate-density lipoproteins (IDL), LDL, and HDL (Table 1)[2,5,15,16].

Each lipoprotein class fulfills a specialized physiological role. Chylomicrons are responsible for transporting dietary triglycerides from the intestinal lumen to peripheral tissues. VLDL particles distribute endogenously synthesized triglycerides from the liver to peripheral sites. LDL functions as the principal carrier of cholesterol to peripheral cells, while HDL mediates reverse cholesterol transport by mobilizing excess peripheral cholesterol back to the liver for biliary excretion.

Table 1: Classification of Lipoproteins.

Lipoprotein	Major Lipid Component	Key Apolipoproteins	Primary Function	Atherogenic Potential
Chylomicrons	Triglycerides	ApoB-48, ApoC-II, ApoE	Transport dietary lipids from intestine	Low
VLDL	Triglycerides	ApoB-100, ApoC-II, ApoE	Transport endogenous triglycerides	Moderate
IDL	Triglycerides & cholesteryl esters	ApoB-100, ApoE	VLDL remnant metabolism	Moderate
LDL	Cholesteryl esters	ApoB-100	Cholesterol delivery to peripheral tissues	High
HDL	Phospholipids, cholesterol	ApoA-I, ApoA-II	Reverse cholesterol transport	Protective

Note: **Apo**: Apolipoprotein; **HDL**: High-density lipoprotein; **IDL**: Intermediate-density lipoprotein; **LDL**: Low-density lipoprotein; **VLDL**: Very-low-density lipoprotein.

Functional Pathways of Lipoprotein Transport

Lipoprotein metabolism is meticulously regulated via three primary transport pathways.

Exogenous Pathway

The exogenous pathway commences with the intestinal absorption of dietary lipids. Enterocytes synthesize chylomicrons, which subsequently enter the systemic circulation via the lymphatic network. The triglycerides packaged within chylomicrons undergo hydrolysis by LPL, liberating free fatty acids for cellular energy utilization or adipocyte storage. The resultant chylomicron remnants are then rapidly cleared from the circulation by the liver through ApoE-mediated receptor endocytosis [13,14].

Endogenous Pathway

The endogenous pathway is driven by the hepatic assembly and secretion of VLDL particles, which transport endogenous triglycerides to peripheral tissues. Progressive triglyceride hydrolysis remodels VLDL into IDL, and ultimately into LDL. LDL particles deliver cholesterol to peripheral tissues and hepatocytes primarily via LDL receptor-mediated endocytosis. Perturbations in LDL receptor functionality, ApoB structural integrity, or PCSK9 regulation precipitate impaired LDL clearance, culminating in hypercholesterolemia [15,16].

Reverse Cholesterol Transport

Reverse cholesterol transport is predominantly orchestrated by HDL particles. HDL effluxes excess cholesterol from peripheral tissues, including lipid-laden macrophages residing within the arterial intima. Within the HDL particle, LCAT esterifies free cholesterol, while cholesteryl ester transfer protein (CETP) facilitates the equimolar exchange of cholesteryl esters and triglycerides between HDL and ApoB-containing lipoproteins. Ultimately, the transferred cholesterol is cleared by the liver and excreted in the form of bile acids [17,18].

Key Enzymes Regulating Lipoprotein Metabolism

Several key enzymes exert critical control over lipoprotein remodeling and systemic lipid homeostasis (Table 2). LPL catalyzes the hydrolysis of triglycerides sequestered in chylomicrons and VLDL, thereby facilitating lipid clearance. Hepatic lipase modifies HDL and IDL particles, significantly influencing hepatic cholesterol turnover. Furthermore, LCAT is indispensable for HDL maturation, whereas CETP governs the dynamic distribution of cholesterol across distinct lipoprotein fractions. Intracellularly, acyl-CoA:cholesterol acyltransferase (ACAT) dictates cholesterol esterification, a process central to macrophage foam cell formation [16,18]. Pathological alterations in the activities of these enzymes can predispose individuals to lipid accumulation, atherogenesis, and systemic metabolic dysfunction (Table 2).

Clinical and Experimental Implications

Dysregulated lipoprotein metabolism forms the fundamental pathological basis for numerous cardiometabolic disorders. Elevated concentrations of circulating LDL and oxidatively modified LDL actively promote endothelial dysfunction and macrophage foam cell generation, acting as the primary instigators of atherosclerosis. Moreover, increased levels of VLDL and small, dense LDL

Table 2: Enzymes Regulating Lipoprotein Metabolism.

Enzyme	Primary Function	Lipoprotein Affected	Clinical Relevance
Lipoprotein lipase (LPL)	Hydrolyzes triglycerides	Chylomicrons, VLDL	TG clearance
Hepatic lipase	Lipoprotein remodeling	HDL, IDL	HDL metabolism
LCAT	Cholesterol esterification	HDL	HDL maturation
CETP	Lipid exchange	HDL ↔ LDL/VLDL	Translational relevance
ACAT	Cholesterol esterification	Macrophages	Foam cell formation

Note: **ACAT**: Acyl-CoA cholesterol acyltransferase; **CETP**: Cholesteryl ester transfer protein; **HDL**: High-density lipoprotein; **IDL**: Intermediate-density lipoprotein; **LCAT**: Lecithin-cholesterol acyltransferase; **LDL**: Low-density lipoprotein; **LPL**: Lipoprotein lipase; **TG**: Triglycerides; **VLDL**: Very-low-density lipoprotein.

particles are hallmark features of insulin-resistant states and diabetic dyslipidemia. Conversely, diminished HDL concentrations compromise reverse cholesterol transport and attenuate its inherent atheroprotective properties [7,9].

These distinct, species-specific variations in lipoprotein metabolism must meticulously guide animal model selection during experimental design. Models inherently lacking CETP activity, such as wild-type mice, inadequately recapitulate human HDL dynamics. In contrast, CETP-expressing species—including rabbits, hamsters, and non-human primates—offer substantially improved translational relevance for the preclinical evaluation of HDL-targeted therapeutic interventions [15,19,20].

ATHEROSCLEROSIS – PATHOGENESIS AND RELEVANCE TO HYPERLIPIDEMIA

Atherosclerosis is a chronic, progressive inflammatory disease of the arterial wall, characterized by lipid accumulation, immune cell infiltration, and fibrous tissue formation. It constitutes the primary pathological basis for ischemic heart disease, ischemic stroke, and peripheral artery disease, which collectively account for a substantial proportion of global cardiovascular mortality. Dyslipidemia—specifically, elevated levels of LDL-C and triglycerides coupled with reduced HDL-C—serves as a principal driver of atherogenesis. Hyperlipidemia contributes fundamentally to disease initiation and progression by promoting oxidative stress, endothelial dysfunction, foam cell formation, and deleterious vascular remodeling [15–17].

Pathophysiological Stages of Atherogenesis

The development of atherosclerosis proceeds through a continuum of overlapping pathophysiological stages intricately linked to underlying lipid abnormalities (Table 3) [15–18].

Endothelial Dysfunction

Elevated circulating LDL particles infiltrate the arterial intima, where they are subjected to oxidative modification. The resultant oxidized LDL (oxLDL) triggers potent pro-inflammatory and prothrombotic signaling cascades, culminating in endothelial activation. Activated endothelial cells upregulate the expression of adhesion molecules, such as VCAM-1 and ICAM-1, thereby facilitating the recruitment and firm adhesion of circulating leukocytes [21,22].

Monocyte Recruitment and Foam Cell Formation

Monocytes adhere to the activated endothelium, transmigrate into the intimal space, and differentiate into mature macrophages. These macrophages avidly internalize oxLDL via scavenger receptors, including CD36 and SR-A1, driving their transformation into lipid-laden foam cells. The progressive accumulation of these foam cells yields fatty streaks, which represent the earliest macroscopically visible atherosclerotic lesions [23].

Smooth Muscle Cell Proliferation and Fibrous Cap Formation

Vascular smooth muscle cells (VSMCs) migrate from the tunica media into the intima, where they undergo phenotypic switching and subsequent proliferation. These cells synthesize substantial quantities of extracellular matrix components, predominantly collagen and elastin, contributing to the formation of a fibrous cap. This fibrous cap serves to stabilize the atheromatous plaque by sequestering the highly thrombogenic, lipid-rich necrotic core from the luminal bloodstream [24].

Table 3: Key Stages in the Pathogenesis of Atherosclerosis and Associated Lipid Abnormalities.

Stage	Pathological Event	Associated Lipid Abnormality	Experimental Relevance
Endothelial dysfunction	Increased permeability	Elevated LDL	Early disease modeling
Fatty streak formation	Foam cell accumulation	Oxidized LDL	Common in rodents
Plaque progression	SMC proliferation	Persistent dyslipidemia	Intermediate models
Plaque destabilization	Fibrous cap thinning	Inflammatory lipids	Large animals
Plaque rupture	Thrombosis	Lipoprotein(a), LDL	Rare in rodents

Note: **LDL**: Low-density lipoprotein; **SMC**: Smooth muscle cell.

Necrotic Core Formation and Plaque Rupture

Unrelenting localized inflammation, coupled with foam cell apoptosis and secondary necrosis, leads to the progressive expansion of the necrotic core. Concurrently, macrophage-derived matrix metalloproteinases (MMPs) enzymatically degrade the extracellular matrix of the fibrous cap, significantly compromising plaque stability. Plaque rupture eventually exposes the highly thrombogenic necrotic core to circulating coagulation factors, triggering acute thrombosis that can precipitate severe clinical events, including myocardial infarction or ischemic stroke[25].

Immunological Mechanisms in Atherosclerosis

Atherosclerosis is increasingly characterized as a complex immunometabolic disorder governed by both innate and adaptive immune responses. In the innate arm, macrophages, dendritic cells, and neutrophils heavily contribute to localized cytokine production and the propagation of the necrotic core. Pro-inflammatory cytokines, notably IL-1β, TNF-α, and MCP-1, serve to amplify lesion progression. Adaptive immune responses are equally critical; for instance, Th1-polarized T cells secrete interferon-γ (IFN-γ), which potently activates macrophages while simultaneously inhibiting collagen synthesis by VSMCs, thereby fostering plaque instability. Consequently, targeting inflammatory pathways has emerged as a vital complementary strategy in cardiovascular therapeutics, a paradigm exemplified by the CANTOS trial, which demonstrated a significant reduction in cardiovascular events following IL-1β inhibition, independent of lipid-lowering efficacy[17,26,27].

Role of Lipoproteins in Atherogenesis

Distinct lipoprotein classes contribute differentially to plaque pathogenesis. LDL serves as the primary atherogenic particle, with its oxidative modification acting as a requisite initiating event in lesion formation. Conversely, HDL exerts profound atheroprotective effects, primarily mediated through reverse cholesterol transport and intrinsic anti-inflammatory properties. Lipoprotein(a) [Lp(a)], which contains the highly homologous apolipoprotein(a), possesses dual pro-atherogenic and pro-thrombotic properties and is frequently elevated in patients with familial hyperlipidemia. Importantly, species-specific variations in baseline lipoprotein profiles significantly dictate susceptibility to atherosclerosis, thereby determining the translational relevance of specific animal models employed in drug screening[28].

Relevance in Animal Models

The accurate biological reproduction of atherosclerotic pathology is a prerequisite for the rigorous evaluation of antihyperlipidemic agents. When subjected to high-fat diets, ApoE$^{-/-}$ and LDLR$^{-/-}$ murine models reliably develop atherosclerotic lesions localized to the aorta and aortic root; however, the architectural complexity of these plaques remains somewhat limited relative to human disease[28]. In contrast, WHHL rabbits and cholesterol-fed New Zealand White rabbits consistently develop foam cell-rich vascular lesions that closely parallel early-stage human plaques[10]. Larger models, including pigs and non-human primates, are capable of exhibiting advanced plaques characterized by distinct necrotic cores, calcification, and mature fibrous caps, thereby offering superior physiological parallels to human atherosclerosis[28,29].

Limitations in Modelling Atherosclerosis

Despite their undeniable utility, animal models harbor inherent translational limitations. Spontaneous plaque rupture and subsequent occlusive thrombosis remain exceedingly rare events in most non-human species. Fundamental discrepancies in lipoprotein metabolism, immunologic repertoire, and relatively abbreviated lifespans further constrain the fidelity of long-term disease modeling. Specifically, the complete absence of CETP expression in rodents fundamentally restricts their utility in reliably replicating human HDL biology. Consequently, rational model selection must be meticulously guided by the specific pathophysiological features and therapeutic mechanisms under active investigation to ensure maximal translational relevance[29].

ANIMAL MODELS FOR SCREENING ANTIHYPERLIPIDEMIC AGENTS

Animal models constitute the cornerstone of preclinical research for the discovery and evaluation of antihyperlipidemic agents. These models enable the rigorous assessment of lipid-lowering efficacy, mechanistic pathways, safety profiles, and pharmacodynamic responses prior to clinical translation[8,30]. The selection of an appropriate model depends fundamentally on the specific research objective, disease complexity, and the metabolic pathway targeted by the therapeutic intervention. Based on the method of induction and genetic background, animal models of hyperlipidemia can be broadly categorized into diet-induced, chemically induced, genetically modified, microbiota-modulated, and emerging alternative models (Figure 2).

Diet-Induced Hyperlipidemia Models

Diet-induced models are among the most widely utilized systems for studying hyperlipidemia and its associated metabolic disorders. These models typically employ high-fat diets (HFD), high-cholesterol diets (HCD), or combinatorial diets enriched with saturated fats, sucrose, or fructose to induce dyslipidemia. Rodents fed an HFD or HCD exhibit elevated plasma triglycerides, total cholesterol, and LDL-C, alongside reduced HDL-C. These metabolic alterations are frequently accompanied by insulin resistance, hepatic steatosis, and low-grade systemic inflammation, closely mimicking the features of human metabolic syndrome[31]. However, diet composition varies considerably across studies with respect to fat source, cholesterol content, and duration of feeding. Such heterogeneity introduces significant variability and complicates cross-study comparisons. Furthermore, rodents require supraphysiological dietary cholesterol to induce hypercholesterolemia, inherently limiting their ability to fully replicate human lipid metabolism[32].

Chemically Induced Hyperlipidemia Models

Chemical induction models utilize pharmacological agents to disrupt systemic lipid homeostasis. Triton WR-1339 (tyloxapol) is commonly employed to inhibit lipoprotein lipase activity, resulting in an acute elevation of plasma triglycerides and cholesterol. Similarly, poloxamer 407 induces hyperlipidemia through the dual inhibition of lipoprotein clearance and the upregulation of hepatic lipid synthesis[33]. These models are particularly advantageous for the rapid, short-term screening of lipid-lowering compounds and for mechanistic studies involving triglyceride metabolism. However, chemically induced hyperlipidemia fails to replicate the chronic inflammatory and complex vascular features characteristic of human dyslipidemia. Consequently, their translational relevance for evaluating long-term cardiovascular outcomes remains highly limited[34,35].

Genetically Modified Animal Models

Genetically modified models provide invaluable insights into inherited dyslipidemias and the specific molecular pathways regulating lipid metabolism. Apolipoprotein E-deficient ($ApoE^{-/-}$) and LDL receptor-deficient ($LDLR^{-/-}$) mice are extensively utilized in atherosclerosis research due to their profound propensity to develop hypercholesterolemia and vascular lesions[36]. $ApoE^{-/-}$ mice develop spontaneous atherosclerosis even when maintained on standard chow diets, whereas $LDLR^{-/-}$ mice require dietary cholesterol supplementation to accelerate lesion formation. Despite their robust utility, these models differ substantially from human lipid metabolism, particularly regarding lipoprotein distribution and the absence of CETP activity[37]. Recent advances in CRISPR/Cas9 technology have enabled the generation of humanized models expressing human ApoB, PCSK9, or CETP, thereby significantly improving their translational relevance. Nonetheless, the high cost and technical complexity associated with generating and maintaining such models continue to limit their widespread adoption[38].

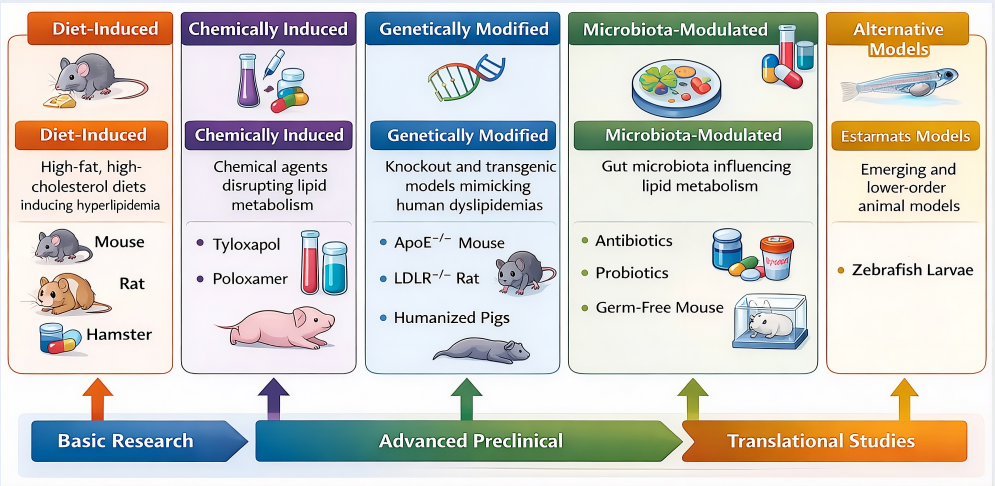

Figure 2: Classification of animal models utilized for antihyperlipidemic drug screening. The models are broadly categorized based on their induction methodologies and physiological characteristics, encompassing diet-induced, chemically induced, genetically modified, microbiota-modulated, and emerging alternative platforms.

Microbiota-Modulated Models

Emerging evidence highlights the critical role of the gut microbiota in systemic lipid metabolism and cardiovascular risk. Interventions utilizing probiotics, prebiotics, and synbiotics have been shown to modulate lipid profiles via bile acid metabolism, short-chain fatty acid production, and the regulation of host gene expression[39,40]. Animal models incorporating microbiota modulation, including antibiotic-treated or germ-free rodents, are increasingly used to elucidate complex host–microbe interactions in hyperlipidemia. While highly promising, such studies often exhibit significant methodological variability in microbial strain selection, dosing regimens, and intervention duration, which complicates reproducibility and translational interpretation[41]. Therefore, critical appraisal of these studies is essential, particularly when sample sizes are restricted or appropriate control groups are lacking.

Alternative and Emerging Models

Zebrafish (*Danio rerio*) have gained considerable attention as alternative models for lipid research due to their optical transparency, genetic manipulability, and rapid lipid metabolism. Zebrafish larvae fed high-cholesterol diets exhibit pronounced lipid accumulation and vascular lipid deposition, providing an efficient platform for the high-throughput screening of prospective antihyperlipidemic compounds. However, fundamental evolutionary differences in cardiovascular anatomy, lipoprotein composition, and immunological responses severely limit their applicability for modeling advanced, complex atherosclerosis. Consequently, zebrafish models are optimally positioned for early-stage discovery and screening rather than for definitive translational studies[42].

COMPARATIVE UTILITY OF SMALL AND LARGE ANIMAL MODELS

Animal models exhibit considerable variation regarding their physiological concordance with humans, associated ethical considerations, financial costs, and overall suitability for specific research objectives. Consequently, a rigorous comparative evaluation of small versus large animal models is imperative to ensure rational model selection[43,44].

Small Animal Models

Small animals—predominantly mice, rats, and hamsters—are extensively utilized due to their cost-effectiveness, ease of handling, rapid reproductive cycles, and the vast availability of genetic manipulation tools. These models are particularly advantageous for mechanistic investigations, target validation, and early-stage pharmacological screening[43]. However, they possess substantial limitations. Notably, most rodents inherently lack CETP activity, exhibit disparate baseline lipoprotein distributions compared to humans, and rarely manifest spontaneous plaque rupture or occlusive thrombosis. Fur-

thermore, a pervasive sex bias remains in preclinical research, as numerous studies are conducted exclusively on male cohorts, thereby significantly restricting the clinical generalizability of the findings (Figure 3).

Large Animal Models

Large animal models, including rabbits, pigs, and non-human primates, provide a superior physiological resemblance to human lipid metabolism, vascular architecture, and atherosclerotic disease progression. Rabbits naturally express CETP and readily develop cholesterol-enriched lipoproteins alongside foam cell-dense vascular lesions. Pigs and non-human primates are capable of developing complex, advanced atherosclerotic plaques characterized by mature fibrous caps and necrotic cores[44]. Despite these distinct translational advantages, the utility of large animal models is frequently constrained by prohibitive costs, extended study durations, heightened ethical scrutiny, and restricted biological availability. Additionally, stringent regulatory requirements and substantial infrastructural demands further limit their routine implementation in lipid research (Figure 3)[38,44].

Strategic Integration of Models

Employing a tiered, strategic approach that integrates both small and large animal models substantially enhances the probability of translational success. Small animals are optimally suited for initial hypothesis generation, fundamental mechanistic exploration, and preliminary efficacy screening. Conversely, large animals are requisite for late-stage translational validation, comprehensive safety assessments, and precise dose optimization. A judicious, sequentially aligned combination of both model archetypes—tailored specifically to the given research phase (e.g., early discovery versus advanced preclinical validation)—ensures scientific robustness, ethical compliance, and maximal translational relevance (Figure 3)[45].

COMPARATIVE PREDICTIVE VALUE AND COST–BENEFIT CONSIDERATIONS OF EMERGING VERSUS TRADITIONAL MODELS

Emerging experimental platforms, such as zebrafish and microbiota-modulated models, offer complementary advantages to traditional rodent, rabbit, and large-animal systems; however, they differ substantially in their predictive value for late-stage clinical translation. Zebrafish models facilitate high-throughput, cost-effective screening and enable the rapid phenotypic assessment of lipid metabolism and vascular lipid deposition, rendering them particularly valuable for early-phase discovery and mechanistic exploration. Nevertheless, fundamental interspecies differences in cardiovascular anatomy, lipoprotein composition, and hemodynamics inherently limit their capacity to predict complex human clinical outcomes, such as spontaneous plaque rupture or long-term cardiovascular risk.

Microbiota-modulated models effectively capture host–microbe–drug interactions, which are increasingly recognized as critical determinants of systemic lipid metabolism and therapeutic responsiveness. While these models enhance the biological relevance of metabolic modulation studies, their translational predictability remains constrained by significant inter-laboratory variability, diet-dependent confounding effects, and overarching challenges in protocol standardization.

In contrast, traditional rodent and rabbit models—despite incurring higher costs and a greater ethical burden—provide more robust pharmacokinetic and pharmacodynamic relationships, aligning more closely with stringent regulatory expectations. Large-animal models, particularly porcine and non-human primate systems, offer the highest degree of translational fidelity, albeit at a substantially greater financial, infrastructural, and ethical cost. Collectively, these comparative considerations underscore that emerging models are optimally positioned as early-phase, hypothesis-generating tools, whereas traditional models remain indispensable for confirmatory efficacy testing and definitive translational validation within a structured, decision-guided drug development framework.

CRITICAL APPRAISAL OF EVIDENCE FROM ANIMAL MODELS

While animal models have substantially advanced our understanding of lipid metabolism and atherosclerosis, their predictive value for clinical outcomes varies considerably. Consequently, a critical appraisal of methodological rigor, biological relevance, and translational reliability is essential when interpreting preclinical findings (Table 4)[46].

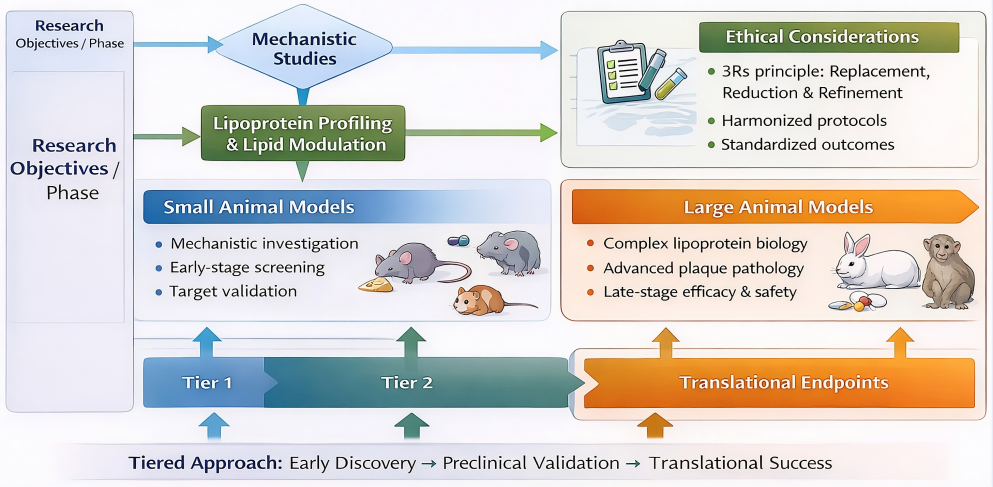

Figure 3: **Translational framework linking animal model selection to research objectives.** This tiered, decision-guided approach illustrates the strategic integration of small animal models for early-stage mechanistic investigation and target validation, followed by the deployment of large animal models for advanced preclinical efficacy, safety assessment, and translation to clinical endpoints.

Table 4: Key Parameters Used in Evaluating Antihyperlipidemic Agents.

Parameter Category	Specific Endpoints	Translational Importance
Biochemical	TC, LDL-C, HDL-C, TG	Primary efficacy
Histological	Plaque size, lipid deposition	Disease severity
Molecular	Inflammatory markers, gene expression	Mechanistic insight
Functional	Endothelial function	Cardiovascular relevance
Safety	Liver enzymes, body weight	Toxicity assessment

Note: **HDL-C**: High-density lipoprotein cholesterol; **LDL-C**: Low-density lipoprotein cholesterol; **TC**: Total cholesterol; **TG**: Triglycerides.

Quality Grading of Evidence

The strength of evidence derived from animal studies was qualitatively graded based on experimental reproducibility, consistency across species, mechanistic plausibility, and alignment with human pathophysiology. Models demonstrating conserved lipid pathways, endogenous CETP activity, and plaque characteristics comparable to human atheromas were considered to provide strong evidence (++ evidence). Conversely, models primarily suited for mechanistic or exploratory studies were graded as providing moderate evidence (+ evidence). Rodent models, for instance, offer robust mechanistic insights but demonstrate limited translational accuracy for evaluating HDL-targeted therapies. In contrast, rabbit and non-human primate models provide a stronger predictive value for LDL-centric interventions, although their utility is frequently constrained by ethical and logistical considerations[47].

Methodological Limitations in Primary Studies

Many of the primary studies included in this review report positive lipid-lowering outcomes without adequately addressing underlying experimental limitations. Common methodological issues include small sample sizes, an absence of sex-based analyses, abbreviated intervention durations, and insufficient reporting of randomization or blinding procedures. These factors collectively inflate the risk of bias and can lead to the overestimation of therapeutic efficacy[48]. Furthermore, in microbiota-modulation studies, profound heterogeneity in microbial strain selection, dosing regimens, and treatment duration further complicates data interpretation. The pervasive absence of standardized outcome measures across independent laboratories remains a major contributor to the irreproducibility often observed in lipid research.

Publication Bias and Reporting Gaps

Publication bias represents a significant yet frequently underappreciated limitation within preclinical lipid research. Studies that demonstrate positive or novel findings are disproportionately more likely to be published, whereas neutral or negative results often remain underreported. This inherent bias can profoundly distort the perceived efficacy of emerging antihyperlipidemic strategies, particularly those evaluated in genetically modified or novel dietary models[49]. Selective outcome reporting, coupled with incomplete methodological descriptions, further diminishes study reproducibility. Greater adherence to standardized reporting frameworks, such as the ARRIVE guidelines, alongside the preregistration of animal studies, could substantially improve the transparency and scientific credibility of the field.

ETHICAL CONSIDERATIONS AND THE 3RS PRINCIPLE

Ethical responsibility remains paramount in animal-based research, particularly concerning chronic metabolic disease models that necessitate prolonged dietary manipulation and invasive experimental procedures. The fundamental principles of Replacement, Reduction, and Refinement (3Rs) establish an essential ethical and scientific framework for rigorous model selection and study design[50,51].

Replacement Strategies

Whenever feasible, alternative methodologies—such as *in vitro* lipid assays, organ-on-a-chip platforms, computational modeling, and zebrafish larval systems—should be prioritized to replace higher-order animals during early-stage pharmacological screening. Integrating these alternative approaches substantially diminishes animal utilization while simultaneously facilitating rapid and efficient hypothesis testing[51].

Reduction of Animal Use

Rigorous statistical planning, the implementation of longitudinal study designs, and the utilization of shared control cohorts can significantly curtail the number of animals required without compromising statistical power or data integrity. Furthermore, the integration of non-invasive imaging modalities and the serial measurement of circulating biomarkers actively minimize the need for repeated animal sacrifice[52].

Refinement of Experimental Procedures

Refinement strategies are explicitly designed to minimize animal suffering and optimize overall welfare. These strategies encompass the provision of enriched housing conditions, the use of highly refined dietary formulations, the establishment of stringent humane endpoints, and the administration of robust analgesic protocols. Importantly, methodological refinement not only ensures ethical compliance but also bolsters data reliability by mitigating stress-induced metabolic and physiological variability[53]. Collectively, strict ethical adherence and scientific rigor operate interdependently, ultimately reinforcing the translational validity of preclinical findings.

TRANSLATIONAL GAPS, CHALLENGES, AND FUTURE DIRECTIONS

Despite decades of extensive preclinical research, the successful translation of antihyperlipidemic therapies from animal models to clinical efficacy remains inconsistent. Several translational gaps persist, underscoring the critical need for more predictive and integrative modeling strategies[54].

Failure of Promising Therapeutics

Numerous pharmacological agents that demonstrated robust efficacy in animal models have ultimately failed to achieve favorable clinical outcomes. CETP inhibitors represent a prominent example of this paradigm; substantial HDL elevation observed in preclinical and early-phase clinical studies did not consistently translate into a reduction in cardiovascular risk and, in certain instances, paradoxically increased adverse events[55]. These clinical failures highlight the profound limitations of relying exclusively on biochemical surrogate endpoints—such as isolated alterations in LDL-C or HDL-C—without adequately evaluating plaque stability, systemic inflammation, or the long-term risk of clinical cardiovascular events.

Over-Reliance on Surrogate Endpoints

Preclinical investigations frequently prioritize the biochemical reduction of circulating lipids as their primary efficacy endpoint. However, the lowering of lipid levels does not invariably correlate with atherosclerotic plaque regression or a proportional decrease in clinical cardiovascular events. Integrating non-invasive vascular imaging, robust inflammatory biomarkers, and functional assessments of

plaque stability into preclinical protocols may substantially improve translational accuracy[56].

Need for Standardization and Decision-Guided Model Selection

The pervasive lack of standardized experimental protocols governing diet composition, intervention duration, and specific endpoint measurements remains a major obstacle to ensuring cross-study comparability. Consequently, implementing a decision-tree framework that closely aligns specific research questions with the most appropriate animal models is essential. For example, mechanistic studies are optimally conducted using genetically modified rodents; lipoprotein profiling necessitates CETP-expressing species; and the comprehensive evaluation of plaque stability requires large-animal models. Such structured, evidence-based model selection fundamentally enhances both reproducibility and predictive value.

Future Directions

Future research in antihyperlipidemic pharmacology should prioritize the following key areas:

- The development and utilization of humanized and multi-gene animal models.
- The integration of multi-omics-based biomarkers to comprehensively profile metabolic responses.
- The implementation of rigorously sex-inclusive experimental designs.
- Strict ethical compliance with the 3Rs principles.
- The adoption of multimodal endpoints that extend significantly beyond basic lipid quantification.

Collectively, these strategic approaches will support a more reliable, predictive, and ethically responsible translational pipeline (Table 5).

CONCLUSION

Animal models remain indispensable tools for advancing antihyperlipidemic drug discovery and understanding the complex biology of lipid metabolism and atherosclerosis. This integrative review highlights the strengths and limitations of commonly used animal models, ranging from diet-induced and chemically induced systems to genetically modified, microbiota-modulated, and large-animal models. Each category offers distinct advantages depending on the specific research objective, disease stage, and therapeutic mechanism under investigation.

While small animal models provide cost-effective and mechanistically informative platforms for early-stage screening, their limited ability to replicate human lipoprotein profiles and plaque complexity restricts direct clinical extrapolation. In contrast, large animal models demonstrate superior translational relevance but are constrained by ethical, logistical, and financial considerations. Importantly, repeated translational failures, including those observed with CETP inhibitors, underscore the limitations of relying solely on lipid-centric surrogate endpoints.

Future progress in antihyperlipidemic research will depend on rational, question-driven model selection, improved methodological standardization, incorporation of sex-inclusive designs, and integration of multi-omics and imaging-based endpoints. Adherence to ethical principles, particularly the 3Rs, must remain central to preclinical study design. Collectively, these strategies will enhance reproducibility, predictive accuracy, and the successful translation of preclinical findings into clinically meaningful cardiovascular therapies.

ABBREVIATIONS

3Rs: Replacement, Reduction, and Refinement; **ACAT**: Acyl-CoA cholesterol acyltransferase; **ApoA-I**: Apolipoprotein A-I; **ApoB-100**: Apolipoprotein B-100; **ApoE**: Apolipoprotein E; **ARRIVE**: Animal Research: Reporting of In Vivo Experiments; **CETP**: Cholesteryl ester transfer protein; **CRISPR**: Clustered Regularly Interspaced Short Palindromic Repeats; **CVDs**: Cardiovascular diseases; **HCD**: High-cholesterol diets; **HDL**: High-density lipoprotein; **HDL-C**: High-density lipoprotein cholesterol; **HFD**: High-fat diets; **ICAM-1**: Intercellular Adhesion Molecule 1; **ICH**: International Council for Harmonisation; **IDL**: Intermediate-density lipoproteins; **IL-1β**: Interleukin-1 beta; **LCAT**: Lecithin-cholesterol acyltransferase; **LDL**: Low-density lipoprotein; **LDL-C**: Low-density lipoprotein cholesterol; **LDLR**: Low-density lipoprotein receptor; **LPL**: Lipoprotein lipase; **MCP-1**: Monocyte chemoattractant protein-1; **MeSH**: Medical Subject Headings; **OECD**: Organisation for Economic Co-operation and Development; **PCSK9**: Proprotein convertase subtilisin/kexin type 9; **PRISMA**: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **TC**: Total cholesterol; **TG**: Triglycerides; **TNF-α**: Tumor necrosis factor-alpha; **VCAM-1**: Vascular cell adhesion molecule 1; **VLDL**: Very-low-density

Table 5: Comparative Translational Value of Common Animal Models.

Model Category	Translational Value	Major Strength	Key Limitation	Ethical Consideration†
Rodents	Moderate	Genetic manipulability	Lack CETP	Low burden
Rabbits	High	CETP expression	Cost	Moderate
Pigs	Very High	Plaque similarity to humans	Infrastructure demands	High
Primates	Highest	Human-like lipid metabolism	Ethical constraints, cost	Very High
Zebrafish	Low–Moderate	High-throughput screening	Limited cardiovascular anatomy	Minimal

Note: **CETP**: Cholesteryl ester transfer protein. †*Ethical consideration categories reflect relative animal welfare burden based on invasiveness of procedures, lifespan, housing complexity, sentience, and regulatory oversight requirements. "Minimal" indicates short-lived, low-sentience models with non-invasive endpoints; "Low" indicates limited distress and short study duration; "Moderate" involves higher physiological burden or longer-term studies; "High" and "Very High" reflect increased sentience, procedural invasiveness, long-term housing, and heightened ethical and regulatory scrutiny.*

lipoprotein; and **WHHL**: Watanabe heritable hyperlipidemic.

ACKNOWLEDGMENTS

The authors would like to thank the Hon. Chancellor, Pro-Chancellor, Vice-Chancellor, Head of the Department of Pharmacy, and the Dean, Faculty of Pharmacy, Integral University, for providing the necessary facilities to complete this work. The University assigned a unique manuscript communication number (IU/R&D/2024-MCN0002443).

AUTHOR'S CONTRIBUTIONS

JAA designed the structure of the manuscript and drafted the initial version. MK performed the literature review. SMH edited and critically reviewed the manuscript. FA formatted the manuscript according to journal guidelines. JA, SA, and NP contributed to data curation. All authors read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that generative AI and AI-assisted technologies were not used in the writing of this manuscript prior to submission, except for minor language editing and grammatical corrections.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. World Health Organization. Cardiovascular diseases (CVDs). Geneva: WHO; 2021. Accessed 22 Jan 2026. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2019;73(24):e285–e350. Available from: https://doi.org/10.1016/j.jacc.2018.11.003.
3. Libby P. The changing landscape of atherosclerosis. Nature. 2021;592(7855):524–533. Available from: https://doi.org/10.1038/s41586-021-03392-8.
4. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet. 2017;390(10113):2627–2642. Available from: https://doi.org/10.1016/S0140-6736(17)32129-3.
5. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41(1):111–188. Available from: https://doi.org/10.1093/eurheartj/ehz455.
6. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: a report of the American College of Cardiology/American Heart Associ-

ation Task Force on Clinical Practice Guidelines. Circulation. 2019;140(11):e596–e646. Available from: https://doi.org/10.1161/CIR.0000000000000678.
7. Getz GS, Reardon CA. Animal models of atherosclerosis: high-dimensional phenotypic mapping enables exploration of novel phenotypes. Arterioscler Thromb Vasc Biol. 2012;32(5):1104–1115. Available from: https://doi.org/10.1161/ATVBAHA.111.237693.
8. Poznyak AV, Bharadwaj D, Prasad G, Grechko AV, Sobenin IA, Orekhov AN. Anti-inflammatory therapy for atherosclerosis: focusing on cytokines. Braz J Med Biol Res. 2020;53(11):e10453. Available from: https://doi.org/10.1590/1414-431X202010453.
9. Tall AR. CETP inhibitors and HDL metabolism: quo vadis? N Engl J Med. 2006;355(23):2583–2584. Available from: https://doi.org/10.1056/NEJMe068245.
10. Shiomi M, Ito T, Shiraishi M. The Watanabe heritable hyperlipidemic rabbit model: its characteristics and application. J Atheroscler Thromb. 2013;20(1):1–10. Available from: https://doi.org/10.5551/jat.15390.
11. Ishibashi S, Goldstein JL, Brown MS, Herz J, Burns DK, Hammer RE. Massive xanthomatosis and atherogenesis without cholesterol esterification. J Clin Invest. 1994;93(5):1885–1893. Available from: https://doi.org/10.1172/JCI117179.
12. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010;8(6):e1000412. Available from: https://doi.org/10.1371/journal.pbio.1000412.
13. Huang LH, Elvington A, Randolph GJ. The role of the lymphatic system in cholesterol transport. Front Pharmacol. 2015;6:182. Available from: https://doi.org/10.3389/fphar.2015.00182.
14. Vuorio T, Kovanen PT, Watts GF, W˚astedt C, Baass A, Boren J, et al. Lymphatic vessels and atherosclerosis. Cardiovasc Res. 2021;117(12):2433–2445. Available from: https://doi.org/10.1093/cvr/cvab122.
15. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, et al. Atherosclerosis. Nat Rev Dis Primers. 2019;5(1):56. Available from: https://doi.org/10.1038/s41572-019-0106-z.
16. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233–241. Available from: https://doi.org/10.1038/35025203.
17. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Catapano AL, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459–2472. Available from: https://doi.org/10.1093/eurheartj/ehx144.
18. Quehenberger O, Dennis EA. The human plasma lipidome. N Engl J Med. 2011;365(19):1812–1823. Available from: https://doi.org/10.1056/NEJMra1104901.
19. Zadelaar S, Kleemann R, Verschuren L, de Vries-van der Weij J, van der Hoorn JWA, Princen HMG, et al. Mouse models for atherosclerosis and pharmaceutical modifiers. Arterioscler Thromb Vasc Biol. 2007;27(8):1706–1721. Available from: https://doi.org/10.1161/ATVBAHA.107.142570.
20. van der Vaart JI, van Eenige R, Rensen PCN, Kooijman S. Overview of mouse models of atherosclerosis. Vasc Biol. 2024;6(1):e230017. Available from: https://doi.org/10.1530/VB-23-0017.
21. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685–1695. Available from: https://doi.org/10.1056/NEJMra043430.
22. Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med. 1999;340(2):115–126. Available from: https://doi.org/10.1056/NEJM199901143400207.
23. Tabas I, Bornfeldt KE. Macrophage phenotype and function in different stages of atherosclerosis. Nat Rev Immunol. 2016;16(6):393–406. Available from: https://doi.org/10.1038/nri.2016.43.
24. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circ Res. 2016;118(4):692–702. Available from: https://doi.org/10.1161/CIRCRESAHA.115.306361.
25. Libby P, Pasterkamp G. Requiem for the 'vulnerable plaque'. Eur Heart J. 2015;36(43):2984–2987. Available from: https://doi.org/10.1093/eurheartj/ehv349.
26. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017;377(12):1119–1131. Available from: https://doi.org/10.1056/NEJMoa1707914.
27. Ketelhuth DFJ, Hansson GK. Adaptive response of T and B cells in atherosclerosis. Nat Rev Immunol. 2016;16(7):403–418. Available from: https://doi.org/10.1038/nri.2016.28.
28. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res. 2014;114(12):1852–1866. Available from: https://doi.org/10.1161/CIRCRESAHA.114.302721.
29. Bentzon JF, Falk E. Pathological intimal thickening as a disease process of the vessel wall. Circ Res. 2010;107(5):589–602. Available from: https://doi.org/10.1161/CIRCRESAHA.110.218271.
30. Chen X, Zhou Y, Yang J, Yang R, Xue S, Wang Y, et al. Animal Model Screening for Hyperlipidemic ICR Mice. Int J Mol Sci. 2025;26(5):2142. Available from: https://doi.org/10.3390/ijms26052142.
31. Paigen B, Holmes PA, Mitchell D, Albee D. Comparison of atherosclerotic lesions and serum cholesterol levels of inbred, genetically engineered, and combined strains of mice. Atherosclerosis. 1987;68(3):231–240. Available from: https://doi.org/10.1016/0021-9150(87)90245-7.
32. Buettner R, Schölmerich J, Bollheimer LC. High-fat diets: linking the diet to fatty liver. Obesity (Silver Spring). 2007;15(4):798–808. Available from: https://doi.org/10.1038/oby.2007.608.
33. Schurr PE, Schultz JR, Tennent DM. Triton-induced hyperlipidemia in rats as an analogue of familial type II hyperlipoproteinemia. J Lipid Res. 1976;17(1):39–45. Available from: https://doi.org/10.1016/S0022-2275(20)40109-8.
34. Johnston TP, Korolenko TA, Saenko Y, Kolaeva SG, Ryzhkova AV, Pirro M. Poloxamer 407-induced hyperlipidemia. J Pharmacol Toxicol Methods. 2010;62(1):14–25. Available from: https://doi.org/10.1016/j.vascn.2010.03.002.
35. Loginova VM, Tuzikov FV, Tuzikova NA, Korolenko TA. Comparative characteristics of lipemia models induced by injections of Triton WR-1339 and poloxamer 407 in mice. Bull Exp Biol Med. 2013;155(2):284–287. Available from: https://doi.org/10.1007/s10517-013-2133-1.
36. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb. 1994;14(1):133–140. Available from: https://doi.org/10.1161/01.ATV.14.1.133.
37. Meir KS, Leitersdorf E. Atherosclerosis in the apolipoprotein-E-deficient mouse: a decade of progress. Arterioscler Thromb Vasc Biol. 2004;24(6):1006–1014. Available from: https://doi.org/10.1161/01.ATV.0000125702.11501.92.
38. Sato A, Hirano Y, Kawakami R, Ishihara T, Inoue T, Nakajima T, et al. Generation of familial hypercholesterolemia in primates via CRISPR/Cas9. Sci Rep. 2023;13(1):15649. Available from: https://doi.org/10.1038/s41598-023-42763-1.
39. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. Circ Res. 2017;120(7):1186–1196. Available from: https://doi.org/10.1161/CIRCRESAHA.117.310715.
40. Huang Z, Liu J, Tang Q. Hypolipidemic effects of Lactobacillus plantarum in high-fat diet-induced hyperlipidemia in mice. Microb Pathog. 2024;186:106258. Available from: https://doi.org/10.1016/j.micpath.2023.106258.
41. Liu Y, Zhang R, Wang H. Lactobacillus paragasseri improves lipid metabolism through regulating gut microbiota in hyperlipidemic mice. Front Microbiol. 2022;13:840209. Available from: https://doi.org/10.3389/fmicb.2022.840209.
42. Chen Y, Zheng J, Leng Y, Zhang Y, Li Y, Li Y, et al. Lactobacillus

fermentum E15 alleviates hyperlipidemia in zebrafish through modulating lipid metabolism and gut microbiota. Front Nutr. 2025;12:1522982. Available from: https://doi.org/10.3389/fnut.2025.1522982.

43. Van Eck M, Herijgers J, Van Berkel TJC, Havekes LM. Golden Syrian hamsters as a model for studies of lipoprotein metabolism. Atherosclerosis. 2003;171(2):285–293. Available from: https://doi.org/10.1016/S0021-9150(03)00295-7.
44. Li J, Liu Y, Jin M, et al. Generation of LDLR-knockout pigs via CRISPR/Cas9 and somatic cell nuclear transfer. J Lipid Res. 2023;64(5):100387. Available from: https://doi.org/10.1016/j.jlr.2023.100387.
45. Tall AR, Rader DJ. Trials and tribulations of CETP inhibitors. Circ Res. 2018;122(1):106–112. Available from: https://doi.org/10.1161/CIRCRESAHA.117.311978.
46. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet. 2012;380(9841):572–580. Available from: https://doi.org/10.1016/S0140-6736(12)60312-2.
47. Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity for preclinical animal research? Br J Pharmacol. 2018;175(21):4062–4071. Available from: https://doi.org/10.1111/bph.14276.
48. Sena ES, van der Worp HB, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. Stroke. 2010;41(3):e34–e40. Available from: https://doi.org/10.1161/STROKEAHA.109.569707.
49. Begley CG, Ellis LM. Raise standards for preclinical cancer research. Nature. 2012;483(7391):531–533. Available from: https://doi.org/10.1038/483531a.
50. Russell WMS, Burch RL. The Principles of Humane Experimental Technique. London: Methuen; 1959.
51. Hartung T. Food for thought ... on alternative methods for chemical safety testing. ALTEX. 2010;27(1):3–14. Available from: https://doi.org/10.14573/altex.2010.1.3.
52. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol. 2020;18(7):e3000417. Available from: https://doi.org/10.1371/journal.pbio.3000417.
53. van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. Can animal models of disease reliably inform human studies? PLoS Med. 2010;7(3):e1000245. Available from: https://doi.org/10.1371/journal.pmed.1000245.
54. Collins FS, Tabak LA. NIH plans to enhance reproducibility. Nature. 2014;505(7485):612–613. Available from: https://doi.org/10.1038/505612a.
55. Ioannidis JPA. Why most published research findings are false. PLoS Med. 2005;2(8):e124. Available from: https://doi.org/10.1371/journal.pmed.0020124.
56. Freedman LP, Cockburn IM, Simcoe TS. The economics of reproducibility in preclinical research. PLoS Biol. 2015;13(6):e1002165. Available from: https://doi.org/10.1371/journal.pbio.1002165.