Unlocking the Potential of Pig Models in Atherosclerosis Research: Insights, Applications, and Future Directions

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Abstract

Background: Choosing an adequate model for studying human diseases, such as atherosclerosis, poses significant challenges. While small animals like mice and rats have been widely employed, the suitability of these models for specific research goals and objectives may vary. Furthermore, differences in lipid physiology and platelet quantities between rodents and humans can impact the translational relevance of findings. Large animal models, particularly swine, offer physiological similarities to humans and present a more accurate representation of clinical complications such as acute myocardial infarction and stroke. Objectives: This review aims to comprehensively evaluate the utility of the swine model in atherosclerosis research by examining the physiological and cardiovascular similarities between swine and humans. It also seeks to explore the significance of hyperlipidemia and atherosclerosis in pigs, considering both natural and genetically engineered mutant pig models. Additionally, the review aims to provide an overview of the potential applications of swine models in atherosclerosis regression research, thereby highlighting the advantages and limitations of employing swine in atherosclerosis studies. Conclusion: This review offers insights into the potential of the swine model as a valuable and versatile tool for expanding the horizons of atherosclerosis research, emphasizing the need for further exploration and utilization of large animal models in cardiovascular research.

Keywords: Atherosclerosis, LDL oxidation, Inflammation, Animal models, Plaque progression

Significance | This review describes the understanding of atherosclerosis through animal models aids in revealing disease mechanisms, testing treatments, and improving therapeutic strategies for humans.

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Introduction

Among the cardiovascular diseases widely prevalent worldwide, atherosclerosis stands out as one of the most frequent and dangerous conditions, resulting in numerous complications and consequences such as thrombosis, ischemia, and myocardial infarction. The pathogenesis of atherosclerosis proceeds gradually under the influence of many factors. The disease development process begins with the emergence of atherosclerotic plaques. The growth rate of these plaques is determined by various factors, including blood biochemistry (Frostegård, 2013). The rate of plaque development and the probability of formation depend primarily on the concentration of LDL in the blood. At high concentrations, LDL tends to accumulate in specific areas of the blood vessels. As a consequence, they undergo oxidation, causing an immune response and leading to the onset of inflammation (Rhoads & Major, 2018).

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Subsequently, as the plaque begins to develop and grow, a permanent inflammatory process occurs in response to the activation of damage-associated patterns (DAMPs). The ongoing growth of the plaque eventually leads to the narrowing and complete occlusion of blood flow (thrombosis). As a result inflammatory processes, the plaque can, after reaching a certain size or complete occlusion of the blood flow, be partially destroyed and detached from the vessel walls, often leading to serious cardiovascular complications and diseases (Fioranelli et al., 2018; Rai & Agrawal, 2017).

To better understand the mechanics and biochemistry of these processes and their molecular mechanisms, researchers have developed animal models of atherosclerosis. These models have primarily been used to investigate the effects of cholesterol, fat, and LDL oxidation processes. LDL oxidation elicits oxidative stress and triggers an inflammatory reaction in vascular cells, specifically endothelial cells (ECs) (Khatana et al., 2020). This process enhances the expression of adhesion molecules, including intracellular and vascular adhesion molecules (ICAM-1 and VCAM-1), E-selectin, and P-selectin, as well as chemotactic proteins such as monocyte chemoattractant protein 1 (MCP-1). These cytokines facilitate the recruitment of monocytes from the bloodstream, across the endothelium, and into the arterial wall (Cook-Mills et al., 2011). Within the arterial wall, monocytes differentiate into macrophages, which internalize oxLDL and transform into foam cells. This transformation serves as the foundation for plaque development as foam cells release numerous inflammatory mediators within the blood vessel wall. The inflammatory response further enhances the incorporation of circulating T-cells and monocytes, amplifying arterial inflammation and inducing migration of vascular smooth muscle cells (VSMCs) from the tunica media into the sub-endothelial space. In this location, VSMCs undergo phenotypic changes, proliferate, and secrete extracellular matrix proteins that contribute to plaque expansion (Mehu et al., 2022).

Advanced human plaques exhibit distinct features, including a substantial necrotic core, a high abundance of lipid-laden and activated macrophages, a scarcity of VSMCs and their derived collagen, impaired efferocytosis, and ongoing inflammation. These characteristics are accompanied and followed by neovascularization within the plaque, hemorrhages, and the development of a fragile fibrous cap insufficiently separating the underlying thrombogenic plaque from the bloodstream (Kojima et al., 2017; Kavurma et al., 2017). The rupture of this fragile fibrous cap triggers intra-luminal thrombosis, leading to sudden arterial blockages and cessation of blood flow, causing life-threatening acute ischemic events such as myocardial infarction (MI), stroke, and sudden death. Furthermore, vessel wall calcification, involving the deposition of complexes containing calcium ions, is a parallel phenomenon that varies considerably across different clinical cohorts. Calcification holds particular significance, especially in the presence of diabetes, as it serves as a clinical indicator of atherosclerosis and contributes to the formation of blood clots and occlusion of blood vessels. Extensive research has been conducted to investigate the impact of calcification on plaque progression and vulnerability. The rigidity associated with extensive calcification reduces the suitability of affected vessels for stenting, which is the predominant treatment for coronary artery occlusion (Mughal et al., 2011).

Animal models play a crucial role in studying cholesterol and atherosclerosis as they provide valuable insights into the pathogenesis, progression, and potential treatments for these conditions (Jenkins et al., 2019). Genetically modified mice are widely used due to their genetic similarities to humans and their ability to develop atherosclerotic lesions. Several strains of mice with specific genetic alterations are available, such as ApoE-deficient mice and LDL receptor-deficient mice (Poznyak et al., 2020). These strains are prone to developing hypercholesterolemia and atherosclerosis when fed a high-fat diet. Rabbits are particularly useful for studying diet-induced atherosclerosis, as they are highly susceptible to atherosclerotic plaque formation when fed a cholesterol-rich diet. Rabbits can develop complex, advanced lesions that closely resemble those found in humans, making them valuable for studying plaque progression and regression (Fan et al., 2015). Non-human primates, such as monkeys, share genetic and physiological similarities with humans, making them valuable for studying the effects of cholesterol and atherosclerosis in a more clinically relevant context. However, the use of non-human primates is relatively limited due to ethical considerations and the high costs associated with their maintenance (Phillips et al., 2014).

These animal models provide researchers with the ability to investigate various aspects of cholesterol metabolism, atherogenesis, and therapeutic interventions. They allow for controlled experiments and enable the assessment of disease progression, lipid metabolism, immune responses, and the effects of novel drugs or interventions on atherosclerosis (Zhang et al., 2021). Nonetheless, it's important to recognize that while animal models provide valuable insights, the findings should be carefully extrapolated to human conditions, as there may still be differences between species in terms of disease progression and treatment response (Swearengen, 2018; Storey et al., 2021). Understanding atherosclerosis pathology justifies the need for diverse animal models because it is a complex disease influenced by a wide range of genetic, environmental, and lifestyle factors. By using diverse animal models, researchers can better replicate the heterogeneity of human populations and understand how different genetic backgrounds, diets, and environments contribute to atherosclerosis development. This allows for a more comprehensive study of the disease and helps in the development of more effective preventive

and therapeutic strategies. Additionally, different animal models can help researchers understand specific aspects of atherosclerosis, such as the role of inflammation, lipid metabolism, or vascular function, providing a more holistic view of the disease process. This diverse approach ultimately enhances our understanding of atherosclerosis and aids in the development of more personalized and targeted treatments.

While animal models, including pigs, have contributed significantly to our understanding of various diseases, including atherosclerosis, there are several limitations and challenges in extrapolating findings from these models to human conditions. Differences in physiology and genetics: Animals and humans have differences in physiology, metabolism, and genetics that can impact how diseases develop and progress. For example, the lipid metabolism in pigs may not fully mirror that of humans, potentially leading to variations in the formation and progression of atherosclerotic lesions (Lee et al., 2017). Response to interventions: The way animals respond to interventions, such as pharmaceutical drugs or surgical procedures, can differ from human responses. This can lead to misleading results when testing potential treatments for atherosclerosis and other conditions (Bray et al., 2020). Complexity of human diseases: Human diseases often have multifaceted causes and manifestations that may not be completely captured in animal models. Atherosclerosis, for instance, can be influenced by a wide range of genetic, environmental, and lifestyle factors, making it challenging to completely replicate in animal models (Spronk et al., 2018). Translation to clinical trials: While animal studies can provide valuable insights, the translation of findings to successful clinical trials in humans is complex. Many potential treatments that show promise in animal models fail to demonstrate the same efficacy or safety in human trials. Ethical and regulatory considerations: There are ethical and regulatory challenges involved in the use of animal models, requiring careful consideration of animal welfare and adherence to guidelines for humane research practices. Despite these limitations, animal models, including pigs, remain crucial for preclinical research and testing. However, it's important to recognize the challenges in extrapolating findings from these models to human conditions and to approach translation to clinical practice with careful consideration and acknowledgment of these limitations.

2. Methodology

2.1 Literature Review:

A comprehensive literature search was conducted using electronic databases such as PubMed, MEDLINE, and Google Scholar. Keywords including "atherosclerosis," "swine model," "pig model," "hyperlipidemia," "atherosclerosis regression," and "cellular models" were used to identify relevant articles published up to the present. The search was limited to English-language articles and encompassed original research studies, review articles, and meta-analyses.

2.2 Selection Criteria:

Articles were screened based on their relevance to the utilization of swine models, including natural and genetically modified pigs, in atherosclerosis research. Additionally, literature discussing the advantages, limitations, and potential applications of swine models in the context of atherosclerosis and hyperlipidemia studies was included. The search also encompassed material highlighting less typical models, such as pigs, and emerging cellular models in atherosclerosis research.

2.3 Data Synthesis and Analysis:

Data extracted from the identified literature were systematically synthesized to provide a comprehensive overview of the physiological similarities between swine and humans, with a specific focus on atherosclerosis and hyperlipidemia. The advantages and limitations of swine models in the context of atherosclerosis research were critically evaluated, and key findings were synthesized to highlight the potential contributions of swine models to advancing our understanding of atherosclerosis pathogenesis and treatment. Furthermore, emerging cellular models and their relevance to atherosclerosis research were examined to provide a holistic perspective on the subject.

2.4 Comparative Analysis:

A comparative analysis was conducted to juxtapose the utility of swine models with traditional small animal and cellular models in atherosclerosis research. This involved evaluating the translational relevance, pathophysiological alignment, and unique contributions of swine models and less typical models to elucidating novel facets of atherosclerosis biology.

The rigor and comprehensiveness of the literature review underpin the findings and conclusions drawn in this review article, shedding light on the underexplored potential of swine models and alternative cellular systems in atherosclerosis research.

2.5 Small versus Large Animal Models

Small animal models, primarily rodents and rabbits, are commonly employed in studies on atherosclerosis and thrombosis due to various factors. These include their cost-effectiveness, wide availability, fewer ethical concerns compared to larger animals (especially nonhuman primates), and their diminutive size, which reduces the amount of agents required for in vivo screening. These traits enable the rapid assessment of new agents on a sufficiently large number of animals (Lee et al., 2017). The utility of intravital microscopy in murine models of thrombosis is noteworthy as it allows for the study of microvascular thrombosis in structures like

mesenteric arteries, providing valuable insights into the characterization of specific cell types involved in thrombus formation and the evaluation of the effects of various inflammatory pathological conditions, such as obesity, on vascular reactivity (Bray et al., 2020). Additionally, the dynamics of thrombus formation can be observed with exceptional resolution in these thin, transparent vessels, offering insight into the specific cellular and molecular interactions in developing thrombi. Furthermore, rodent models present numerous opportunities for immunological approaches, including the use of monoclonal antibodies. Genetically modified mouse models with specific defects in platelet function facilitate the identification of individual roles and interactions of platelet proteins in thrombus formation (Spronk et al., 2018).

However, studies using small animal models may yield conflicting results. For instance, studies involving mice with low tissue factor (TF) levels by different groups have led to opposing conclusions about the respective source of TF in vascular thrombus formation (Kretz et al., 2010). One group highlights the contribution of vascular-associated TFs, while the other emphasizes the significant thrombogenic stimulus from blood-borne TFs. Other studies in large animal models of thrombosis have supported the hypothesis that TFs formed in the vessel wall are a major contributor to arterial thrombus formation and propagation. However, blood-borne TFs may also contribute, depending on the trigger lesion and shear rate (Zwicker et al., 2011). Hence, it is important to consider the results of microvascular and macrovascular models of thrombosis together. For this purpose, it is necessary to complement rodent microvascular models of thrombosis with macrovascular models more akin to human coronary, carotid, and cerebral arteries. Large animal models allow a more precise representation of the type of thrombus formation associated with clinical complications of cardiovascular disease, such as acute myocardial infarction (AMI) and stroke (Diaz et al., 2012).

Nonetheless, it is crucial to acknowledge the disparities between rodents and humans, particularly concerning their physiological traits and platelet quantities, which can significantly influence the relevance and interpretation of research outcomes. One prominent difference lies in the contrasting lipid physiology of rodents and humans. Mice, for example, possess a lipid profile that differs from humans, with the majority of their cholesterol being transported in high-density lipoprotein (HDL)-like particles (Masson-Meyers et al., 2020). In contrast, humans primarily transport cholesterol in low-density lipoprotein (LDL) particles. This dissimilarity in lipid metabolism can impact the development and progression of atherosclerosis in rodent models, often necessitating genetic modifications to induce atherosclerosis in mice. Another noteworthy difference is the variation in platelet quantities between rodents and humans (Oppi et al., 2019). Mice typically exhibit a higher platelet count compared to humans, with approximately two to three times more platelets per microliter of blood. This difference in platelet numbers can influence the dynamics of thrombus formation and the interpretation of experimental results. Moreover, the characteristics of platelets themselves, such as size, receptor expression, and response to activation, may vary between rodents and humans, further complicating the translation of findings from rodent models to human conditions (Nagy et al., 2019).

Similarities between primates and humans offer valuable insights into the pathophysiology of atherosclerosis and thrombosis. Primates share a significant degree of genetic and physiological similarity with humans, extending to the structure and function of the cardiovascular system, including the composition of blood vessels, platelet characteristics, lipid metabolism, and clotting mechanisms (Jebari-Benslaiman et al., 2022). These shared features enhance the translational relevance of primate studies. Primates possess an immune system comparable to humans, allowing researchers to investigate immune-mediated processes involved in atherosclerosis and thrombosis. This includes studying the role of inflammatory cells, cytokines, and immune responses in the development and progression of these cardiovascular conditions (Varki et al., 2009). Moreover, primates, particularly those kept in controlled research settings, can be exposed to diets and lifestyle factors similar to those of humans, enabling the study of the impact of specific dietary components, such as cholesterol and fat, as well as lifestyle factors like physical activity and stress, on the development of atherosclerosis and thrombosis (Matei et al., 2022).

Hyperlipidemia and Atherosclerosis in Pigs

Pig Lipoproteins

The pig is often used as an experimental model for atherosclerosis due to its physiological and cardiovascular similarities to humans. In pigs, LDL and HDL are the primary plasma lipoproteins. Unlike in humans, pig HDL exhibits greater homogeneity in size and density. However, similar to humans, pig HDL is composed of a complex mixture of approximately 250 lipids and over 500 protein components, as confirmed by mass spectrometry (Tsang et al., 2016). Although pigs lack the cholesteryl ester transfer protein (CETP), they can transfer cholesteryl ether unidirectionally from HDL to LDL. Unlike humans, pigs do not possess the apoE allelic variants but do express apoB100 exclusively in the liver, with pig enterocytes demonstrating efficient apoB mRNA editing similar to humans. Hypercholesterolemia in pigs can be induced through dietary changes or occur naturally due to genetic variations (Lanfranco et al., 2020).

Feeding pigs a diet rich in cholesterol and fat leads to the emergence of β-VLDL, a cholesterol-rich lipoprotein containing apoE, and high-density cholesterol-rich lipoprotein (HDLc) as a result of elevated LDL levels. Studies conducted on Yorkshire minipigs fed cholesterol-rich diets revealed the presence of two HDL subspecies, HDL1 and HDL2, with plasma cholesterol and LDL subspecies showing variations based on dietary cholesterol content (Harris et al., 2004). Genetic hyperlipidemia in pigs with naturally occurring apoB100 and LDL receptor allelic variants has been explored through selective breeding, resulting in diverse pig strains worldwide. Studies examining apoB allotypic epitopes have identified numerous variants, including functionally significant ones. Additionally, rare allotypic variants in other LDL-associated apoproteins have been observed. Significant variations in Lpb allotypes exist among different pig species, with Lpb5 associated with progressive atherosclerosis due to elevated LDL levels (Vaseghi et al., 2021).

Pigs carrying the Lpb5 allele also exhibit allotypic Lpr and Lpu lipoprotein antigens. However, the presence of the Lpb5 allele alone does not contribute to phenotypic heterogeneity. Distinct subtypes, such as Lpb5.1 and Lpb5.2, are distinguished by their severity of hypercholesterolemia. LDL from Lpb5 pigs is more buoyant compared to LDL from normal lipidemic pigs, and this disparity is influenced by the Lpu unidentical apoprotein allelic variant. Fractional catabolic rate (FCR) studies revealed that LDL from Lpb5 pigs exhibits lower sensitivity to the LDL receptor, leading to reduced clearance compared to normal LDL (Aiello et al., 1994). Notably, a mutation in the LDL receptor, involving an arginine-to-cysteine substitution at position 84, was identified in Lpb5 pigs, suggesting the interplay between apoB and LDL receptor mutations in hypercholesterolemia genesis. Although familial hypercholesterolemia is primarily associated with the Lpb5 allotype, other allotypes may also contribute to hypercholesterolemia. Variability was observed in three families with high, intermediate, and normal cholesterol levels, where LDL cholesterol, apoB, apoE, and apoC-III levels displayed a gradient. In addition to naturally occurring genetic models, atherosclerotic pig models have been developed through genetic manipulation of the apoE and LDL receptor genes, which will be further discussed in subsequent sections (Widhalm et al., 2007).

Diet-Induced Atherosclerosis

Pig models have proven to be valuable in studying atherosclerosis due to the similarities in the morphology and development of atherosclerotic lesions compared to humans. These lesions in pigs display complex characteristics such as necrotic cores, neovascularization, calcification, and thin fibrous caps. The distribution of atherosclerotic plaques in the vascular tree, including the coronary arteries, closely resembles that seen in humans, which is clinically significant (Mushenkova et al., 2019). The focal development of lesions in pigs has been examined by assessing the high permeability of lesion-prone areas of the aortic arch using Evans blue dye in Yorkshire-Landrace pigs fed a diet rich in cholesterol and lard. The areas stained blue, indicating increased permeability, appear even before gross or microscopic detection of lesions. Within 2 to 6 weeks of consuming the atherogenic diet, there is enhanced adhesion of blood monocytes to the endothelium in the blue areas compared to the less permeable white areas of the hyperlipidemic pigs' aortas and the blue areas of the normolipidemic pigs' aortas (Richardson et al., 1982).

Immunohistochemical analysis reveals the presence of ApoB in the extracellular areas of the blue stained areas but not in the white areas or blue areas of the normolipidemic pigs' aortas. By 12 weeks of the diet, the monocytes primarily reside within the intima, which is thickened with edema and contains collagen and elastin. The endothelial cells covering the blue areas show a distinctive profile with short, cuboidal morphology and a relatively high abundance of endoplasmic reticulum and lysosomes, in contrast to the flattened, elongated profile observed in normal endothelium (Alban et al., 2015). Extended periods of feeding this pig model have demonstrated the presence of more advanced lesions in various arterial regions, including the infrarenal abdominal aorta, aortic arch, carotid arteries, and proximal portion of the coronary arteries, although their presence can vary. After 6 months of feeding, advanced coronary lesions exhibit extracellular lipid pools, lipid-rich necrotic cores, a thin fibrous cap, macrophages, and calcification. Inflammation markers are also evident in the coronary artery lesions (Gertz et al., 2013).

The differential permeability observed in the aortic arch using Evans blue dye, influenced by hemodynamic forces, provides an excellent opportunity to explore the genetic control of early atherogenesis using advanced techniques such as single-cell RNA sequencing. Diabetes, a prevalent risk factor in the progression of atherogenic vascular disease, has been investigated in the Yorkshire pig model by inducing type 1 diabetes using streptozotocin at the initiation of a high-fat diet. Although diabetes does not alter plasma cholesterol levels, it leads to increased plasma triglyceride levels (Chiu & Chien, 2011). After 12 weeks of the diet, the area of the aorta stained by Sudan IV, indicating neutral lipids, is twice as extensive in diabetic animals compared to non-diabetic animals. Additionally, the aortas of diabetic animals contain twice as much cholesterol. Coronary lesions were predominantly observed in the proximal segment of the artery, occurring within the first 2-3 mm, and by 20 weeks of the diet, the extent of coronary artery stenosis was significantly greater in diabetic vessels (Ho et al., 2022).

Utilizing the same diabetic pig model, researchers investigated the correlation between coronary lesions and hemodynamics. Areas characterized by persistent low shear stress on the endothelium, as determined through angiography, intravascular ultrasound (IVUS), and computational hemodynamics, exhibited eccentric thin-capped plaques. These lesions displayed reduced smooth muscle cells that transitioned phenotypically toward myofibroblasts, decreased expression of procollagen type I mRNA and collagen content, and increased levels of mRNA and proteins associated with various metalloproteinases (Papafaklis et al., 2015).

Regarding the mechanisms underlying early atherogenesis, Gerrity employed electron microscopy to demonstrate the migration of monocytes into the intima and their transformation into foam cells. Upon LDL entry into the intima, it undergoes oxidation facilitated by intimal cells, subsequently promoting its uptake by scavenger receptors on lesion macrophages (Thim, 2010). The degree of LDL oxidation within the lesion was assessed in minipigs fed a cholesterol-enriched diet for 6, 14, and 24 weeks. Despite no further increase in plasma LDL levels after 6 weeks of the diet, LDL continued to accumulate within the intima. By the 24th week of the diet, nearly all LDL present in the intima of the left anterior descending coronary artery was oxidized, as quantitatively measured through immunohistochemistry, and was associated with monocytes/macrophages (Ludvigsen et al., 2015).

As the lesion progresses, the artery undergoes compensatory enlargement, a phenomenon previously described by Glagov and colleagues in human coronary arteries (Glagov et al., 1997). The Wilensky group examined lesions in hypercholesterolemic type 1 diabetic pigs after 1, 3, 6, and 9 months of the diet (Wilensky et al., 2008). However, it is worth noting that the diet included cholate to enhance hypercholesterolemia. Notably, no lesions were observed after 1 month of the diet. By 3 months, a significant proportion of the coronary arteries (more than half) and the thoracic artery (90%) exhibited lesions, whereas only 20% of the carotid arteries had lesions. By 6 months and beyond, all samples from the coronary arteries and thoracic artery showed lesions. By 9 months, the coronary arteries displayed the most complex and advanced lesions. Gene expression analysis was performed on 59 genes known to be involved in atherosclerosis and plaque stability or influence atherogenesis (Osborn & Jaffer, 2013). After 3 months of the diet, genes related to cholesterol metabolism, insulin response, and inflammation were significantly upregulated, with a more pronounced increase observed in the coronary artery compared to the carotid and thoracic aortas. This study provided dynamic evaluation of lesion progression and gene expression by examining three vascular sites at four different time points. However, it had limitations, including the assessment of histology and gene expression in different arterial samples and the use of whole arteries for gene analysis, which may not reflect differences between lesion and non-lesion areas (Fahed et al., 2022).

Nonetheless, this model offers valuable insights with the potential for further application of advanced methodologies. Studies demonstrated that type I diabetes increased atherosclerosis in hypercholesterolemic pigs. The metabolic syndrome, characterized by obesity, impaired glucose tolerance, insulin resistance, and hypertension, is often associated with atherosclerosis. Ossabaw minipigs exhibited features of metabolic syndrome and coronary microcirculatory dysfunction when fed an atherogenic diet (Zhang & Lerman, 2016). Atherosclerosis was more diffuse in male Ossabaw minipigs, extending throughout the coronary vessels, while in Yucatan minipigs, lesions were mainly confined to the proximal portion of the left anterior descending artery. The inclusion of cholate in the diet may introduce confounding factors, but similar outcomes were observed in female Ossabaw pigs without cholate (Susser & Rayner, 2022). The Glagov phenomenon, characterized by compensatory expansion of coronary arteries, was observed in Ossabaw pigs fed the cholate-containing diet for 4 months (Bonthu et al., 1997).

Different pig strains have been used in diet-induced atherosclerosis studies, including Gottingen minipigs, domestic Swedish Landrace swine, Yucatan pigs, Ossabaw pigs, and Bama pigs. Variations in lesion distribution and severity among these strains highlight the importance of genetic background in atherosclerosis development. Gender is also a modulator of atherosclerosis, with studies predominantly conducted in male pigs. In the comparison of male and female Gottingen minipigs, abdominal aortic lesions were similar between the sexes, but the female pigs exhibited higher cholesterol content in their coronary arteries despite initially higher LDL levels (Matthan et al., 2018).

2.2.2 Natural and Genetically Engineered Mutant Pigs

Pig models of atherosclerosis can be induced through genetic mutations or genetic engineering, in addition to dietary induction. The Rapacz familial hypercholesterolemia model, characterized by the Lpb5 allele and Ldlr R84C mutation, is one such genetic model. Lesion development in this model is relatively slow when low-fat chow is provided, with lesions appearing in the coronary arteries and peripheral iliac and femoral arteries by 12 months of age (Artinger et al., 2009). Over time, the coronary lesions become stenotic and exhibit necrotic cores, calcium deposits, cholesterol clefts, and neovascularization by 2 years. The peripheral arteries, on the other hand, become smooth muscle-rich and fibrous. By 3 years, the coronary lesions become even more complex with fibrous caps, extensive vascularization, and possible signs of rupture. The

complexity of these lesions correlates with the duration and severity of hypercholesterolemia (Stefanadis et al., 2017). However, this model requires a prolonged time course for lesion development, which can be costly for experimental purposes. To address this, the model has been downsized by crossing into the Gottingen minipig.

In recent years, understanding of LDL receptor regulation has advanced with the discovery of PCSK9, a protease that is primarily secreted from the liver and acts to degrade the LDL receptor. PCSK9 binds to the LDL receptor, leading to its lysosomal degradation rather than recycling back to the cell surface. This results in reduced receptor density on the cell surface and impaired clearance of plasma LDL (Farnier, 2013). Variants of PCSK9 with gain of function (GOF) or loss of function (LOF) have been identified. A major GOF variant involves the substitution of aspartic acid 374 with tyrosine in the catalytic domain of human PCSK9, leading to enhanced degradation of LDL receptors and increased plasma LDL levels (Sarkar et al., 2022).

Taking advantage of this knowledge, researchers have generated transgenic Yucatan minipigs expressing PCSK9-D374Y, a GOF variant, under the control of a liver-specific promoter to induce hypercholesterolemia. These transgenic pigs were fed a diet rich in lard and supplemented with cholesterol for up to 1 year to further increase cholesterol levels. Atherosclerosis was observed in the left anterior descending coronary artery, aorta, and iliofemoral arteries of the transgenic pigs compared to nontransgenic pigs. Histological examination of the most advanced lesion in each vascular site revealed extensive lesions in the coronary artery, characterized by necrotic cores, intraplaque vascularization, hemorrhage, and calcification (Yuan et al., 2018).

The adeno-associated virus (AAV) approach to LDL receptor reduction in pigs involves the use of AAV vectors to deliver gene therapies that target the LDL receptor pathway. AAVs are small, non-pathogenic viruses that have been widely studied and utilized for gene delivery due to their ability to efficiently infect various cell types and exhibit long-term gene expression (Wang et al., 2019). In the context of LDL receptor reduction, the AAV approach aims to modulate the expression or function of the LDL receptor gene in pig models.

One strategy is to introduce a specific gene sequence into the AAV vector that can interfere with the normal expression or activity of the LDL receptor. This could involve the expression of RNA molecules, such as small interfering RNA (siRNA) or short hairpin RNA (shRNA), that can bind to the LDL receptor mRNA and inhibit its translation or promote its degradation (Somanathan et al., 2014). Another approach is to introduce a therapeutic gene into the AAV vector that encodes a modified LDL receptor or a related protein capable of reducing LDL cholesterol levels. This therapeutic gene can be designed to enhance LDL receptor activity or to mimic its function, thereby promoting the uptake and clearance of LDL particles from the bloodstream (Jiang et al., 2018).

Once the AAV vector carrying the desired genetic material is administered to the pigs, it can target specific cells, such as hepatocytes in the liver, which play a crucial role in LDL metabolism. The AAV vector delivers the therapeutic gene or RNA molecules, allowing for sustained expression or activity within the target cells. By modulating the LDL receptor pathway, this approach aims to reduce LDL cholesterol levels in pigs, mimicking aspects of human cholesterol metabolism and providing insights into the development and treatment of atherosclerosis (Maestro et al., 2021).

In studies involving the AAV approach to LDL receptor reduction in pigs, apoE is often considered due to its interaction with the LDL receptor. The expression or function of apoE may be modulated to influence LDL receptor activity or cholesterol metabolism. For example, some studies may aim to overexpress apoE in combination with LDL receptor modulation to enhance LDL clearance and reduce LDL cholesterol levels further. Additionally, certain genetic variations or mutations in the apoE gene can affect its function and lead to altered cholesterol metabolism. The apoE4 variant, for instance, has been associated with increased LDL cholesterol levels and an increased risk of atherosclerosis (Tsang et al., 2016).

2.2.3 Atherosclerosis Regression

Experimental atherosclerosis research has two primary objectives: understanding the disease's mechanisms and pathogenesis and exploring potential reversal methods. Several studies have examined the regression of diet-induced atherosclerosis in pigs and factors correlated with this process (Gimbrone & García-Cardeña, 2016). For instance, Gottingen minipigs were induced with hypercholesterolemia using a diet containing 15% beef tallow and 1.5% cholesterol for 6 months, followed by a switch to a standard chow diet for an additional 9 months (Kobari et al., 1991). Blood cholesterol levels quickly returned to baseline, and fatty streak lesions in the thoracic aorta regressed, although fibrous plaques in the abdominal aorta remained resistant to regression. Another study investigated the effect of fish oil or its constituent polyunsaturated fatty acids (PUFAs) on lesion regression in Yucatan minipigs, finding significant reductions in fatty streak lesions in the ascending aorta, thoracic aorta, and carotid arteries after a regression period with a standard diet, with or without fish

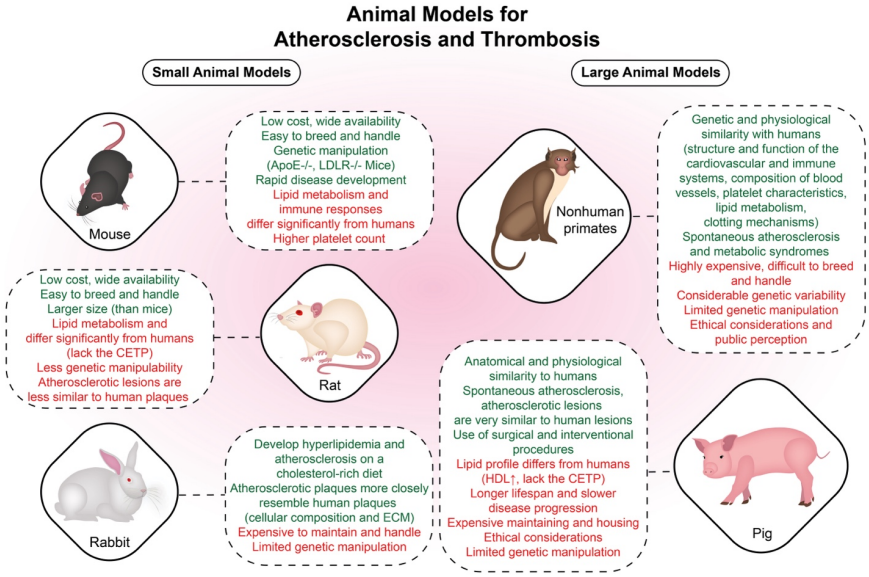

Figure 1. Overview of Small and Large Animal Models for Atherosclerosis and Thrombosis. Abbreviations: LDLR-/- low-density lipoprotein receptor-deficient; ApoE-/- apolipoprotein E-deficient; CETP cholesteryl ester transfer protein; HDL high density lipoprotein; ECM extracellular matrix.

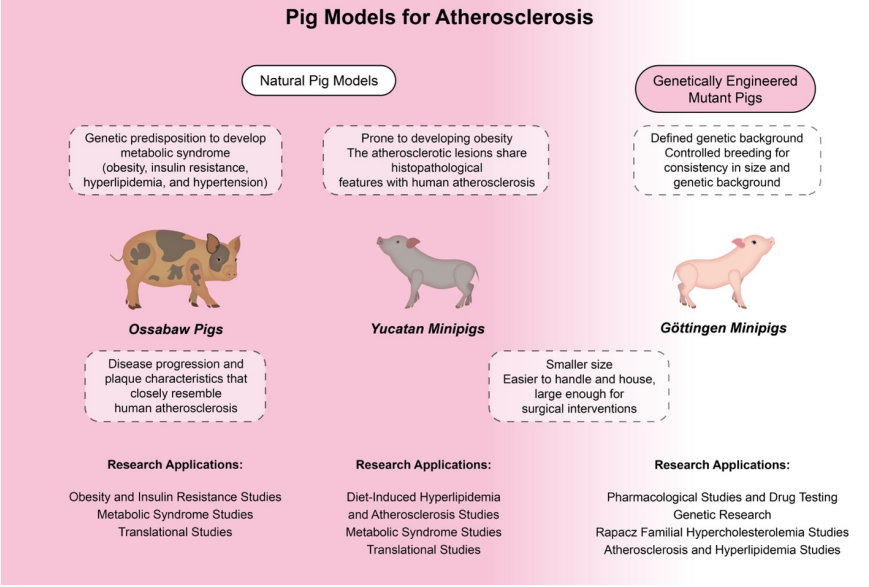

Figure 2. Characteristics and Research Applications of the Most Common Natural and Genetically Engineered Pig Models for Atherosclerosis.

oil supplementation (Linton et al., 2019). However, the ratio of n-3 to n-6 fatty acids in the diet did not influence these changes, and the reduction in coronary artery lesions did not reach statistical significance (Zhao et al., 2021).

Overall, the minipig model is valuable for studying experimental atherosclerosis, particularly with advancements in molecular approaches for genetic modulation without extensive crossbreeding. However, it is important to note that human atherosclerotic cardiovascular disease is a complex, multifocal vascular disease that develops over a lifetime, which makes it challenging to precisely simulate the timescale of disease progression (Getz & Reardon, 2022).

Conclusion

In conclusion, this review provides an extensive examination of the utility of swine models in advancing our understanding of atherosclerosis and hyperlipidemia, shedding light on their relevance to translational research and potential contributions to therapeutic development. By systematically evaluating the physiological, pathological, and genetic similarities between swine and humans, we have demonstrated that swine models offer a unique platform for studying atherosclerosis pathogenesis, regression, and therapeutic interventions. Moreover, we have critically assessed the advantages and limitations of swine models in comparison to traditional small animal models and emerging cellular systems, emphasizing the need for a comprehensive approach to atherosclerosis research.

The novelty of this review lies in its comprehensive analysis of the key features that make swine models an invaluable tool in atherosclerosis research. By assimilating and synthesizing the available literature, we have highlighted the potential of swine models to bridge the translational gap between preclinical studies and clinical outcomes, thereby accelerating the translation of experimental findings into meaningful clinical applications. This review not only consolidates existing knowledge but also identifies unexplored avenues for utilizing swine models in addressing critical gaps in our understanding of atherosclerosis, ultimately opening new possibilities for therapeutic innovation and personalized medicine.

Synthesizing the results with relevant literature, we reaffirm that swine models mirror the multifaceted aspects of human atherosclerosis and hyperlipidemia, thereby offering a reliable platform for studying disease progression and evaluating potential therapeutic strategies. Our critical analysis suggests that swine models provide unique opportunities to investigate complex pathophysiological mechanisms, such as the interplay between lipid metabolism, inflammation, plaque stability, and interventional devices, with implications for designing more effective interventions.

Furthermore, by exploring the potential mechanisms underlying the observed associations between swine models and human atherosclerosis, this review underscores the relevance of the model in recapitulating intricate disease features, including plaque composition, vulnerability, and response to therapeutic interventions. The anatomical and physiological similarities between swine and humans, coupled with the ability to induce atherosclerosis through various dietary and genetic manipulations, position swine models as indispensable for unraveling the complex interplay of genetic, environmental, and lifestyle factors that drive atherosclerosis progression.

Looking ahead, future research should delve into refining the use of swine models to investigate the efficacy and safety of novel therapeutic modalities, including gene editing, targeted drug delivery, and regenerative medicine approaches. Building upon the foundation laid by previous studies, efforts should be directed toward elucidating the interventional potential of swine models in evaluating the clinical relevance of emerging diagnostic modalities, such as imaging techniques and biomarker identification, to guide personalized atherosclerosis management.

Moreover, the integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, in swine models holds promise for unraveling the molecular underpinnings of atherosclerosis, identifying potential disease modifiers, and devising precision medicine strategies tailored to individual risk profiles. Additionally, leveraging advanced imaging technologies, such as intravascular ultrasound, optical coherence tomography, and magnetic resonance imaging, in combination with swine models can provide invaluable insights into the dynamic aspects of plaque biology, aiding in the development of targeted therapies and precision interventions.

In conclusion, this review highlights the pivotal role of swine models in atherosclerosis research and emphasizes the need for continued exploration of their potential to inform clinical practice. By leveraging the strengths of swine models and integrating cutting-edge methodologies, we can unlock novel dimensions of atherosclerosis and propel the development of transformative interventions aimed at mitigating the burden of cardiovascular disease.

Please note that this conclusion is a generic example and may not align precisely with your specific review article. If you have a specific topic or findings in mind, feel free to provide more details, and I can tailor the conclusion to better match your review.

Author contributions

Original draft preparation was carried out by A.V.P., with V.N.S., D.F.B., M.A.P., A.V.C., A.A.L., T.I.K., and A.N.O. contributing to the review and editing process. All authors have thoroughly read and approved the final version of the manuscript for publication.

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Competing financial interests

The authors have no conflict of interest.

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