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Evaluating the Efficacy and Pathophysiology of 28-Day Diet-Induced Atherosclerosis Models: A Comprehensive Review

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ABSTRACT

Atherosclerosis is a chronic inflammatory disease that usually develops over a prolonged period. Rapid animal models are therefore valuable for studying early lesion formation, testing interventions, and evaluating vascular mechanisms within a practical experimental timeframe.This review examines 28-day or four-week diet-induced atherosclerosis protocols and closely related short-term models. The review considers dietary composition, animal species and strains, supplementary mechanical or chemical interventions, lipid outcomes, inflammatory responses, lesion morphology, histopathology, reproducibility, and translational relevance. Four-week protocols can produce marked dyslipidaemia, endothelial dysfunction, inflammatory activation, intimal expansion, macrophage accumulation, and early atherosclerotic-like lesions. The most rapid and reproducible outcomes are generally obtained when an atherogenic diet is combined with genetic susceptibility or a supplementary vascular injury.A 28-day diet-induced model is useful for investigating early atherogenesis and accelerated vascular responses. However, the protocol should be selected according to the scientific question because diet-only models, genetically susceptible models, and injury-assisted models do not reproduce identical aspects of human atherosclerosis.

1. Introduction

Atherosclerosis is a progressive arterial disease characterised by lipid accumulation, endothelial dysfunction, inflammatory-cell recruitment, smooth-muscle-cell responses, extracellular-matrix remodelling, and plaque development. Experimental animal models are essential for studying these processes and for evaluating candidate preventive or therapeutic interventions [1].

Conventional dietary models may require several months before clearly measurable lesions develop. Long protocols increase cost, animal use, experimental duration, and the possibility of confounding changes unrelated to the intended intervention. These limitations have encouraged the use of accelerated or short-term protocols designed to generate measurable vascular abnormalities within approximately 28 days [2].

A four-week protocol is not a single standardised model. Its effectiveness depends on the species, genetic background, diet composition, cholesterol concentration, presence of cholate or other dietary additives, baseline lipid metabolism, and use of a supplementary vascular injury. In susceptible mice and rabbits, carefully selected dietary or combined protocols can produce detectable lipid deposition and intimal changes within this period [3].

Many rapid protocols use genetically susceptible animals, including apolipoprotein E-deficient mice or LDL-receptor-deficient animals. Genetic susceptibility is frequently combined with a Western-type or cholesterol-enriched diet to intensify hypercholesterolaemia and inflammatory activation [4, 5]. Other studies add balloon injury, perivascular cuffing, endothelial denudation, laser injury, or local inflammatory stimulation to accelerate lesion formation. These interventions may be experimentally useful, but they also mean that the resulting phenotype represents diet-associated atherogenesis combined with tissue injury rather than diet alone [6].

This review evaluates the efficacy and pathophysiology of 28-day diet-induced atherosclerosis models. It distinguishes evidence from protocols specifically lasting approximately four weeks from evidence derived from related short-term models. The review focuses on model design, dietary protocols, efficacy outcomes, pathophysiological mechanisms, species and strain effects, biomarkers, histopathology, reproducibility, translational relevance, limitations, and future directions.

2. Review Methodology

The review was based on a combined scholarly source table containing 166 unique records retrieved from searches of SciSpace, Google Scholar, and PubMed. The search strategy covered 28-day, four-week, short-term, high-fat, high-cholesterol, cholesterol-enriched, Western-type, and atherogenic dietary protocols. Studies were considered particularly relevant when they reported experimental outcomes within approximately four weeks or used a short-term protocol designed to accelerate atherosclerotic lesion development.

Data of interest included animal species and strain, genetic status, diet composition, dietary duration, supplementary vascular interventions, plasma lipid changes, inflammatory markers, endothelial function, lesion area, intimal thickness, macrophage content, and histological features. Studies with longer durations were used only when they provided useful comparative information about lesion progression or model limitations. Protocols combining diet with balloon injury or other vascular injury were interpreted separately from diet-only models. A short-term ApoE-deficient rat protocol combining high-fat feeding and balloon injury illustrates the importance of this distinction because the diet and mechanical injury jointly contribute to the observed phenotype [7].

3. Model Design and Dietary Protocols

3.1 Dietary composition

Rapid diet-induced atherosclerosis requires a dietary challenge sufficiently strong to produce hypercholesterolaemia or other metabolic disturbances within a short period. Common approaches include high-fat diets, cholesterol-enriched diets, Western-type diets, and diets containing animal fat or margarine. In Wistar rats, a margarine-based diet has been used to produce dyslipidaemia and atherosclerotic-like changes over four weeks [8].

In genetically susceptible mice, Western-type diets are commonly used to intensify lipid accumulation and inflammatory signalling. ApoE-deficient mice are especially sensitive to dietary lipid loading, whereas the response of wild-type animals is generally slower and less consistent. In rabbits, cholesterol is often added to the diet in combination with a vegetable oil or another fat source. The magnitude of the response depends on cholesterol concentration, dietary fat, feeding duration, and the rabbit strain [3].

Diet composition should be reported in sufficient detail to permit replication. Important variables include total fat percentage, cholesterol concentration, type of fat, presence or absence of cholate, protein and carbohydrate composition, energy density, feeding schedule, and whether the diet is started before or after an intervention. A nominally similar label such as “high-fat diet” may represent substantially different metabolic challenges across laboratories.

3.2 Supplementary vascular interventions

Perivascular cuffing is one strategy for accelerating lesion formation in susceptible mice. Placement of a cuff around an artery produces local changes in haemodynamics, vascular permeability, and inflammatory recruitment. When combined with a cholesterol-rich diet in ApoE*3-Leiden mice, cuffing accelerates local intimal lesion development [1].

Balloon or catheter injury is another common strategy. Endothelial denudation exposes the subendothelial compartment and stimulates platelet activation, inflammatory recruitment, smooth-muscle-cell migration, and neointimal formation. In rabbits, dietary cholesterol combined with arterial deendothelialisation can produce substantial vascular lesions within a short period [3]. Similar injury-assisted protocols have been used in rats and genetically modified animals [7].

Some models use local inflammatory stimulation to produce more advanced plaque-like changes. In a rabbit iliac-artery model, local delivery of inflammatory proteins was used to promote advanced plaque characteristics within a short experimental period [9]. Such models may be valuable for investigating inflammation and plaque vulnerability, but they should not be interpreted as equivalent to a purely diet-induced model.

Angiotensin II infusion and other systemic or chemical stimuli may also accelerate lesion formation in susceptible mice. These approaches can increase vascular inflammation or alter haemodynamics, but the additional stimulus should be described explicitly because it changes the biological interpretation of the model [6].

4. Efficacy Outcomes

4.1 Lipid and metabolic outcomes

The most immediate measure of a successful four-week protocol is a reproducible metabolic response. Depending on species and genotype, the response may include increased total cholesterol, low-density lipoprotein cholesterol, triglycerides, non-high-density lipoprotein cholesterol, or circulating inflammatory mediators. In ApoE-deficient mice with severe hypercholesterolaemia, short-term dietary exposure has been associated with substantial plasma lipid elevation and accelerated atherosclerotic development [4].

Rabbits can also develop marked hypercholesterolaemia after several weeks of cholesterol-enriched feeding. The degree of elevation is influenced by the cholesterol dose, fat source, animal strain, and whether vascular injury is performed [3]. In rats, the response to a high-fat or margarine-based diet may

be more variable because wild-type rats are relatively resistant to spontaneous atherosclerotic lesion formation; therefore, lipid measurements should not be used as the only indicator of lesion development [7, 8].

4.2 Lesion development and morphometry

Morphometric endpoints are necessary to establish whether a metabolic response has translated into vascular pathology. Common outcomes include lesion area, intimal area, intima-to-media ratio, percentage of Oil Red O-positive surface, stenosis, macrophage-positive area, and plaque composition. In ApoE*3-Leiden mice, a cholesterol-rich diet combined with perivascular cuffing produced accelerated local intimal expansion [1].

In rabbits, deendothelialisation combined with dietary cholesterol can generate intimal thickening and lipid-rich lesions within approximately four weeks [3]. Injury-assisted models may also produce neointimal stenosis and fibrous plaque-like changes. For example, rabbit models using vascular injury have been developed to study stenosis and plaque disruption-related features [10].

A meaningful efficacy assessment should report the vascular site examined, the method used for lesion quantification, the denominator used for percentage calculations, the number of sections analysed, and whether measurements were performed blinded to treatment group. Without these details, comparisons across models are difficult.

5. Pathophysiology

5.1 Lipid retention and foam-cell formation

Atherogenic diets increase circulating apoB-containing lipoproteins or alter lipoprotein clearance. Retention and modification of these particles in the arterial intima promote endothelial activation and monocyte recruitment. Recruited monocytes differentiate into macrophages, internalise modified lipoproteins, and form foam cells. In short-term protocols, these changes may be observed before the development of a mature fibrous cap or a complex necrotic core.

5.2 Inflammation and immune responses

Inflammation is a central feature of rapid diet-induced models. Western-type feeding in ApoE-deficient mice has been associated with increased expression of IL-17-secreting T cells during early lesion progression [2]. In rats exposed to a margarine-based diet, increased inflammatory and oxidative-stress markers have also been reported [8]. Local inflammatory-protein delivery in rabbits can further intensify plaque inflammation and macrophage accumulation [9].

The inflammatory response is influenced by lipid burden, endothelial injury, innate immune activation, adaptive immune-cell recruitment, and the composition of the vascular microenvironment. Consequently, a model driven primarily by dietary hypercholesterolaemia may produce a different immune profile from a model driven by mechanical denudation or local cytokine stimulation.

5.3 Endothelial dysfunction and oxidative stress

Endothelial dysfunction is an early functional manifestation of atherogenesis. It may precede large visible lesions and can be assessed using vasorelaxation assays, nitric-oxide bioavailability, adhesion-molecule expression, oxidative-stress markers, or vascular permeability measurements. Short-term dietary models may therefore be useful even when lesion burden remains modest. In rabbit models, diet-associated endothelial dysfunction has been examined in relation to vascular injury and circulating or tissue-associated factors [11].

5.4 Neointimal growth and vascular remodelling

Mechanical injury promotes platelet deposition, inflammatory-cell recruitment, smooth-muscle-cell migration, extracellular-matrix deposition, and neointimal growth. This response may resemble selected components of human atherosclerosis but also overlaps with restenosis biology. The distinction is important when interpreting a four-week model that combines atherogenic feeding with balloon injury. Such a model may be highly effective for generating measurable stenosis while being less suitable for studying spontaneous plaque initiation.

6. Species and Strain Considerations

6.1 Mice

ApoE-deficient mice are widely used because they develop hypercholesterolaemia and atherosclerotic lesions more rapidly than many wild-type strains. Western-type feeding can increase the speed and magnitude of early lesion development, while perivascular cuffing can localise and accelerate pathology [1, 2, 4]. LDL-receptor-deficient models provide a complementary approach in which dietary cholesterol strongly influences circulating lipoproteins and lesion burden [5, 6].

6.2 Rats

Wild-type rats are relatively resistant to spontaneous diet-induced atherosclerosis compared with ApoE-deficient mice or rabbits. Specialised diets can nevertheless induce dyslipidaemia and early vascular abnormalities, particularly when combined with balloon injury. Genetically modified ApoE-deficient rats may provide a more responsive platform for short-term studies [7, 8].

6.3 Rabbits

Rabbits are useful for short-term atherosclerosis research because they respond to dietary cholesterol and have vessels large enough for deendothelialisation, balloon injury, local infusion, and repeated vascular assessment. Japanese White and New Zealand White rabbits have been used in rapid dietary and injury-assisted protocols [3, 9, 10]. Their response can be strong, but cholesterol feeding may produce non-physiological lipid concentrations unless the dietary dose is carefully selected.

6.4 Swine

Swine have greater anatomical and physiological similarity to humans than rodents and are particularly relevant for coronary interventions. LDL-receptor-deficient swine combined with balloon injury and a high-cholesterol diet have been used to generate accelerated coronary atherosclerosis [5]. Their cost, housing requirements, and ethical burden restrict routine use, but they may be valuable for translational validation.

7. Biomarkers and Histopathology

A robust 28-day study should combine systemic, functional, and tissue-level endpoints. Systemic endpoints include total cholesterol, triglycerides, LDL cholesterol, glucose, insulin, and inflammatory mediators. Vascular endpoints include endothelial-dependent relaxation, arterial stiffness, vascular reactivity, and thrombogenicity. Tissue endpoints include lesion area, intimal thickness, Oil Red O staining, macrophage markers such as CD68 or RAM11, smooth-muscle-cell markers, collagen, lipid-core characteristics, and inflammatory-cell distribution.

Histological interpretation should distinguish fatty streaks, early fibrocellular lesions, neointimal hyperplasia, fibrous plaques, and advanced or rupture-prone plaque-like structures. Macrophage accumulation may be prominent in both diet-only and injury-assisted models, but the distribution and morphology of macrophages can differ. Inflammatory-protein stimulation in rabbits, for example, has been used to increase macrophage-rich plaque features [9].

The choice of biomarker should match the biological question. A lipid endpoint is appropriate for assessing dietary efficacy, whereas macrophage content and cytokine expression are more informative for inflammatory mechanisms. A single biomarker should not be interpreted as proof of atherosclerosis without supporting morphological evidence.

8. Reproducibility and Translational Relevance

The principal strength of the 28-day model is experimental efficiency. It can produce measurable metabolic and vascular changes in a short period and can therefore support screening studies, mechanistic experiments, and pilot evaluations. However, reproducibility depends on standardising diet composition, animal age, sex, genotype, housing, microbiological status, feeding schedule, injury technique, sampling site, and histological analysis.

Standardisation is especially important because a four-week model may be described as “diet-induced” even when it includes a substantial mechanical or chemical component. Reports should therefore state the contribution of each component separately. Recommendations to improve reproducibility in animal atherosclerosis research include clearer reporting of protocol details, appropriate controls, prespecified endpoints, and consistent lesion quantification [12].

Translational relevance is also endpoint-specific. A diet-only mouse model may be useful for studying lipid-driven inflammation, whereas an injury-assisted rabbit or swine model may be more suitable for studying neointimal growth, stenosis, or device-related vascular responses. No single 28-day model reproduces the full natural history of human atherosclerosis.

9. Limitations

First, many four-week protocols generate early lesions rather than mature human-like plaques with a large necrotic core, thin fibrous cap, extensive calcification, and spontaneous rupture. Second, injury-assisted models introduce biological processes that may confound interpretation of diet-induced disease. Third, extreme cholesterol concentrations can produce pathology that is mechanistically useful but not fully representative of typical human exposure.

Fourth, species differences in lipid metabolism, lipoprotein distribution, immune responses, and arterial structure limit direct comparison. Fifth, the short duration may be insufficient to assess long-term plaque stability, regression, treatment durability, or cardiovascular events. Sixth, differences in diet

formulation and lesion quantification can produce apparently conflicting results even when the nominal protocol duration is identical.

10. Future Directions and Research Gaps

Future studies should report dietary composition in full and use harmonised definitions for a 28-day protocol. Comparative experiments should include diet-only, genetic-susceptibility, and injury-assisted groups whenever possible. Serial measurements of lipids, endothelial function, inflammatory mediators, and lesion development would help distinguish early metabolic changes from established vascular pathology.

Further work should also evaluate sex, age, strain, microbiome status, and genetic background as sources of variability. Advanced imaging, spatial transcriptomics, single-cell approaches, and longitudinal vascular measurements may improve mechanistic resolution. Translational studies should compare short-term model endpoints with human plaque features rather than assuming that a large lesion or high cholesterol concentration automatically indicates clinical similarity. Standardisation and transparent reporting remain essential for improving reproducibility [12].

11. Conclusion

A 28-day diet-induced atherosclerosis model can rapidly generate hypercholesterolaemia, endothelial dysfunction, inflammation, macrophage accumulation, intimal expansion, and early atherosclerotic-like lesions. Its efficacy is greatest when the diet, genetic background, species, and supplementary intervention are matched to the research question. Diet-only protocols are useful for studying metabolic and inflammatory initiation, whereas injury-assisted protocols are more effective for producing rapid neointimal growth or advanced plaque-like features. The model should therefore be described precisely rather than treated as a uniform experimental system. Careful protocol reporting, appropriate controls, multimodal endpoints, and strict distinction between diet-driven and injury-driven pathology are necessary to maximise scientific and translational value.

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