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Visualizing Atherosclerotic Burden in ApoE-/- Mice Treated with Multiple Doses of Celastrol via ORO en face Staining

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Abstract

Atherosclerosis is an inflammatory disease marked by lipid accumulation in large arteries, leading to cardiovascular disease and stroke. The ApoE$^{-/-}$ mouse is widely used to study plaque development and test interventions. This study aimed to assess the anti-atherosclerotic effect of celastrol using standardized Oil Red O (ORO) en face staining. ApoE$^{-/-}$ mice (n=24) on a high-fat diet were treated with varying doses of celastrol. ORO *en face* staining revealed significant plaque reduction in celastrol-treated groups. These findings suggest that celastrol may attenuate plaque development and warrant further mechanistic research to support its clinical translation in atherosclerosis treatment.

Keywords: atherosclerosis; celastrol; lipid; Oil Red O

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1.0 Introduction

Atherosclerosis is an inflammatory disease affecting the large arteries where the lipid-rich plaques accumulate within arterial walls. This progressive disease is the primary cause of cardiovascular disease (CVD) and stroke (Gusev & Sarapultsev., 2023). Murine models are often used to study the formation of plaque and the effects of interventions. ApoE-/- mice, widely recognized as models of atherosclerosis, exhibit impaired lipid clearance and accelerated plaque formation due to the absence of apolipoprotein E, making them valuable for evaluating potential therapies (Sijbesma et al., 2023). Current preclinical therapy of atherosclerosis has grown tremendously over the past few years, and celastrol is an addition to the list of prospective drugs showing plaque-attenuation activity. Celastrol, a

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quinone methide triterpenoid derived from the roots of *Tripterygium wilfordii*, exhibits a range of pharmacological properties, including anti-inflammatory, antioxidative, and lipid-lowering effects, making it a promising candidate for the treatment of atherosclerosis (Zhao et al., 2023). Nevertheless, it is essential to ascertain the optimum dose of celastrol in ApoE $^{-}$/$^{-}$ mice with a high-fat diet (HFD) to determine its therapeutic potential. In addition to this, the method of plaque visualization and quantification is essential in determining the accuracy and reproducibility of data to properly measure the atherosclerotic burden, especially in regions such as the root of the aorta and its branches, where the lesions typically develop early.

To determine the optimum dose of celastrol and its plaque visualization in ApoE-/- mice on a high-fat diet (HFD), a study was performed in ApoE-/- mice that were fed a high-fat diet for a 12-week duration and co-treated with a few doses of celastrol during the last 4 weeks of a high-fat diet. Celastrol is hypothesized to reduce the atherosclerotic burden in ApoE-/- mice.

This experiment was carried out to elucidate the optimum dose of celastrol that can attenuate plaque by measuring the percentage of plaque clearance attenuated compared to a positive control disease model with vehicle treatment using Oil Red O (ORO) *en face* staining. By investigating the plaque incidence in ApoE-/- mice triggered by a high-fat diet in the celastrol-treated and control groups, we can better understand the role of celastrol in the intervention of atherosclerosis.

2.0 Literature Review

Atherosclerosis is the leading cause of cardiovascular disease (CVD) worldwide. It is a chronic, progressive inflammatory condition marked by the hardening and thickening of arterial walls due to excessive cholesterol and lipid accumulation in medium- and large-sized arteries. The pathogenesis of atherosclerosis involves endothelial dysfunction, lipid retention, immune cell infiltration, and plaque formation. Over time, these plaque deposits can rupture, leading to thrombosis, which is the primary trigger for clinical manifestations such as stroke and myocardial infarction (Gusev & Sarapultsev., 2023).

Cardiovascular disease, driven largely by atherosclerosis, is the most prevalent life-threatening condition globally, significantly affecting quality of life and contributing to high rates of morbidity and mortality. In Malaysia, as in many other countries, CVD remains a critical public health issue. According to the World Health Organization (WHO), cardiovascular diseases account for an estimated 17.9 million deaths annually, making them the leading cause of death worldwide.

2.1 Celastrol as an Intervention for Atherosclerosis

Based on current literature, the mechanisms of atherosclerosis are well studied; current treatments focus largely on controlling risk factors such as hypertension and hyperlipidemia to prevent clinical complications (Aziz & Yadav, 2022). These approaches, although effective at reducing overall cardiovascular risk, do not directly target the inflammatory processes central to disease progression. Therefore, there remains a pressing need for new therapeutic strategies that address these underlying mechanisms.

To fill the current gap, one promising compound under investigation is celastrol, a triterpenoid extracted from *Tripterygium wilfordii* (Thunder God Vine), a plant commonly used in traditional Chinese medicine. Celastrol has demonstrated strong antioxidant and anti-inflammatory properties, making it a potential therapeutic candidate for inflammatory diseases, including atherosclerosis (Li et al., 2022). It suppresses the NF-κB pathway, thereby inhibiting the production of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. In addition, celastrol reduces oxidative stress by neutralizing reactive oxygen species (ROS), contributing to improved endothelial function and decreased lipid oxidation—two critical components in the pathogenesis of atherosclerosis.

Despite its therapeutic potential, celastrol's clinical application is complicated by several pharmacological challenges. Among the challenges is the need to determine an optimal dose that maximizes efficacy while minimizing toxicity. Celastrol exhibits a narrow therapeutic window. While low doses may be insufficient to produce significant effects, high doses or long-term use can result in toxicity, including gastrointestinal disturbances and potential hepatic or renal injury. Therefore, dose optimization is essential to ensure both safety and therapeutic efficacy.

Pharmacokinetic limitations also restrict celastrol's clinical use. The compound has poor water solubility, which affects its absorption and systemic bioavailability (Cascão et al., 2017). It is primarily metabolized in the liver and excreted via the feces and urine. These characteristics complicate its delivery and limit its effectiveness, particularly when administered orally. To address these issues, future studies should explore advanced drug delivery systems or formulation techniques that improve solubility and targeted distribution.

Animal models, such as the ApoE-/- mouse, have been widely used to evaluate celastrol's anti-atherosclerotic effects. Studies using these models have demonstrated significant reductions in plaque burden and inflammatory markers following celastrol treatment (Gu et al., 2013). However, variability in dosing regimens and administration methods across studies makes it difficult to draw standardized conclusions about the optimal therapeutic dose. Hence, it is important to determine the optimum dose to prevent toxicity while still gaining positive effects (Sun et al., 2024).

2.2 *ApoE-/- Knockout Mice (ApoE-/-) as Atherosclerotic Animal Model*

Atherosclerosis in humans has a complicated pathogenesis. These processes, which result in immunometabolic dysregulation, are driven by several risk factors, including aging, hyperlipidemia, hypertension, and diabetes (Libby, 2021). These factors contribute to oxidative stress, chronic inflammation, and endothelial dysfunction, ultimately leading to plaque formation and vascular complications (Hansson & Hermansson, 2011). Due to the complexity of atherosclerosis and the involvement of multiple organ systems and signalling pathways, in vivo models are essential for investigating disease mechanisms and evaluating potential therapeutic interventions (Zhang et al., 2021).

Animal models that closely resemble human pathophysiology are necessary for studying the immunometabolic processes and molecular mechanisms underlying the disease. These models enable researchers to observe disease progression in a controlled environment and assess the impact of genetic, dietary, and pharmacological factors. However, no single model fully replicates all aspects of the human condition. Variations in lipid metabolism, immune response, and plaque structure may limit the translatability of findings (Fenyo & Gafencu, 2013). Therefore, selecting an appropriate model based on research objectives is crucial.

Over the past decades, rabbits, pigs, and genetically modified mice have been employed in atherosclerosis research, each offering unique benefits and limitations. Among these, the Apolipoprotein E knockout (ApoE-/-) mouse is considered one of the most reliable and widely used models. ApoE-/- mice spontaneously develop atherosclerotic lesions and hypercholesterolemia, especially when fed a high-fat diet, making them ideal for studying plaque development and testing anti-atherosclerotic agents (Zhang et al., 2021). An animal model should be chosen based on the research focus. For this study, the ApoE-/- mouse was selected due to its proven ability to mimic human-like lesion formation and its relevance in evaluating therapeutic agents.

As mentioned previously, celastrol, when administered with an appropriate dosing regimen, may offer therapeutic benefits in treating atherosclerosis (Sun et al., 2024). Additionally, accurate quantification of atherosclerotic plaque burden is critical for assessing treatment efficacy. The Oil Red O (ORO) *en face* staining method provides a standardized, sensitive, and reproducible technique to visualize and quantify lipid-rich plaques along the aortic surface. This method offers a comprehensive view of disease progression or regression (Chen et al., 2022). Combined, these tools allow for rigorous evaluation of celastrol's anti-atherosclerotic potential and enhance the translational relevance of preclinical findings.

3.0 Methodology

This section includes the methodology to obtain plaque quantification via ORO en face analysis, starting from the induction of disease in the animal model, animal sacrifice, and followed by the staining method and data analysis.

3.1 Induction of Atherosclerosis in ApoE-/- Mice

Atherosclerosis in ApoE-/- mice is naturally occurring; however, in this study, we utilized Altromin Western Type- high-fat diet (HFD composition: 50% carbohydrates, 21% fat, 20% protein, and 0.21% cholesterol) to speed up the process of plaque deposition. Before inducing atherosclerosis by providing a HFD, 24 mice were randomly distributed into four groups to minimize selection bias and ensure that each group was comparable at baseline. The high-fat diet was started at the age of 8 weeks on male ApoE-/- mice for 12 weeks. This 12-week induction period is adequate to observe the formation of plaque lesions on the tunica intima of the aorta. Few studies found that ApoE -/-mice fed HFD for 10 weeks at 7-8 weeks of age developed plaques throughout the aorta (Feng et al., 2018), including the brachiocephalic and coronary arteries (Kumar et al., 2016). Thus, 12 weeks of HFD duration were optimized to induce plaques throughout the aortic length of mice, including in the aortic sinus. The mice were weighed weekly to ensure an increase in body mass as a result of the high-fat diet. Body scoring, including observation, was also carried out weekly to ensure the mice stayed healthy throughout the experiment period. The health of the mice throughout the 20 weeks was monitored closely to avoid affecting the outcome of the experiment. All experiments were in accordance with the institutional and national rules of animal care and were approved by the appropriate Institutional Animal Care and Use Committee (IACUC) UiTM CARE: 426/2023.

3.2 Celastrol Treatment on Atherosclerotic ApoE-/- mice Induced High Fat Diet

In order to determine the optimum celastrol dosage to attenuate atherosclerosis effectively, this study proposed the comparison of 3 doses, which are 1.5mg/kg, 2mg/kg, and 2.5mg/kg. In a previous study by Cheng et al. (2021) and Gu et al. (2013), they found that celastrol at 2mg/kg decreased plaque size and lesion area, as well as reduced inflammatory markers. In this study, we proposed a lower dose at 1.5mg/kg compared to previous studies and a higher dose at 2.5mg/kg. This is because celastrol at a lower dose may also attenuate atherosclerosis, which allows us to understand the level of alleviation, and by comparing it with a higher dose, such as 2.5mg/kg, we will be able to elucidate if celastrol has a dose-response effect; in which a higher concentration, offers higher alleviation of atherosclerosis. To prepare a working celastrol solution, lyophilized celastrol (≥98% purity, Cat. No. C0869) was purchased from Sigma-Aldrich (St. Louis, MO, USA) was prepared by diluting the stock solution in absolute DMSO, the solution was vortexed and centrifuged at 1000g for 30 seconds to ensure the dosage is accurate. The stock solution was then diluted into concentrations of 1.5mg/kg, 2mg/kg, and 2.5mg/kg with 1X PBS. The working solution was prepared fresh daily prior to administration via intraperitoneal injection (i.p) using an insulin needle to reduce injection site irritation. The treatment of celastrol and vehicle (2% DMSO) was carried out during the last 4 weeks of HFD in ApoE-/- mice aged 16 weeks.

3.3 Harvesting of the Aorta from the Aortic Root to the Iliac from the ApoE-/- Mouse Model.

The mice sacrificed for this experiment were carried out at 20 weeks old, with a post-high-fat diet and treatment. The mice were anesthetized using a ketamine and xylazine cocktail, and the aorta was harvested and placed in 10% neutral buffered formalin for fixation for 48 hours.

Staining of en face Aortic Tissue Using O Red Oil (ORO)

Oil Red O (ORO) staining requires ORO working solution, ultrapure water, and 60% isopropanol. Oil Red O (ORO) working solution was prepared by dilution of the ORO stock solution with ultrapure water and filtered using a filter syringe. The *en face*-prepared aortas were treated with a series of ORO working solutions to stain neutral lipids (plaque), 60% isopropanol to remove excess dye, and ultrapure water to eliminate background staining. This protocol ensured clear and specific red staining of lipid-rich atherosclerotic plaques located on the tunica intima. To visualize the ORO *en face* staining, the image was captured using a stereomicroscope (Olympus SZX7, Olympus Corporation, Tokyo, Japan), and the plaque in each aorta was calculated using Adobe Photoshop (version: 25.3.1, Adobe Systems Inc. USA). The data obtained from Adobe Photoshop was imported into GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) to perform statistical tests and analyze the significance of the data.

4.0 Results

The atherosclerotic lesions are quantified by calculating the total plaque area of the aorta against the full size of the aorta. Figure 1 compares atherosclerotic lesions between the control group and celastrol treatment in ApoE-/- mice.

Fig 1. Plaque incidence in *en face* aortas stained with Oil Red O (ORO). Representative aortas from each treatment group are shown. The atherosclerotic lesion areas are visualized with red ORO staining.

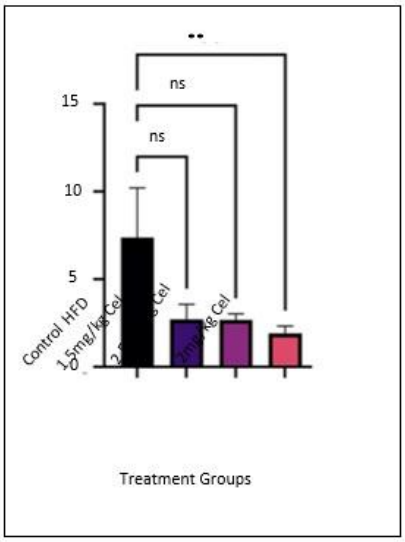

Fig 2. Quantification of plaque lesion in aorta en face samples stained with Oil Red O (ORO). Data are shown as mean ± SEM (n = 6) for each treatment group. **, $p < 0.01$. The lesion areas for each artery were quantified using Adobe Photoshop (version 25.9, Adobe Systems Inc., USA.

En face Oil Red O staining of whole aortas was used to provide an overall assessment of plaque development and to investigate whether celastrol at various doses could attenuate plaque development compared to the control group. This assessment was conducted after a 12-week period of high-fat diet-induced atherosclerosis, with celastrol intervention at various doses during the last 4 weeks of the high-fat diet. The whole aortas were then dissected and subjected to *en face* Oil Red O staining.

5.0 Discussion

In our study, we found that celastrol helped reduce plaque buildup in mice, which is a promising sign for future treatments. This study examined how celastrol, a natural compound from *Tripterygium wilfordii*, affects the buildup of atherosclerotic plaques in ApoE-/- mice fed a high-fat diet. Using a standard Oil Red O *en face* staining method, we found that mice treated with celastrol had less plaque compared to untreated controls. This suggests that celastrol may help reduce the development of atherosclerosis by lowering inflammation and lipid levels.

In this study, we utilized celastrol at three doses to create a comparison of effects on plaque lesion deposition. This is to observe if celastrol at a lower dose may also attenuate atherosclerosis, which allows us to understand the level of alleviation, and by comparing it with a higher dose, such as 2.5mg/kg, we will be able to elucidate if celastrol has a dose-response effect; in which a higher concentration, offers higher alleviation of atherosclerosis. As detailed in the Methods section, the Oil Red O (ORO) *en face* staining technique was applied to enable comprehensive visualization of atherosclerotic plaque burden across the entire surface of each aorta. This method facilitated the subsequent quantification of lipid-rich lesions, which was used as a metric to assess and compare the effectiveness of the different administered doses in attenuating plaque formation against a high-fat diet control group that only received a vehicle of 2% DMSO.

Based on the gross plaque quantification in the whole aorta, ORO *en face* staining showed that celastrol exhibits a plaque-clearing effect across all doses of 1.5mg/kg, 2mg/kg, as well as 2.5mg/kg. However, the most significant plaque-clearing effect was observed at a dose of 2.5mg/kg ($p<0.01$) when compared to the control group. This indicates that in this study, 2.5mg/kg is the dose that maximizes celastrol's anti-atherosclerotic effect. Similar findings were found by Luo *et al.* (2017); the fat deposition in the celastrol group C57BL/6N obese mice was reduced in a dose-dependent manner ($n = 10$, $p < 0.001$). The most significant dose is at 7.5 mg/kg/d via oral gavage, which exhibits fat accumulation similar to the deposition in control mice. Although lower doses exhibit lower plaque-clearing effects, it is worth noting that other in-depth analyses should be studied to ensure proper dosing of celastrol.

Based on Figure 2, the plaque burden can mainly be observed in high concentrations at the branches of the aorta. The aortic arch consists of a brachiocephalic trunk that divides into the right subclavian artery and right common carotid artery; the second branch from the right is the left common carotid artery, and the third branch is the left subclavian artery. The aortic arch is a frequent site for the initiation and accumulation of atherosclerotic plaque due to a combination of anatomical and hemodynamic factors. Unlike the straight sections of the arterial system where blood flows smoothly, the curvature of the aortic arch and the presence of major branch points, such as the brachiocephalic trunk, left common carotid artery, and left subclavian artery, create areas of disturbed and turbulent blood flow (Dong et al., 2024). These regions experience low or oscillatory shear stress, which is known to impair endothelial function and promote a pro-atherogenic environment (Cunningham & Gotlieb, 2005; Cheng et al., 2023). Under such conditions, endothelial cells are more likely to express adhesion molecules and inflammatory mediators that attract circulating monocytes. These monocytes infiltrate the arterial wall, differentiate into macrophages, and take up lipids to become foam cells, initiating the formation of atherosclerotic plaques (Čejková et al., 2016; Moore et al., 2015). These factors collectively make the aortic arch a vulnerable region for the development of atherosclerosis, as observed in both human studies and animal models such as ApoE-/- mice.

The treatment duration and dosing regimen, while effective in reducing plaque burden, require longer-term evaluation to fully understand celastrol's chronic effects and potential toxicity, especially when exploring different routes of celastrol administration. Additionally, Oil Red O *en face* staining reliably quantifies lipid-rich plaques but does not provide details on plaque composition, stability, or inflammation. Future studies should use complementary methods such as immunohistochemistry to better characterize plaque features and treatment impact. This study is limited by the variation of celastrol dosage, currently we were only able to compare between 3 different dosages. Furthermore, this study only calculated the plaque burden on *en face* whole length aorta, ORO histological staining should also be done on sections of aorta to confirm the assessment made by *en face* analysis.

In summary, this study shows that celastrol can reduce plaque buildup in a mouse model of atherosclerosis. ORO *en face* staining is a valuable tool for studying atherosclerosis and testing new treatments. These findings support further research into celastrol as a potential therapy for cardiovascular disease.

6.0 Conclusion & Recommendation

To conclude this study, we found that celastrol attenuates the incidence of atherosclerotic lesions in ApoE-/-mice fed a high-fat diet, with the highest plaque-clearing effect at 2.5mg/kg concentration. However, further investigation into the underlying molecular mechanisms by which celastrol exhibits its anti-atherosclerotic effects is warranted. Future studies should focus on elucidating how different doses of celastrol modulate key inflammatory pathways, lipid-metabolism processes, and oxidative-stress responses that contribute to disease progression. In addition, determining the optimal therapeutic dose at both superficial and molecular levels will be essential for understanding its mechanism of action. Comprehensive investigations are also needed to assess celastrol's long-term safety profile,

including potential toxicological effects, and to evaluate its sustained therapeutic efficacy through well-designed preclinical studies and, ultimately, controlled clinical trials. Such research will help define celastrol's viability as a safe and effective therapeutic agent.

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Paper Contribution to Related Field of Study

This study contributes to the growing body of research on anti-atherosclerotic therapies by providing new evidence for the therapeutic potential of celastrol, a bioactive compound derived from *Tripterygium wilfordii*. Using an established ApoE-/- mouse model and a widely used *en-face* staining protocol, the study demonstrates that celastrol treatment leads to a measurable reduction in atherosclerotic plaque burden. These findings support the hypothesis that celastrol exerts lipid-lowering and possibly anti-inflammatory effects within the vascular system with proper dosing to reflect optimum results. By highlighting celastrol's efficacy in attenuating plaque formation, this work lays the groundwork for further exploration of celastrol as a candidate compound for cardiovascular disease intervention.

References

Aziz, M., & Yadav, K. (2022). Pathogenesis of Atherosclerosis: Review. *Journal of Pharmaceutical Research International*, 54–62.

Cascão, R., Fonseca, J. E., & Moita, L. F. (2017). Celastrol: A Spectrum of Treatment Opportunities in Chronic Diseases. *Frontiers in medicine*, *4*, 69.

Čejková, S., Králová-Lesná, I., & Poledne, R. (2016). Monocyte adhesion to the endothelium is an initial stage of atherosclerosis development. *Current Vascular Pharmacology, 14*(1), 18–28.

Chen, P. Y., Qin, L., & Simons, M. (2022). Imaging and Analysis of Oil Red O-Stained Whole Aorta Lesions in an Aneurysm Hyperlipidemia Mouse Model. *Journal of visualized experiments : JoVE*, (183), 10.3791/61277. https://doi.org/10.3791/61277

Cheng, J., Tian, Z., Li, J., & Hu, H. (2021). Celastrol attenuates atherosclerosis in Apolipoprotein E (apoE) knockout mice fed an atherogenic diet. *International Scholars Journals*, Article ID 55319.

Cheng, H., Zhong, W., Wang, L., Zhang, Q., Ma, X., Wang, Y., Wang, S., He, C., Wei, Q., & Fu, C. (2022). Effects of shear stress on vascular endothelial functions in atherosclerosis and potential therapeutic approaches. *Biomedicine & Pharmacotherapy, 153*, Article 114198.

Cunningham, K. S., & Gotlieb, A. I. (2005). The role of shear stress in the pathogenesis of atherosclerosis. *Laboratory Investigation, 85*(1), 9–23.

Dong, Y., Gao, W., Hong, S., Song, D., Liu, M., Du, Y., Xu, J., & Dong, F. (2024). Evaluation of turbulence index and flow pattern for atherosclerotic carotid stenosis: A high-frame-rate vector flow imaging study. *Ultrasound in Medicine & Biology, 50*(4), 549–556.

Feng, T., Liu, P., Wang, X., Luo, J., Zuo, X., Jiang, X., Liu, C., Li, Y., Li, N., Chen, M., Zhu, N., Han, X., Liu, C., Xu, Y., & Si, S. (2018). SIRT1 activator E1231 protects from experimental atherosclerosis and lowers plasma cholesterol and triglycerides by enhancing ABCA1 expression. *Atherosclerosis*, *274*, 172–181.

Fenyo, I. M., & Gafencu, A. V. (2013). The involvement of the monocytes/macrophages in chronic inflammation associated with atherosclerosis. *Immunobiology, 218*(11), 1376–1384.

Gu, L., Bai, W., Li, S., Zhang, Y., Han, Y., Gu, Y., Meng, G., Xie, L., Wang, J., Xiao, Y., Shan, L., Zhou, S., Wei, L., Ferro, A., & Ji, Y. (2013). Celastrol prevents atherosclerosis via inhibiting LOX-1 and oxidative stress. *PloS One*, *8*(6), e65477.

Gusev, E., & Sarapultsev, A. (2023). Atherosclerosis and Inflammation: Insights from the Theory of General Pathological Processes. International journal of molecular sciences, 24(9), 7910. https://doi.org/10.3390/ijms24097910

Hansson, G. K., & Hermansson, A. (2011). The immune system in atherosclerosis. *Nature immunology*, *12*(3), 204–212.

Kumar, S., Chen, M., Li, Y., Wong, F. H. S., Thiam, C. W., Hossain, M. Z., Poh, K. K., Hirohata, S., Ogawa, H., Angeli, V., & Ge, R. (2016). Loss of ADAMTS4 reduces high-fat diet-induced atherosclerosis and enhances plaque stability in ApoE(-/-) mice. *Scientific Reports*, *6*, 31130.

Li, Z., Zhang, J., Duan, X., Zhao, G., & Zhang, M. (2022). Celastrol: A Promising Agent Fighting against Cardiovascular Diseases. *Antioxidants*, *11*(8), 1597. https://doi.org/10.3390/antiox11081597

Libby, P. (2021). The changing landscape of atherosclerosis. *Nature, 592*, 524–533.

Luo, Y., Duan, H., Qian, Y., Feng, L., Wu, Z., Wang, F., Feng, J., Yang, D., Qin, Z., & Yan, X. (2017).Macrophagic CD146 promotes foam cell formation and retention during atherosclerosis. *Cell Research, 27*(3), 352–372.

Moore, K., Sheedy, F., & Fisher, E. (2013). Macrophages in atherosclerosis: A dynamic balance. *Nature Reviews Immunology, 13*(10), 709–721.

Sijbesma, J. W. A., van Waarde, A., Kristensen, S., Kion, I., Tietge, U. J. F., Hillebrands, J.-L., Bulthuis, M. L. C., Buikema, H., Nakladal, D., Westerterp, M., Liu, F., Boersma, H. H., Dierckx, R. A. J. O., & Slart, R. H. J. A. (2023). Characterization of a novel model for atherosclerosis imaging: the apolipoprotein E-deficient rat. *EJNMMI Research*, *13*(1). https://doi.org/10.1186/s13550-023-01055-5

Sun, Y., Wang, C., Li, X., Lu, J., & Wang, M. (2024). Recent advances in drug delivery of celastrol for enhancing efficiency and reducing the toxicity. *Frontiers in Pharmacology, 15*, Article 1137289.

Zhang, Y., Fatima, M., Hou, S., Bai, L., Zhao, S., & Liu, E. (2021). Research methods for animal models of atherosclerosis (Review). *Molecular medicine reports*, *24*(6), 871.

Zhao, X., Huang, B., Zhang, J. *et al.* (2023). Celastrol attenuates streptozotocin-induced diabetic cardiomyopathy in mice by inhibiting the ACE / Ang II / AGTR1 signaling pathway. Diabetol Metab Syndr 15, 186.