Review

# Effects of Lipophagy on Atherosclerosis

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An excess build-up of lipids in the arterial wall might result into atherosclerosis. Lipophagy is the autophagic degradation of lipids that regulates lipid metabolism in various kinds of cells. Lipophagy replaces intracellular lipid, making it vital for atherosclerosis development and progression. This review focuses on advances in lipid metabolism through lipophagy. The role of lipophagy in vascular endothelial cell injury, macrophage lipid accumulation and vascular smooth muscle cells phenotypic shift has been explained by specifying the lipophagy– atherosclerosis relationship. Novel therapeutic choices can be discovered by understanding the significance of lipophagy in these processes which could be a breakthrough in the treatment of atherosclerosis.

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## Introduction

Lipophagy is a particular kind of autophagy. It uses lysosomal acid lipases to selectively destroy intracellular cholesterol and triglycerides (TGs) that are stored in lipid droplets (LDs). The proteins in LD membranes are sighted and then are concealed by microtubule-associated protein 1 light chain 3 II (LC3 II) for formation of autophagosomes (APs), which later fuses with lysosomes to form autolysosomes (ALs). Eventually, β-oxidation takes place and the engulfed TG-rich lipid droplets decompose into free fatty acids to produce adenosine triphosphate in mitochondria.[1-3] Later, lipophagy hydrolyses cholesterol ester-rich LDs to free cholesterol for efflux, mediated by ATP-binding cassette transporter A1 (ABCA1).[4] Hence lipophagy is important in regulating intracellular lipid accumulation, cholesterol efflux, and supporting energy homeostasis. However, a defective lipophagy might lead to tissue lipid build-up like in atherosclerosis and fatty liver diseases.[5-7]

Atherosclerosis is caused due to lipid accumulation in arterial wall and is a progressive disease.[8] Underlying mechanisms of atherosclerosis remain obscure despite of known risk factors. So, several studies have come up related to the disease: endothelial dysfunction, lipid metabolism, cell apoptosis, genetic and epigenetic factors and oxidative stress.[9-13] Autophagy impairment is noticed in both human and animal atherosclerotic plaques.[14,15] Recent reports have stated that lipophagy damage occurs in foam cells, so lipophagy is linked to atherosclerosis.[16,17] So it is difficult to conclude whether lipophagy is a cause or an outcome of atherosclerosis.

This review mentions the mechanism and function of lipophagy. It outlines the damage of autophagic degradation of lipid, taking place in the atherosclerotic lesions. Moreover, the effect of deficiency in Lipophagy on atherogenic procedure like, macrophage lipid accumulation, VSMCs proliferation and movement and vascular EC dysfunction have also been described. Additionally, we also have focused on advanced methods to reverse lipid metabolism disorders via the regulation of lipophagy in treatment of atherosclerotic disease.[18]

### *Mechanism of Lipophagy*

Autophagy forms and matures certain double membrane structures like phagophores, ALys and Aps. The

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formation of an isolation membrane is a signal of the beginning of autophagy. The elongation and closure of phagophores form APs and then fuse with lysosomes, resulting in ALys. Here lysosomal hydrolytic enzymes are used to reduce the engulfed cargoes.[19,20] Autophagy is regulated by numerous autophagy-related genes (ATGs). So far more than 40 ATGs have been discovered in yeast, the majority of which are mammalian homologs.[21] The formation of phagophores and APs are caused by the interaction of these ATGs and other components such as unc-51-like autophagy-activating kinase 1 (ULK1).[20]

Lipophagy is a selective-autophagy in which APs separate the LDs and get degraded using lysosomes (Fig 1). The first well-defined lipophagic method was cultured in hepatocytes and was explained by Singh et al.[22] According to the authors, autophagy is induced by starvation and promotes the degradation of LDs into free fatty acid (FFAs) in the liver. However, this practice is disturbed by autophagy inhibitor, 3-methyladenine (3-MA) or ATG5 and ATG7 knockdown, causing a significant decline in the breakdown of LDs. Thus, defective lipophagy blocks LD clearance leading to lipid accumulation.[2,23] However, current studies claim that lipophagy can also occur in non-liver tissue and cells, primarily the heart, vascular endothelial cells, macrophages, and others.[24-26]

## *Morphology of lipophagy*

Morphologically, LDs can recognize the type of lipophagy used, by determining how selective contents are being engulfed during the autophagic process. In most cells, the LDs are large subcellular structures with probable diameters of 0.1 to 100 μm. LDs are primarily larger than lysosomal cells in mammalian cells (0.1-1 μm in diameter). However, yeast vacuoles like lysosomes are generally larger than yeast LDs. Recent studies indicate that LDs make contact with vacuoles, specifically at vacuolar junctions/ER; which contradicts previous studies that suggested that LDs entered the vacuoles.[27] In higher eukaryotic cells, LD is formed on double membrane phagophores and they pinch off portions of LD membrane with neutral lipid content. This process is gradual.

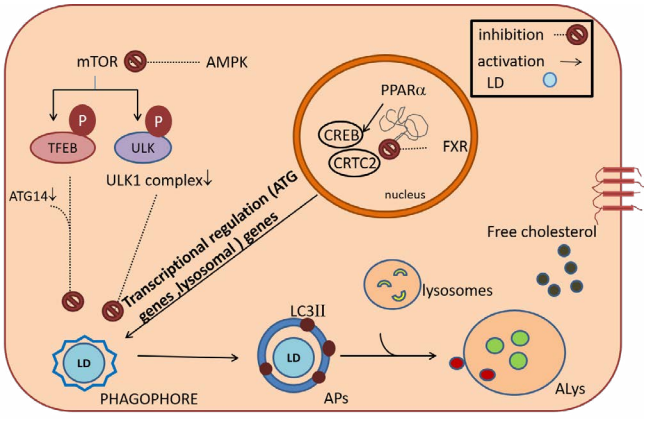


**Fig 1:** Mechanism of Lipophagy

## *Regulation of Lipophagy*

### *Significance of mammalian target of rapamycin*

The mammalian target of rapamycin (mTOR) is an important negative autophagy regulator activated by PI3K/Akt/signaling.[28] mTOR exists in two complexes: mTOR complex 1 (mTORC1) and mTORC2. mTORC1 affects lipophagy by inhibiting phagophore initiation and formation of AP. Reports suggest that MTORC1 activation stimulates phosphorylation of UNC-51-like autophagy that activates kinase1/2 (ULK1/2) and ATG13. This impedes the formation of ULK1 complex (including FIP200, ATG101 and ATG13).[29,30] Moreover, mTORC1 restricts lipophagy using phosphorylation of the transcription factor –EB (TFEB) and ATG14.[31,32] However, phosphorylation of ULK1 at Ser 317 and Ser 777 causes initiation of AMP-activated protein kinase (AMPK) which poses reversed effect on the inhibition of mTOR in lipophagy (Fig 1).[33] Earlier studies suggested that activation of P13K-Akt-mTOR causes impairment of ox-LDL-induced macrophage lipophagy and foam cell formation.[34] However, current studies discovered that AMPK activation inhibits the effect of mTOR on macrophage lipophagy.[35]

### *Farnesoid X receptor/cAMP response element-binding protein axis signaling in lipophagy*

Regulation of lipophagy occurs by two transcriptional regulators: cAMP response element-binding protein (CREB) and Farnesoid X receptor. Lipophagy is seen to be regulated by FXR-CREB signaling.[36] CREB improves lipophagy by increasing autophagic gene expression like ATG7 and ULk1 by stimulating CREB-regulated transcription co-activator 2 (CRTC2) (Fig 1).

Nonetheless, eliminating the enhancement of CREB-mediated lipophagy by activating FXR can stop the production of functional REB-CRTC2 complex. Moreover, reversing the inhibitory effect of FXR on lipophagy is possible by activation of PPARα.[37,38] Thus FXR-CREB signaling is a significant way to regulate lipophagy.

*Role of PLIN protein family in lipophagy*

PLINs family proteins are surface proteins located on LDs and serve as gatekeepers. Their degradation is essential for lipophagy/lipolysis.[39] This family consists of five members, PLIN 1-5 which serve in lipophagy as regulators by binding lipase to LDs. It is confirmed that PLIN2 and PLIN3 can be selectively recognized by chaperone-mediated autophagy (CMA) to degrade, transferring the LDs towards lysosomes for CMA clearance.[40] Accumulation of LDs inside the cells is caused by inhibition of CMA, confirming that degradation of PLIN2 and PLIN3 occurs before the commencement of lipophagy. According to recent data analysis, PLIN2 interacts with HSP70 thus activating AMPK signaling and involving lipophagy regulation.[41] Thus, this data confirms that PLIN s family proteins are vital contributors for the regulation of lipophagy.

*Receptor proteins in lipophagy*

Various selective autophagy receptors might also function as LD receptors. Some of them are nuclear dot protein 5kDa (NDP52), Huntingtin, optineurin, SQSTM1/p62, out of which Huntingtin is responsible for recognizing and degrading various organelles like mitochondria and LDs. Surprisingly cells possessing the mutation Huntingtin repossess no cargo and show large empty APs. Huntingtin acts as a lipophagic receptor.[42] Moreover, a mutation in Huntingtin causes excessive LD accumulation in cells. According to reports LC3 binds with cardiolipin and phospholipid.[43] Hence, we assume that LC3 might directly identify LDs; this partly supports that ATGL promotes LDs degradation by binding to LC3.

*Transcriptional regulation*

According to current evidence, transcription factors like transcription factor EB (TFEB),[44] transcription factors E3 (TFE3)[45] and forkhead homeobox type protein O1 (FoxO1)[46] are important factors in the regulation of lipophagy. As per the report by Settembre *et al*., expression of ATGs and lysosomal gene can be increased by lipophagic activity and lipophagy via TFEB.[47,48] Moreover up-regulation of peroxisome proliferator-activated receptor-γ coactivator-1 alpha (PGC-1α) expression enhances LDs degradation which TFEB promotes. TFE3 acts in a cell-specific manner during lipid metabolism and the hyper-expression of TFE3 in hepatocytes amplifies lipophagy and upgrades liver steatosis.[45] Obesity might be caused due to overexpression of adipocytes.[49] Besides, several other transcription factors might help in the regulation of lipophagy like FoxO1 causes lipophagy using up-regulation of expression in autophagy gene ATG14 and lysosomal acid lipase (LAL) in adipocytes.[46] Thus, transcription factors are significant in the regulation of lipophagy.

*Effect of Lipophagy on Atherosclerosis*

The accumulation of excessive lipid in the arterial walls causes atherosclerosis. Injury in vascular endothelial cells triggers atherogenic processes such as monocyte infiltration and differentiation, VSMC proliferation and movement.[50-52] Infiltrated monocytes form macrophages and engulf large modified lipids and LDL. These excess lipoproteins and lipid molecules within macrophages get stored as LDs which later form foam cells; these develop in atherosclerosis.[53] LDs in these foam cells contain cholesterol ester and free cholesterol. Thus ceasing foam cell production and atherosclerosis development occurs due to LDs degeneration and cholesterol efflux from the cells. According to reports by Ouimet *et al*. autophagic degradation restricts lipid accumulation during lipophagy, thus preventing the occurrence of atherosclerosis.[54] Hence, impaired lipophagy might lead to excess lipid build-up, leading to fatty liver diseases and atherosclerosis.[55] Nonetheless, underlying lipophagic mechanisms in atherosclerosis are unclear.

This part mainly focuses on influence of lipophagy on injured endothelial cell, VSMCs migration and proliferation, and macrophage lipid build-up. These factors can be potential causes in atherosclerosis as they are connected with the development of atherosclerosis.

*Lipophagy in vascular endothelial cells*

Vascular endothelial cells form single layer of flat cells in the interior of blood vessels, which participate in homeostasis. Physiologically, these maintain structure of vessels to control the transport of substances across blood walls, to regulate vascular tone and produce and secrete vasoactive substances. Other characteristics of these cells are cell adhesion, immunity, inflammation, cell signal transduction and so on.[56,57] Certain elements like hyperglycemia and hyperlipidemia cause blood monocyte recruitment, adhesion and infiltration in damaged arterial intima, triggering assimilation of modified macrophagial lipids to produce foam cells and induce VSMC proliferation and migration, which triggers atherogenesis.[58] Defective VEC in athero-prone are can cause lipid amassing due to direct uptake of excess cholesterol-rich lipoprotein, causing the formation of foam cells.[53,59]

Atherogenesis begins with endothelial cell injury. The function of autophagy is self-protection against numerous

detrimental agents. So, in case of damage, endothelial cells attempt to prevent from being harmed.[60,61] The impaired autophagy causes cell death, thus damaging the integrity of endothelium. However, a mechanism for the regulation of for autophagy of endothelial cells is unclear. Hence advanced knowledge regarding mechanisms underlying endothelial cell injury might be useful for therapeutic interventions for atherosclerosis.

Autophagy in vascular endothelial cell is vital in survival and functions. Activation of autophagy shields endothelial cells against damage by advanced glycation end products (AGEs) and ox-LDL.[62-64] These regulate apoptosis of signal-regulating kinase 1 (ASK1)/JNK, silent mating type information regulation 2 homolog 1/ FoxO1 pathways and mammalian target mTORC1/ULK1 65. Moreover, shear stress in arterial wall is significant for regulating autophagy in endothelial cells 66. High shear stress has been observed to provide protective autophagy in vascular endothelial cells,[67,68] whereas low-stress results in activating mTOR pathway that causes autophagy inhibition.[60] Autophagy also plays a vital role in endothelial eNOS expression, arterial aging and thrombosis.[61] A current study claims that autophagy is also visible in ECs atherosclerotic lesions. Vion A-C *et al.* later elucidated that adequate endothelial autophagy prevents senescence, inflammation and apoptosis, thus preventing atherogenesis.[60] However, excessive autophagy might cause plaque instability as a result of autophagic cell death of vascular endothelial cells.[69,70] This concludes that regulating endothelial autophagy could be effective in ameliorating atherosclerosis.

Currently, lipophagy mainly targets excessive accumulation of LDs comprising of cholesterol esters in vascular endothelium, causing inflammation and stress in the endoplasmic reticulum (ER) leading to endothelial dysfunction and injury.[59,71] Lipophagy promotes the degradation of LDs, thus maintaining a protective mechanism for endothelial survival and optimizing accurate functions (Fig 2). This is evident as the epigallocatechin gallate (EGCG) reduces intracellular lipid accumulation in aortic endothelial cells by establishing co-localization of LDs and ALs.[24] This suggests that decreasing lipid accumulation could restrain lipotoxicity in vascular endothelial cells by inducing the degradation of LDs n lipophagy.

### *Macrophage-derived foam cell formation and lipophagy*

In the process of atherosclerosis pathogenesis, monocyte-derived macrophages play vital roles like initiation, evolution and plague rupture.[72] Excessive uptake of modified lipids like ox-LDL and ac-LDL by macrophages

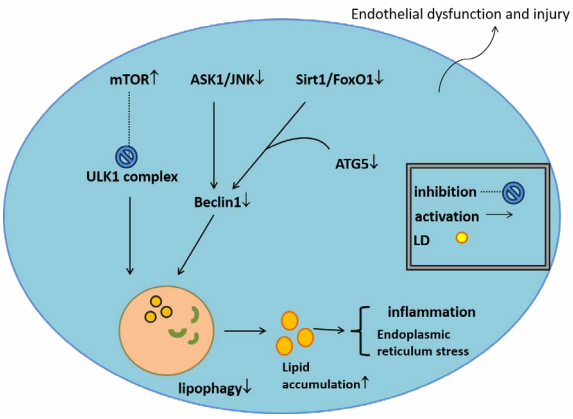


**Fig 2:** Mechanism of endothelial cell lipophagy in atherosclerosis

stimulates atherogenesis. Deficiency in cholesterol efflux and lipophagy add to build-up of cholesterol-rich LDs in these cells which are then called macrophage-derived foam cells and are major factors for atherosclerotic lesions.[17] LD-rich macrophages promote atherosclerosis progression and cleavage of plague by inducing inflammatory responses in the vessels.[73] Macrophages are classified as M1 and M2 phenotypes based on their prompt inflammatory response. M1 phenotype is a pro-inflammatory response in advanced atherosclerotic plaques whereas M2 phenotype is an anti-inflammatory response in early-stage atherosclerotic lesions.[74]
Upcoming reports claim that lipophagy facilitates LD degradation causing cholesterol efflux from macrophages.[5,17,25] Some evidences and mechanisms of lipophagy in formation of macrophage derived foam cell have been discussed in the following context (Fig 3).

As per the current reports, a major role of lipophagy has been spotted in the accumulation ofmacrophages.[18,54] Improved response to lipid treatment has been detected by macrophage lipophagy in both in vivo and in vitro conditions.[5,25] Lipophagy flux was gravely hampered due to altercations in Atg5, causing ineffective degradation of LDs, forming the foam cells.[75] Another analysis claimed that in mice fed with a high-fat diet for a short term showed lipophagy whereas excessive accumulation of LDs and lipid metabolic ailments were visible in those with long-term input.[76] Thus we can conclude that degradation of lipids occurs under lipophagy but the existence of prolonged high-fat input can hamper lipophagy.

Earlier reports showed that high-level ox-LDL (100 μg/mL) elevated accumulation of lipid in macrophage cells by PI3K-Akt-mTOR signaling and decreased co-localization of LDs with LC3-II.[34] Autophagy

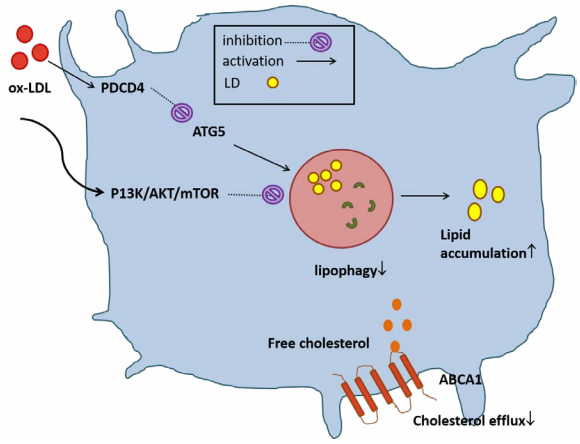


**Fig 3:** Mechanism of atherosclerosis in macrophage

activators like rapamycin and nicotinate-curcumin can reverse the above-mentioned effects of ox-LDL on macrophages. Programmed cell death protein 4 (PDCD4) poses a negative effect of lipophagy in macrophages as mentioned in an analysis by Wang L *et al.* According to their report intracellular LD conglomeration and foam cell formation can be reduced by knockdown of PDCD4, which might promote macrophage lipophagy using up-regulation of ATG5.[77] The outcomes suggest that inhibition of ATG5-mediated lipophagy accelerates the formation of macrophage foam cells.[78] So, PDCD4 could be a potential therapeutic target to prevent and treat atherosclerosis.

Toll-interacting protein (Tollip) regulates macrophage lipophagy that helps in atherogenesis as per the report by Chen *et al.*[5] As per the study, impaired lipophagy, distended atherosclerotic plaques and amplified LDs accumulation in macrophages is visible due to deficiency in Tollip. Thus, lipophagy is accounts for the degradation and clearance of excess lipid in macrophages. This helps regulate macrophage lipophagy and facilitates cholesterol efflux, thus helping treat atherosclerotic diseases.

### *Lipophagy in vascular smooth muscle cells*

Vascular smooth muscle cells (VSMC) conduct phenotypic switching from contractile to synthetic or macrophage-like phenotypes thus hold a significant role in the development of atherosclerosis.[79] Several vascular physiological and pathophysiological processes like repairing vessel injury, development of atherosclerosis, vascular remodeling, embryonic angiogenesis etc. requires Phenotypic shift in vascular smooth muscle cells.[80,81] VSMC-derived foam cells are the major source of foam cells in atherosclerotic lesions (approx. 50% in human plaques) and are formed due to the uptake of excess lipid content by macrophage-like VSMC.[82,83] Migration and proliferation of VSMCs can occur as a result of the phenotypic switch; this promotes the advancement of atherosclerosis. Death of excess VSMC in necrotic core formation and fibrous cap thinning helps to maintain plaque stability.[84] Hence VSMCs shift to pro-atherosclerotic phenotype thus play a vital role in atherosclerosis.

Various stimuli help in achieving a pro-atherosclerotic switch in VSMC phenotype; some of these stimuli include oxidized lipids, metabolic stress, growth factors, reactive oxygen species and cytokines. These factors eventually lead to autophagy in VSMCs, declaring that phenotypic switch in VSMCs includes a major role of autophagy in VSMCs.[85] Thus, we can infer that platelet-derived growth factors promote VSMC autophagy and causes synthetic VSMC phenotype by increasing synthetic markers and reducing contractile protein expression.[86] VSMC viability can be determined by autophagy. Mostly, VSMC survival can be obtained by appropriate autophagy but results like apoptosis and senescence can occur as a result of abnormal autophagy.[87] Ox-LDL and 4-hydroxynonenal (a lipid peroxidation product) induce a defensive mechanism against VSMC apoptosis. Current studies suggest that VSMC senescence and atherogenesis can occur due too deficient VSMC autophagy.[88] Thus we can draw an inference that VSMC autophagy is vital in sustaining normal vascular function and securing the arterial wall against atherosclerosis.

Recent reports show that lipophagy protects against VSMC-derived foam cell formation and atherosclerotic development. Moreover, accumulation of LDs and foam cell formation occurs as a result of activation of phenotypic switching of VSMCs, causing engulfing of lipid. Defective lipophagy leads to foam cell formation and could hamper lipid metabolism in VSMCs (Fig 4). Earlier studies have mentioned that ox-LDL endorses VSMC-derived foam cell production by inhibiting lipophagy which is visible through reduced co-localization LDs with LC3 and enhanced LD accumulation.[16]

### *Preventing and treating atherosclerosis by lipophagy*

In addition to the information given previously in the review, we can say that lipophagy is a potential factor in preventing and treating patients with atherosclerosis. Several pharmacological agents have been discovered to regulate lipophagy, like mTOR inhibitor rapamycin and its derivatives (rapalogs) help promote lipid autophagic degradation and cholesterol efflux and reduce vascular endothelial cell damage.[92] Cholesterol-lowering agents

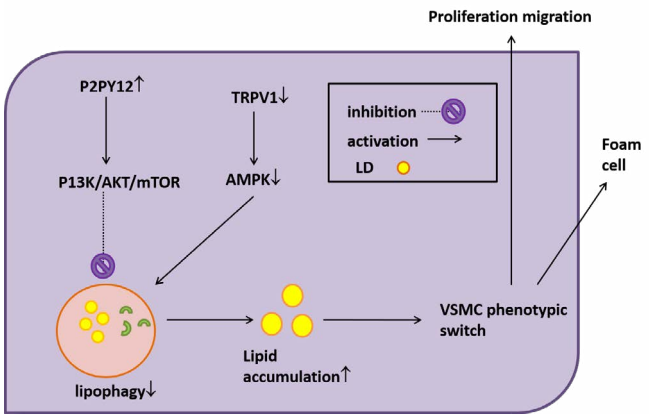


**Fig 4:** Mechanism of VSMC lipophagy in atherosclerosis

inhibit the mTOR pathway thus promoting autophagy. Lipophagy can also be inhibited by proprotein convertase subtilisin/kexin type 9 (PCSk9) via facilitating AKT/mTOR signaling. A mechanism involving PCSK9 inhibitors helps curtail low-density lipoprotein cholesterol level via inhibiting mTOR pathway.[93]

As mentioned earlier, lipophagy inducing drugs like raplogs and rapamycin, PCSK9 and statins facilitate cholesterol efflux and lipid degradation thus preventing atherosclerotic diseases. But clinical drugs that peculiarly propose lipophagic modulation have not yet been discovered and this absence of precision affects their potential clinical benefits. Moreover, the inadequacy of biomarkers that detect lipophagic activity is a major shortfall in assessing the effects of lipophagy-inducing drugs.

## Conclusion

Atherosclerosis occurs due to excess accumulation of foam cells (in form of cholesterol-rich LDs) in the arteries, marking it as a progressive disease. Recent reports, lipophagy mediated LDs degradation helps maintain lipid accumulation and prevent atherosclerosis.[5,17,25] Lipophagic and autophagic depletion is noticed with growing age which might ultimately lead to LDs build-up and if worse, can cause atherosclerosis. Lipophagy is vital for VSMC phenotypic shift and has a major role in EC injury. So regulating lipophagy in cells is a major way for treating atherosclerosis.

Though the benefit of lipophagy regulation for treatment of atherosclerosis is supported by many evidences yet some queries are left unanswered before its actual application. (1) Sometimes, Activation of autophagy can also trigger inflammatory reactions.[94,95](2) Alterations of atherosclerotic cell autophagy during atherosclerosis in animals should be understood to detect the effect of cell lipophagy on atherosclerosis pathogenesis. This could be done by cross-breeding mice with atherosclerosis having ATGs knockout. (3) There is a controversy regarding neutral lipolysis and lipophagy; can improvement in lipophagy lead to inhibition of neutral lipolysis. (4) Transportation of free cholesterol during LD degradation should be done through ABCA1, which could prevent re-esterification from promoting macrophage foam cell formation. (5) More advanced studies on lipophagy in VAMCs and ECs is required for better understanding. Understanding the mechanisms of lipophagy perturbations during this disease helps in recognizing its ultimate potential as a new therapeutic target for the treatment of atherosclerosis.

## Conflict of Interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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## Authors' contributions.

All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

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