

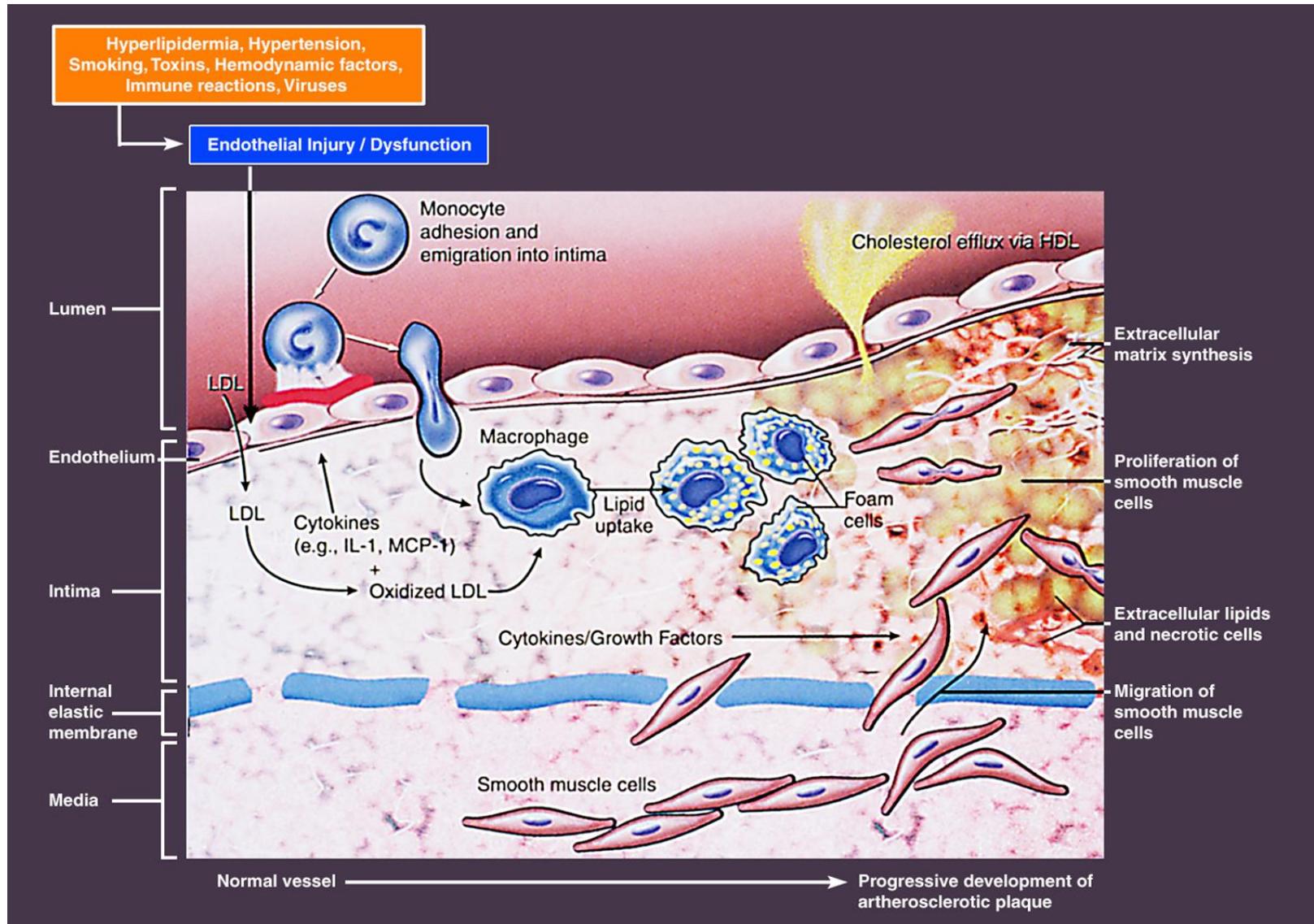
Cholesterol Crystals in Developing Atherosclerosis

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Center for Cardiovascular Research**

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Busan, Korea**

Development of Atherosclerosis



Endothelial Cells in Atherosclerosis

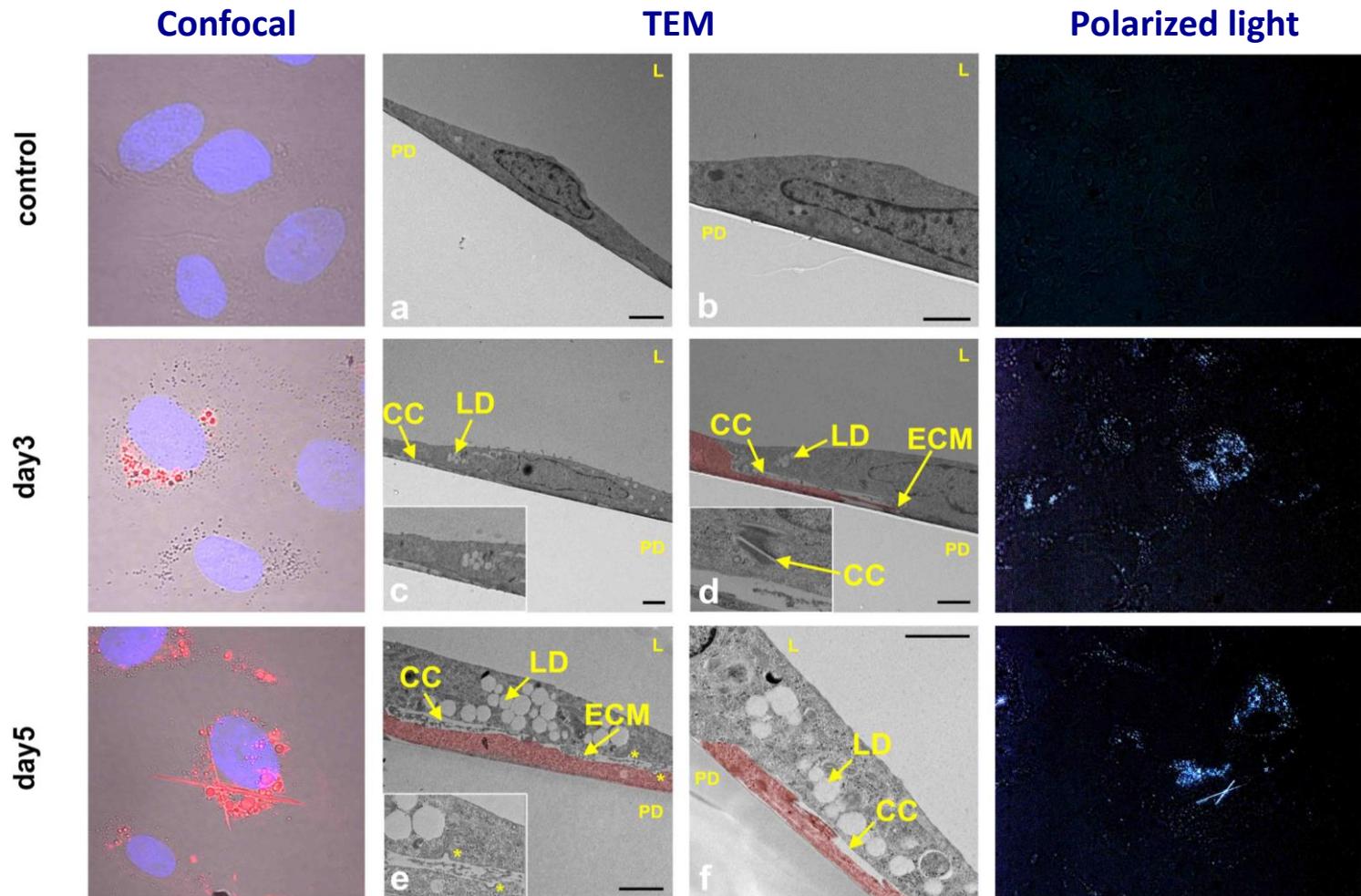
- Provides barrier between the lumen and the vessel wall
- Mediates transmigration of lipid particles and leukocytes
- LDL particles are thought to transcytose through the endothelial layer
- Surprisingly little is known about the processing of LDL by the EC

Endothelial Cells in Atherosclerosis

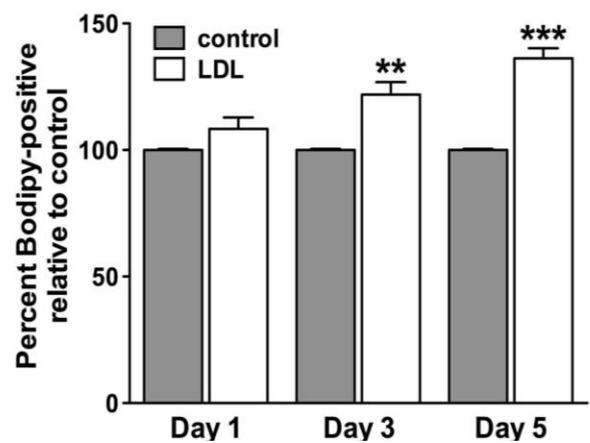
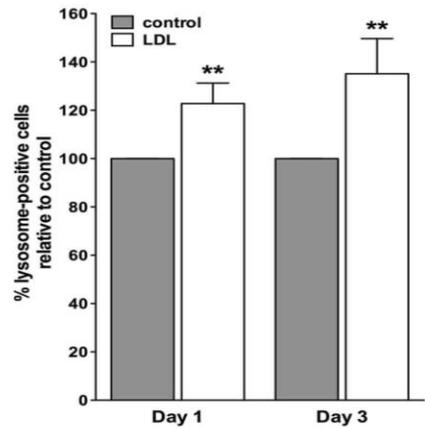
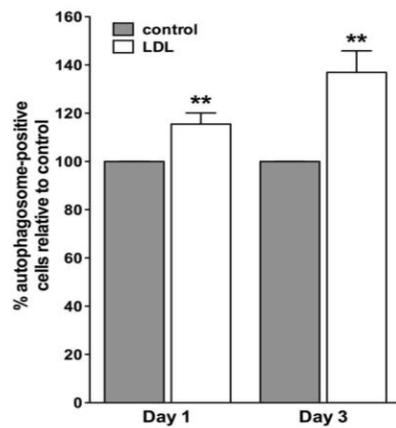
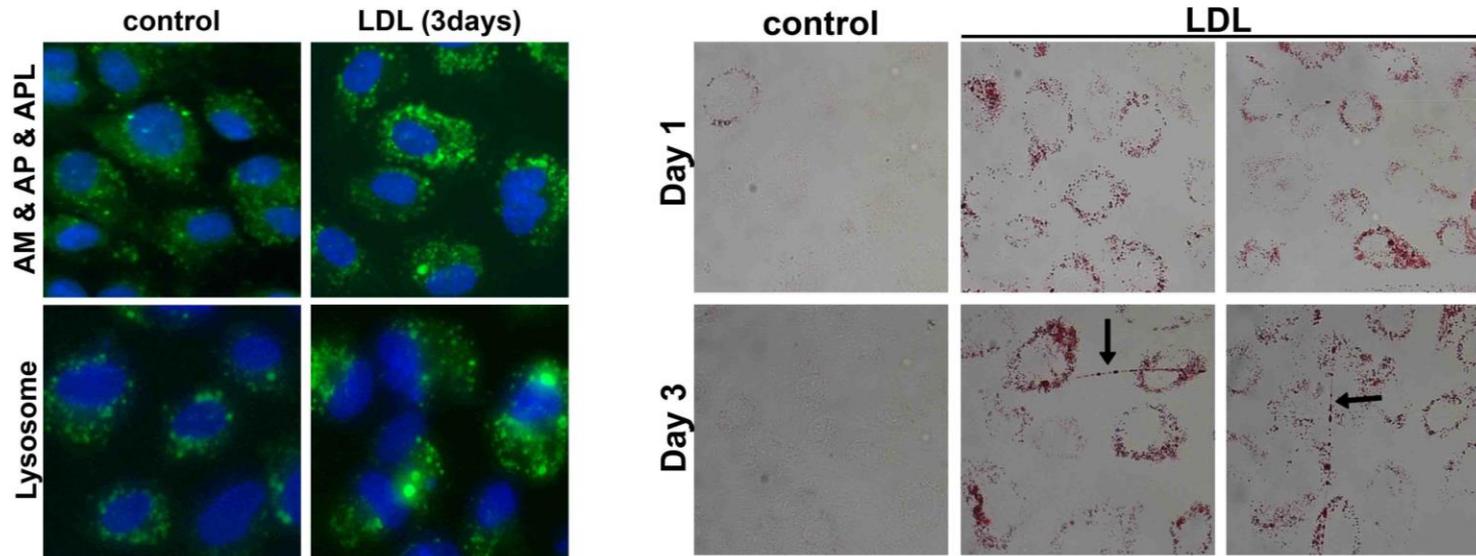
Questions:

1. Do the endothelial cells take up LDL particles under hyperlipidemic conditions?
2. How do the cells process the lipid?

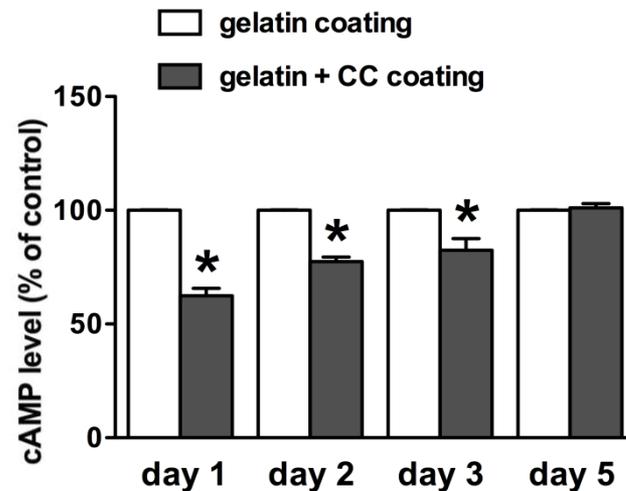
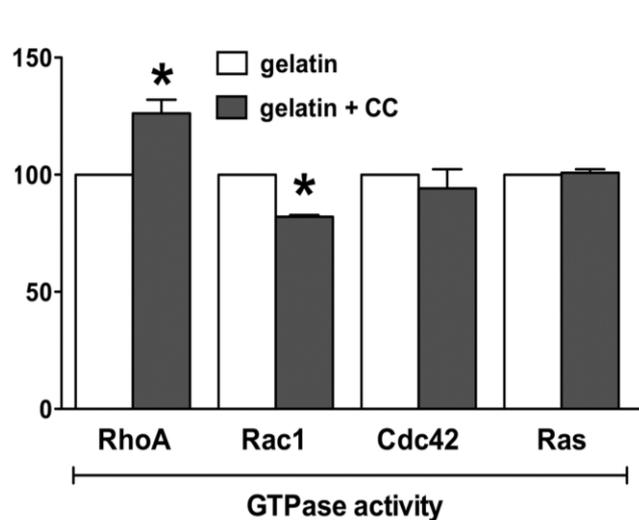
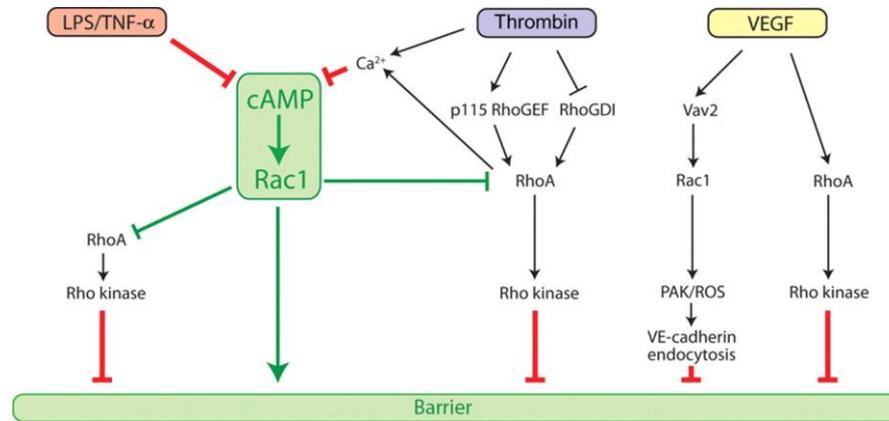
Cholesterol crystals are produced and secreted by human aortic endothelial cells upon LDL treatment



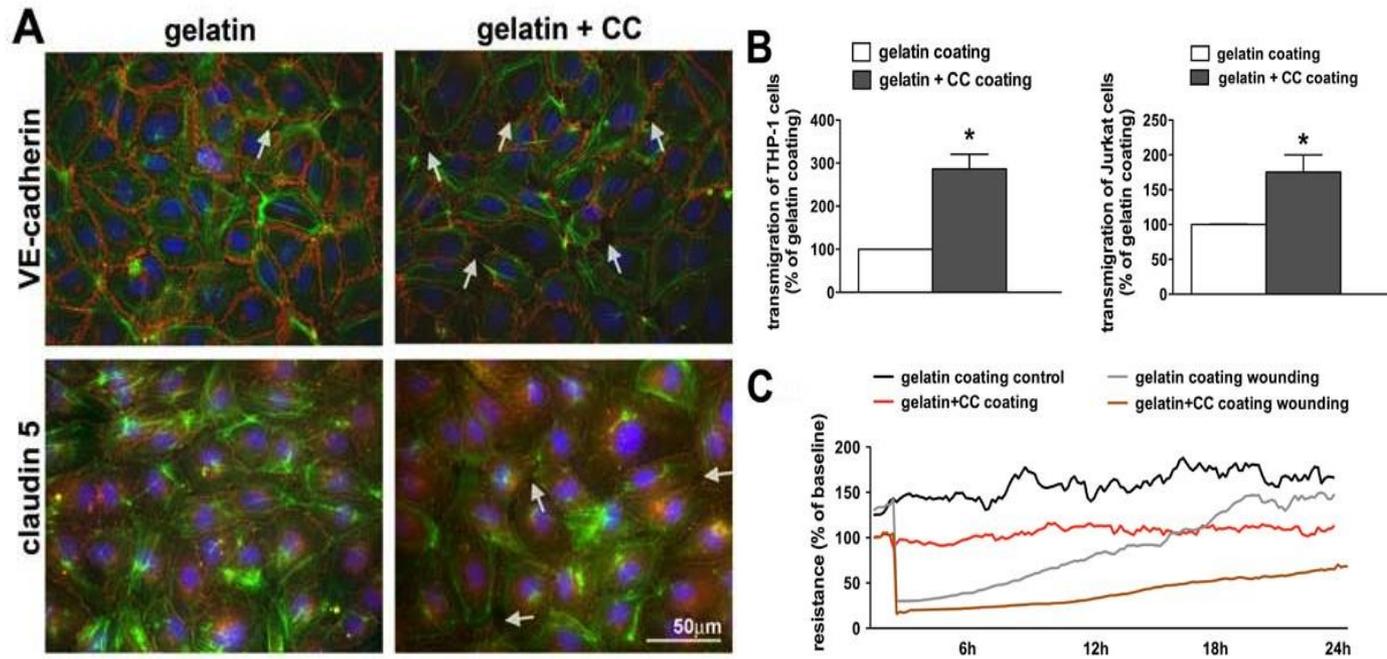
Lipid processing by HAoEC



Cholesterol crystal-induced changes in endothelial signaling



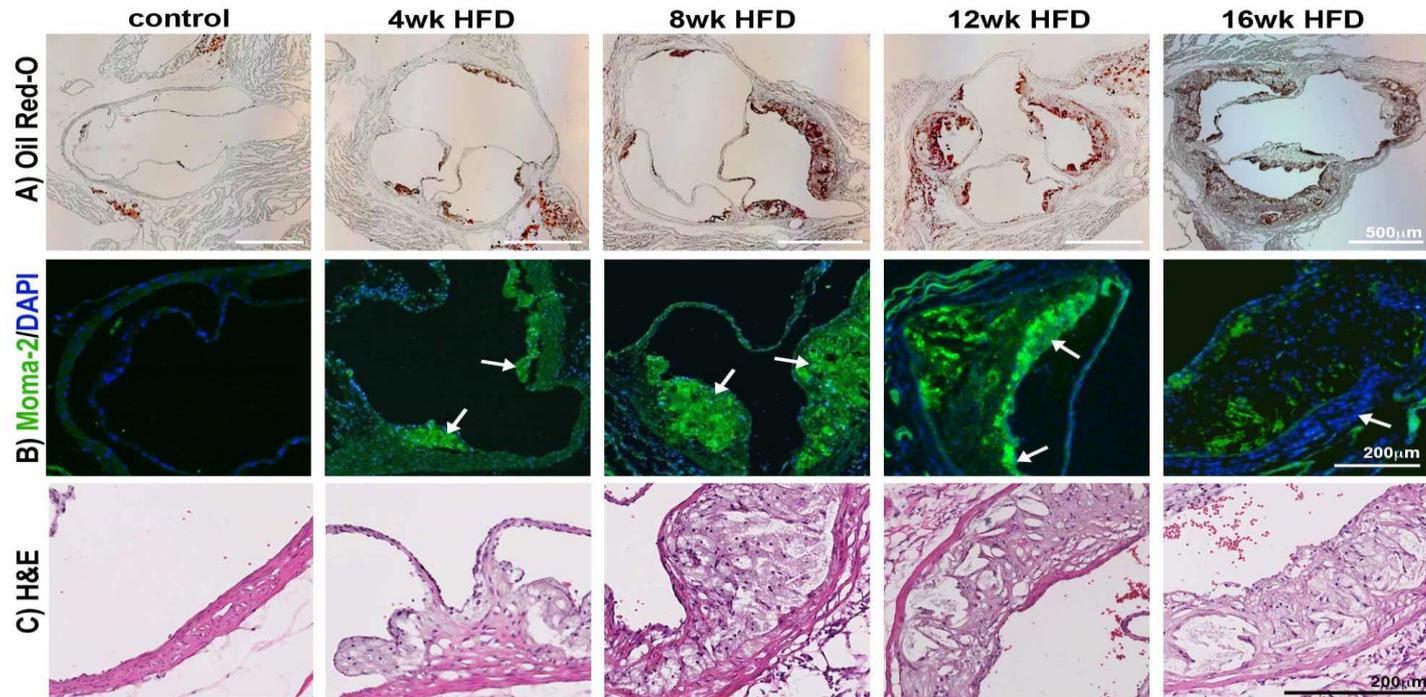
CC induces changes in endothelial function



What about cholesterol crystals in more advanced stages of atherosclerosis?

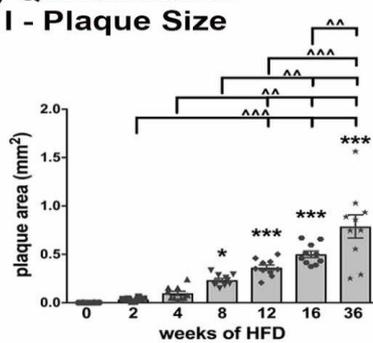
1. Chronicled the ultrastructural changes in atherosclerotic plaque formation through various stages of the disease.
2. Investigated the presence of cholesterol crystals in ultramorphological detail in advancing atherosclerosis.

Progression of atherosclerosis in the LDLR^{-/-} mice

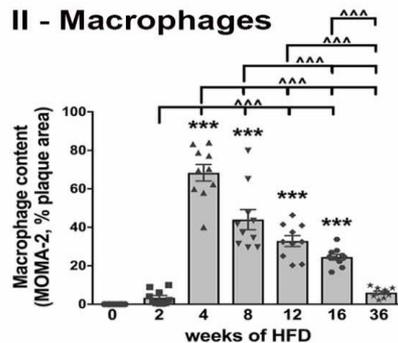


D) Quantification

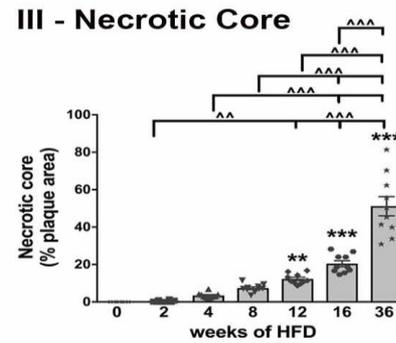
I - Plaque Size



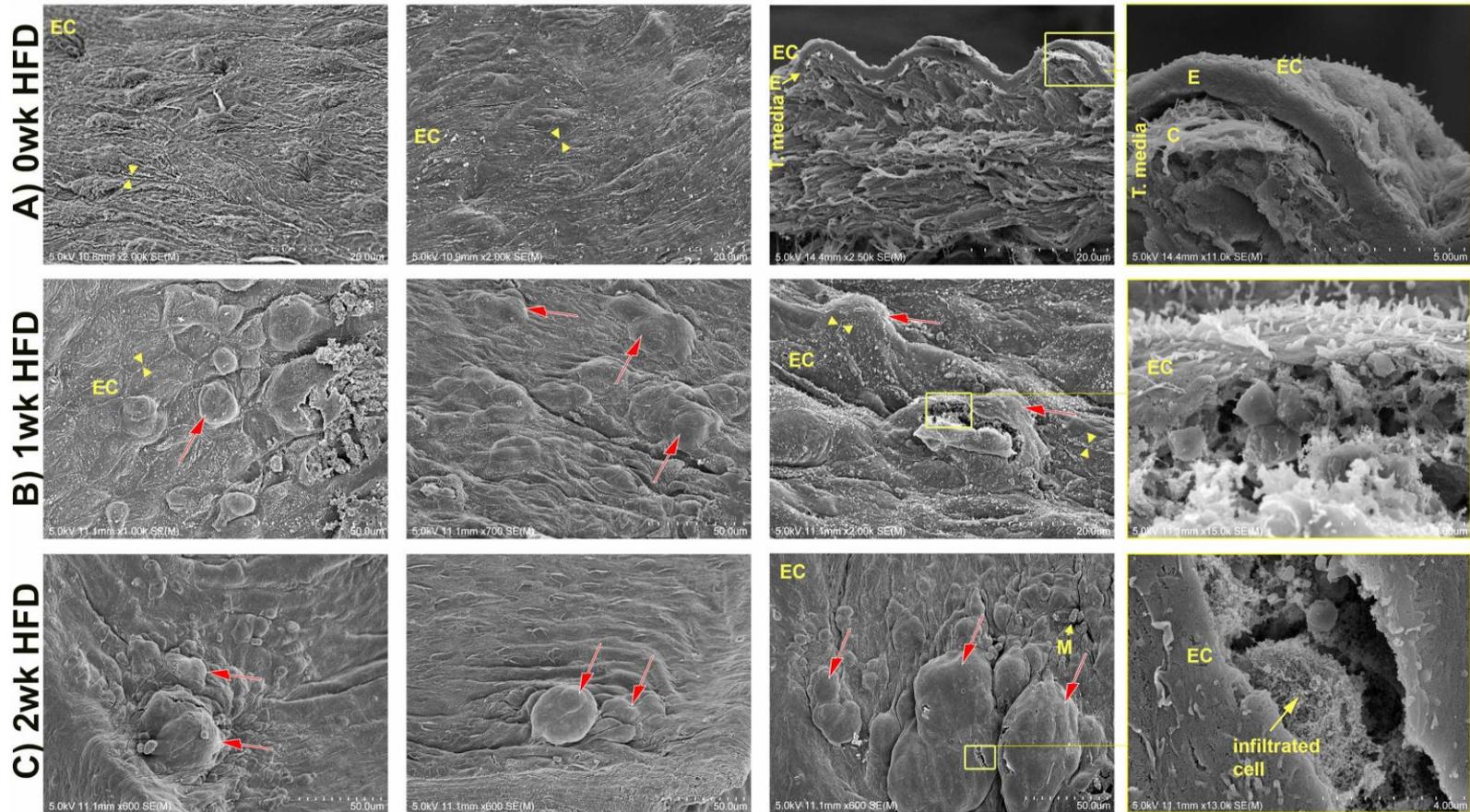
II - Macrophages



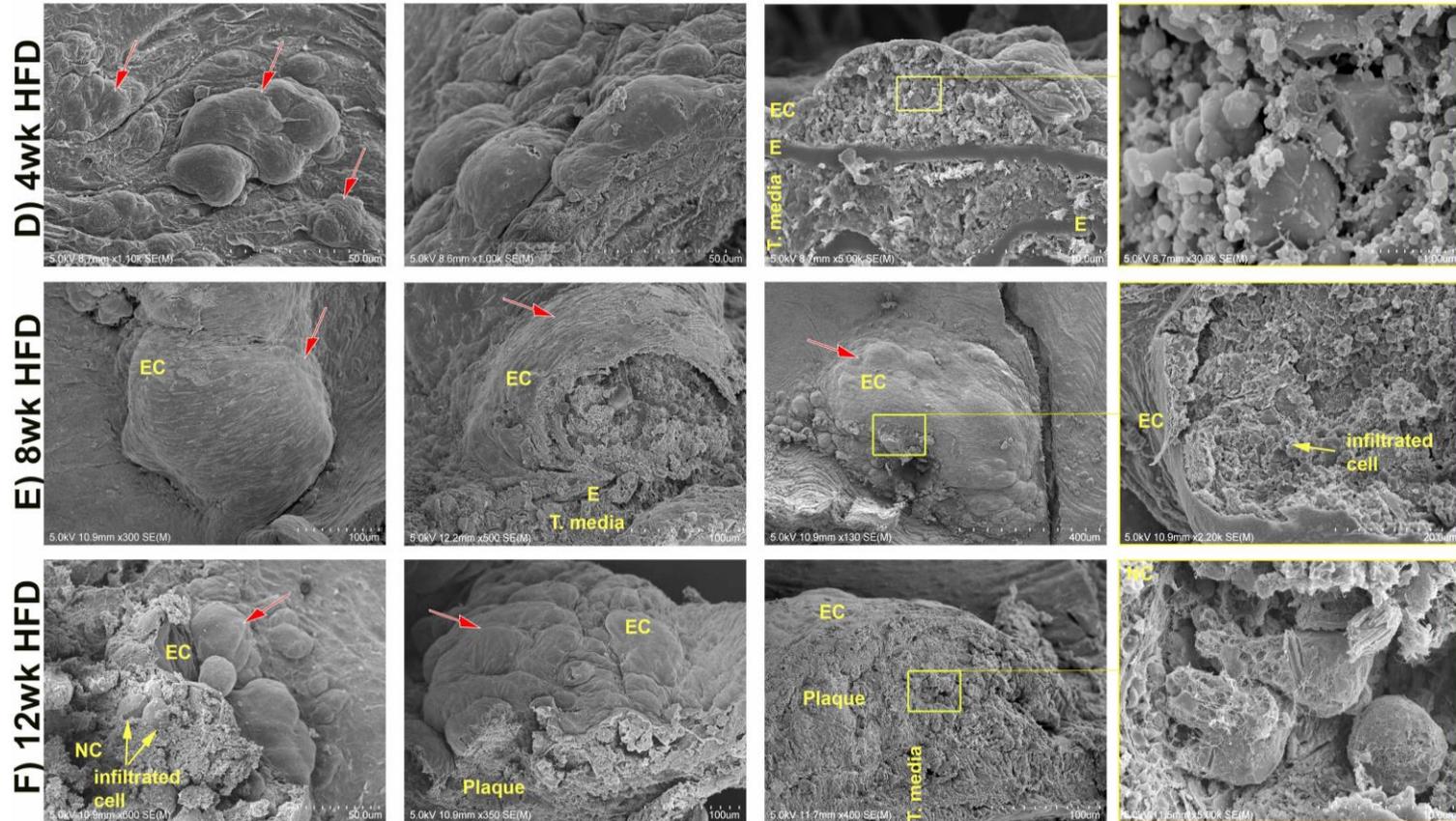
III - Necrotic Core



SEM images of atherosclerotic endothelium early stages

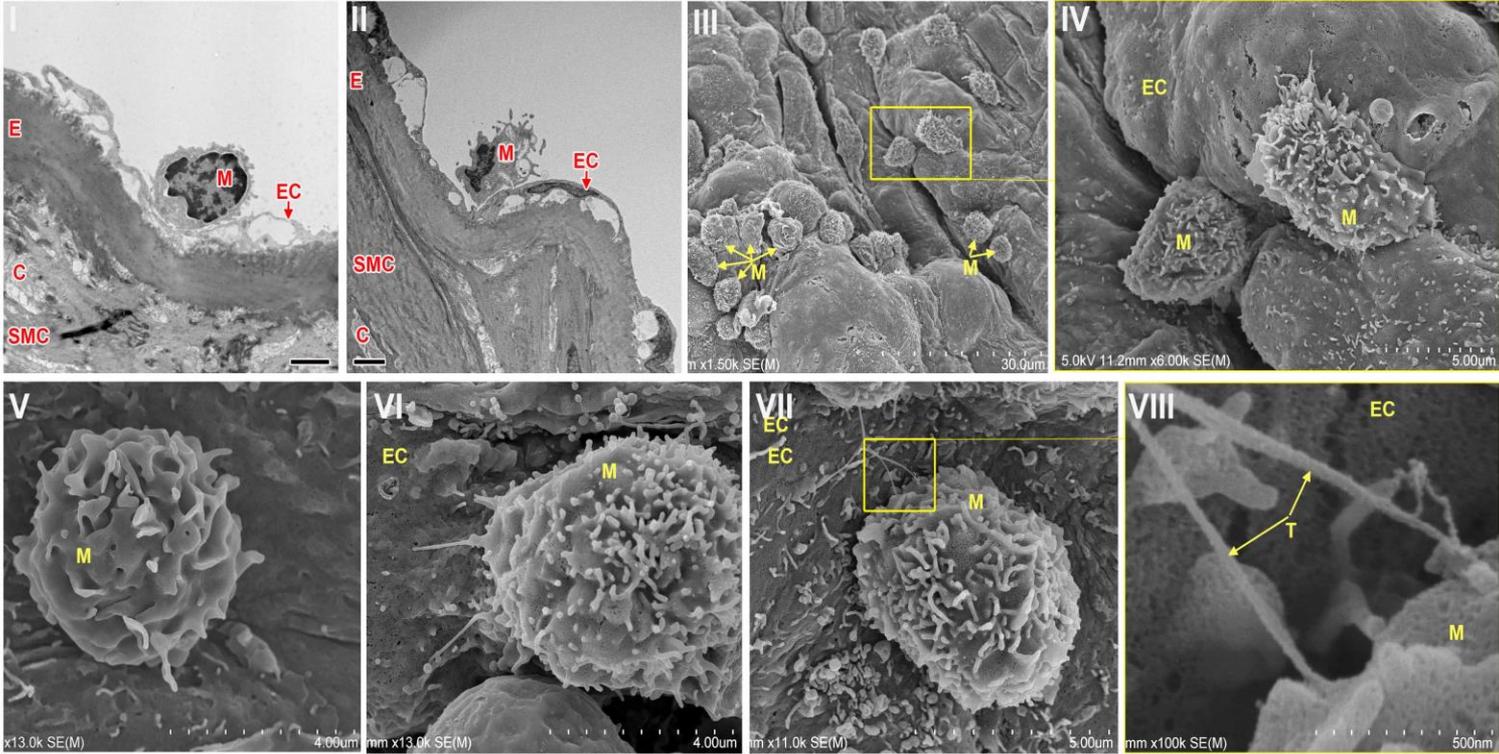


SEM images of atherosclerotic endothelium late stages



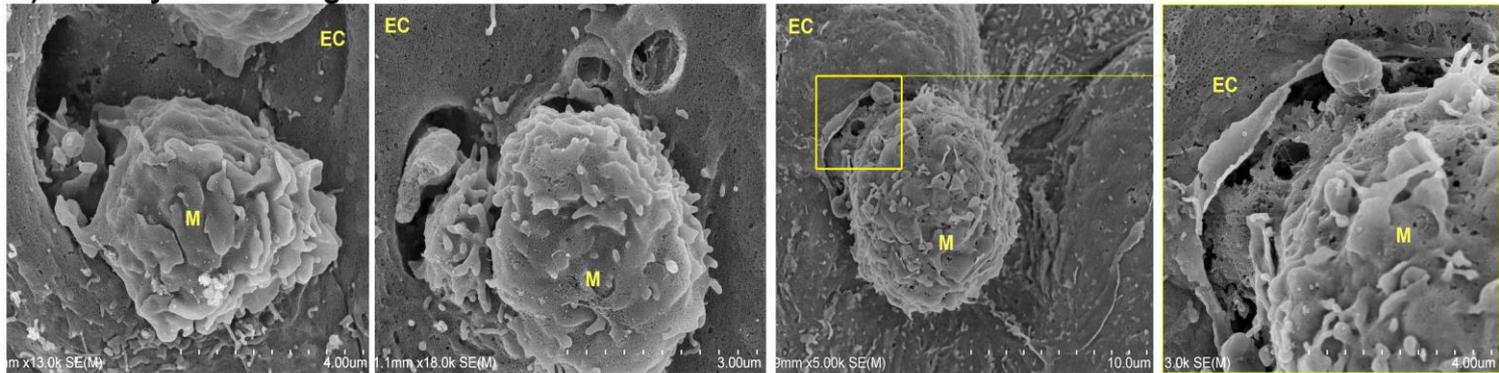
Monocyte adherence to endothelium in early atherosclerosis

A) Monocyte adherence

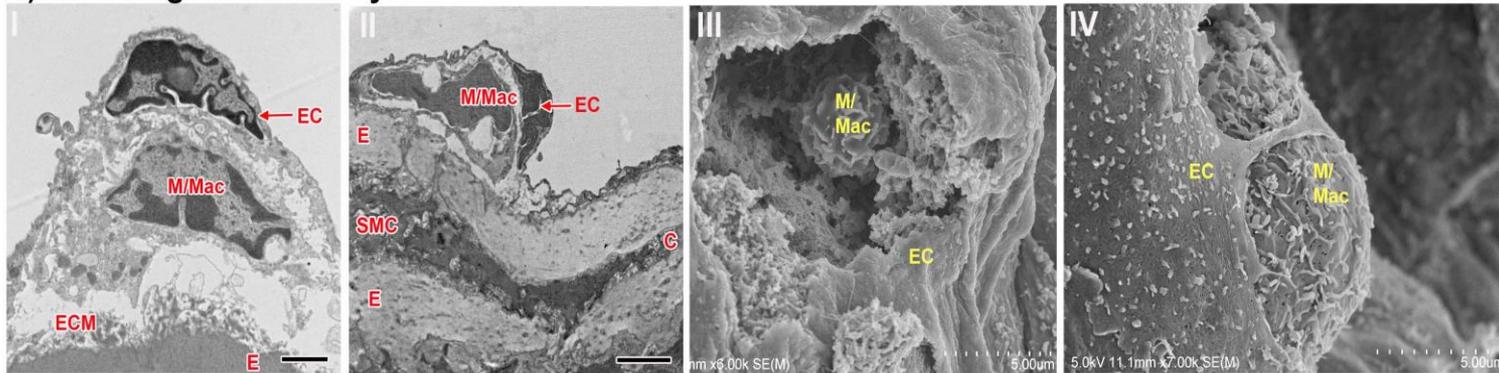


Monocyte diapedesis through the endothelium

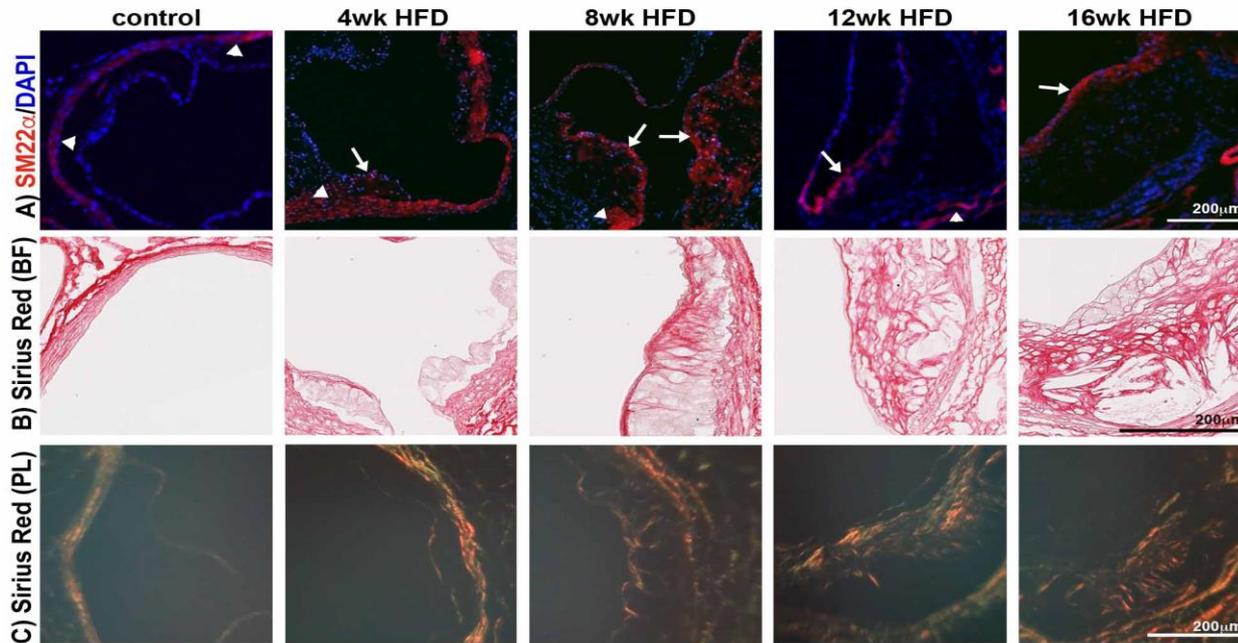
B) Monocyte Transmigration



C) Transmigrated Monocyte

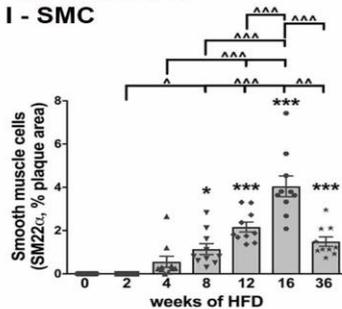


Changes in extracellular matrix composition during atherosclerosis progression

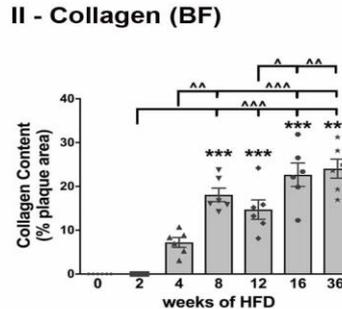


D) Quantification

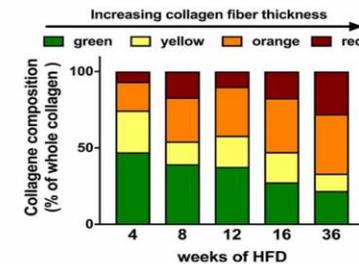
I - SMC



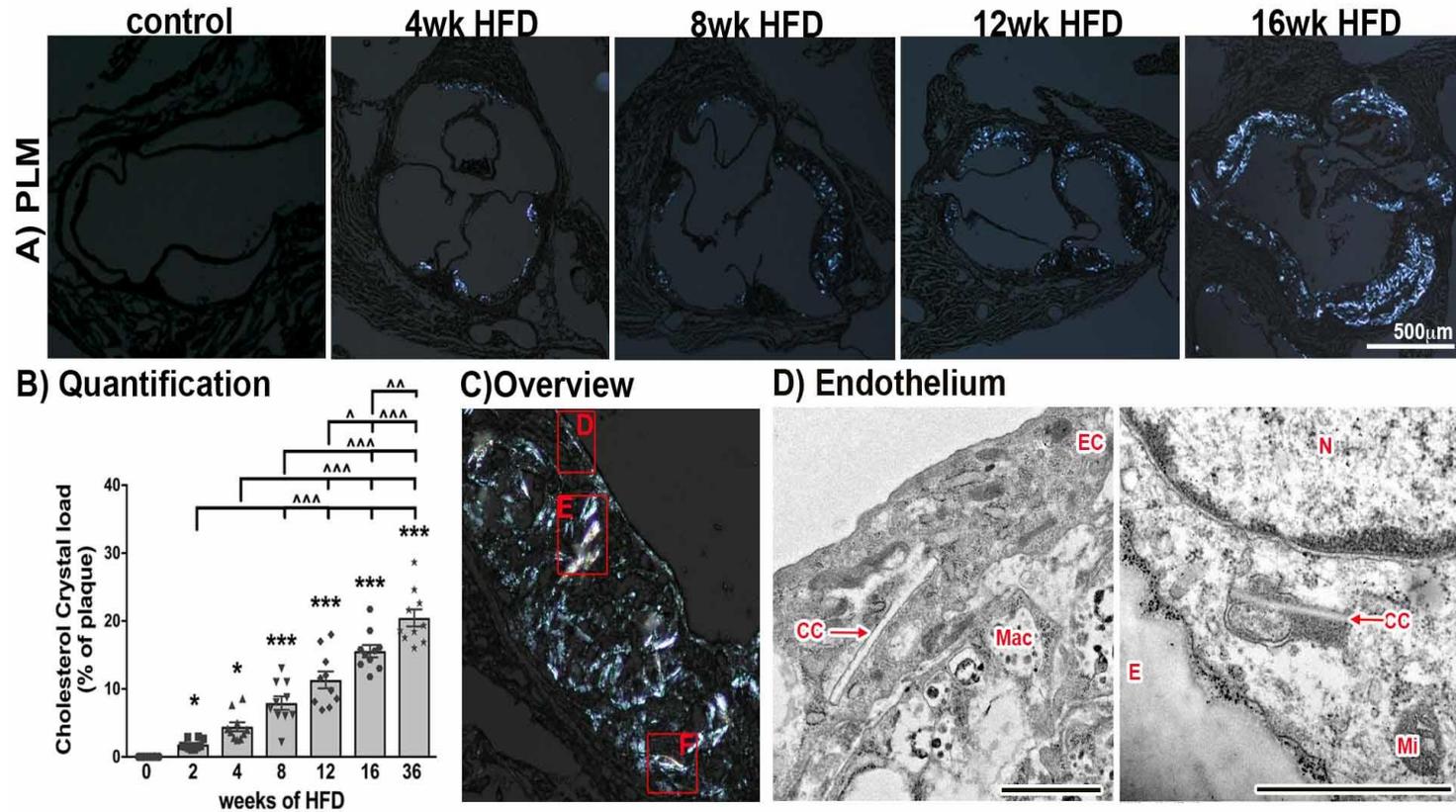
II - Collagen (BF)



III - Collagen (PL)

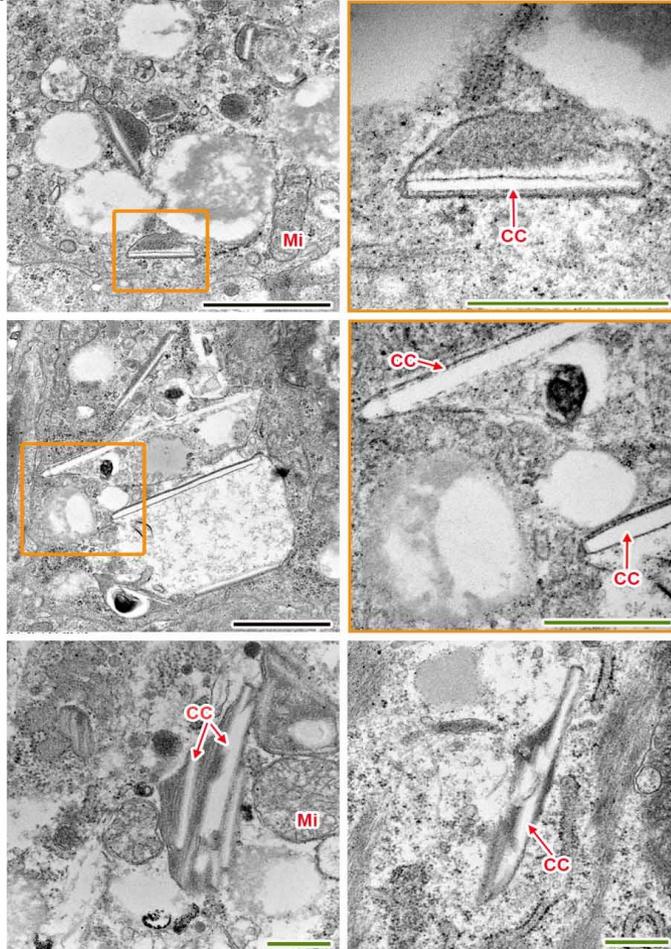


Presence of cholesterol crystals during atherosclerosis progression

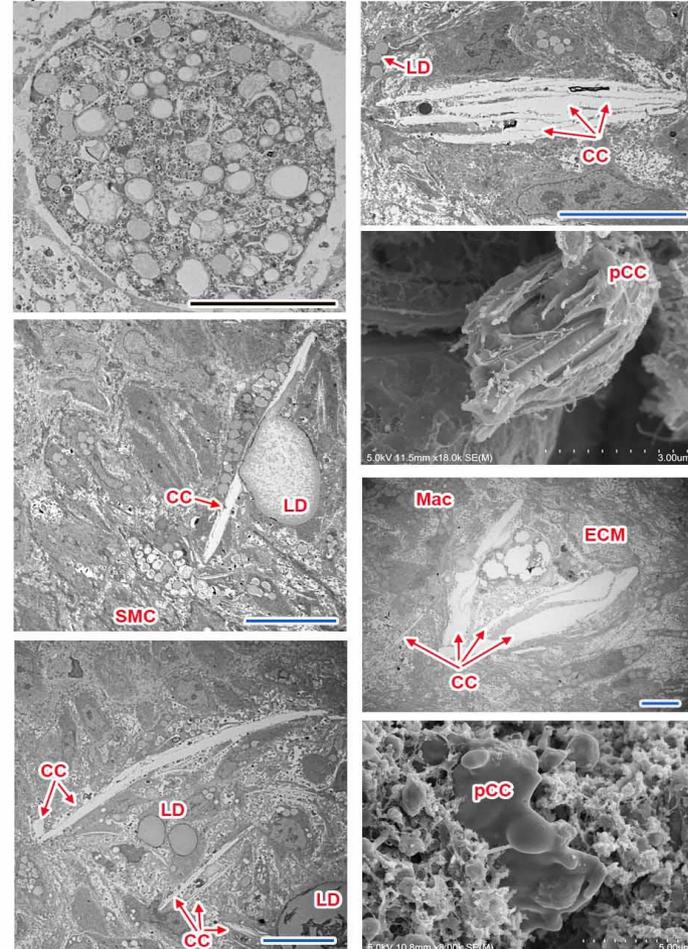


Presence of cholesterol crystals during atherosclerosis progression

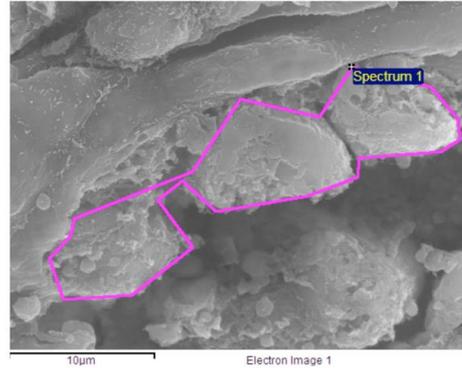
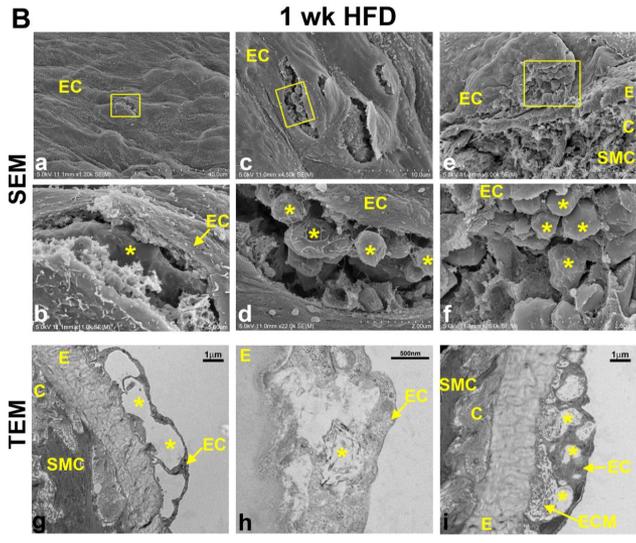
E) Macrophages / Foam cells



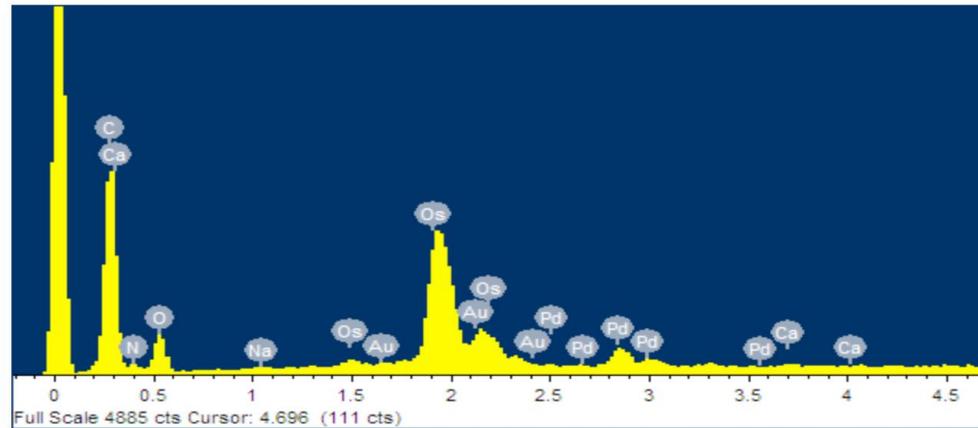
F) Necrotic core



Composition of subendothelial CC

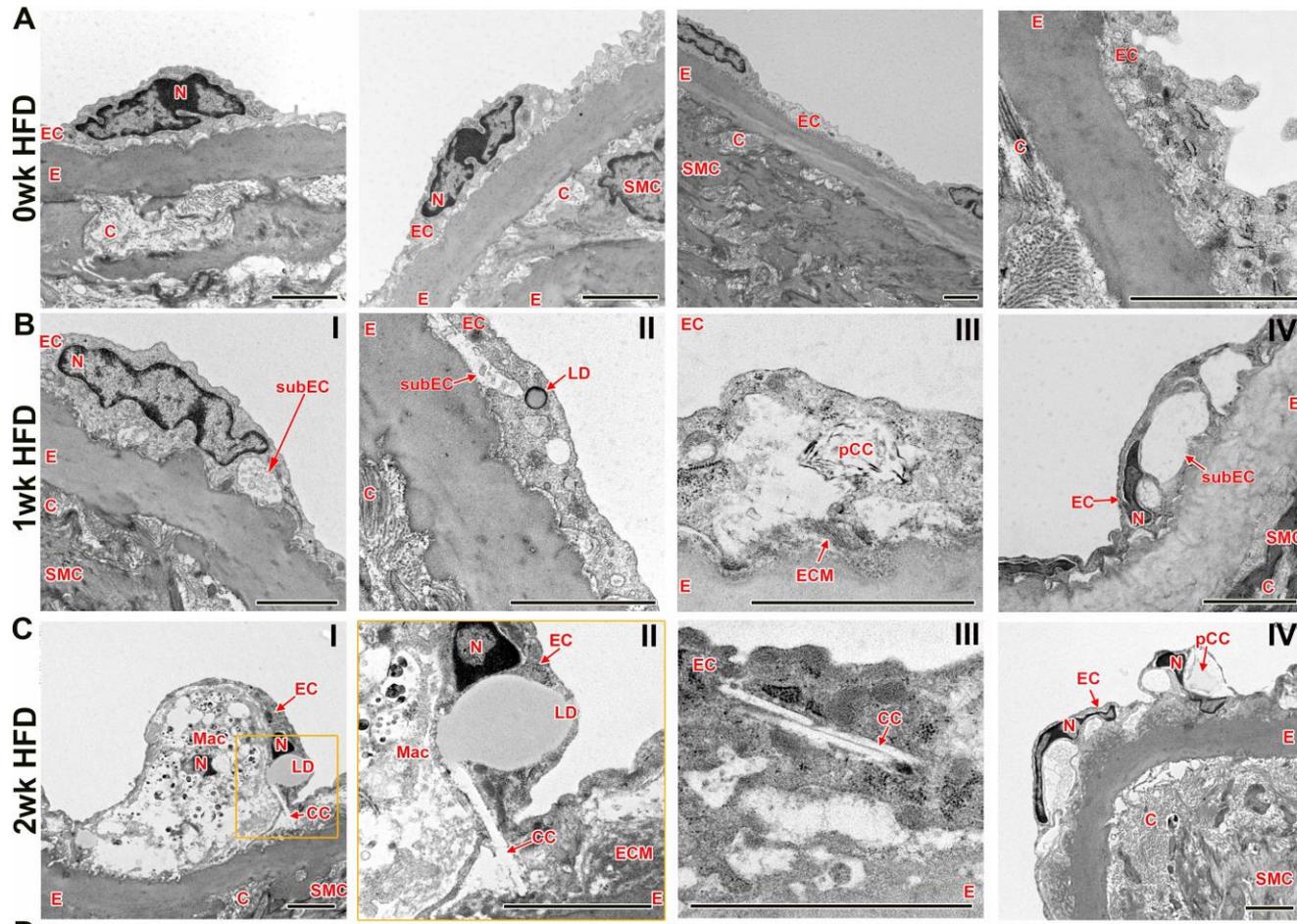


Element	Weight%	Atomic%
C	32.03	56.63
N	12.04	18.25
O	15.12	20.07
Ca	0.00	0.00
Pd	5.87	1.17
Os	29.11	3.25
Au	5.83	0.63
Totals	100.00	

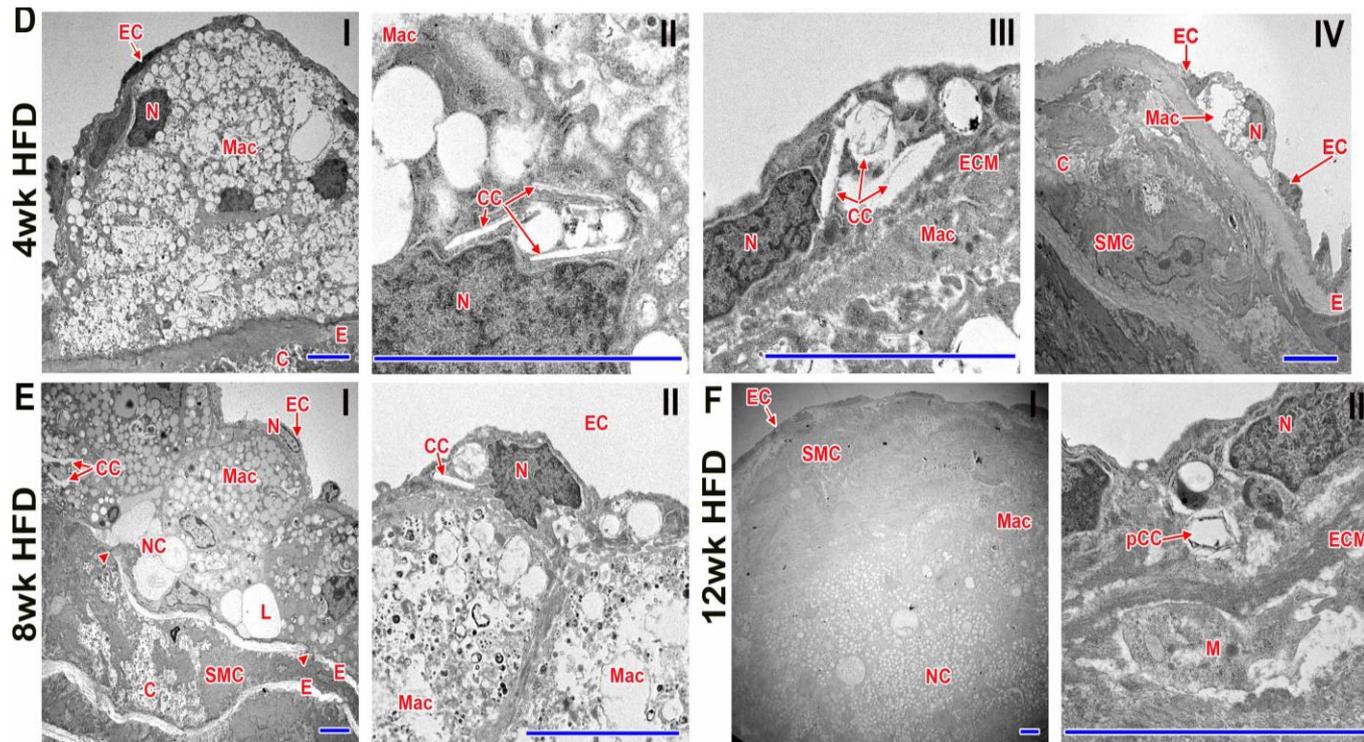


These early cholesterol crystals are composed of C, O and N but not Ca^{2+} .

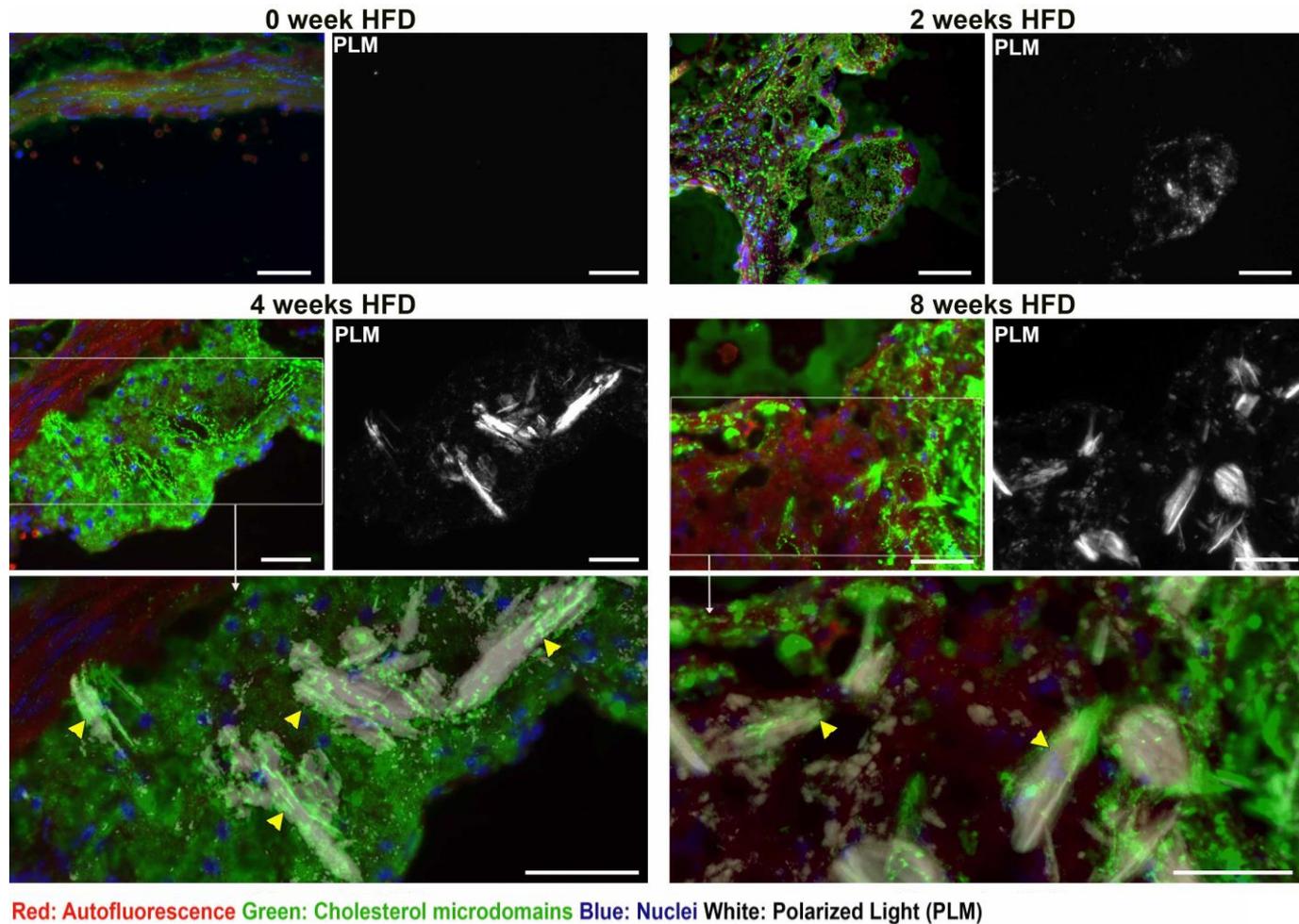
Visualization of atherosclerotic plaque through TEM



Visualization of atherosclerotic plaque through TEM



Localization of CC and cholesterol microdomains



Late cholesterol crystal formation

- Late cholesterol crystal formation most likely occurs in lipid-laden macrophage foam cells
- Cholesterol crystals are also seen in the necrotic core, most likely due to lipid leakage from dead foam cells
- In advanced plaque both needle-shaped and plate-shaped crystals are found
 - Needle-shaped crystals are formed from cholesteryl ester crystallization
 - Plate-shaped crystals are formed from crystallization of free cholesterol

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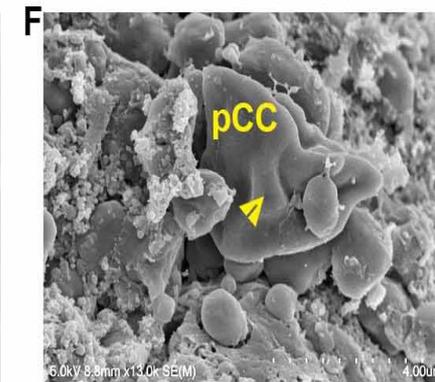
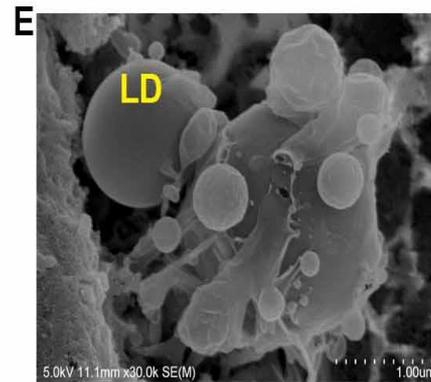
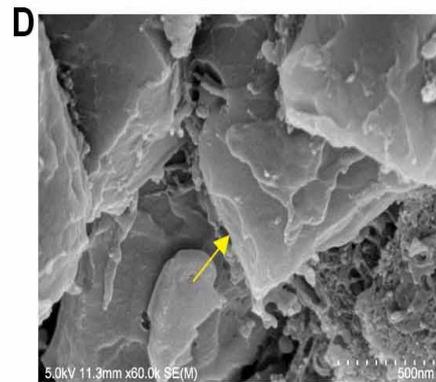
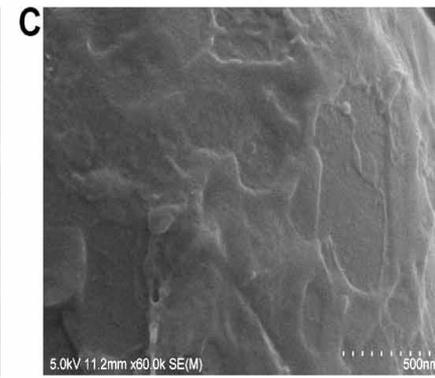
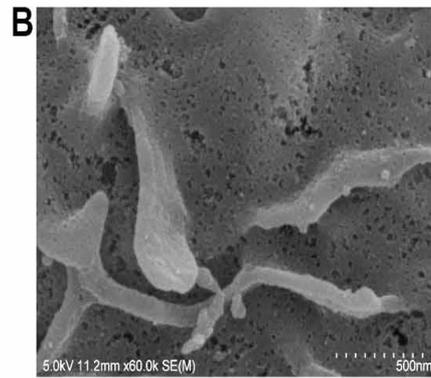
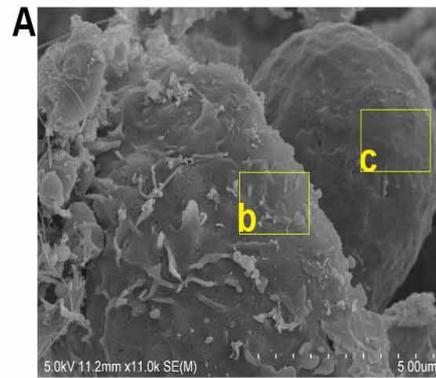
NIH/NHLBI

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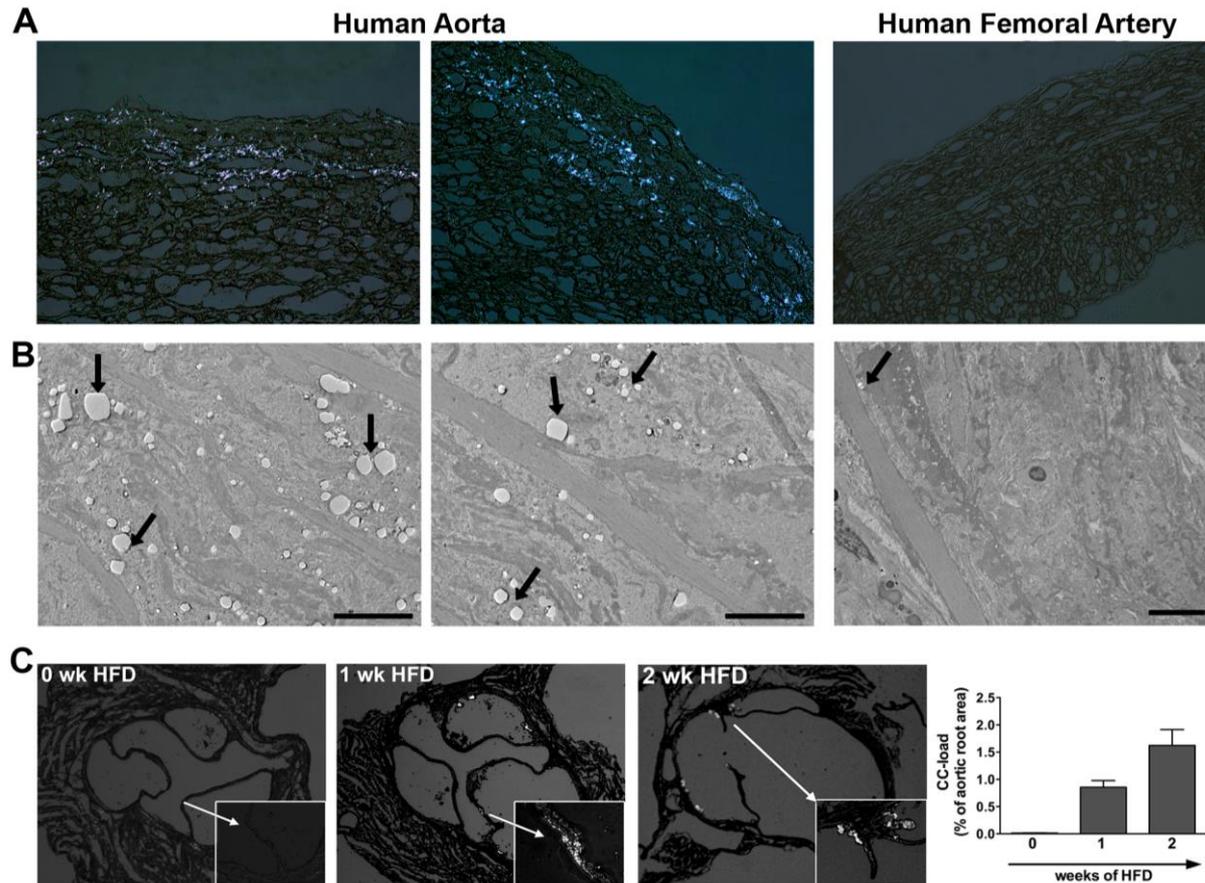


MAHALO

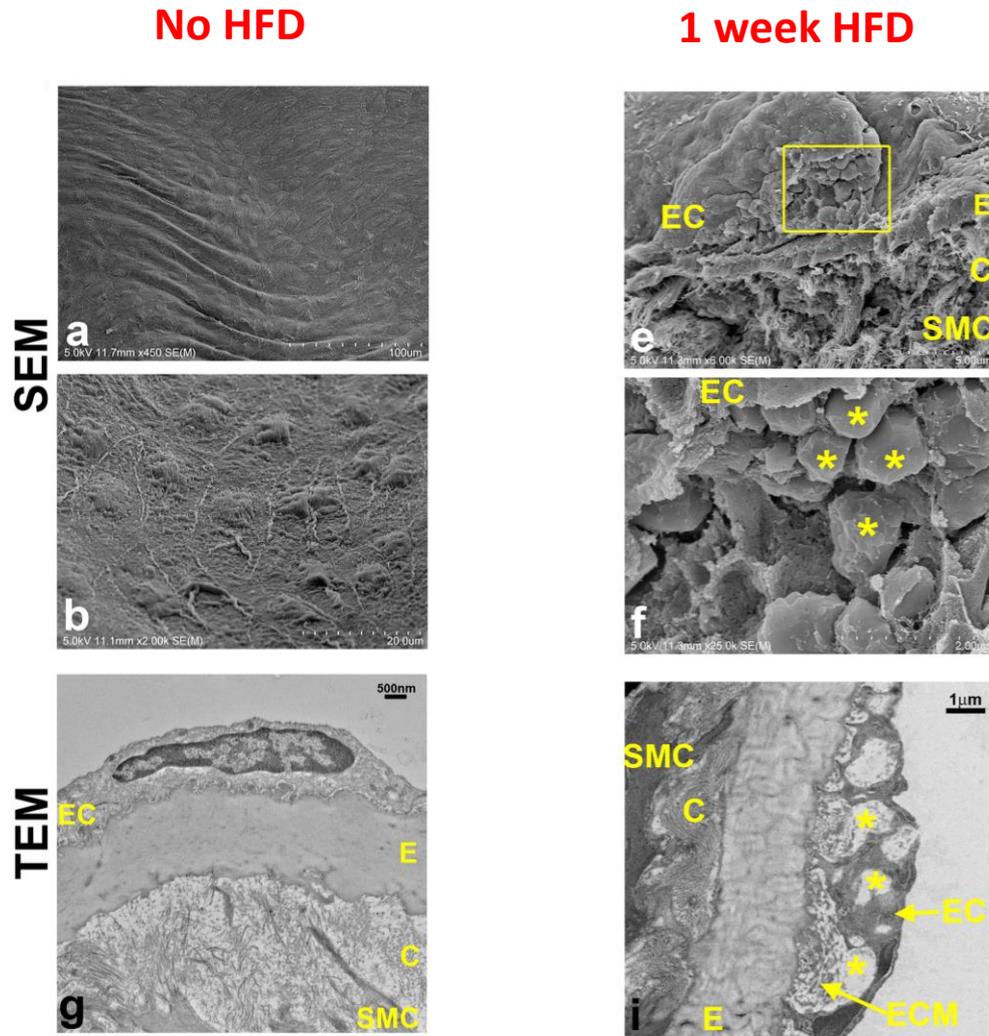




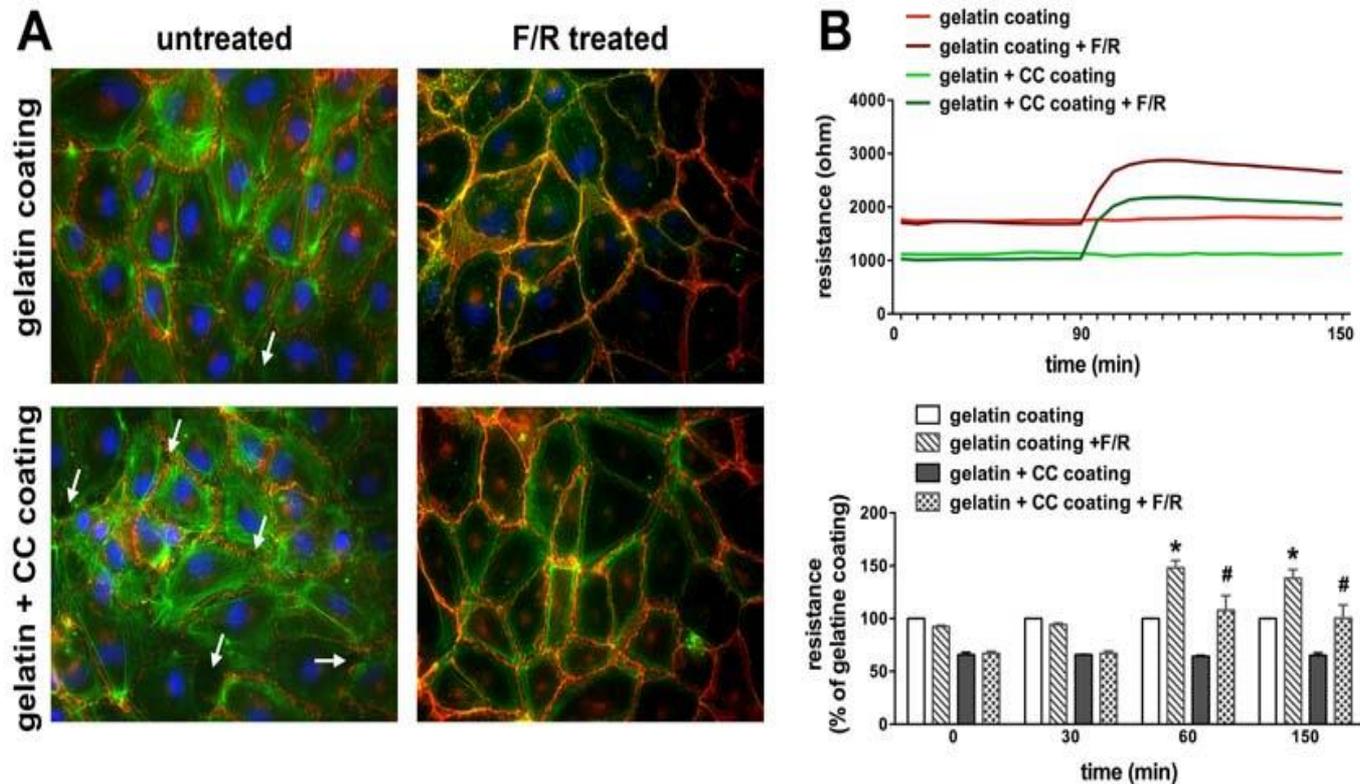
Cholesterol crystals in human and mouse atherosclerotic plaque



Subendothelial CC deposition in *Idlr*^{-/-} mouse aorta

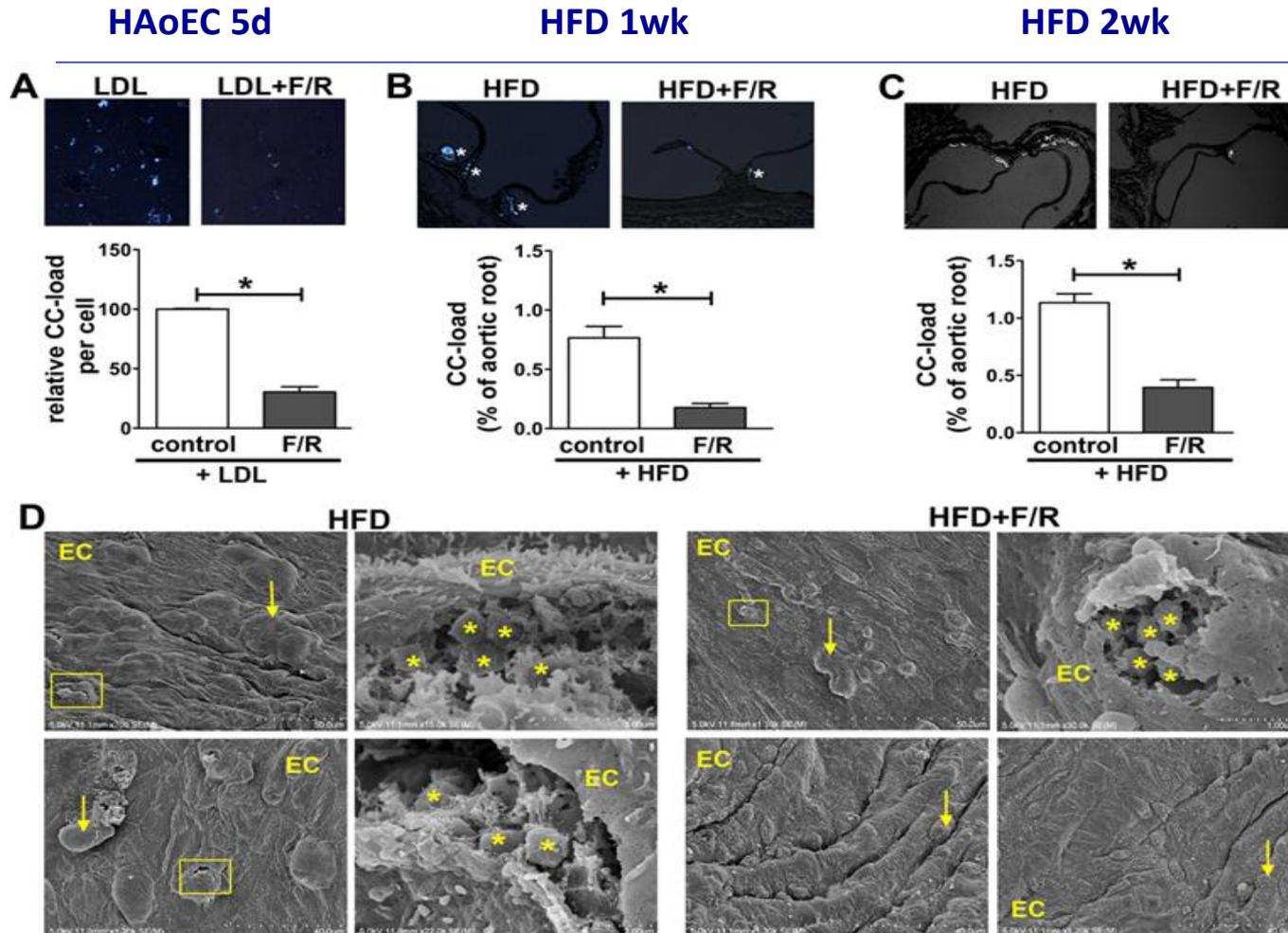


F/R restores the barrier dysfunction caused by CC

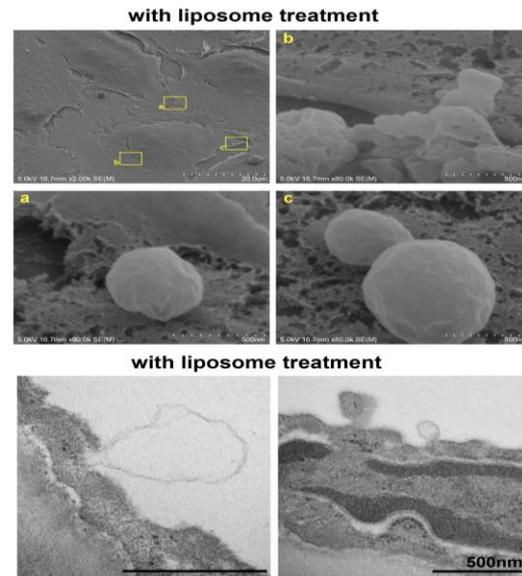
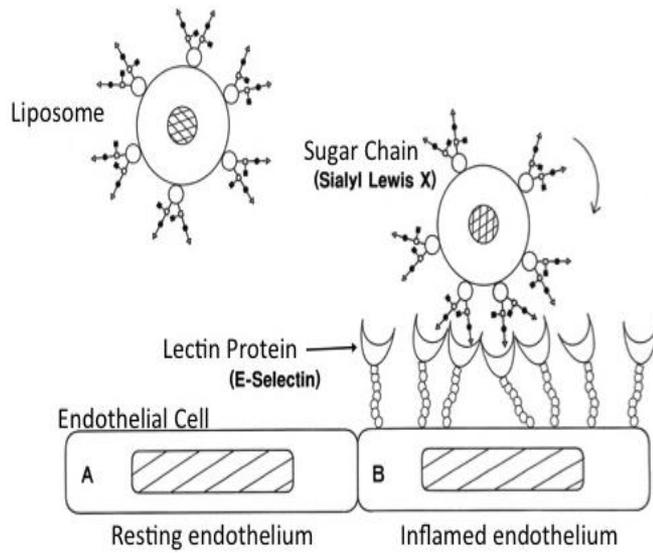


Forskolin/rolipram are well known cAMP-enhancing agents

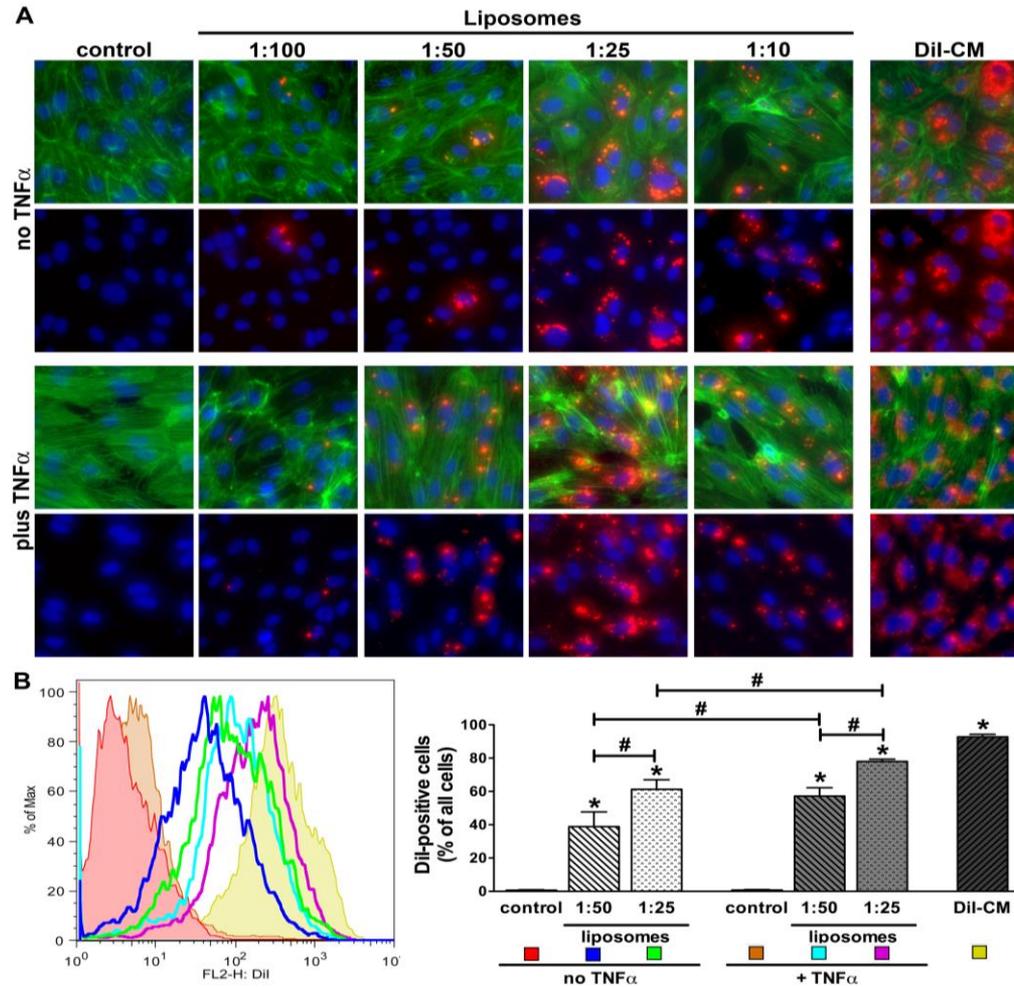
F/R treatment reduces CC formation in *ldlr*^{-/-} mice



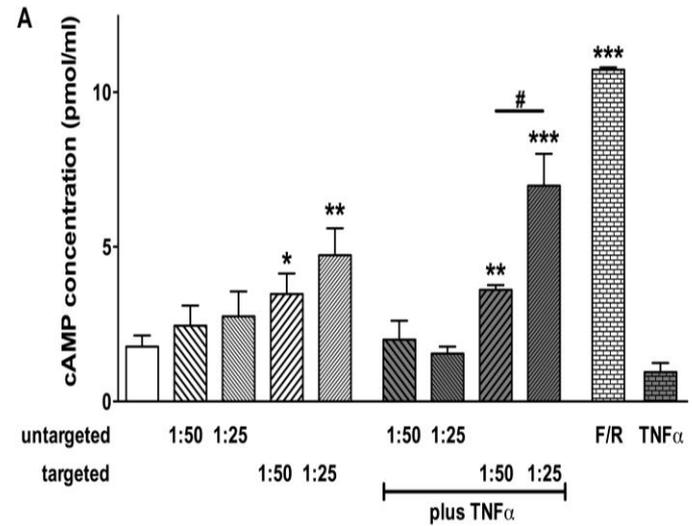
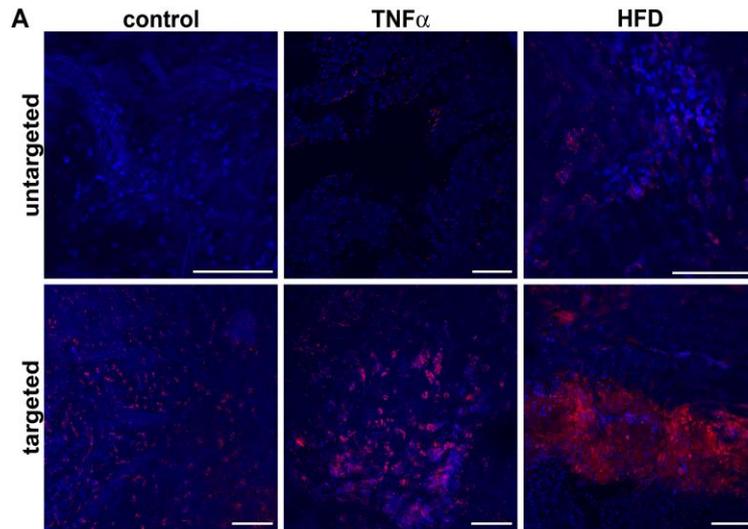
Characterization of F/R-containing liposome



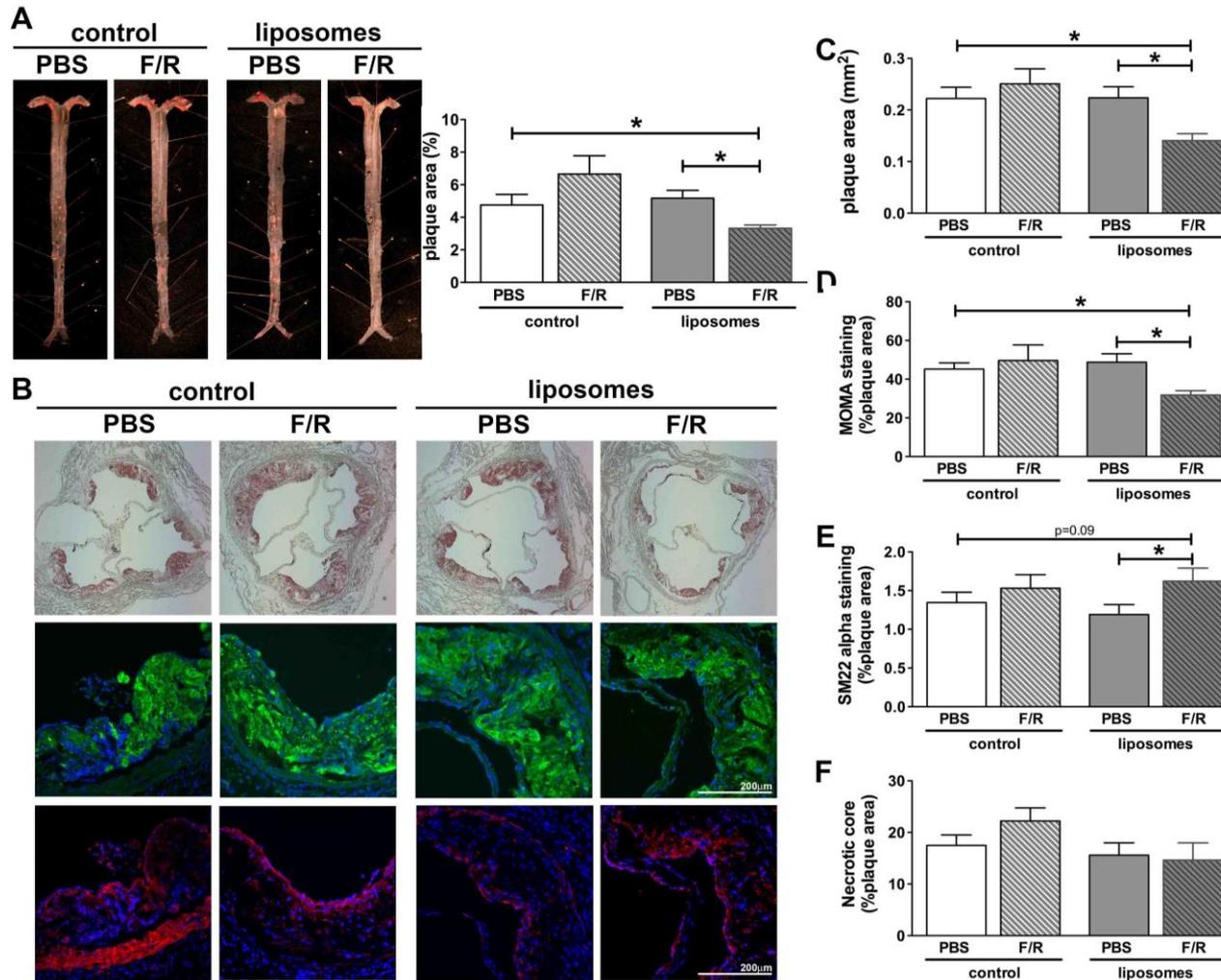
Binding of F/R-liposome to endothelium



Effects of F/R liposome administration *in vivo*



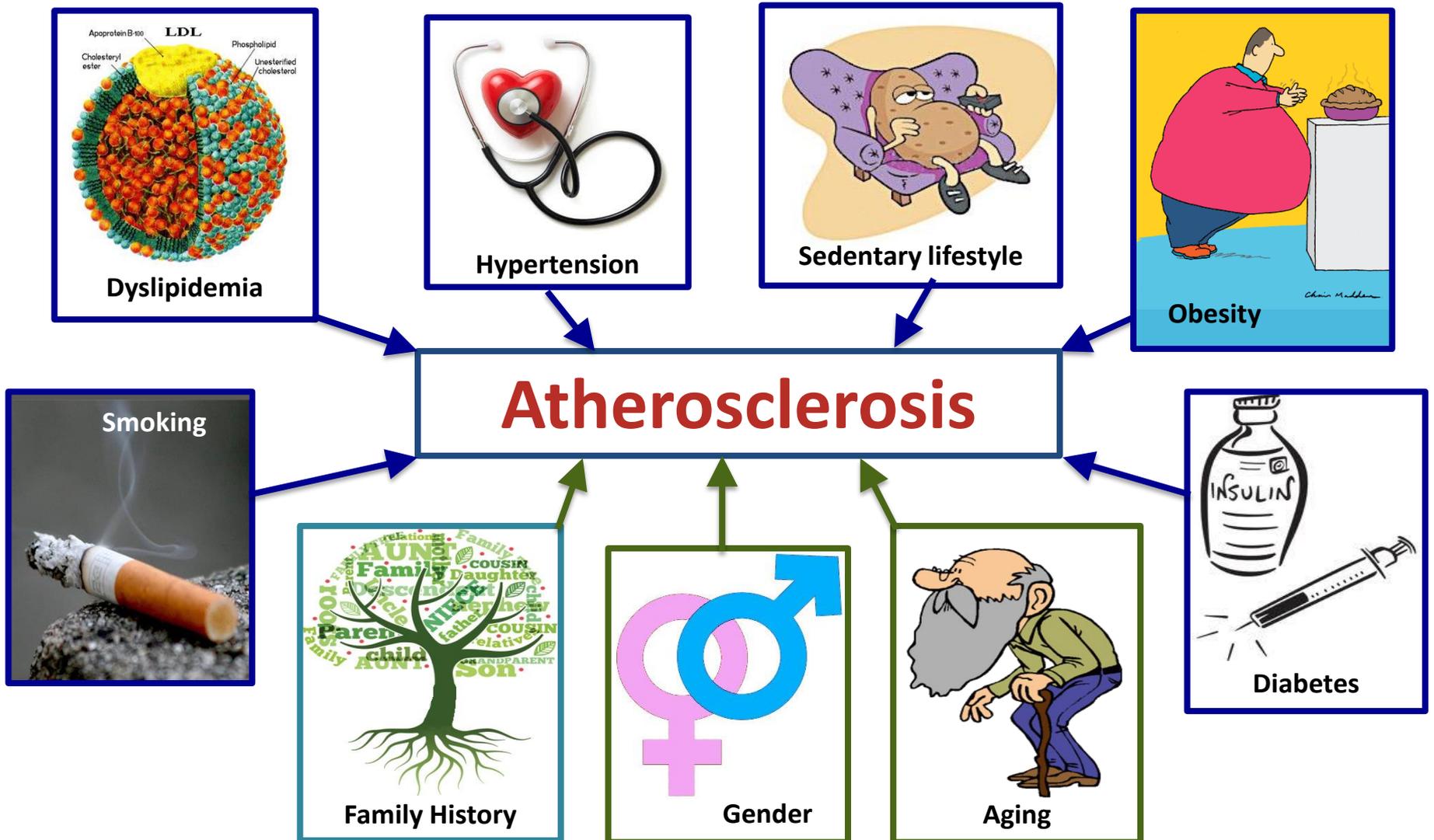
F/R liposome treatment in HFD-fed *apoE*^{-/-} mice



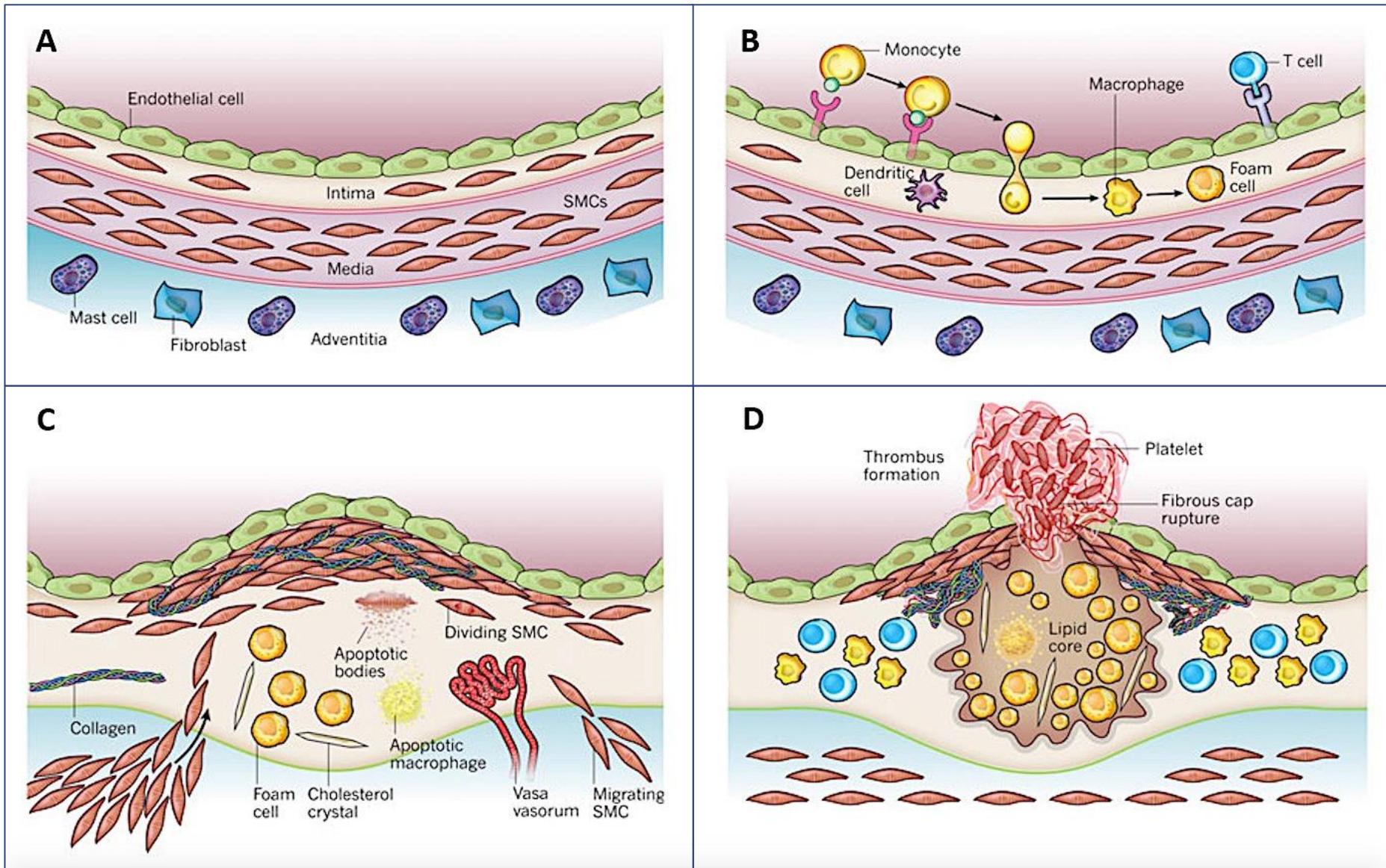
Summary

- F/R effectively improves the endothelial barrier function compromised by CC
- Short-term F/R treatment in HAoEC and in *ldlr*^{-/-} mice resulted in reduced CC formation
- F/R-containing liposomes tagged with sialyl lewis x effectively target the inflamed endothelium
- *ApoE*^{-/-} mice treated with F/R liposomes for 6 weeks displayed significant reduction in the extent of atherosclerosis

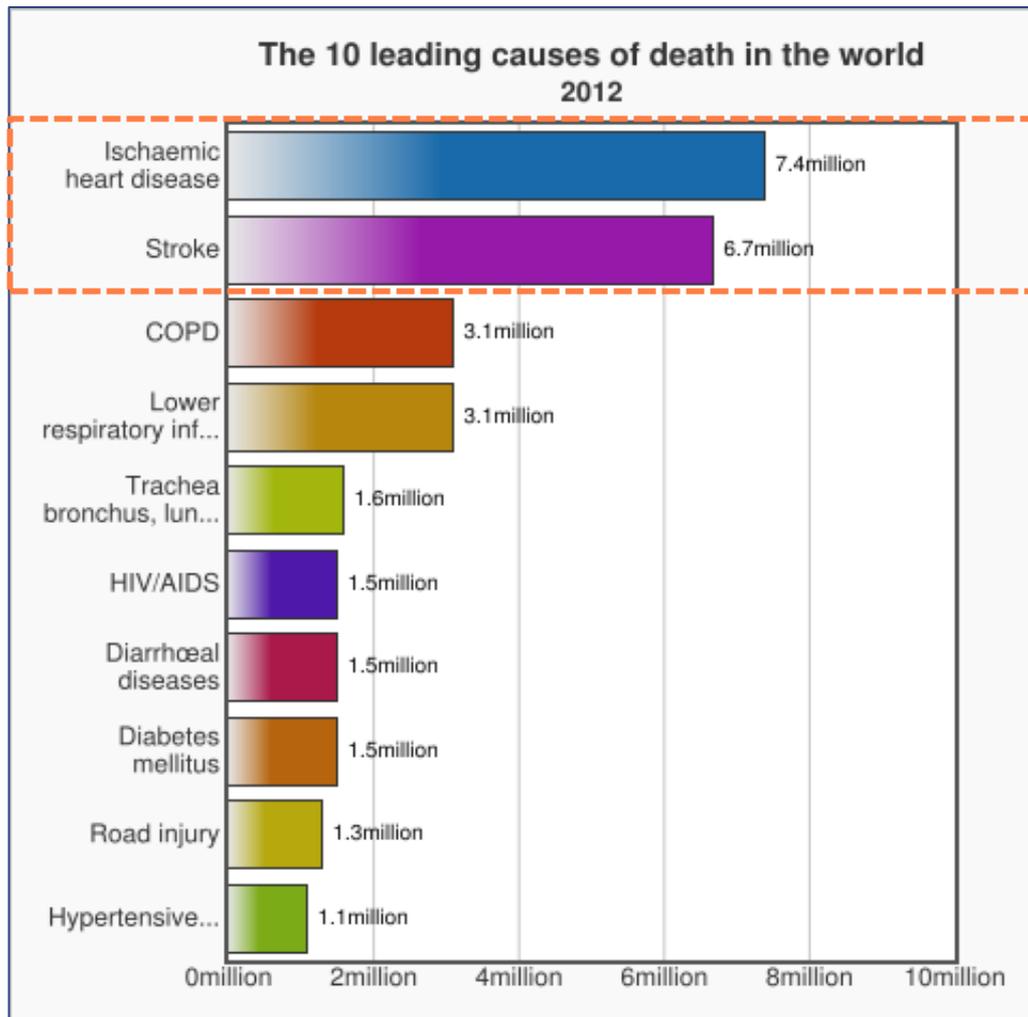
Risk Factors



Stages of Atherosclerosis

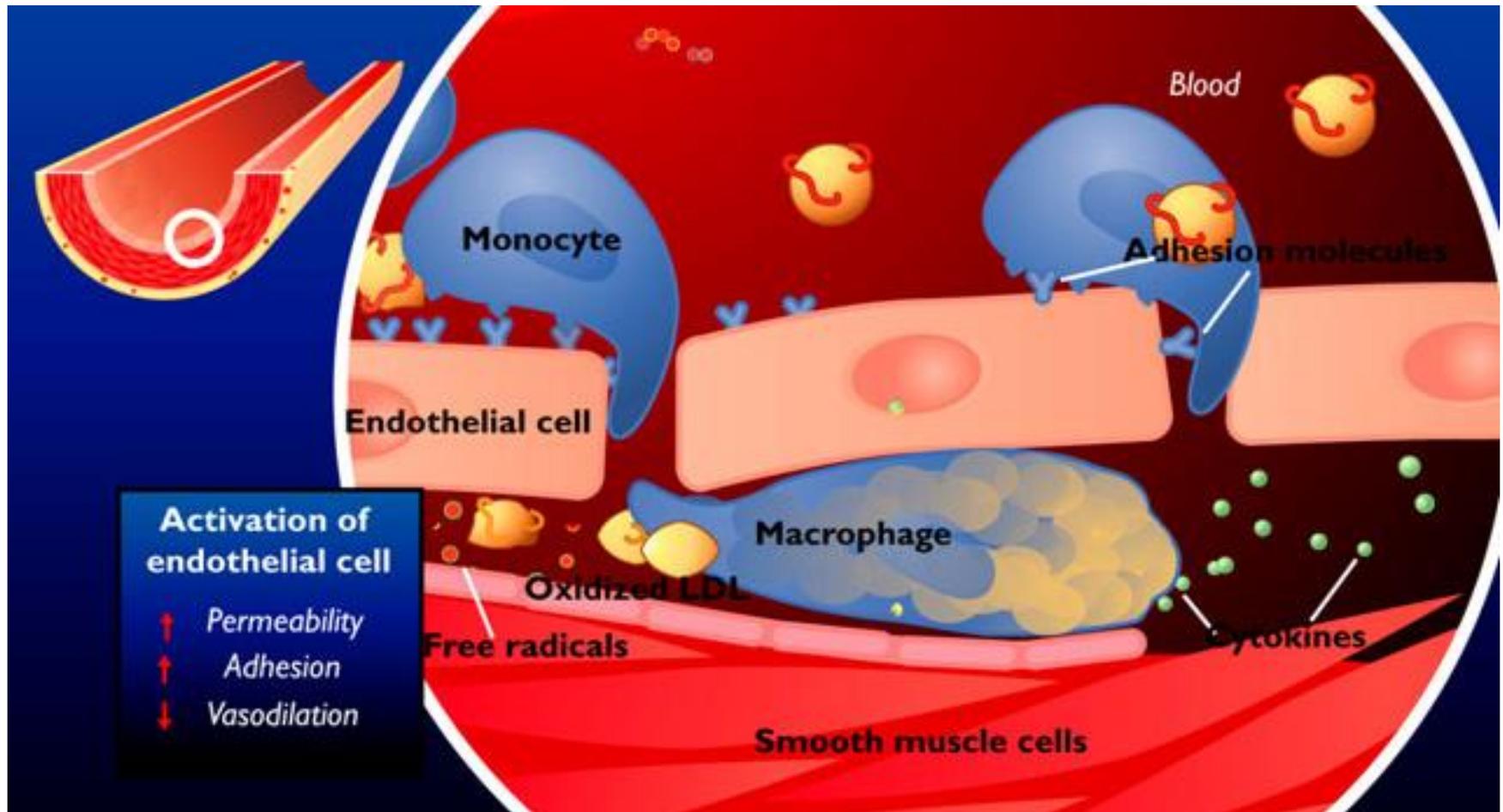


Cardiovascular Diseases (CVD)

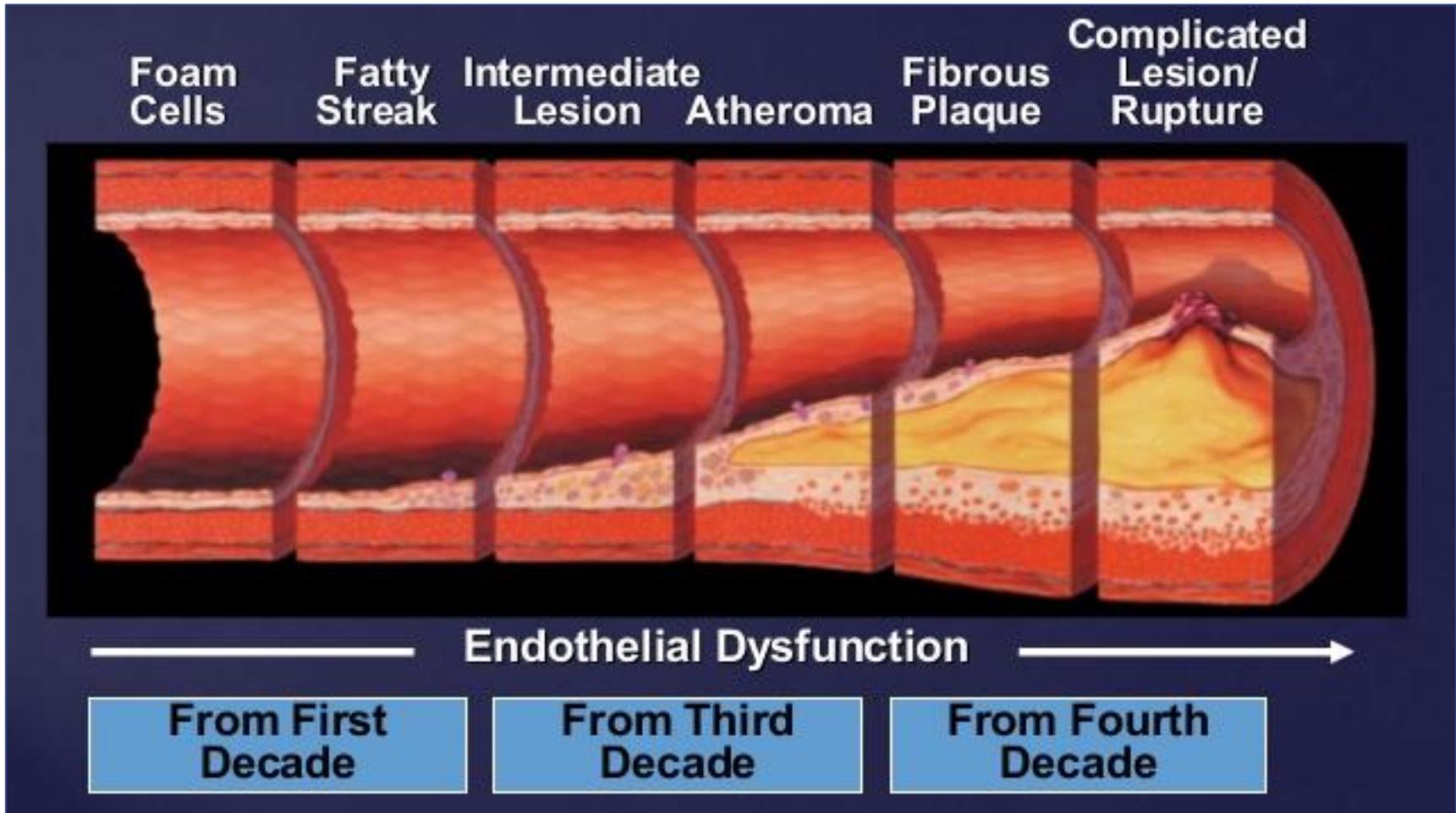


- CVD and stroke are top causes of death in the world – 35% of total
- \$863 billion in annual global economic burden
- 17% of US national healthcare costs

Vascular endothelium modification in atherosclerosis

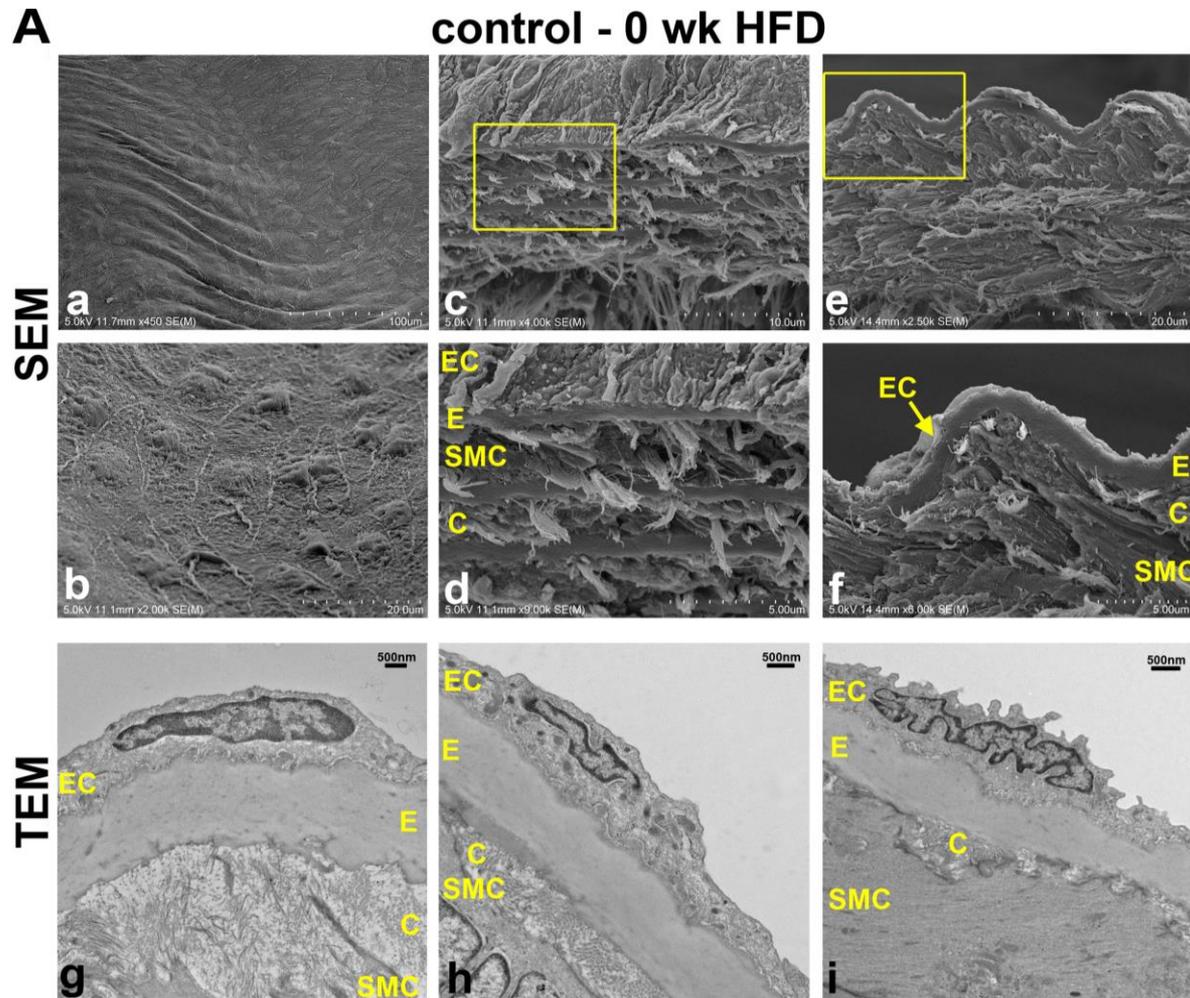


Atherosclerosis Timeline



Subendothelial CC deposition in *Idlr*^{-/-} mouse aorta

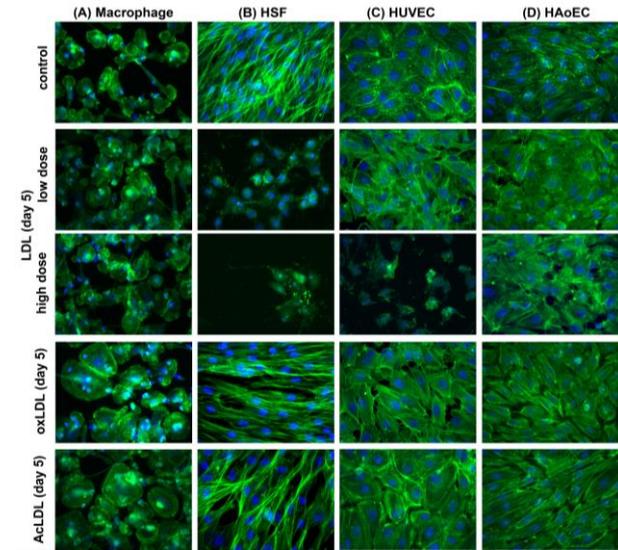
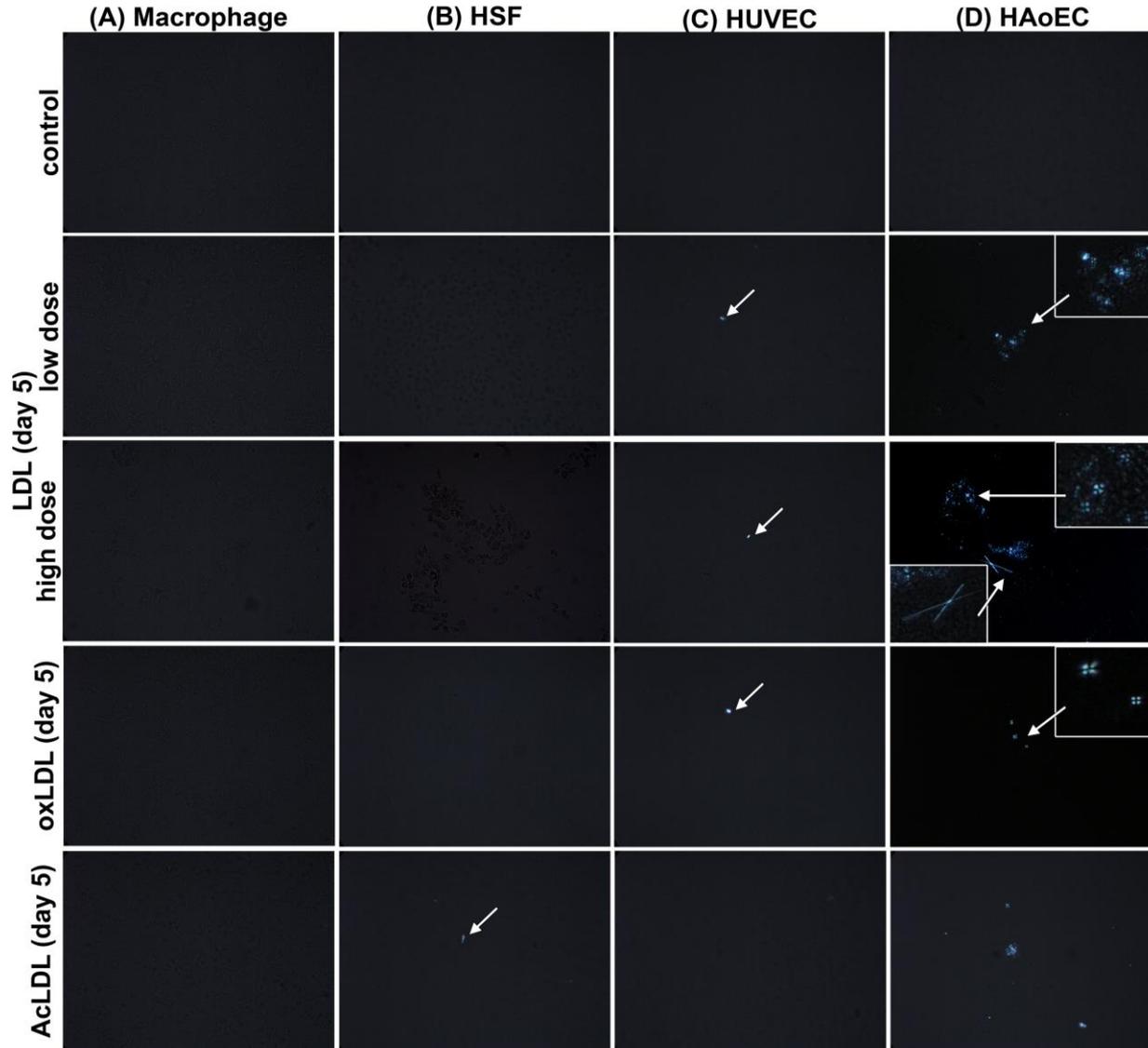
No HFD



Atherosclerosis is an arterial disease of chronic inflammation and hyperlipidemia

- **Intimal thickening that progresses with time**
- **Mononuclear cell infiltrate consisting of monocyte-derived macrophages is very prominent during fatty streak formation**
- **The intimal macrophages and smooth muscle cells are cholesterol loaded**
- **T lymphocytes, dendritic cells, natural killer cells and mast cells accumulate during later stages**
- **The lesion contains cholesterol crystals, necrotic core, fibrous cap (collagen fibers, extracellular matrix)**

Cholesterol crystals are produced and secreted exclusively by aortic endothelial cells upon LDL treatment



- No CC formation can be seen in macrophages, fibroblasts or HUVECs.

Smaller Lipoprotein Particles can Diffuse Through the Endothelial Layer

