

# **Cholesterol Crystals in Developing Atherosclerosis**

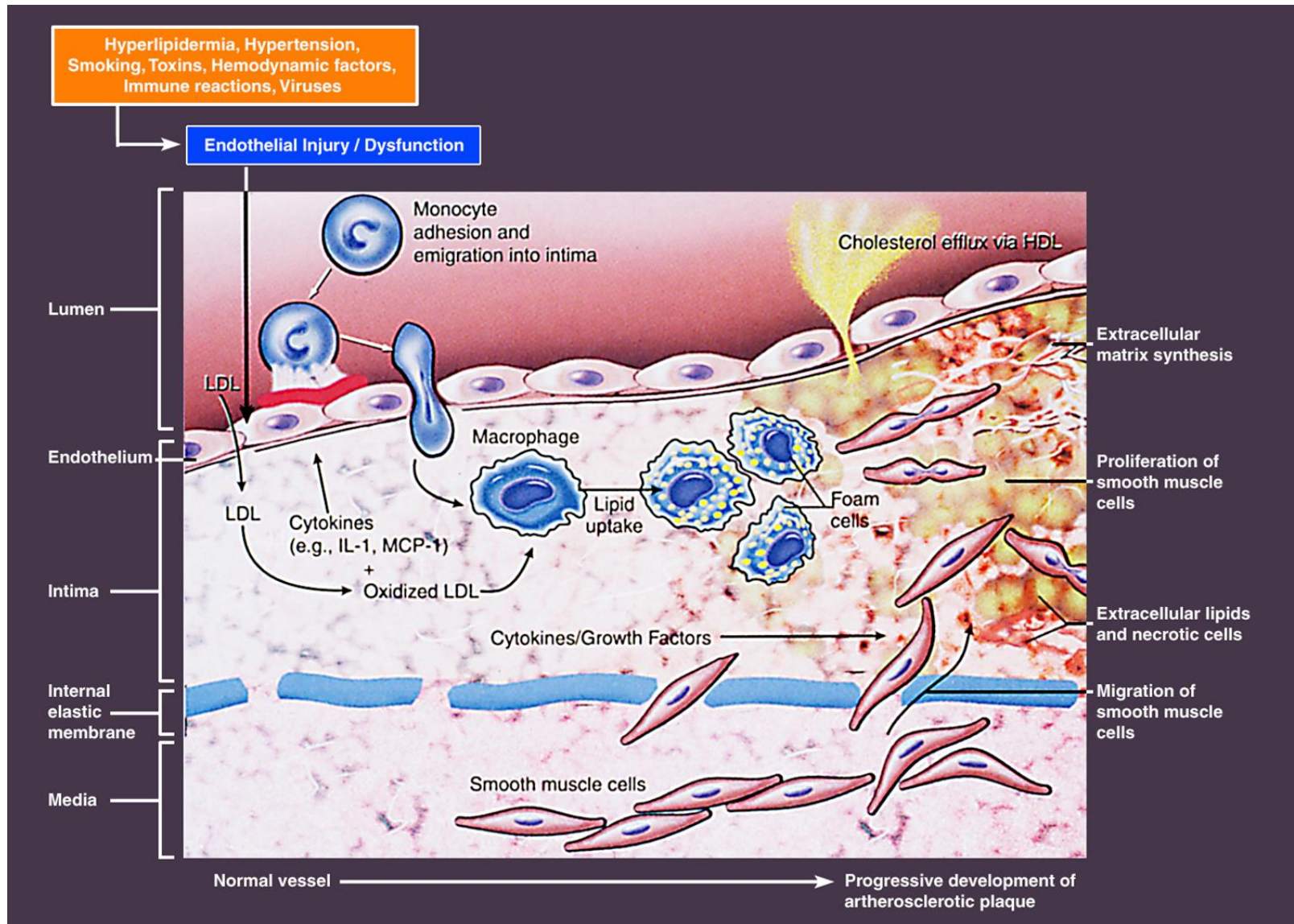
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**University of Hawaii  
John A Burns School of Medicine  
Center for Cardiovascular Research**

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Busan, Korea**

# Development of Atherosclerosis



# Endothelial Cells in Atherosclerosis

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- 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

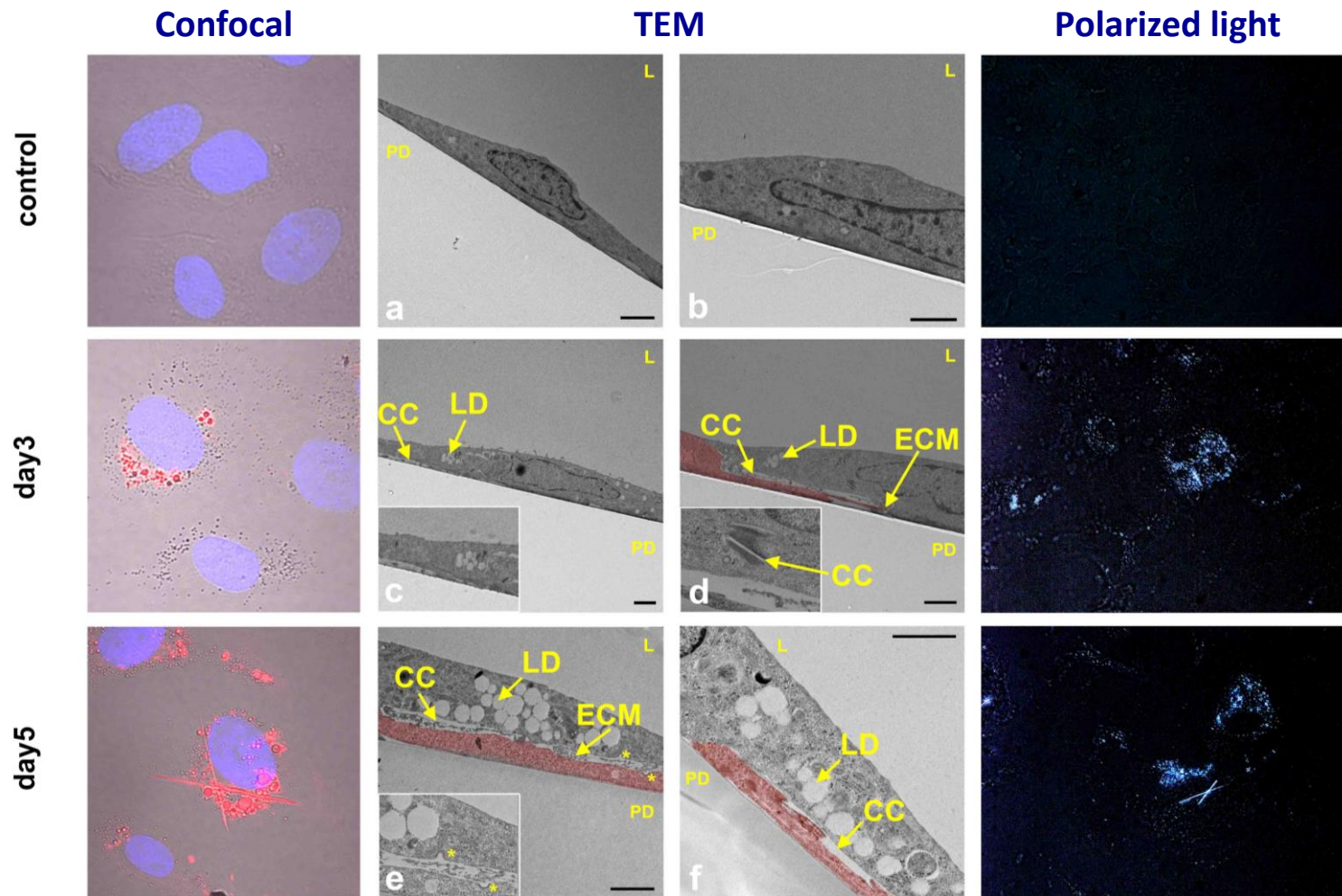
# Endothelial Cells in Atherosclerosis

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Questions:

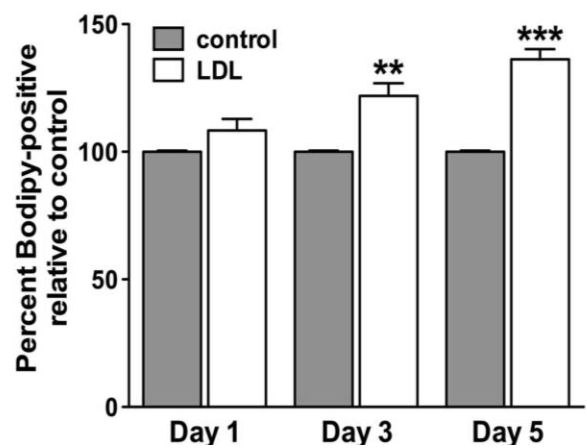
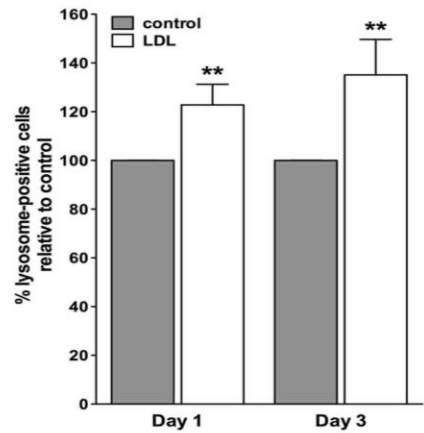
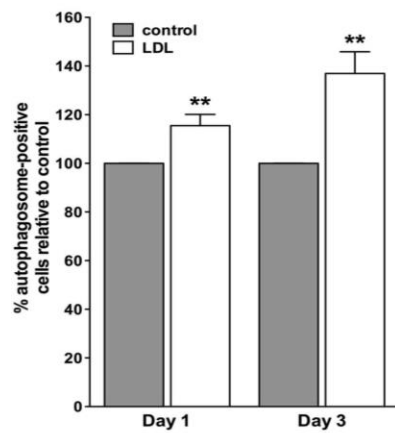
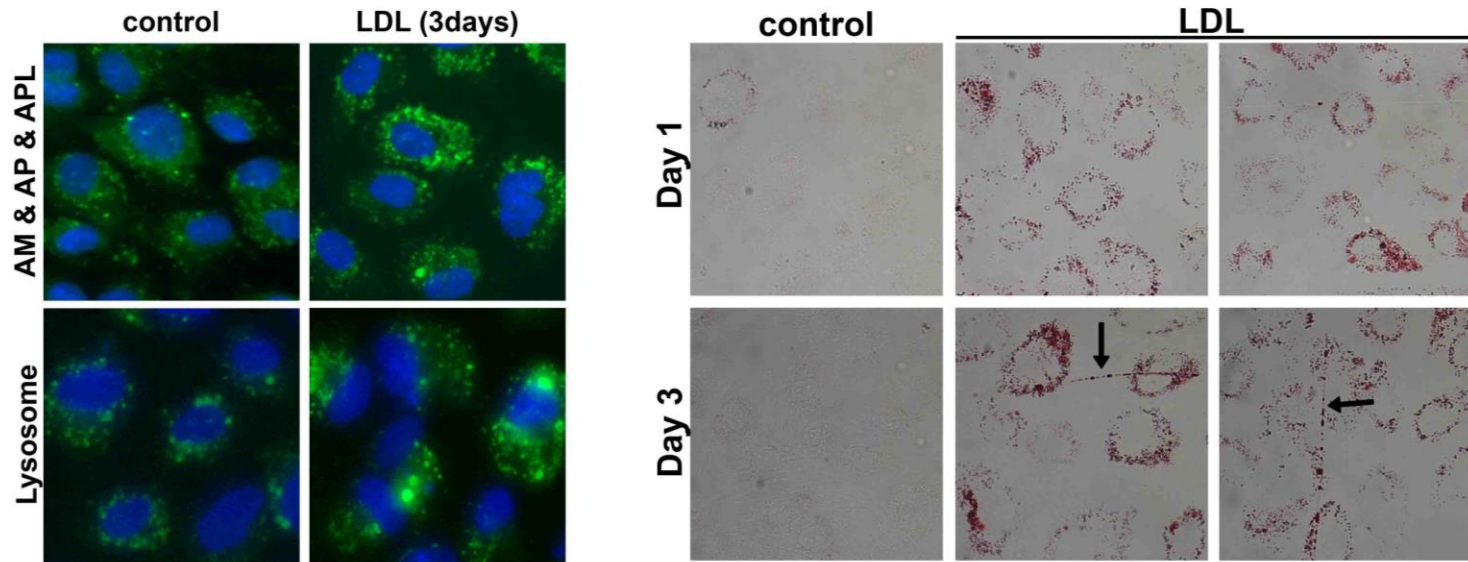
1. Do the endothelial cells take up LDL particles under hyperlipidemic conditions?
2. How do the cells process the lipid?

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

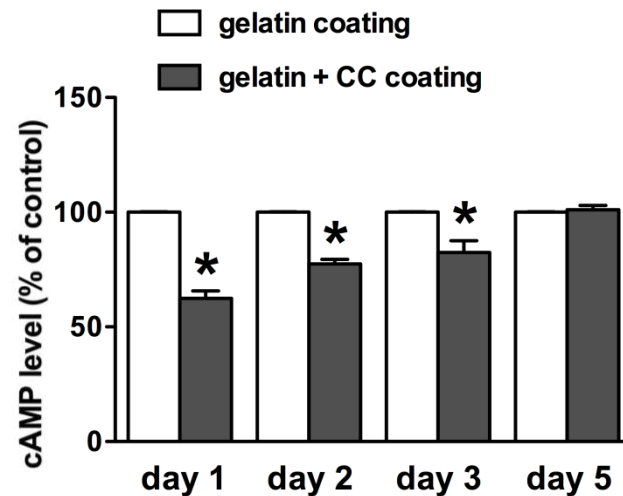
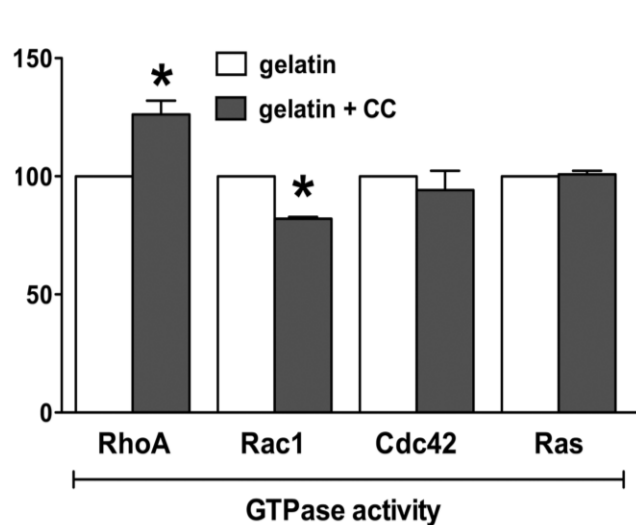
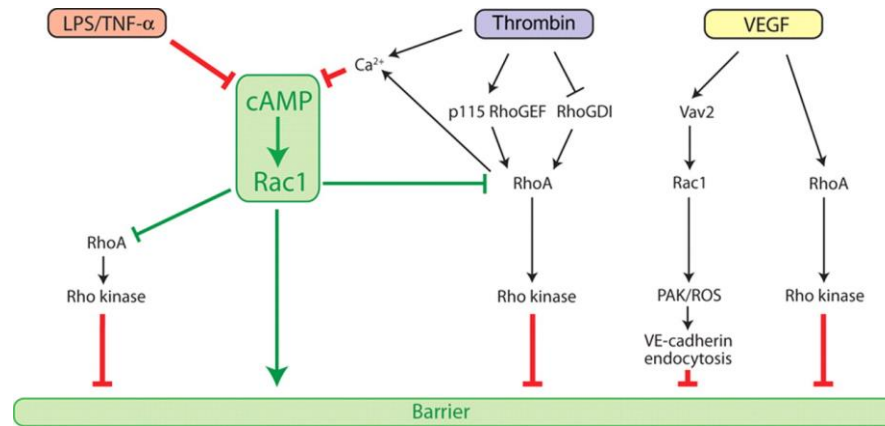




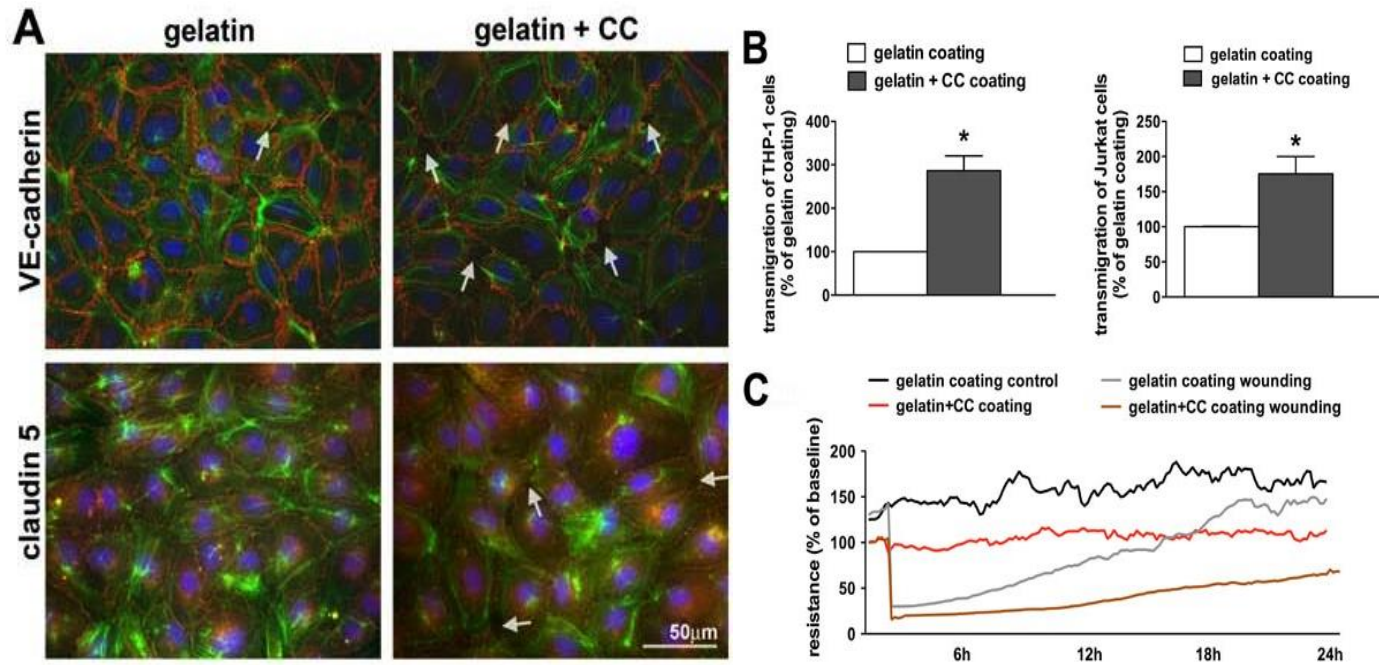
# Lipid processing by HAoEC



# Cholesterol crystal-induced changes in endothelial signaling



# CC induces changes in endothelial function



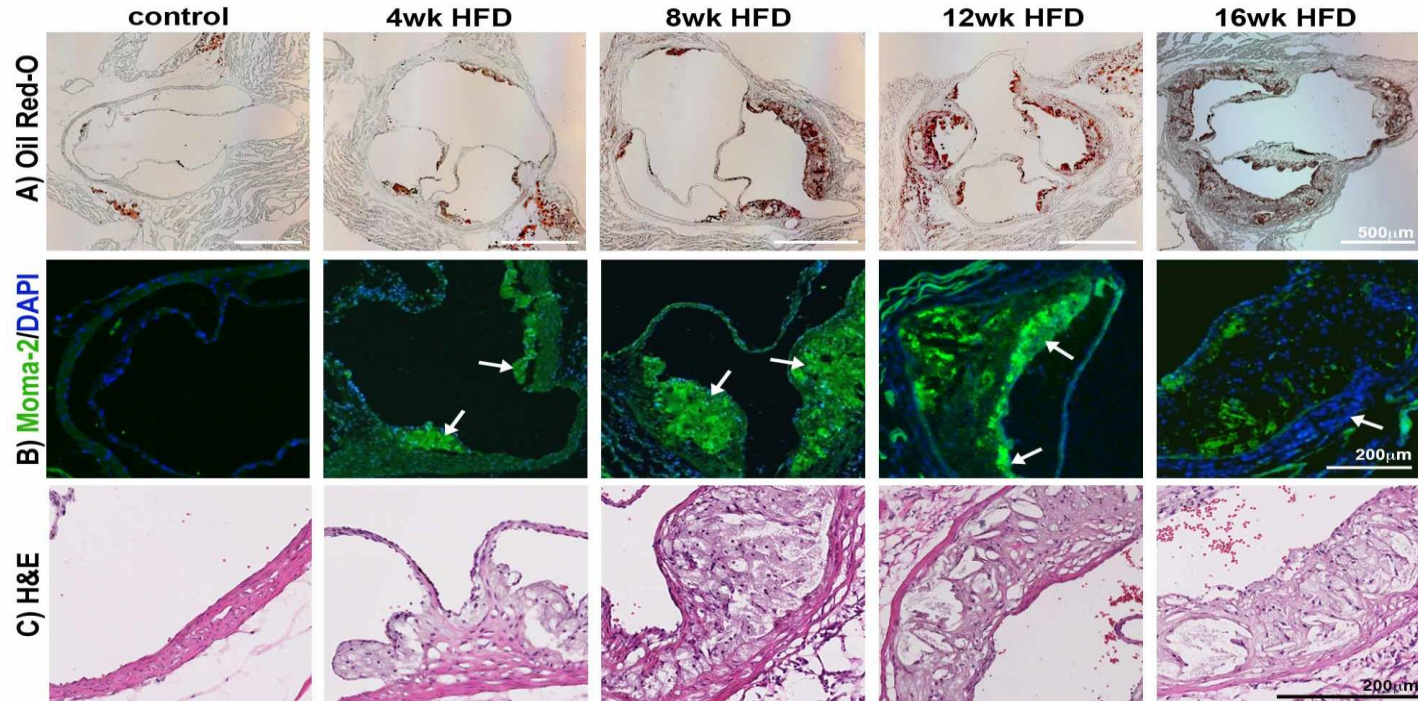


# What about cholesterol crystals in more advanced stages of atherosclerosis?

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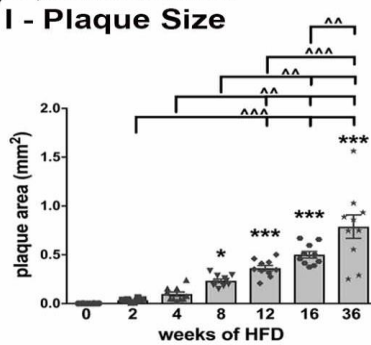
1. Chronicled the ultrastructural changes in atherosclerotic plaque formation through various stages of the disease.
2. Investigated the presence of cholesterol crystals in ultramorphological detail in advancing atherosclerosis.

# Progression of atherosclerosis in the LDLR<sup>-/-</sup> mice

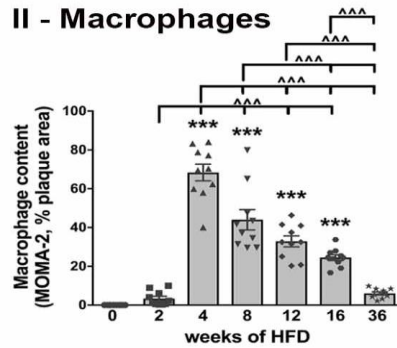


## D) Quantification

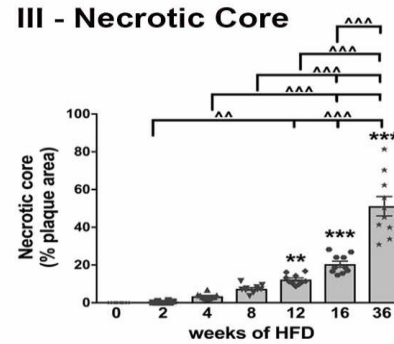
### I - Plaque Size



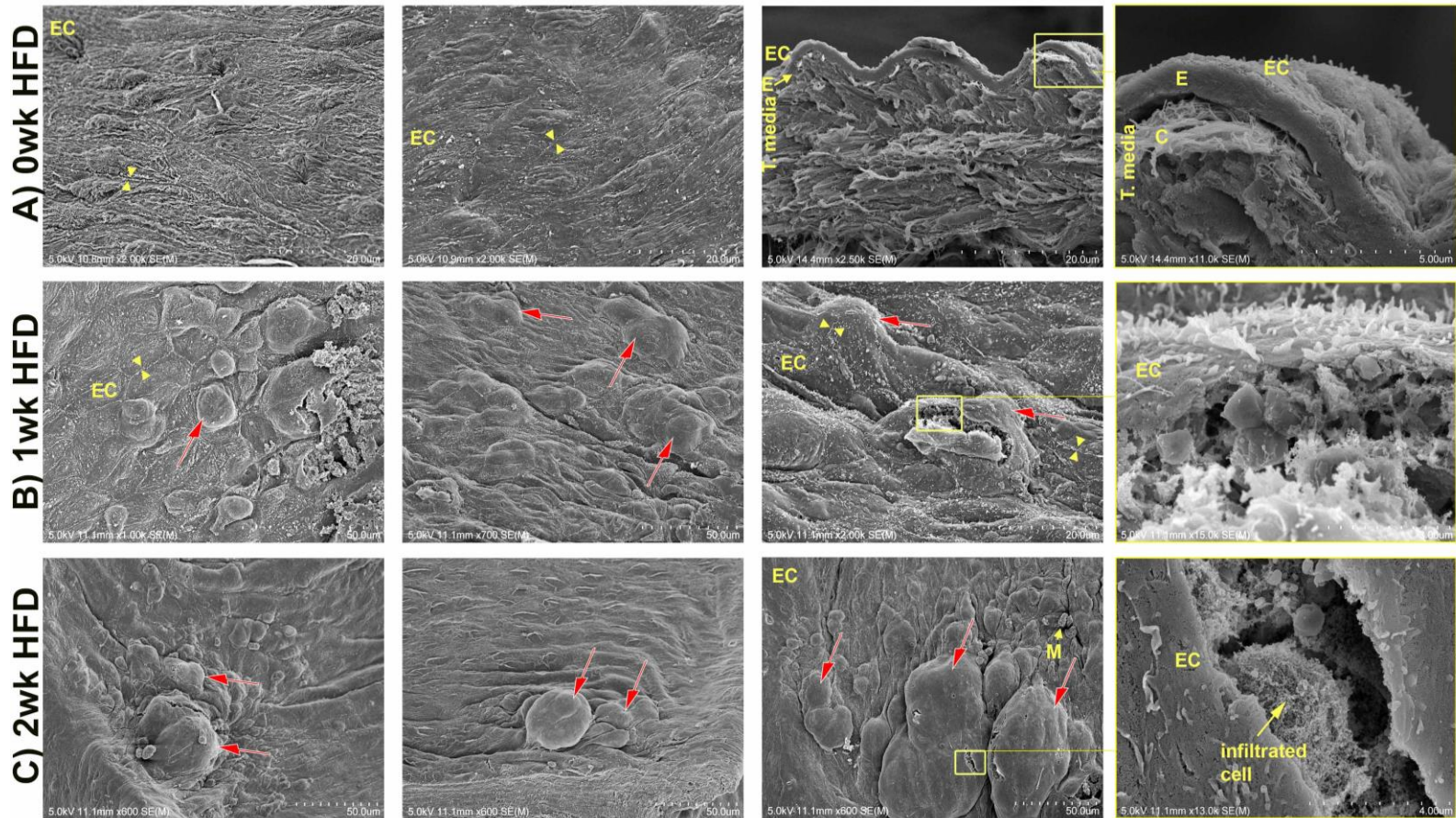
### II - Macrophages



### III - Necrotic Core

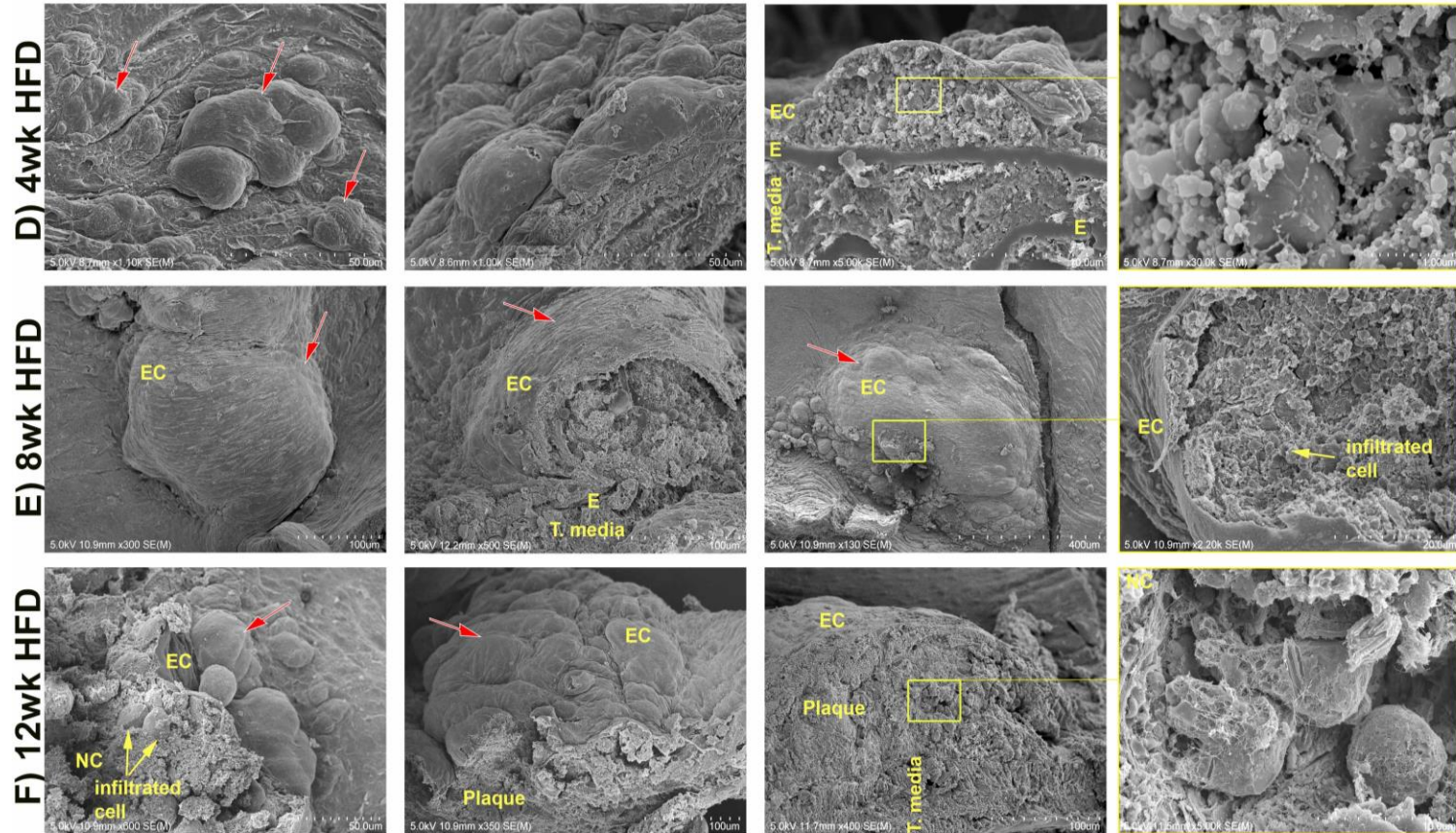


# SEM images of atherosclerotic endothelium early stages



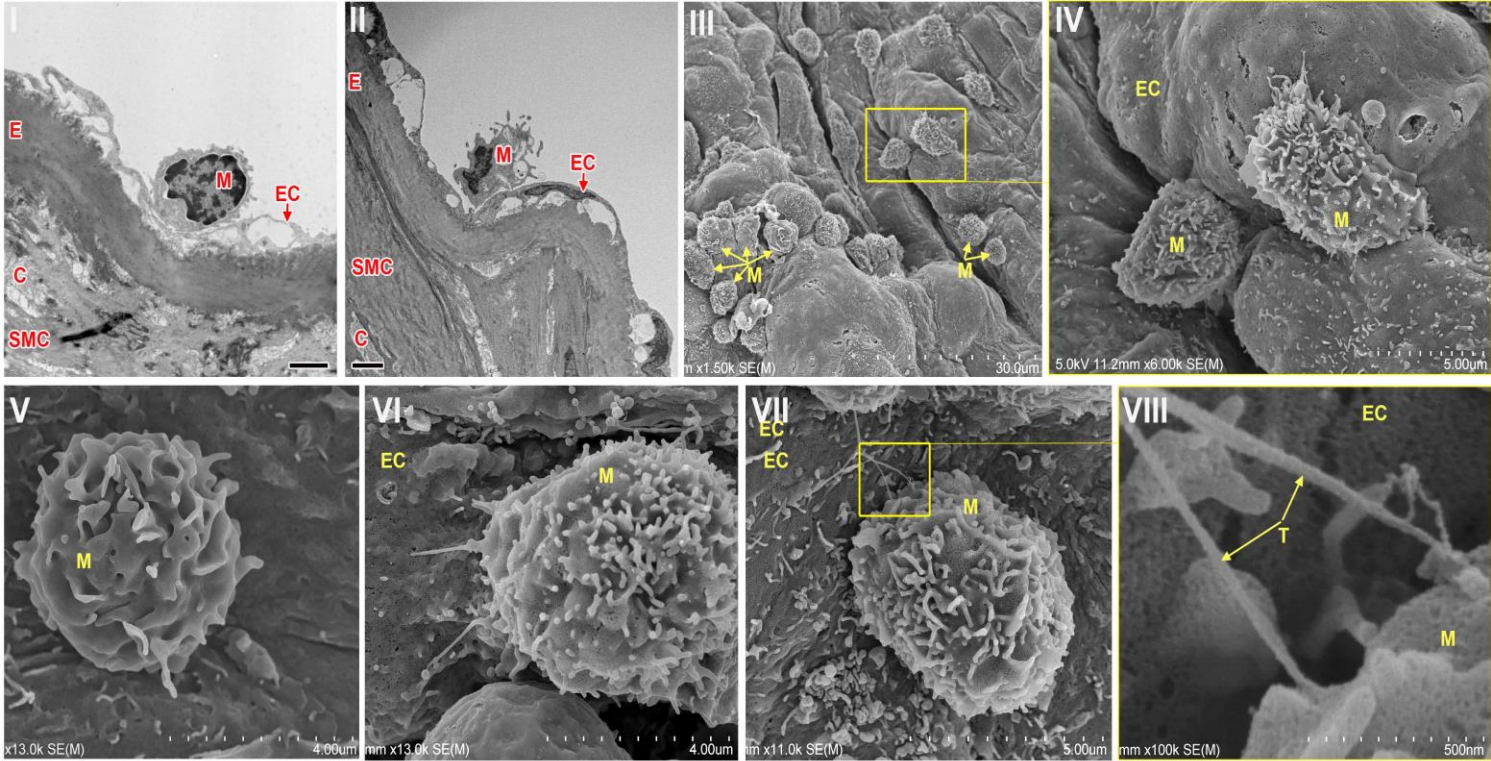


# SEM images of atherosclerotic endothelium late stages



# Monocyte adherence to endothelium in early atherosclerosis

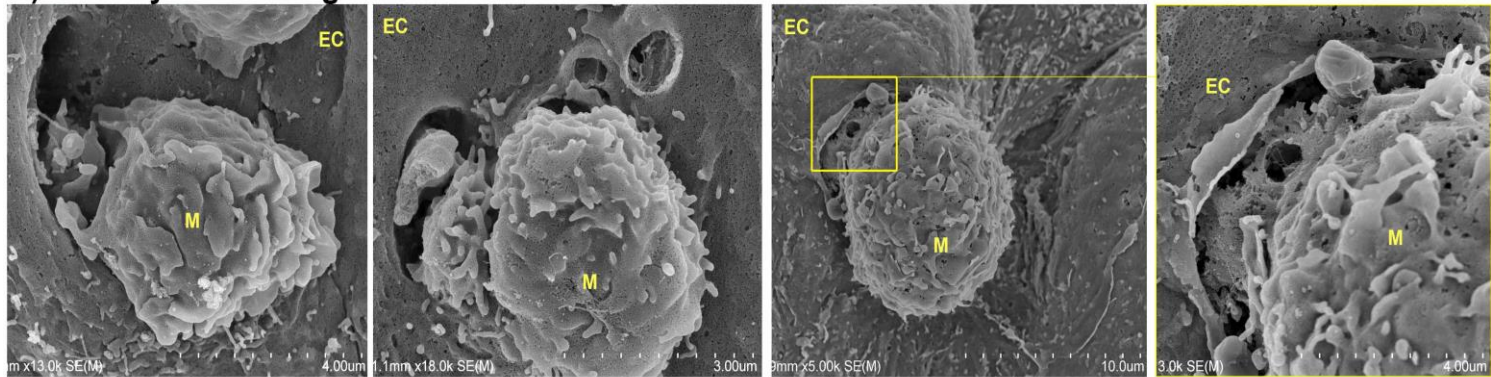
## A) Monocyte adherence



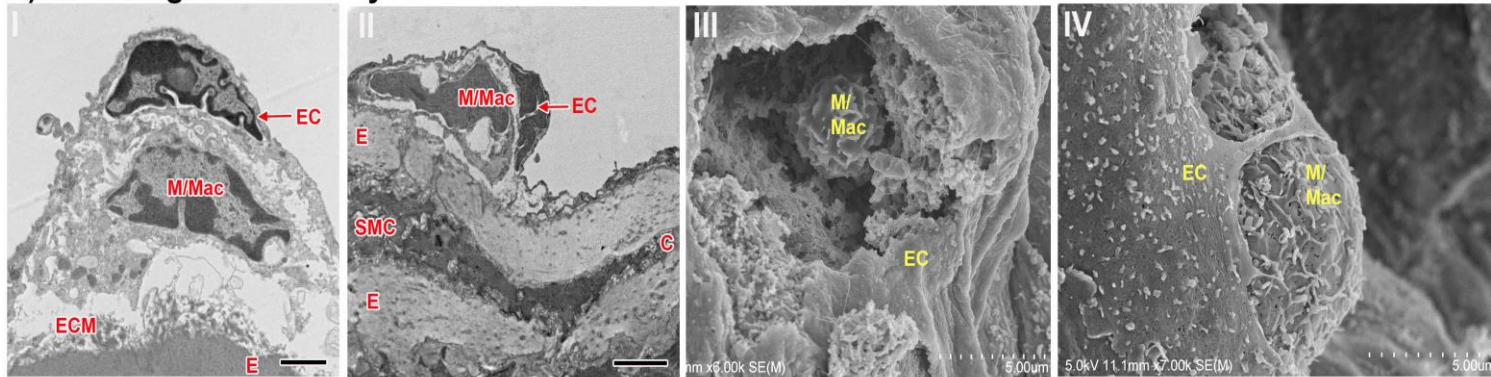


# Monocyte diapedesis through the endothelium

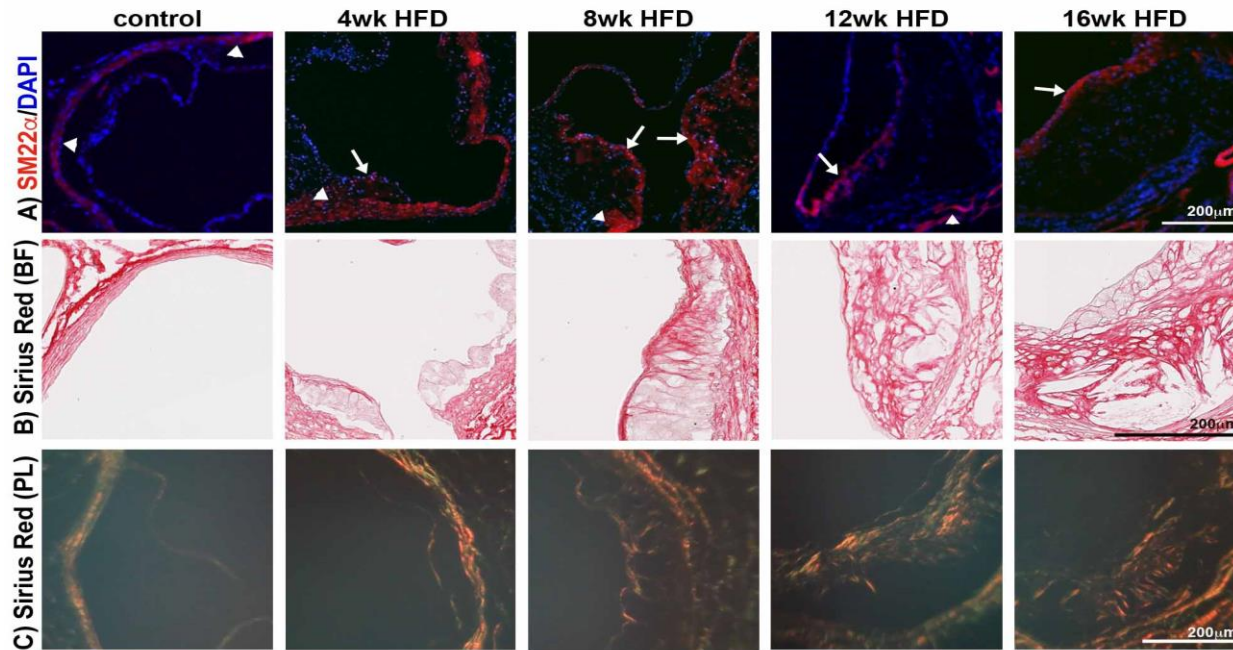
## B) Monocyte Transmigration



## C) Transmigrated Monocyte

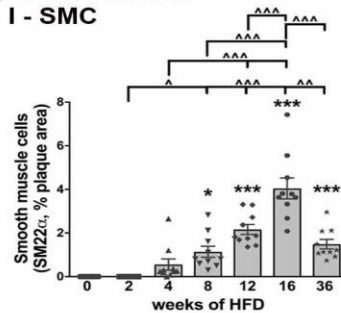


# Changes in extracellular matrix composition during atherosclerosis progression

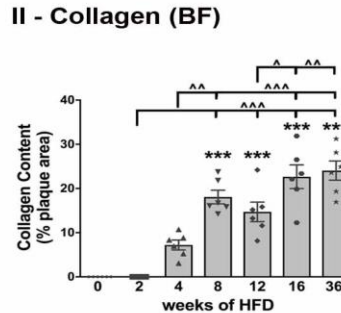


## D) Quantification

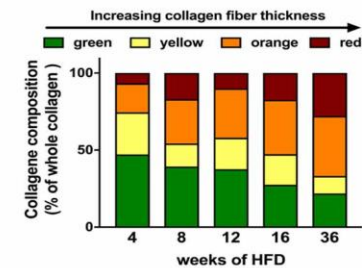
### I - SMC



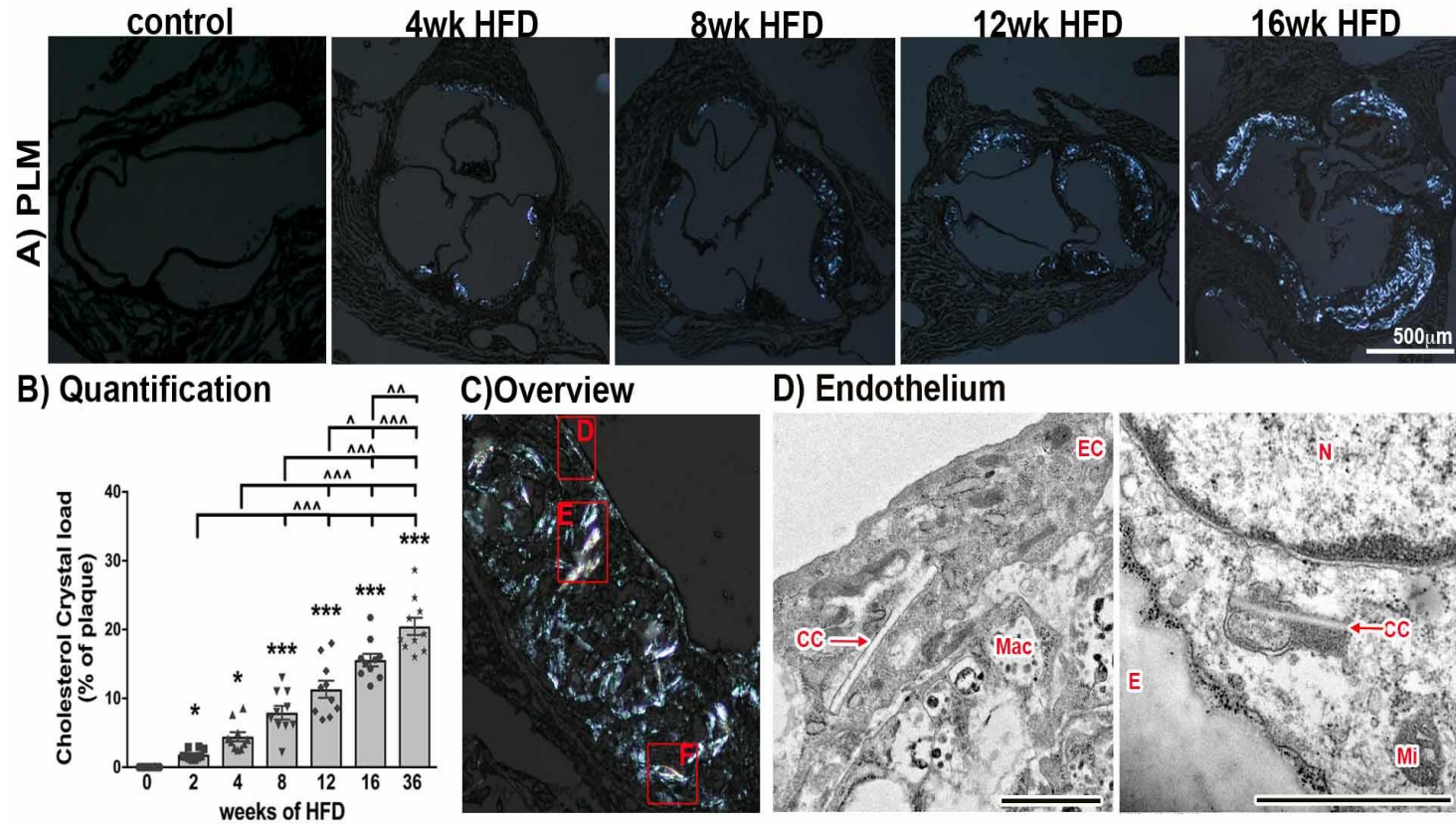
### II - Collagen (BF)



### III - Collagen (PL)



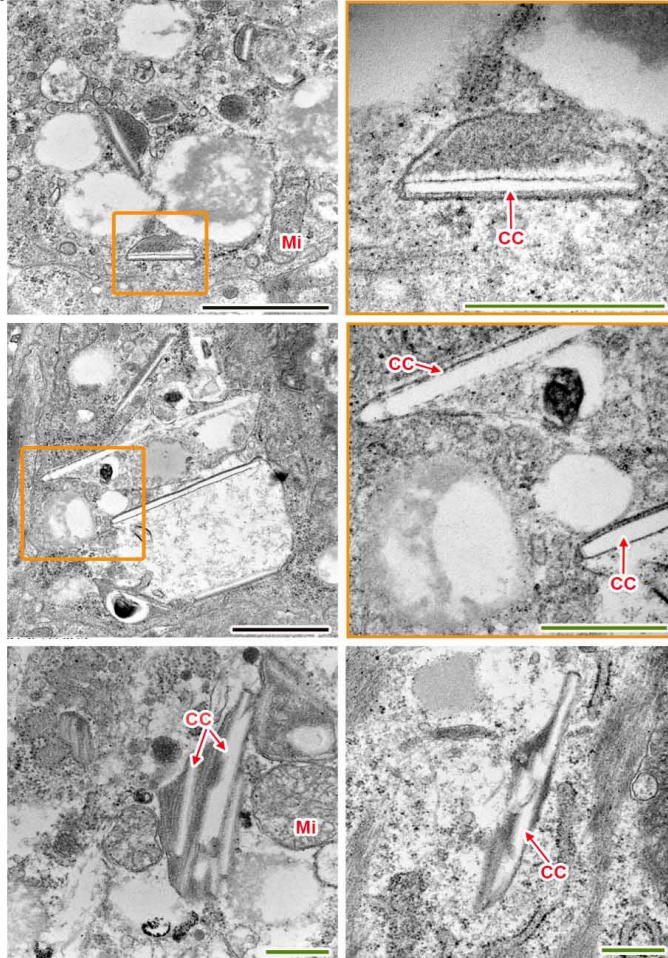
# Presence of cholesterol crystals during atherosclerosis progression



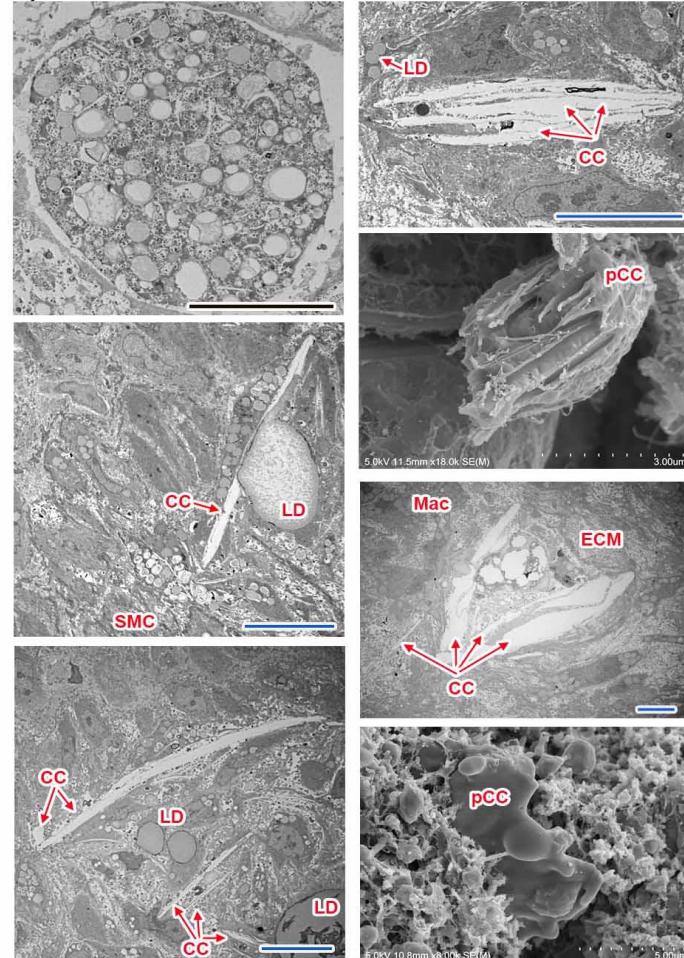


# Presence of cholesterol crystals during atherosclerosis progression

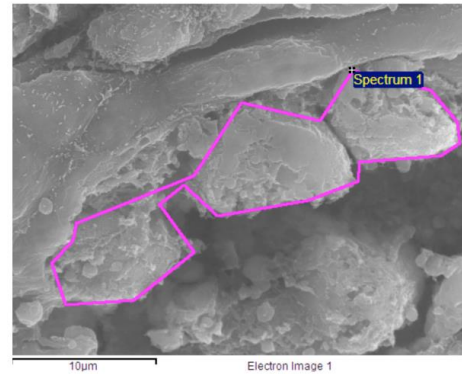
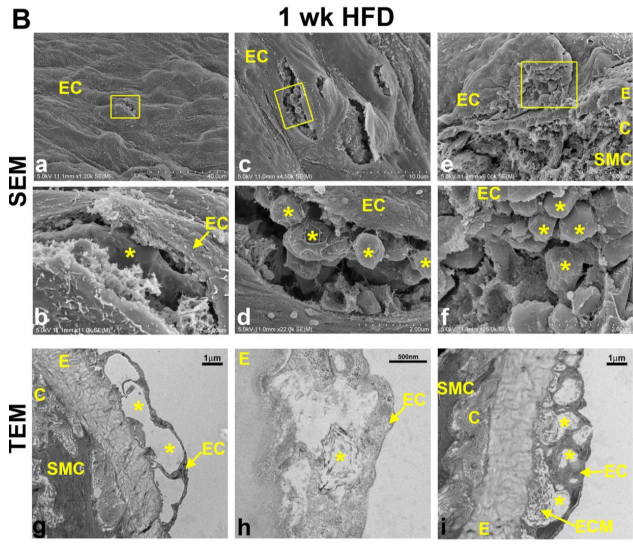
E) Macrophages / Foam cells



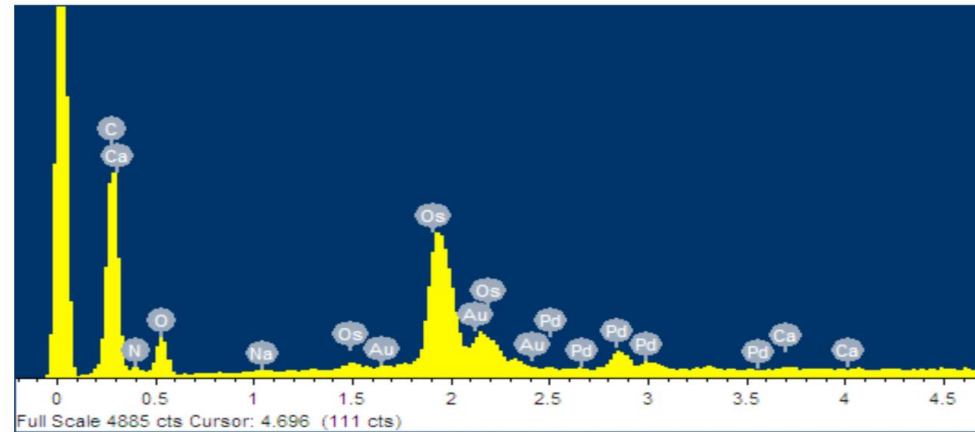
F) Necrotic core



# Composition of subendothelial CC



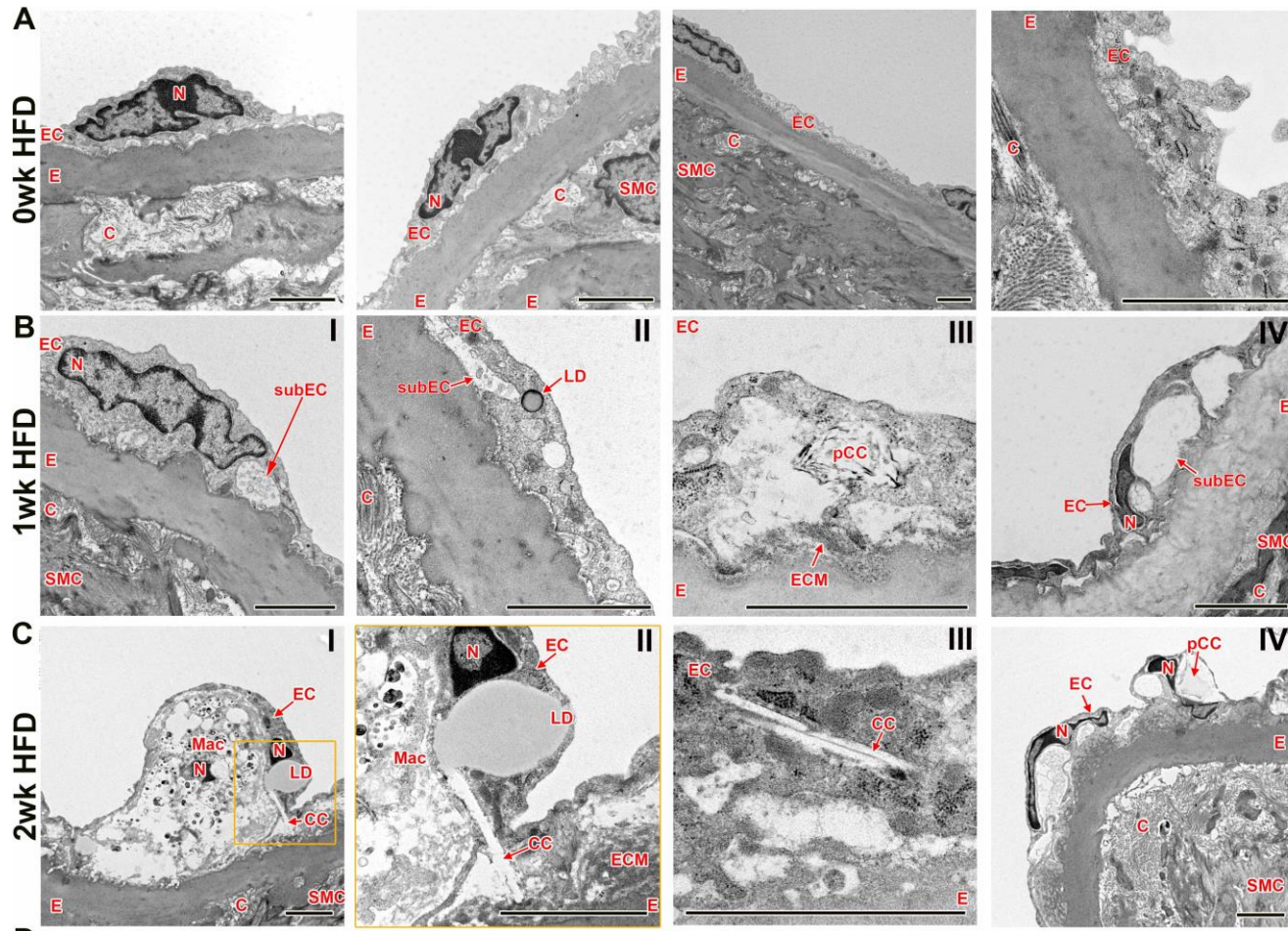
Element	Weight%	Atomic%
C	32.03	56.63
N	12.04	18.25
O	15.12	20.07
Ca	0.00	0.00
Pd	5.87	1.17
Os	29.11	3.25
Au	5.83	0.63
Totals	100.00	



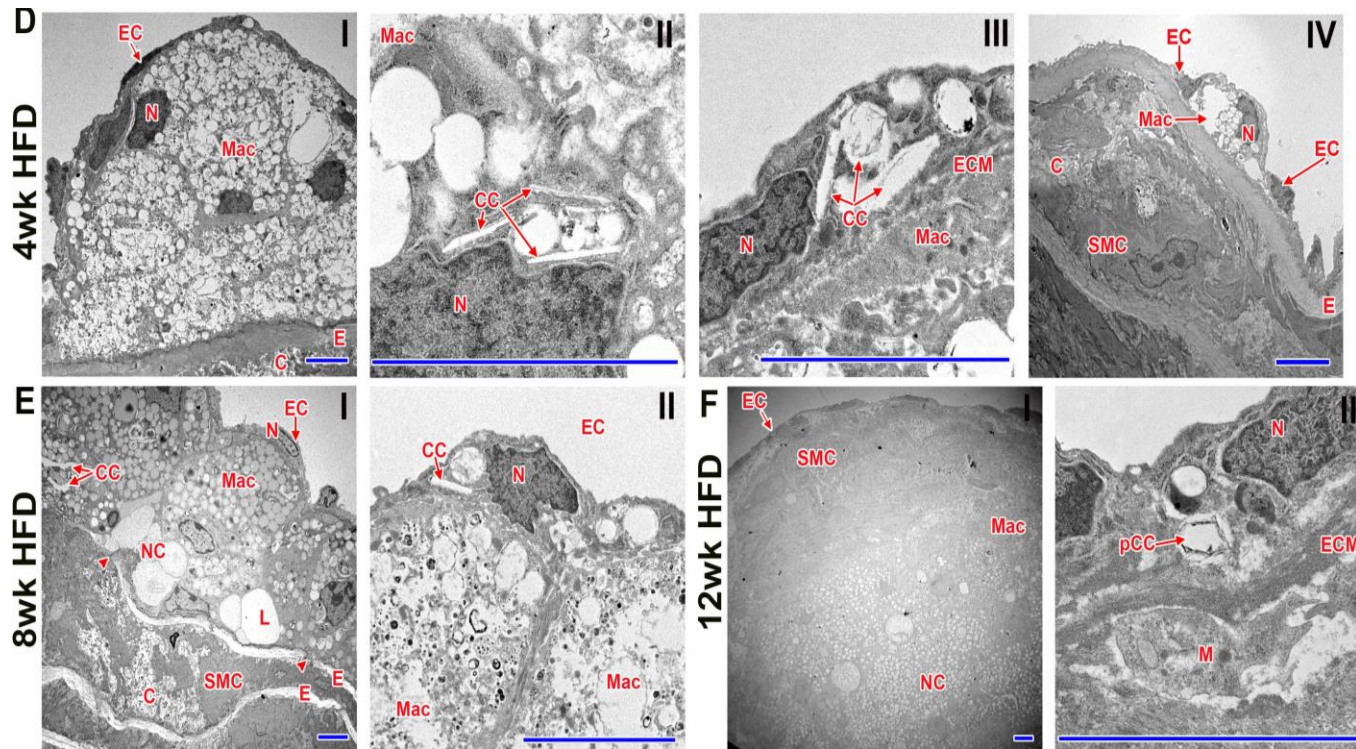
These early cholesterol crystals are composed of C, O and N but not  $\text{Ca}^{2+}$ .



# Visualization of atherosclerotic plaque through TEM

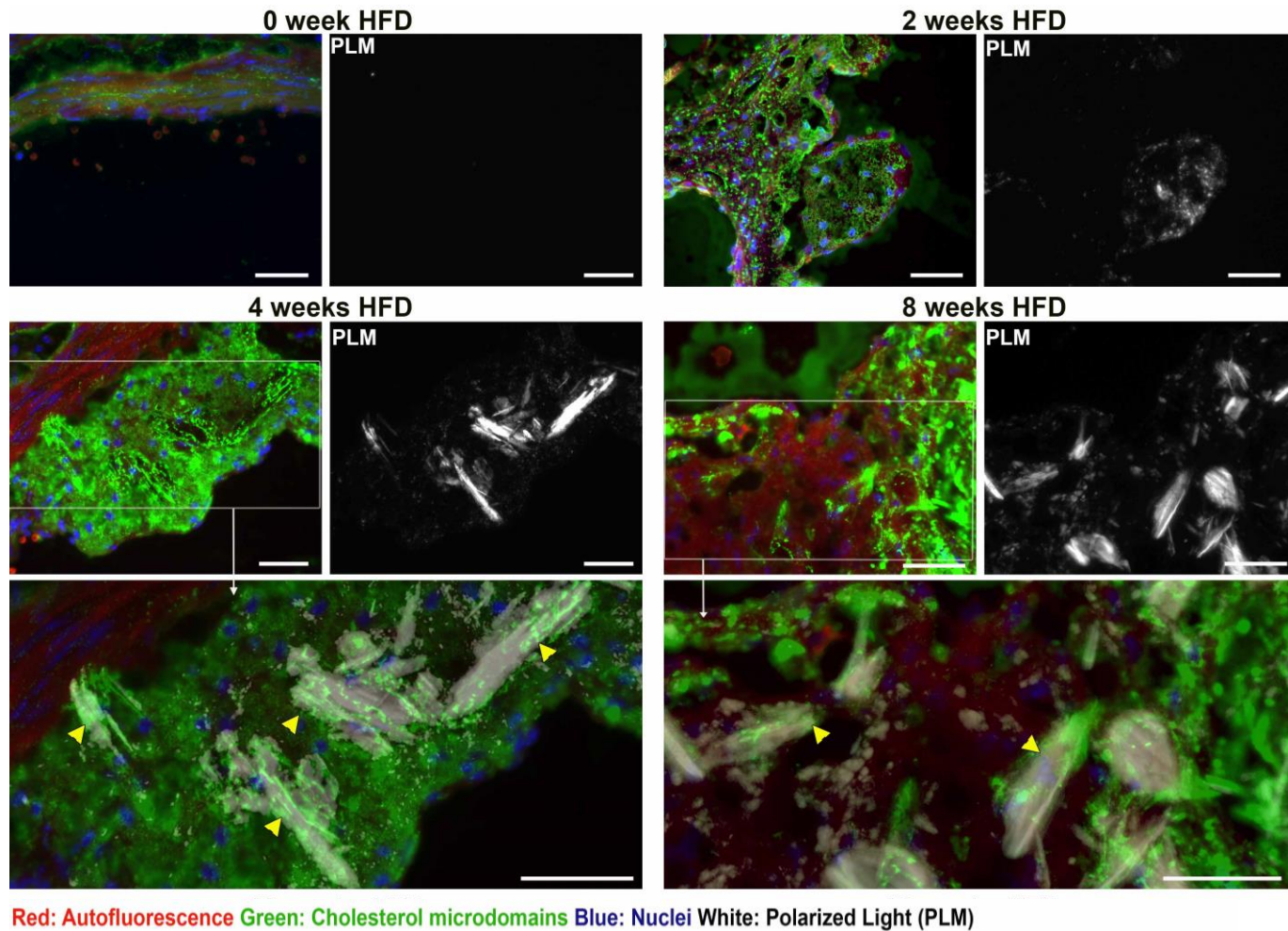


# Visualization of atherosclerotic plaque through TEM



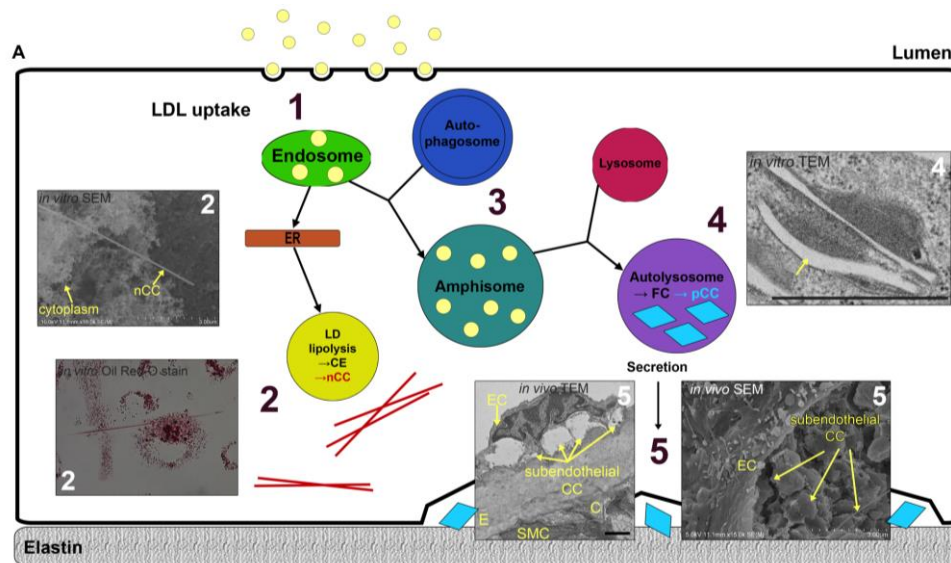


# Localization of CC and cholesterol microdomains



# Early cholesterol crystal formation

- EC robustly take up LDL and process the lipid intracellularly
- When the cells are overwhelmed with cholesterol the EC produce cholesterol crystals that become deposited subendothelially
- CC causes increase in RhoA and reduction in Rac1 and cAMP, all consistent with disturbance of endothelial barrier function
- The increase in endothelial permeability allows transmigration of leukocytes



# Late cholesterol crystal formation

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- 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



# Acknowledgement

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## Collaborators

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- Per Fogelstrand, PhD

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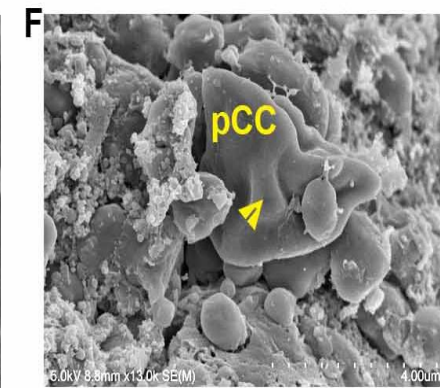
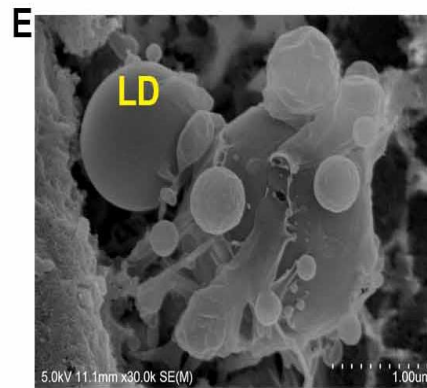
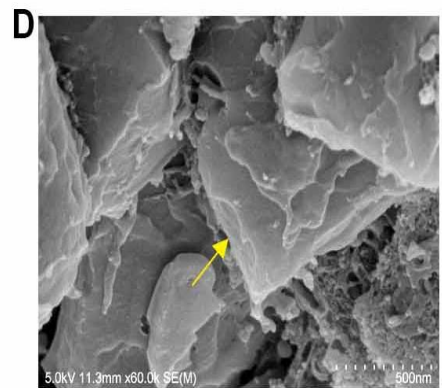
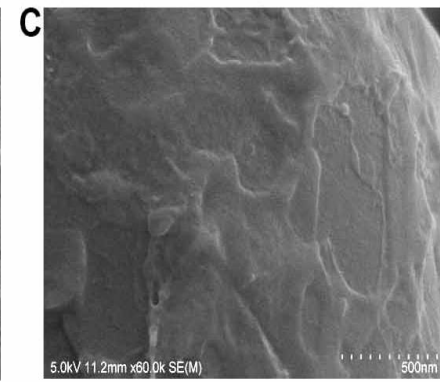
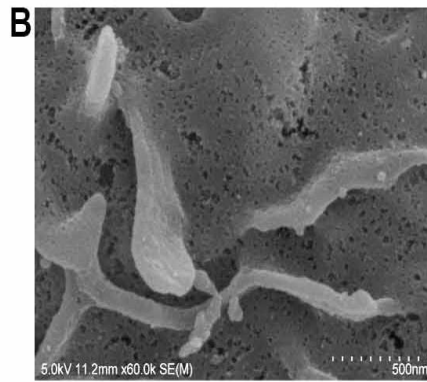
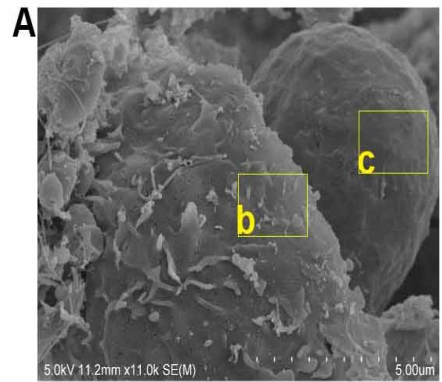
- **Howard Kruth, PhD**



# MAHALO

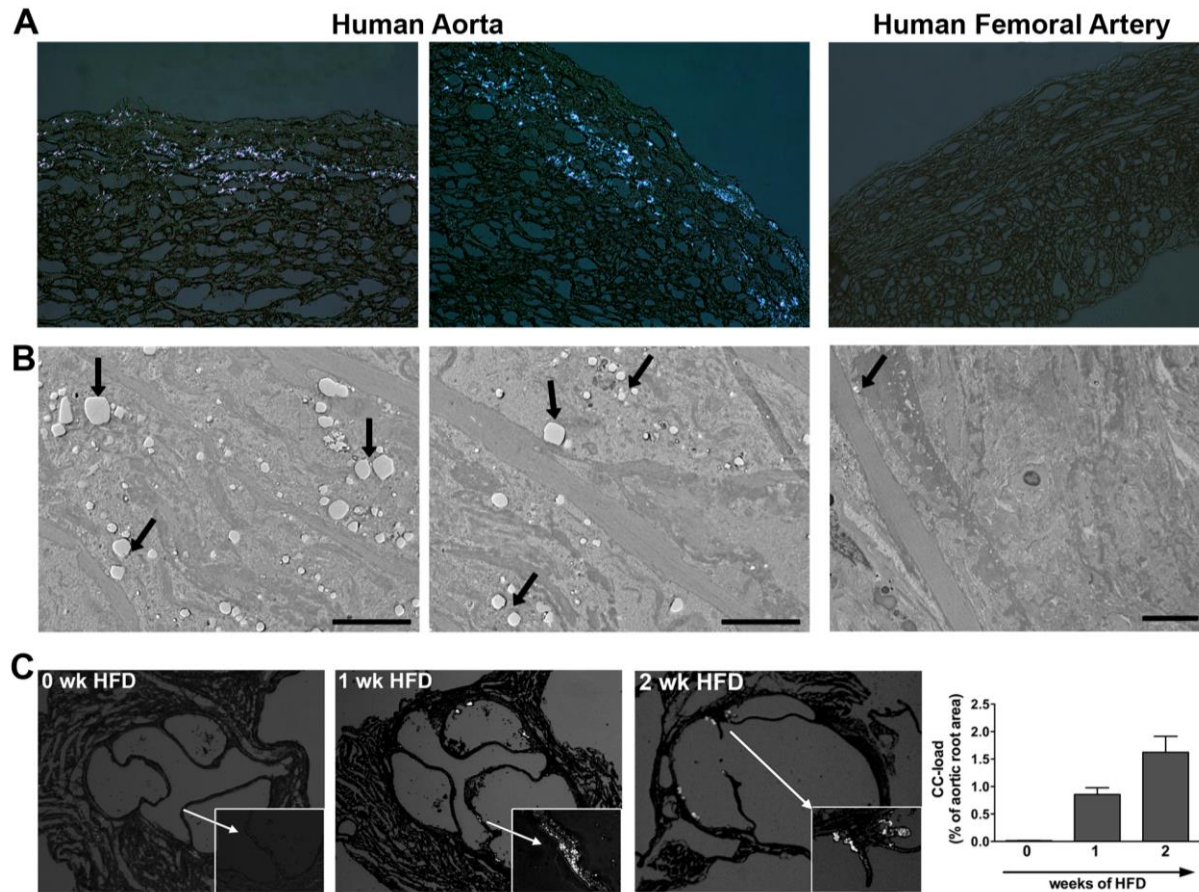




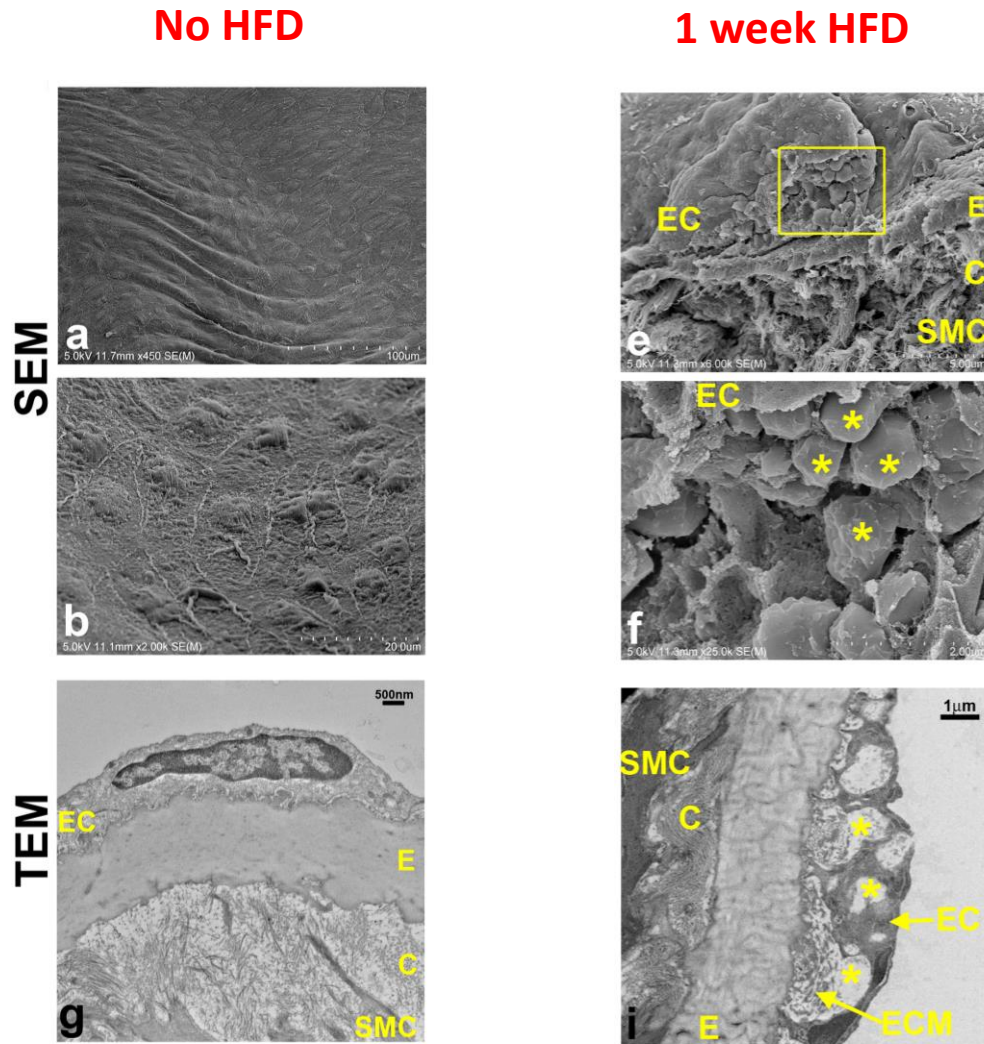




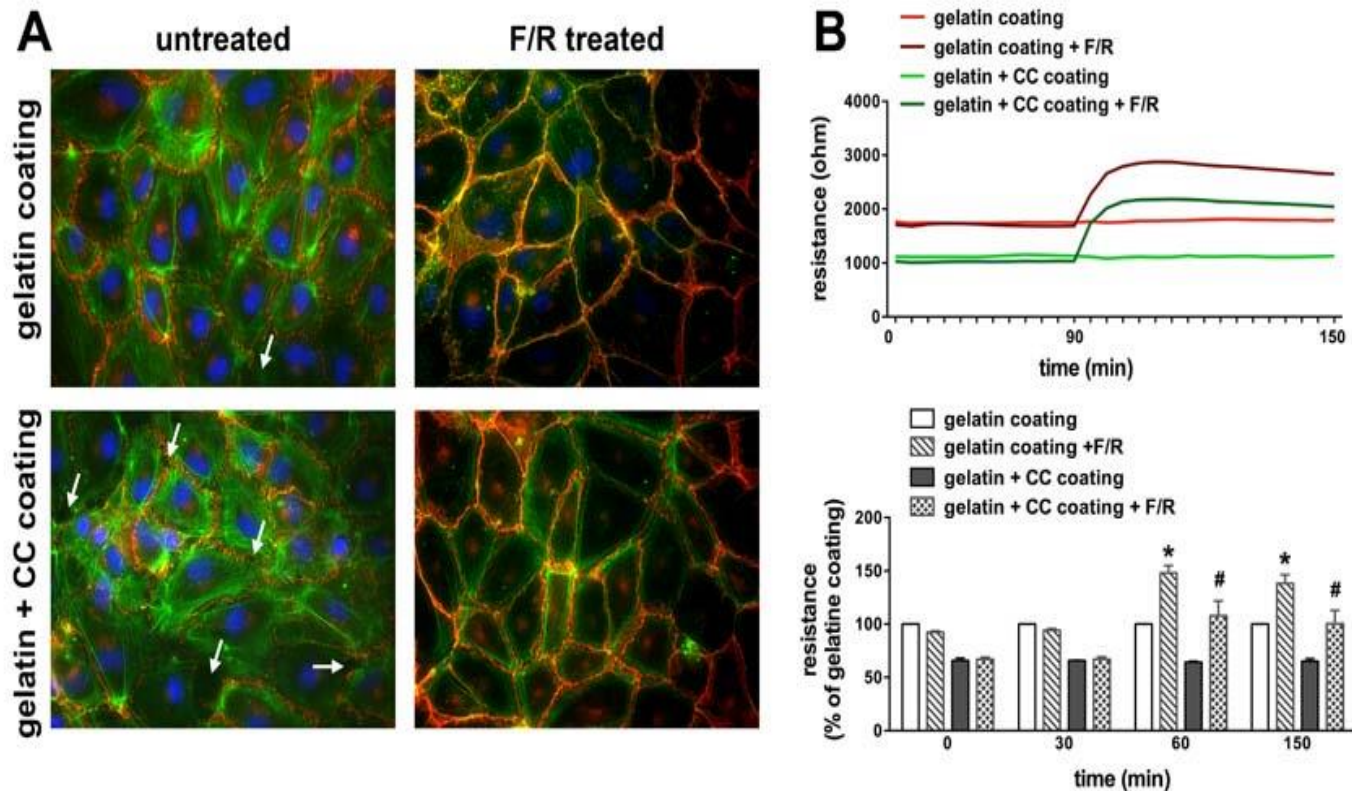
# Cholesterol crystals in human and mouse atherosclerotic plaque



# Subendothelial CC deposition in *Idlr*<sup>-/-</sup> mouse aorta



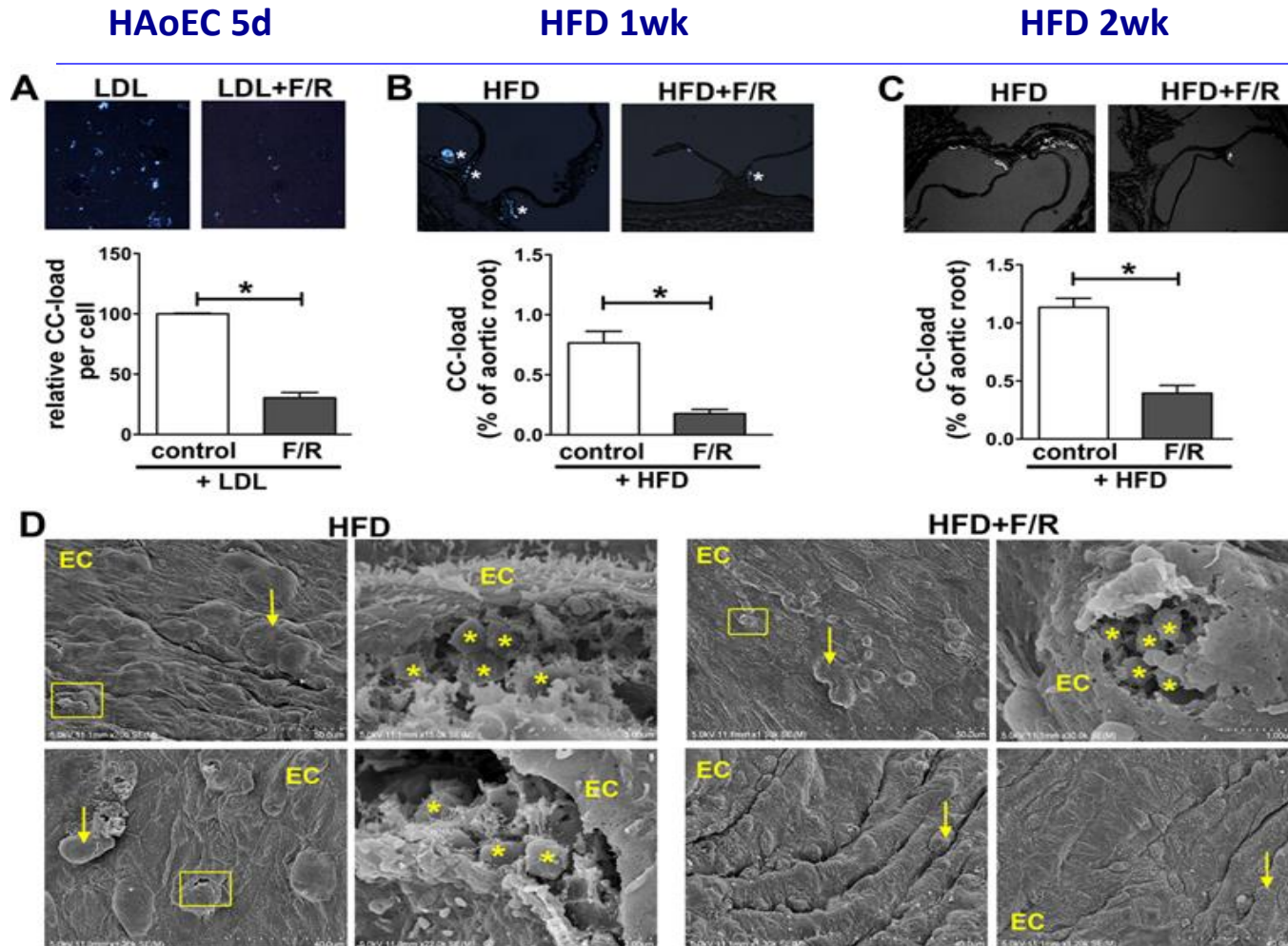
# F/R restores the barrier dysfunction caused by CC



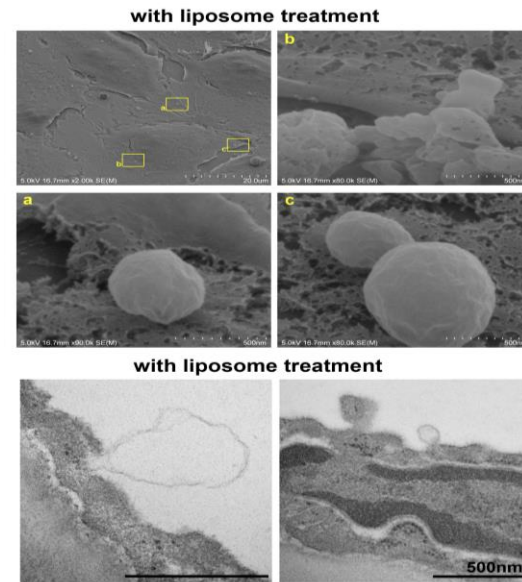
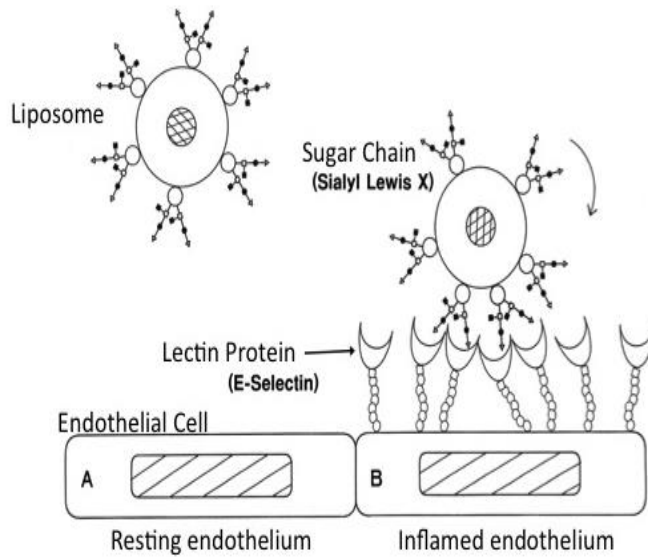
Forskolin/rolipram are well known cAMP-enhancing agents



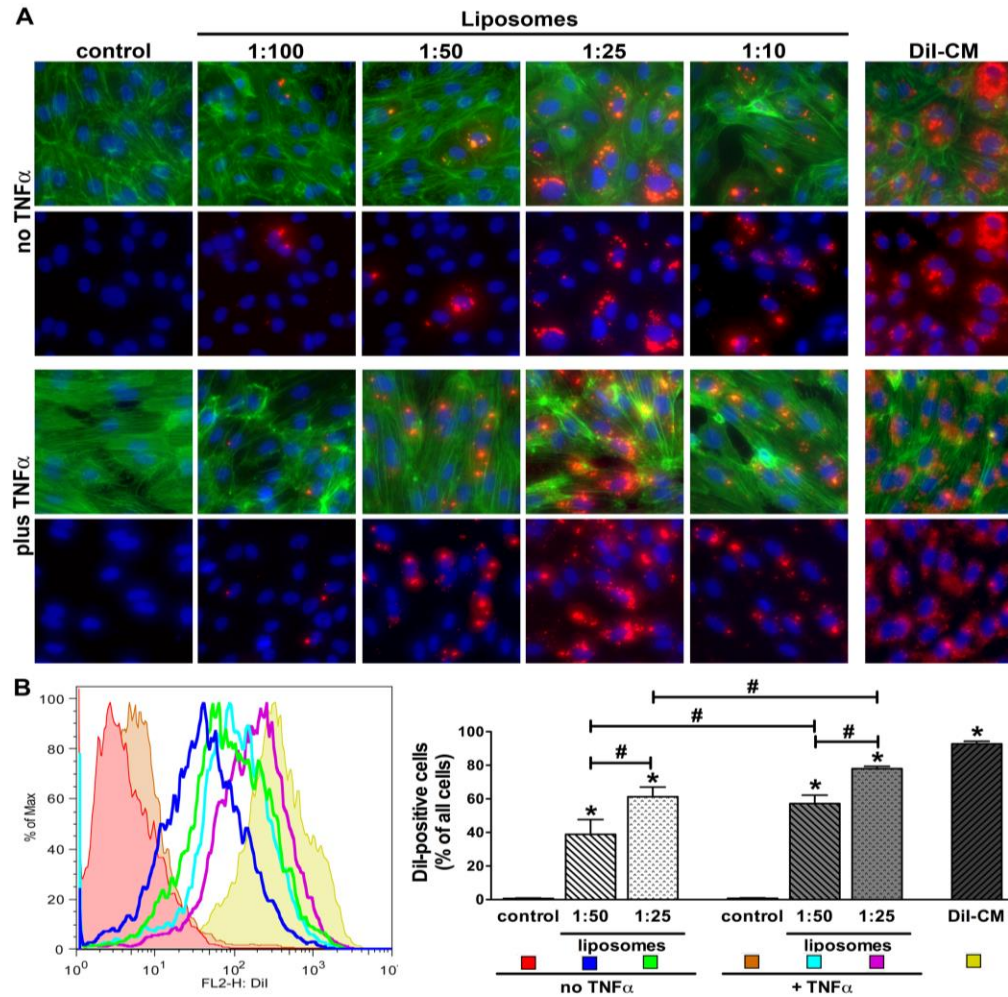
# F/R treatment reduces CC formation in *ldlr*<sup>-/-</sup> mice



# Characterization of F/R-containing liposome

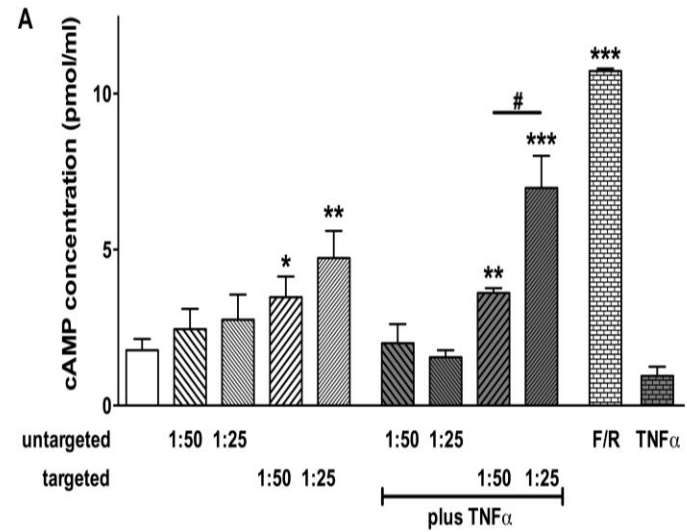
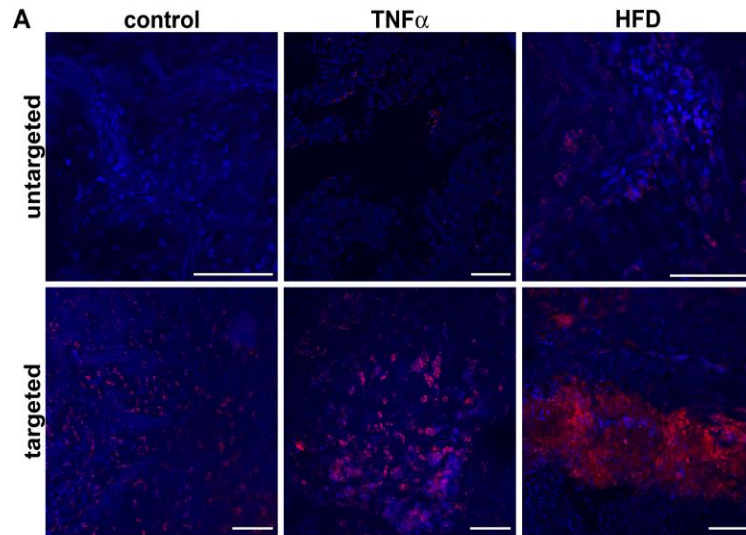


# Binding of F/R-liposome to endothelium

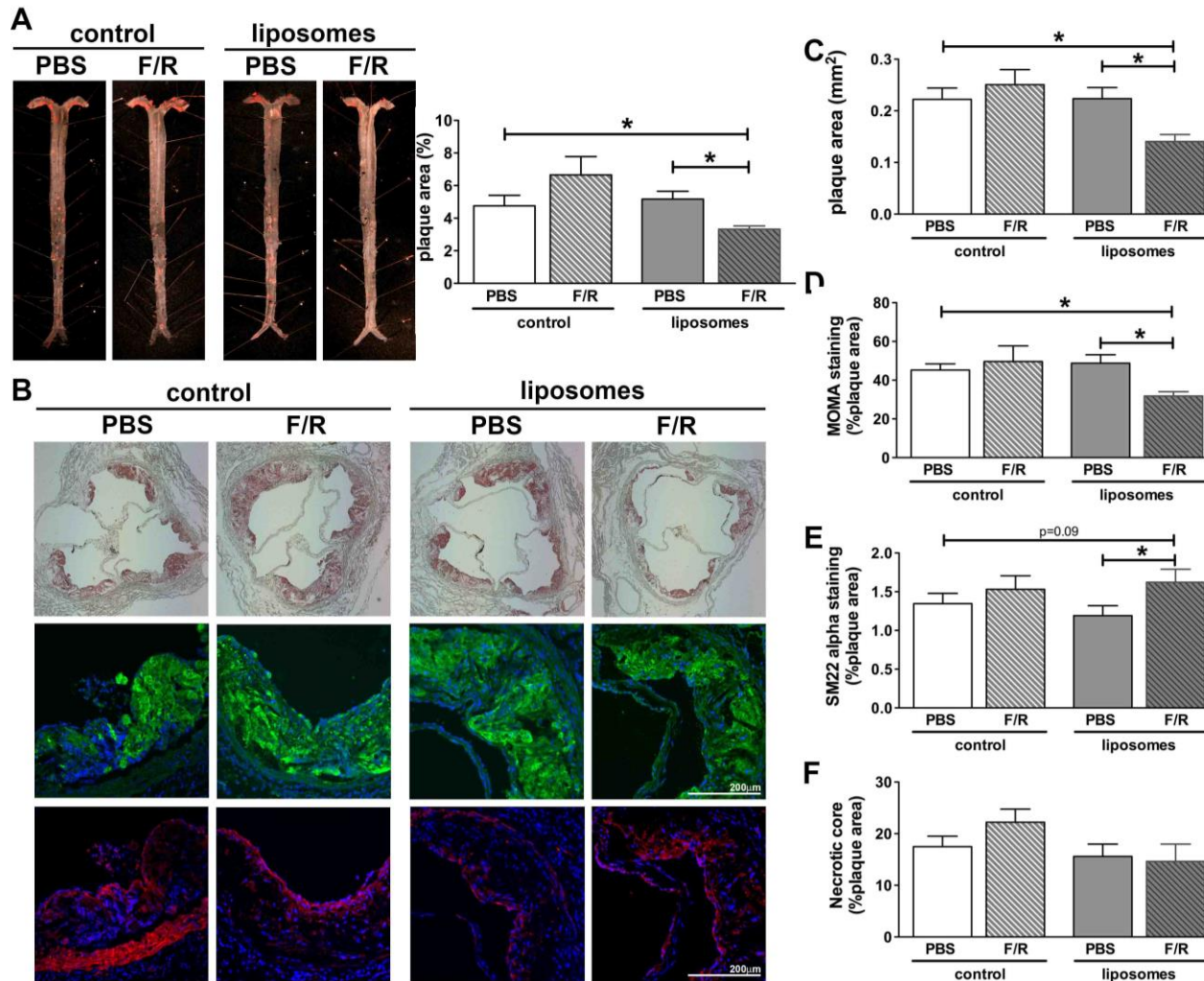




# Effects of F/R liposome administration *in vivo*



# F/R liposome treatment in HFD-fed *apoE*<sup>-/-</sup> mice



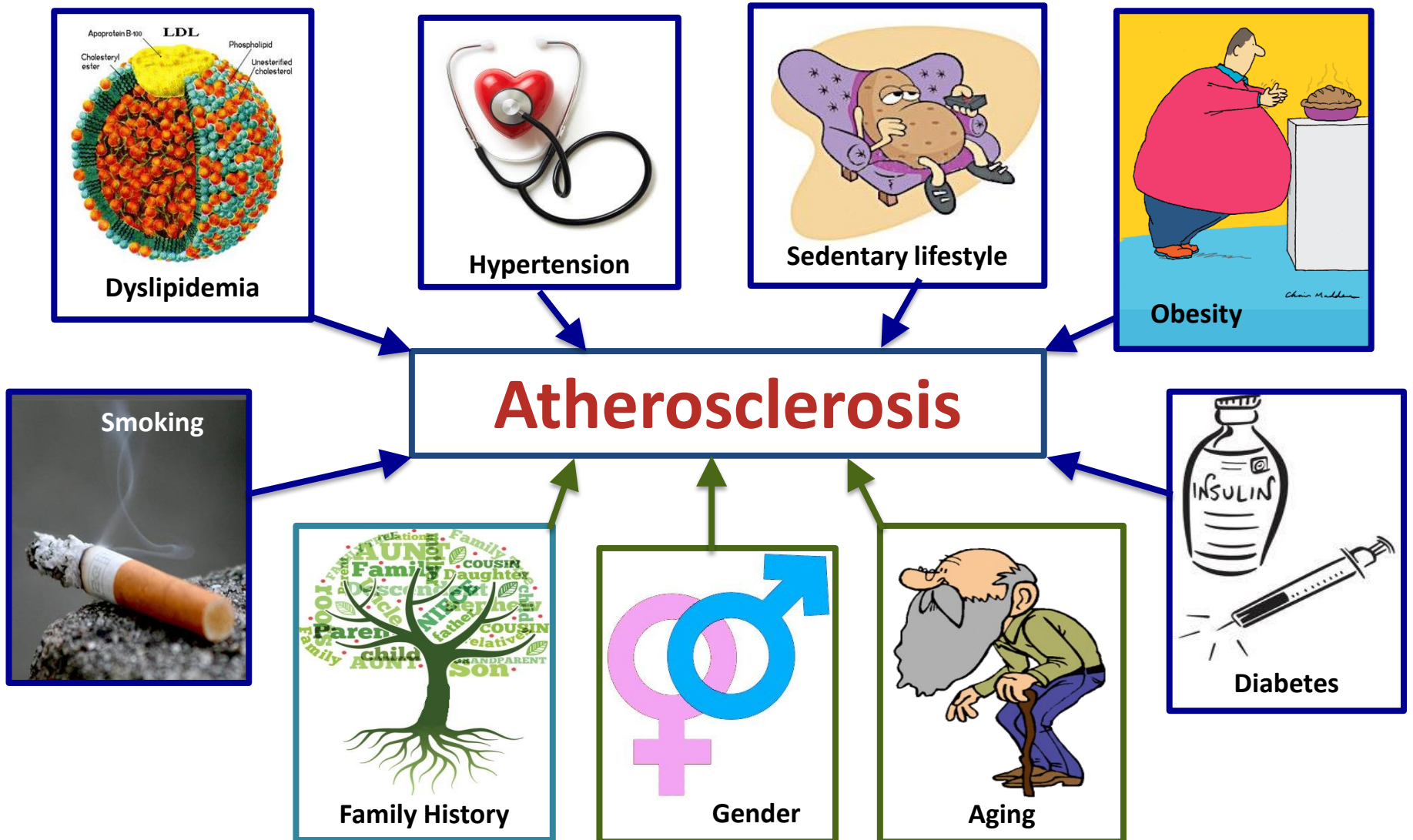
# Summary

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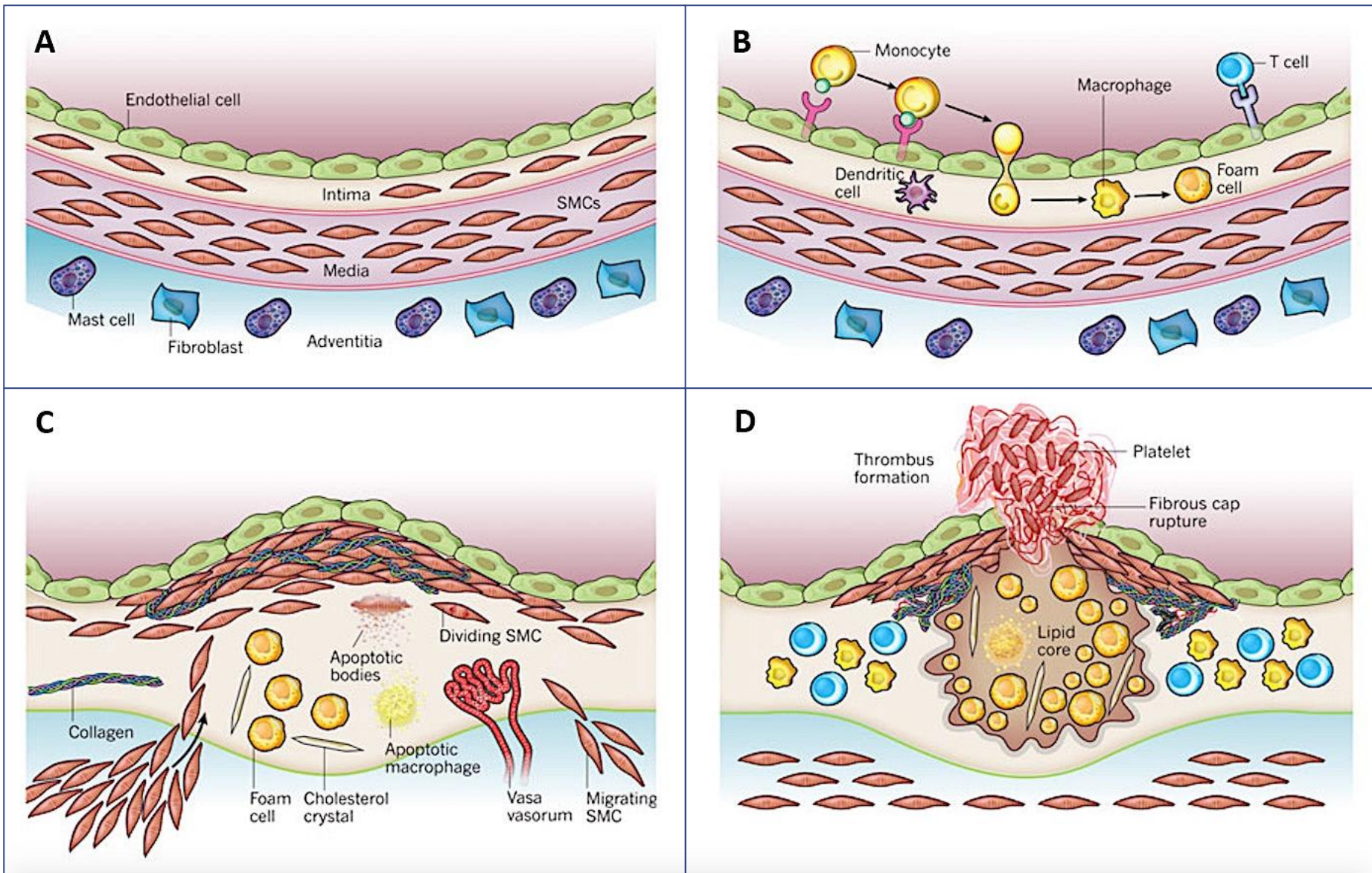
- F/R effectively improves the endothelial barrier function compromised by CC
- Short-term F/R treatment in HAoEC and in *ldlr*<sup>-/-</sup> mice resulted in reduced CC formation
- F/R-containing liposomes tagged with sialyl lewis x effectively target the inflamed endothelium
- *ApoE*<sup>-/-</sup>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



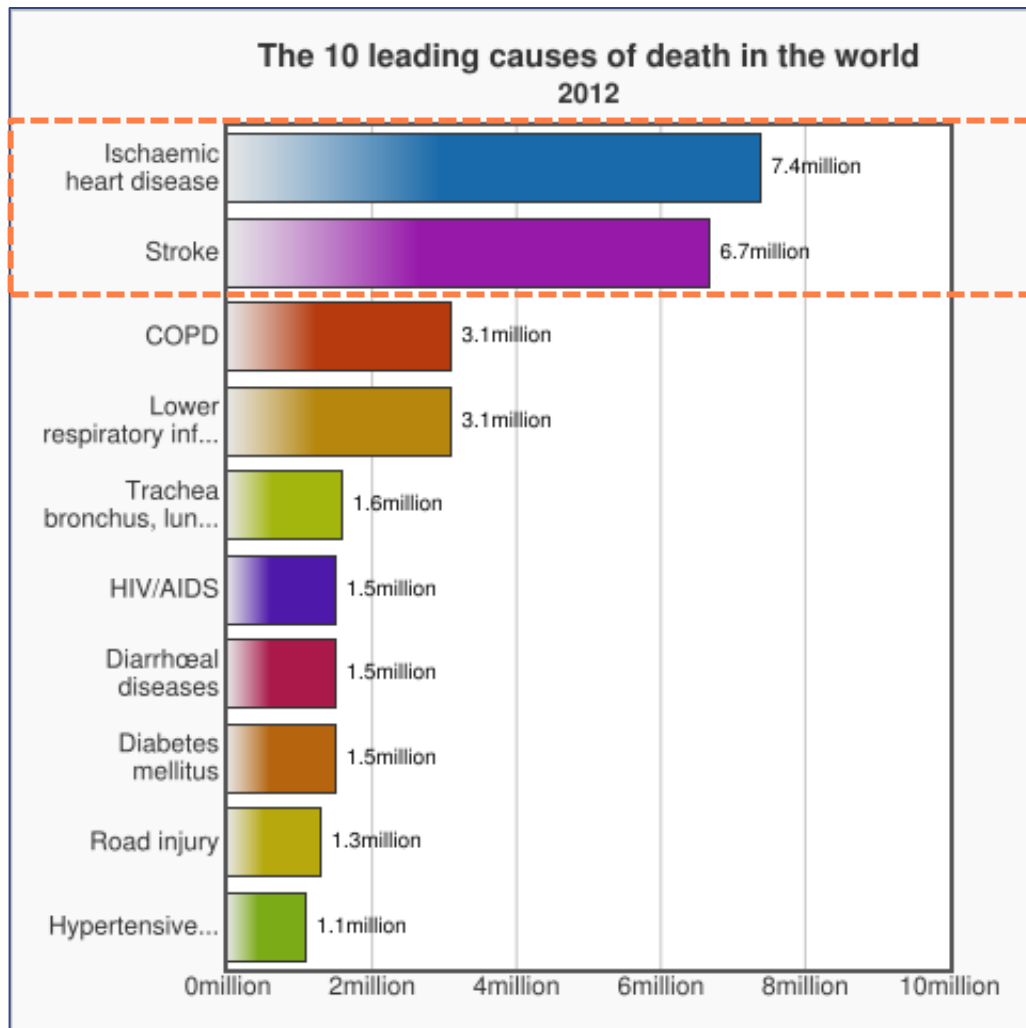
# Risk Factors



# Stages of Atherosclerosis



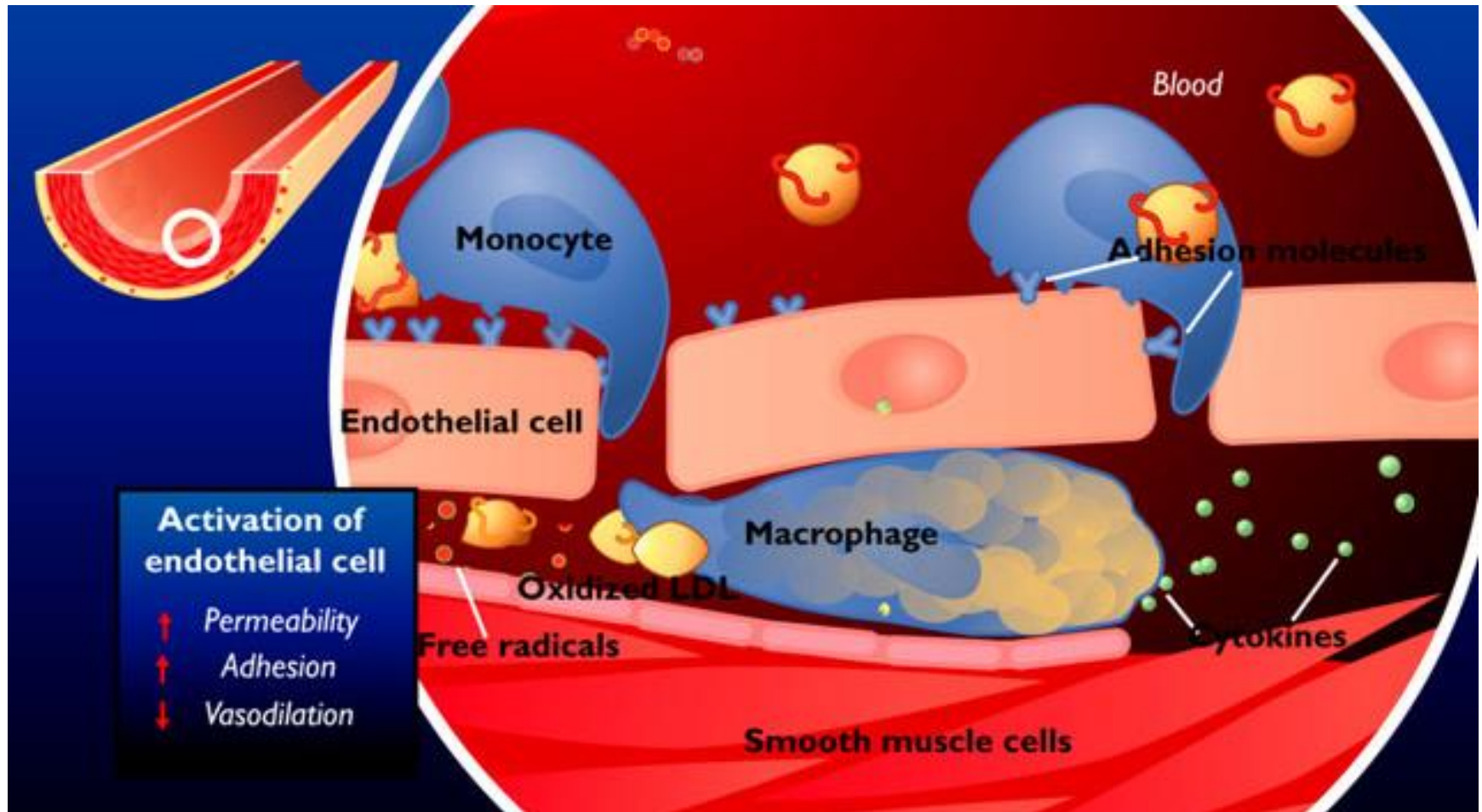
# Cardiovascular Diseases (CVD)



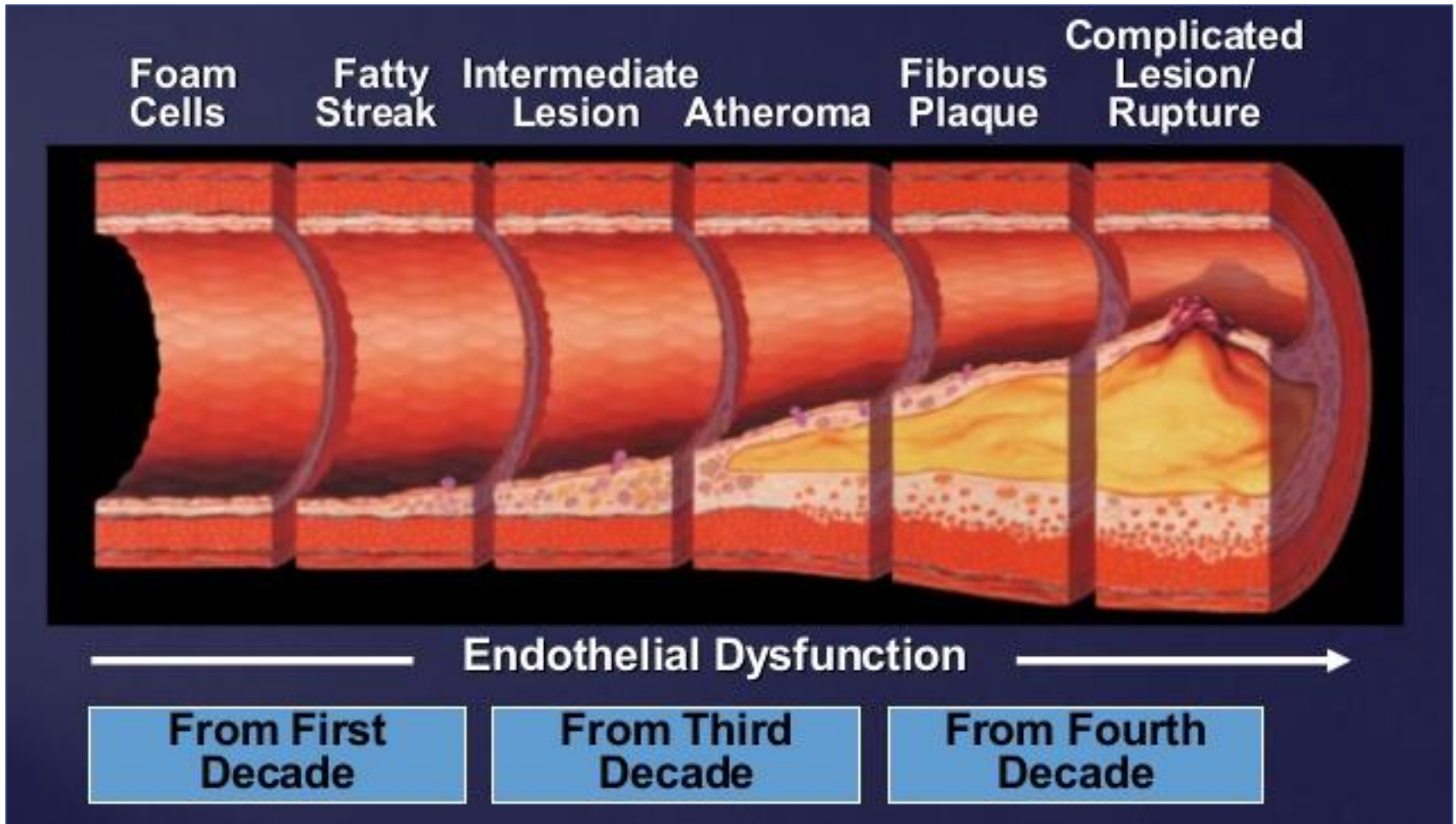
- CVD and stroke are top causes of death in the world – 35% of total
- \$863 billion in annual global economic burden
- 17% of US national healthcare costs



# Vascular endothelium modification in atherosclerosis

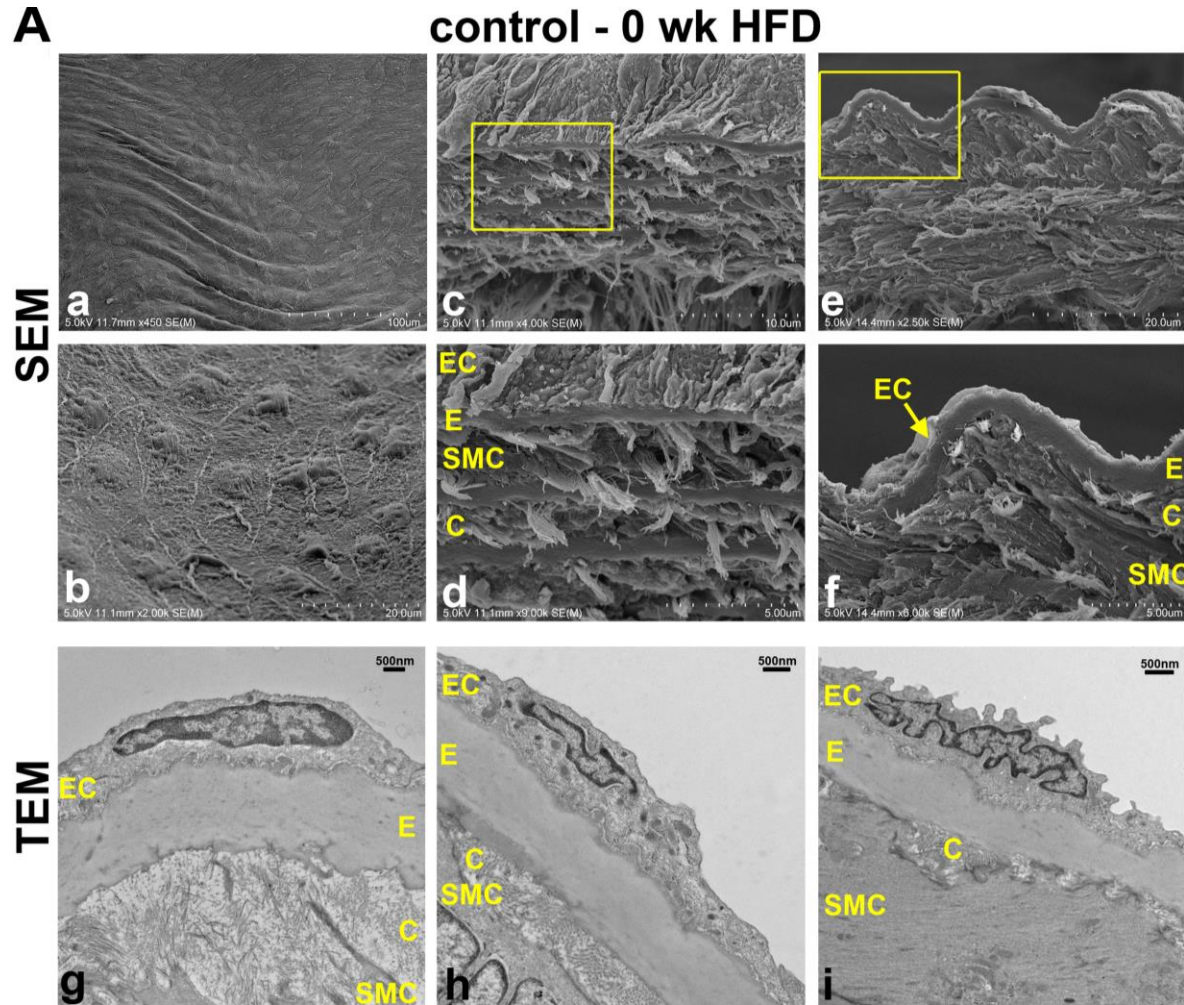


# Atherosclerosis Timeline



# Subendothelial CC deposition in *Idlr*<sup>-/-</sup> mouse aorta

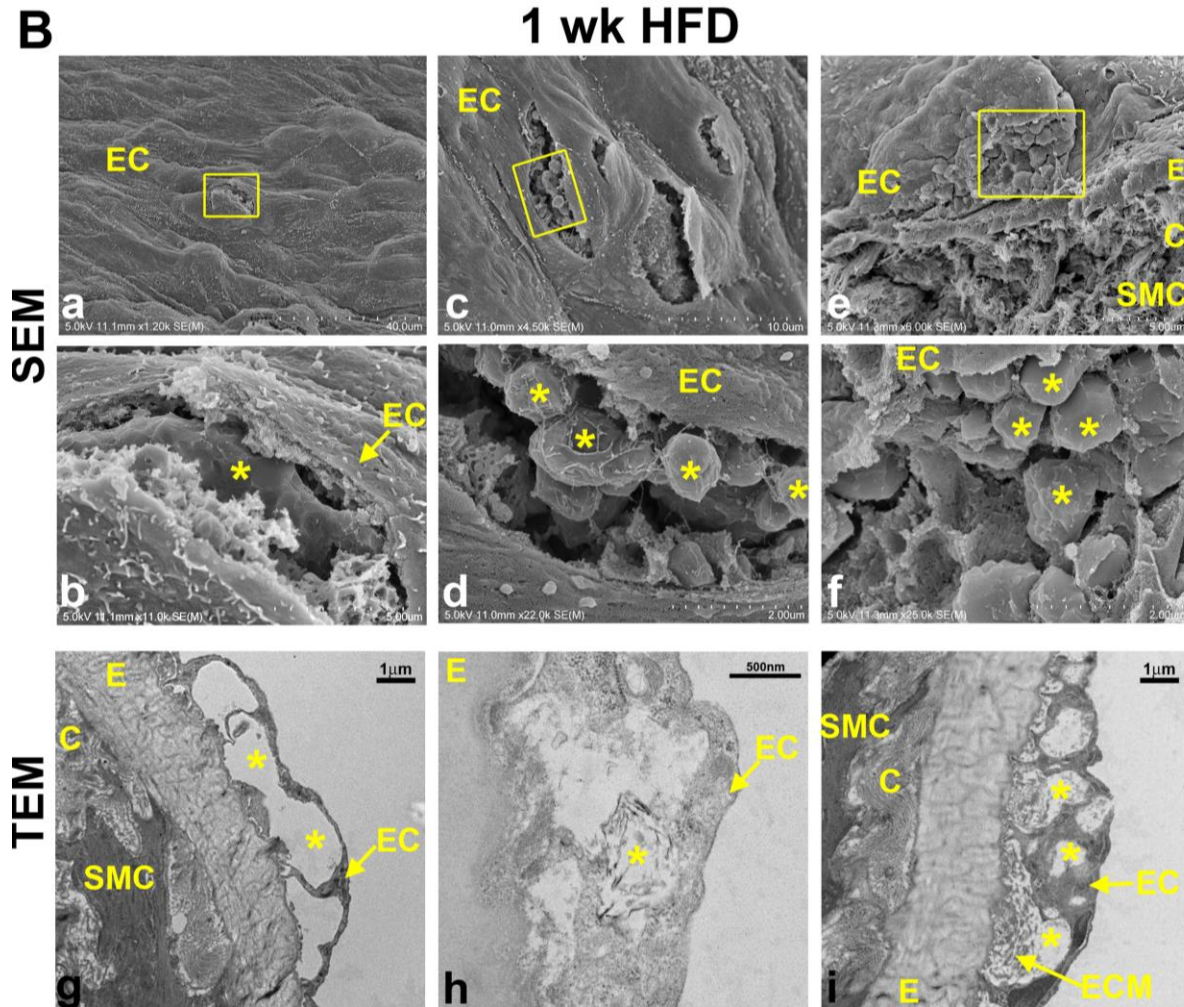
## No HFD





# Subendothelial CC deposition in *Idlr*<sup>-/-</sup> mouse aorta

1 week HFD

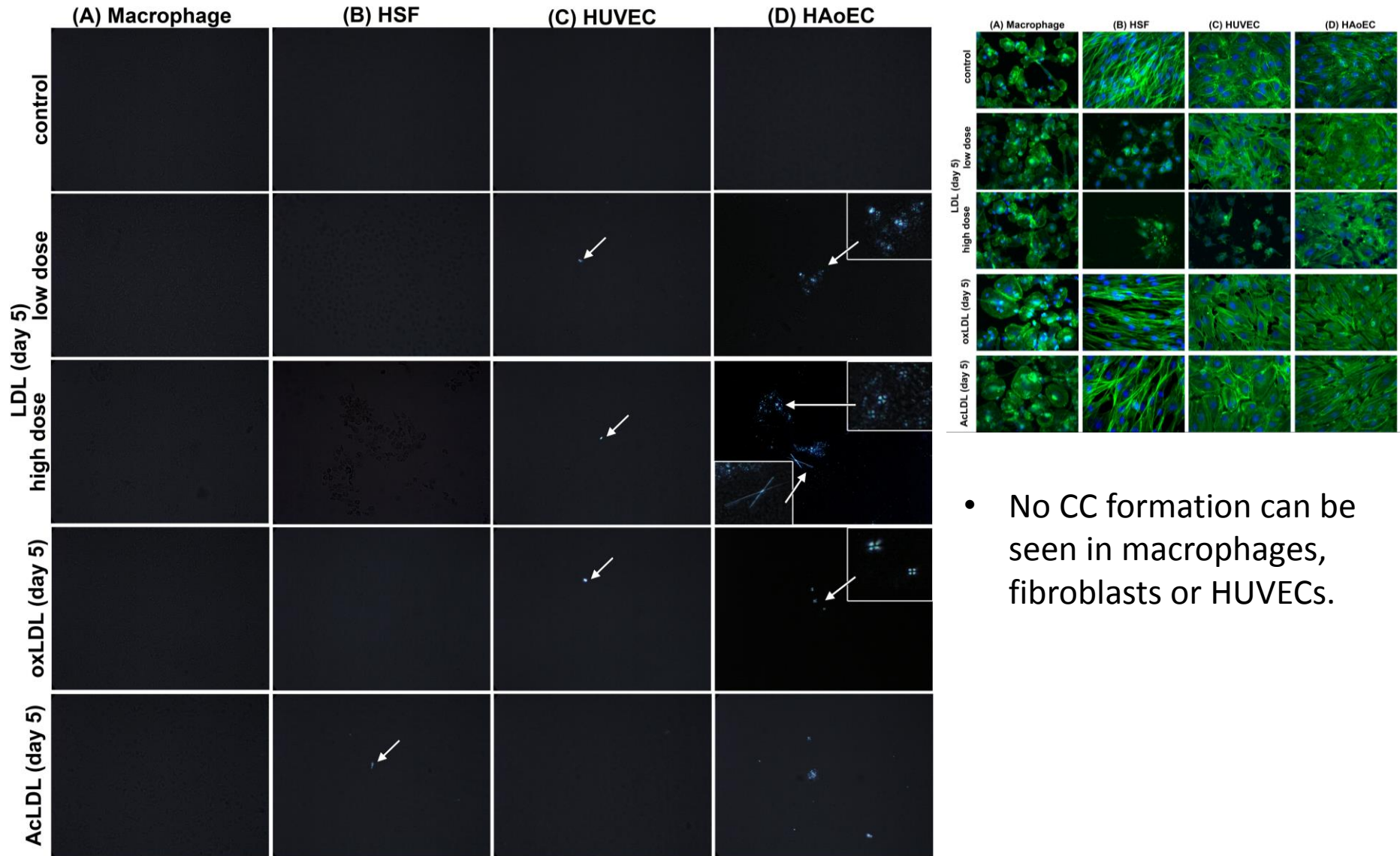


# Atherosclerosis is an arterial disease of chronic inflammation and hyperlipidemia

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- **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





# Smaller Lipoprotein Particles can Diffuse Through the Endothelial Layer

