Endothelial Cells Produce Cholesterol Crystals During Hyperlipidemia

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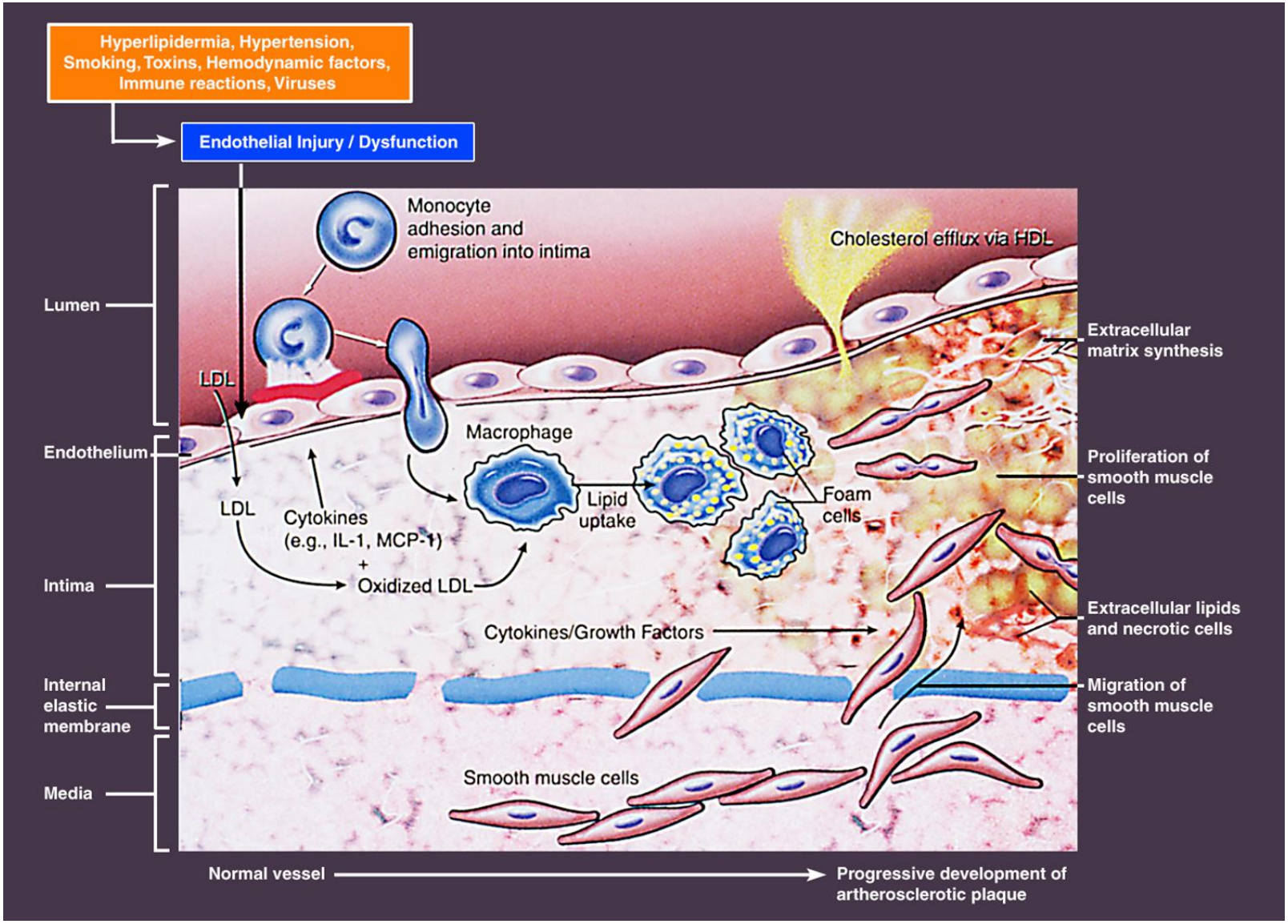
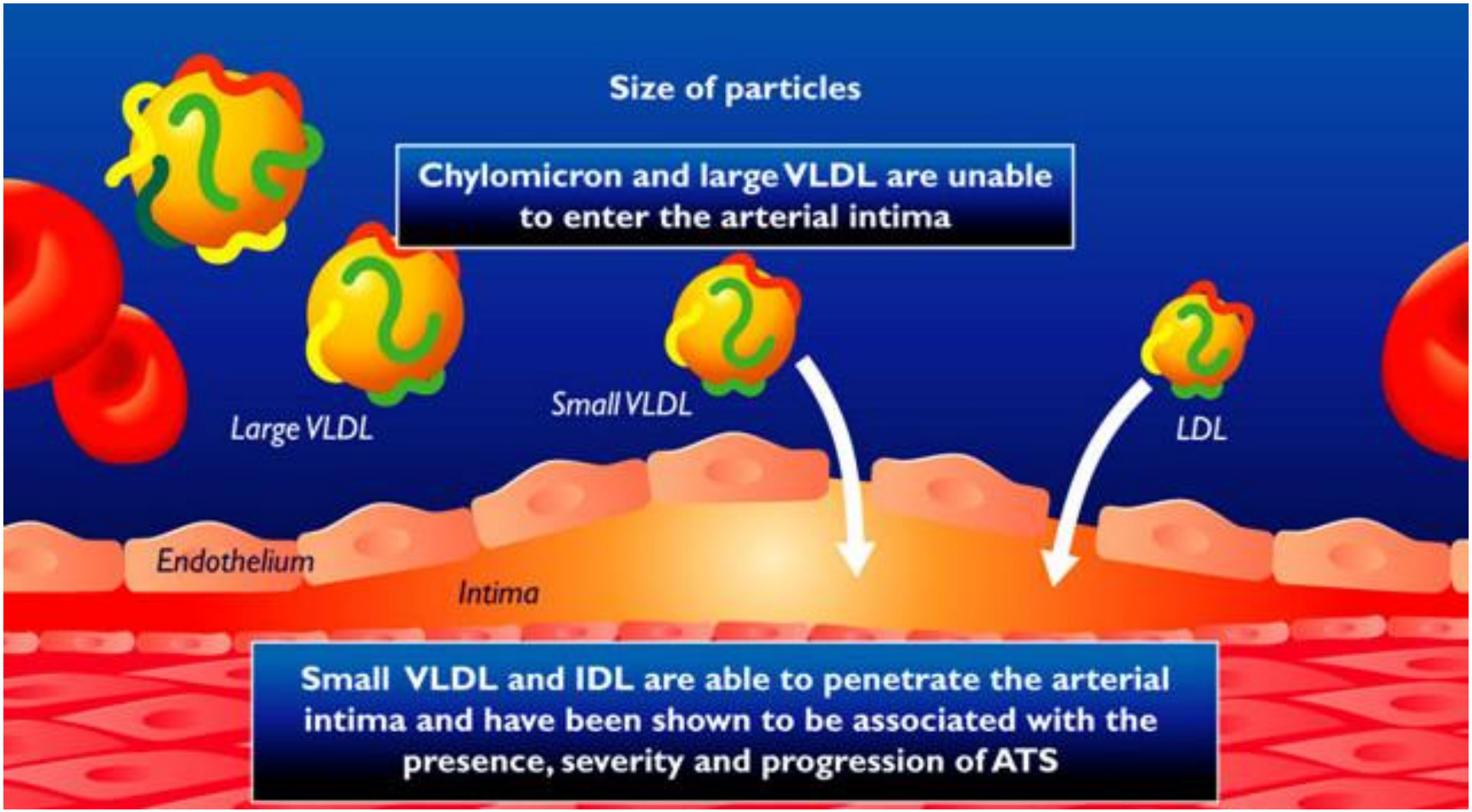
Development of Atherosclerosis



Smaller Lipoprotein Particles can Diffuse Through the Endothelial Layer



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

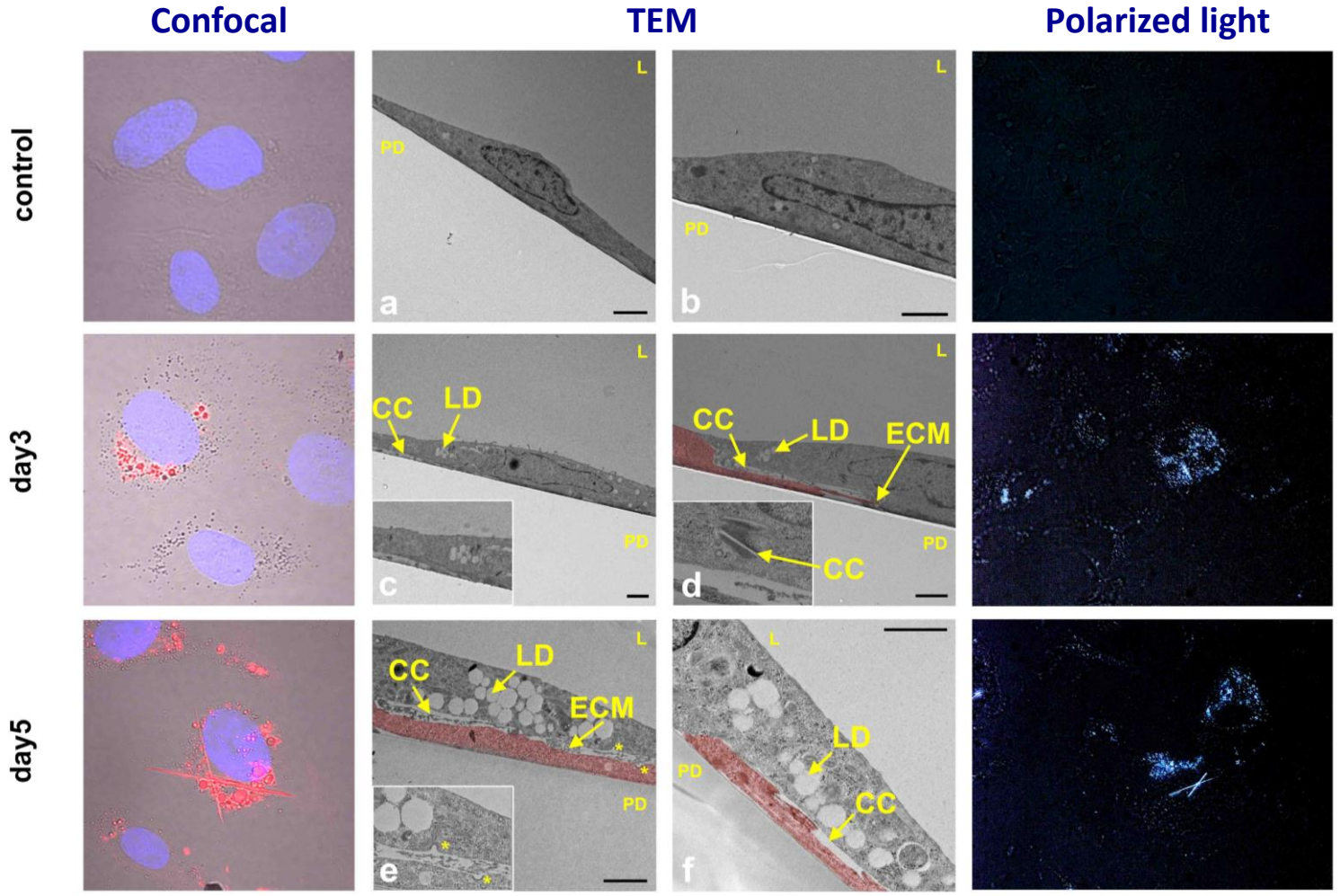
Endothelial Cells in Atherosclerosis

Questions:

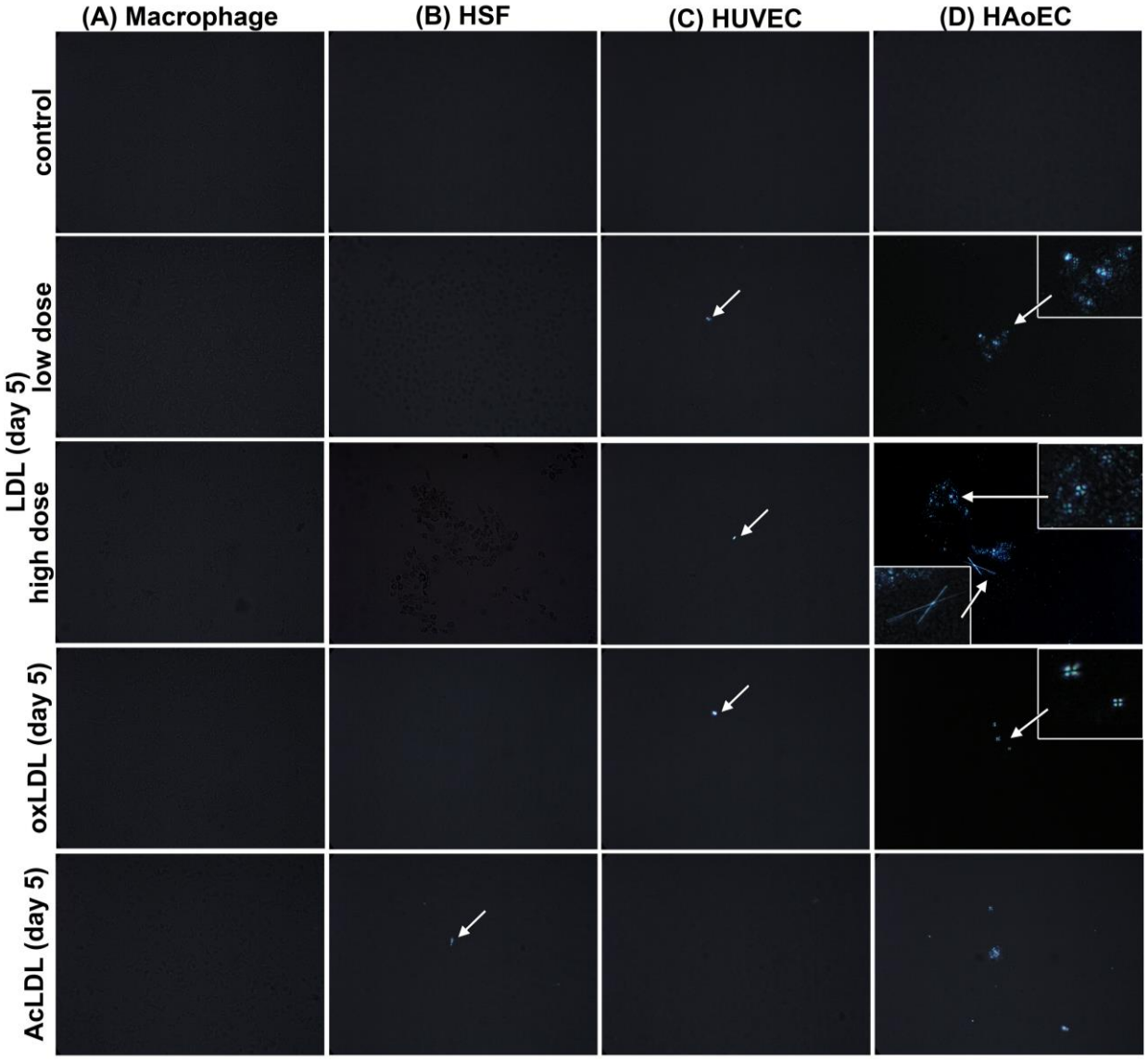
1. Do the endothelial cells take up LDL particles under hyperlipidemic conditions?

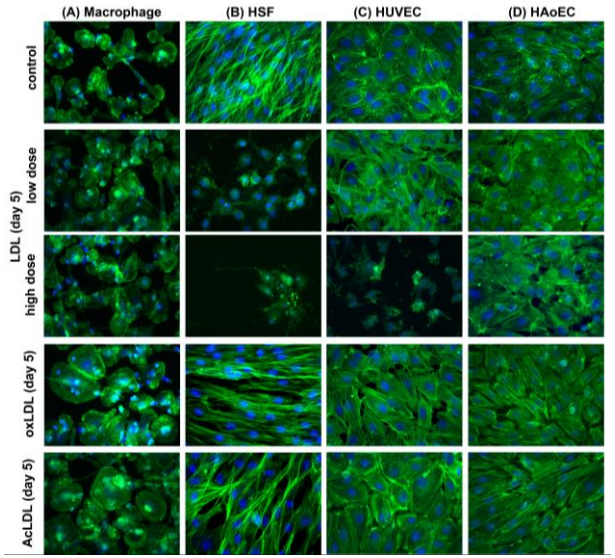
2. How do the cells process the lipid?

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



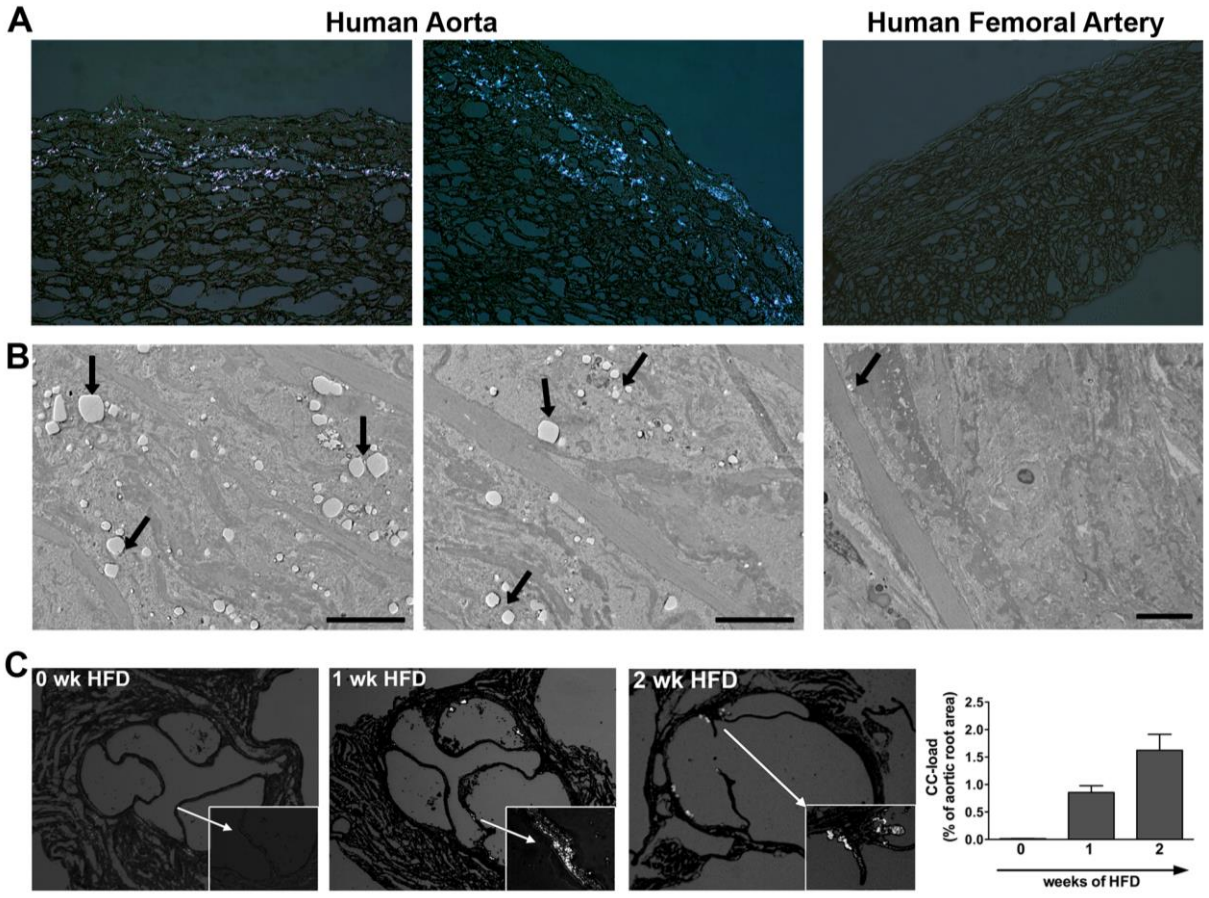
Cholesterol crystals are produced and secreted exclusively by aortic endothelial cells upon LDL treatment



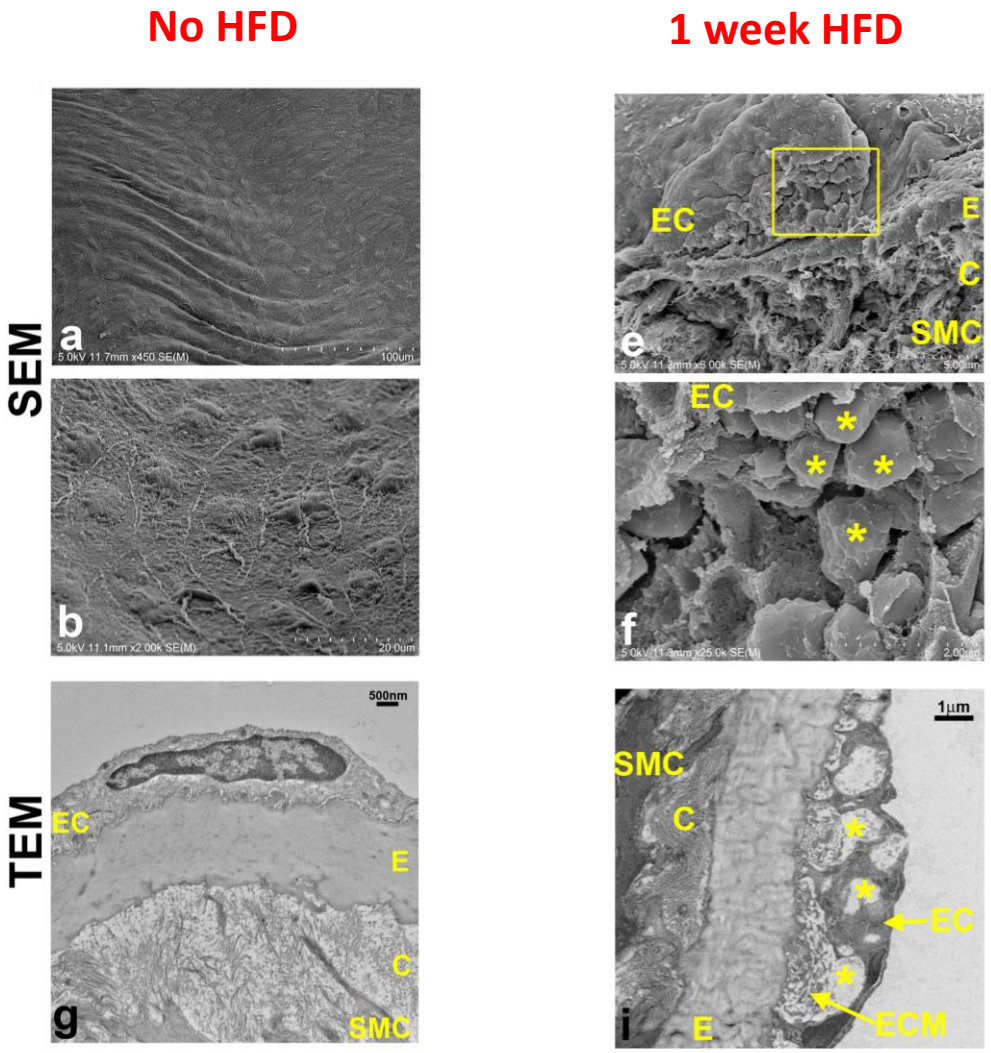


- No CC formation can be seen in macrophages, fibroblasts or HUVECs.

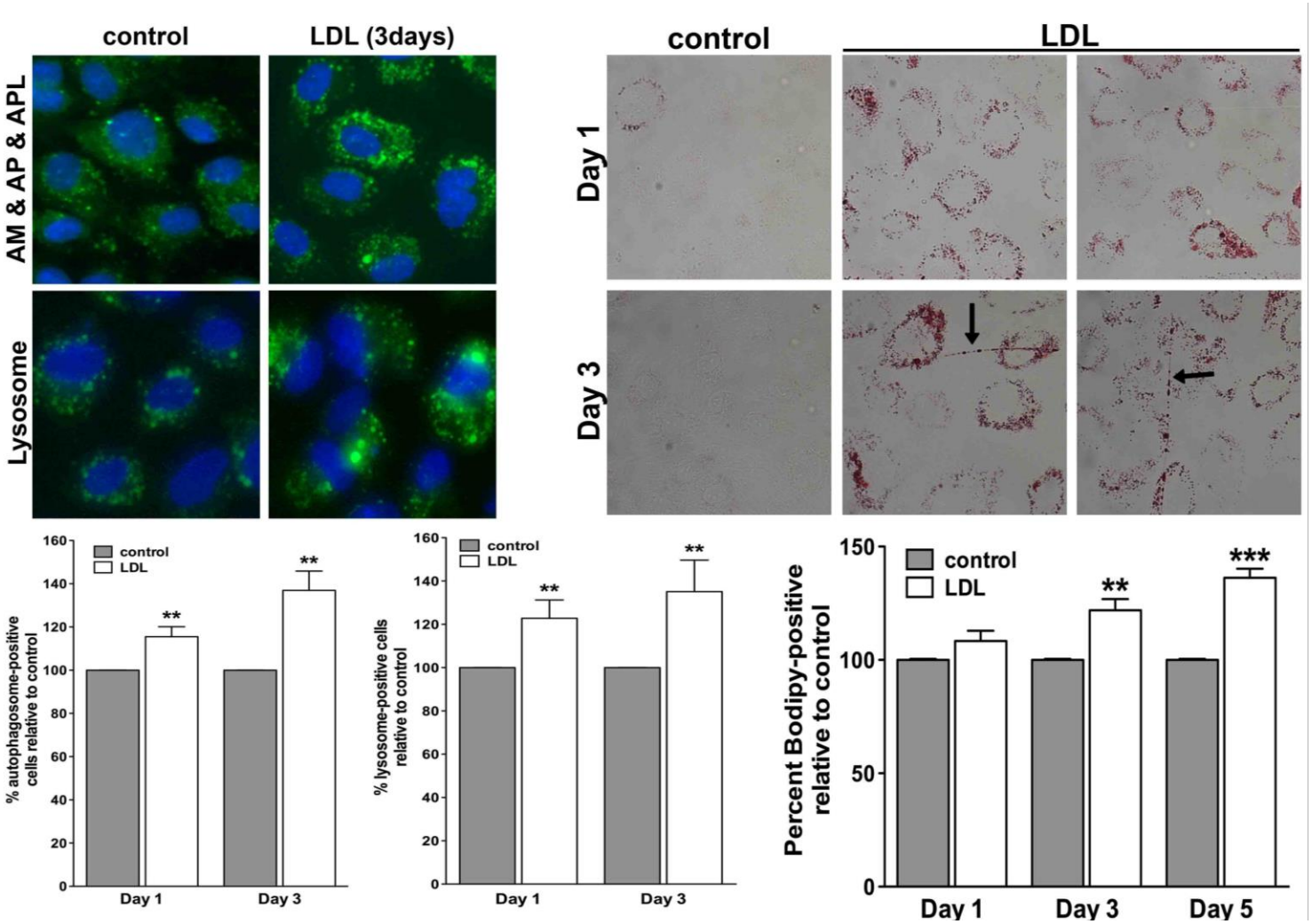
Cholesterol crystals in human and mouse atherosclerotic plaque



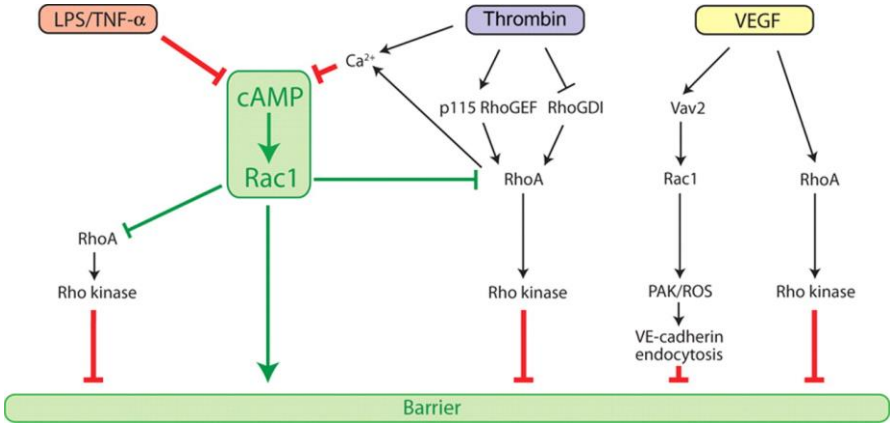
Subendothelial CC deposition in ldlr$^{-/-}$ mouse aorta

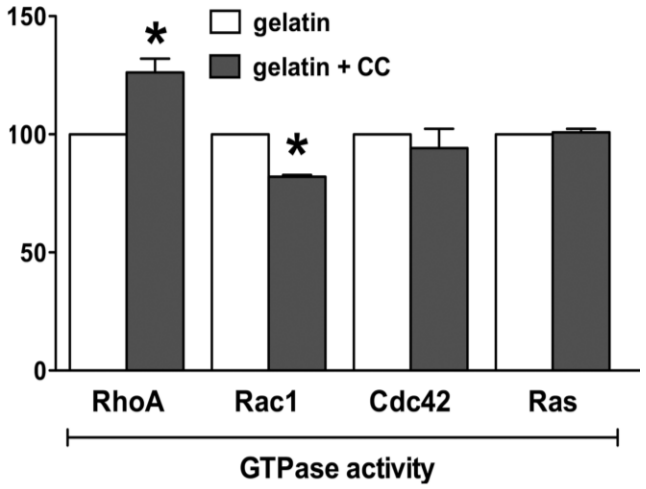


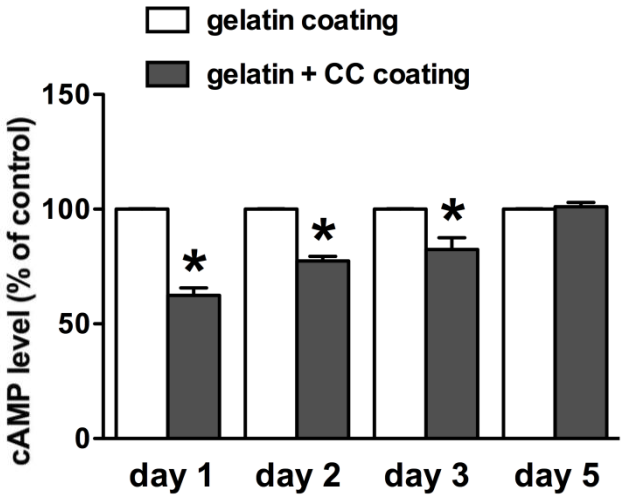
Lipid processing by HAoEC



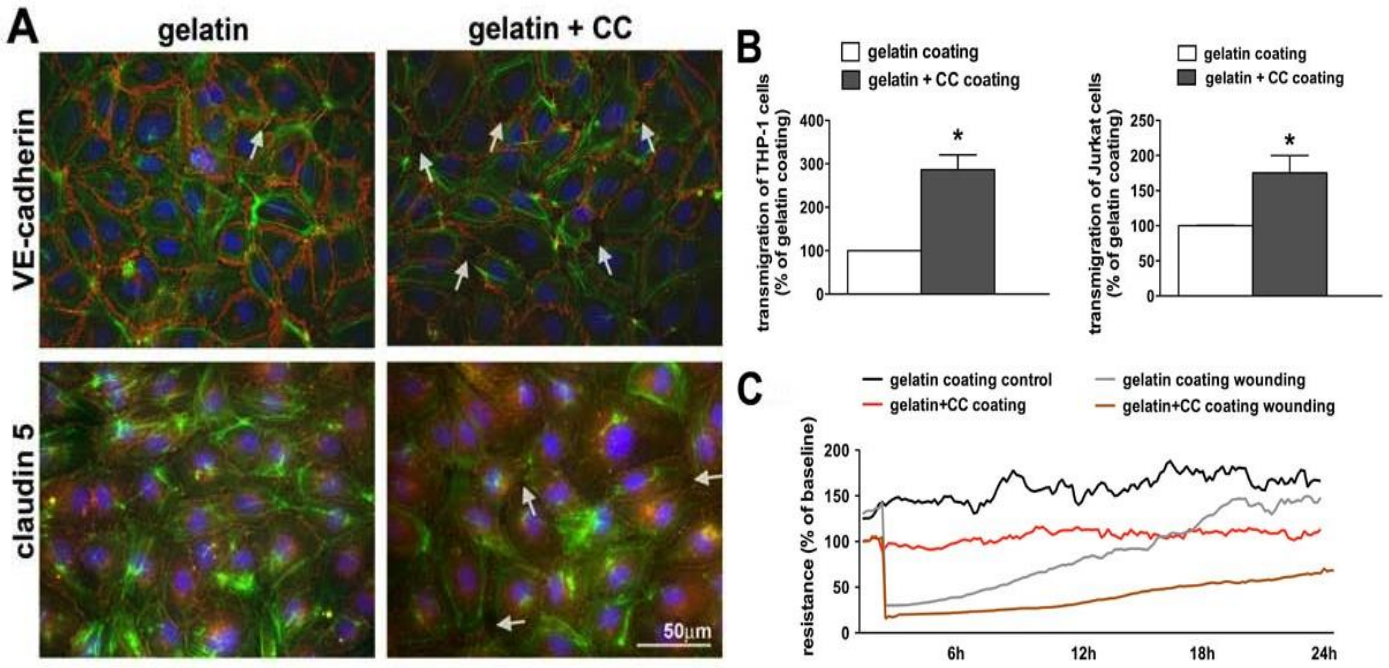
Cholesterol crystal-induced changes in endothelial signaling



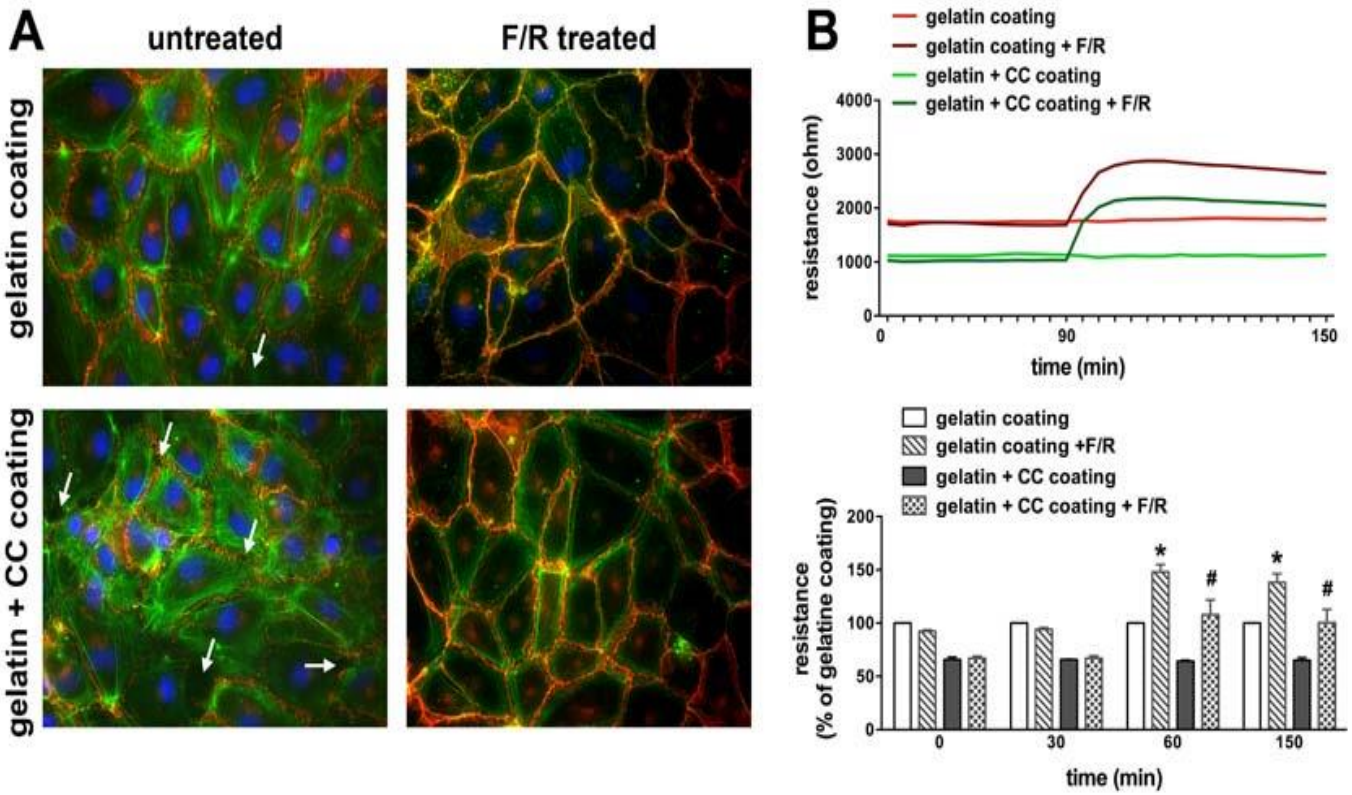




CC induces changes in endothelial function

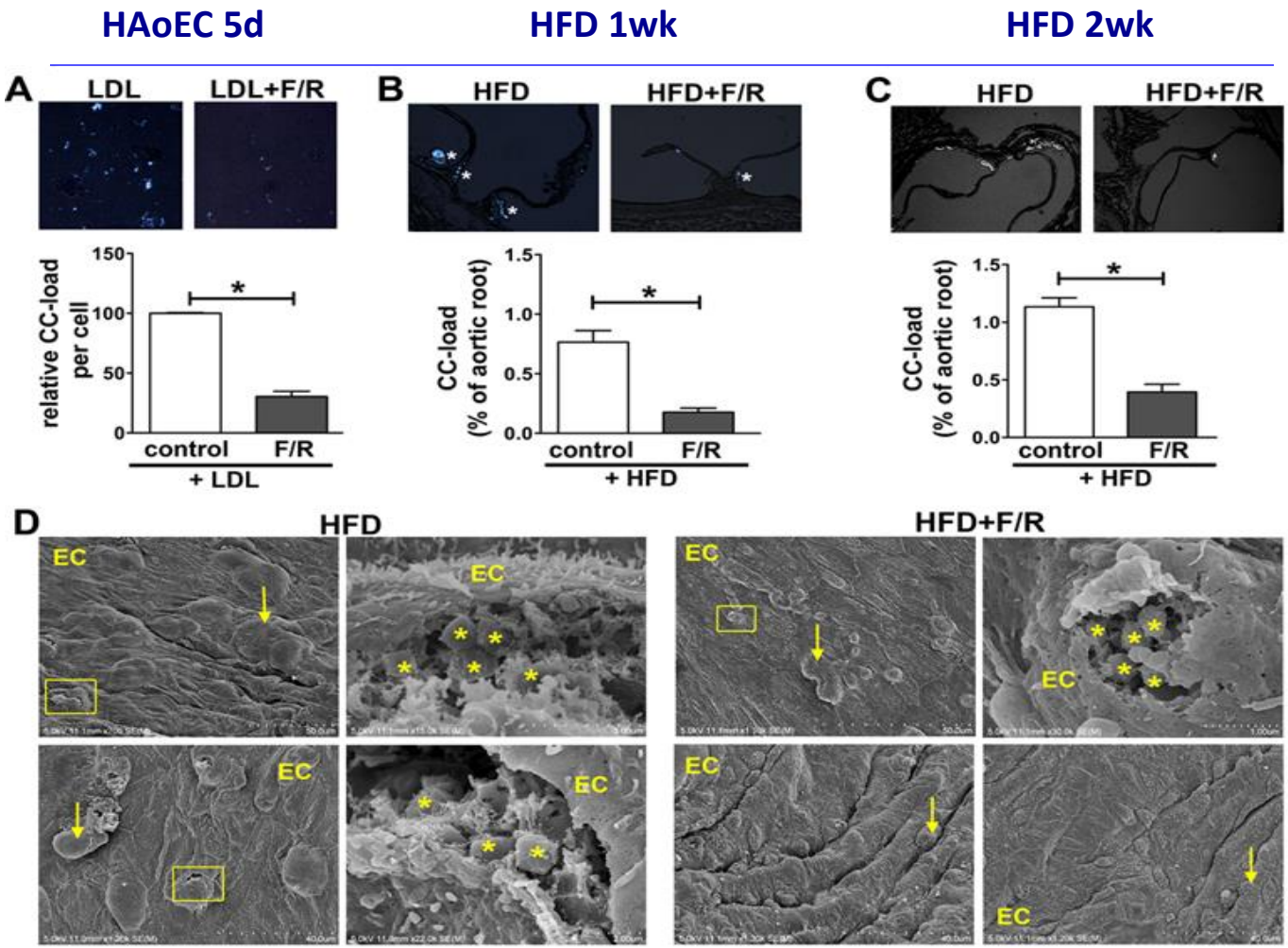


F/R restores the barrier dysfunction caused by CC

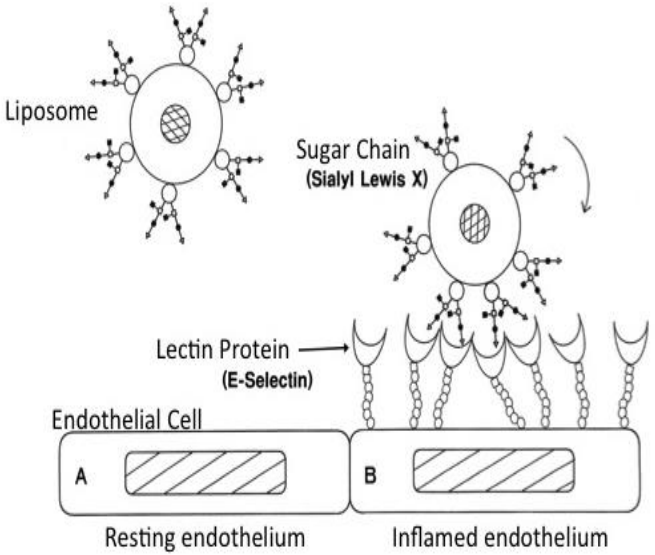


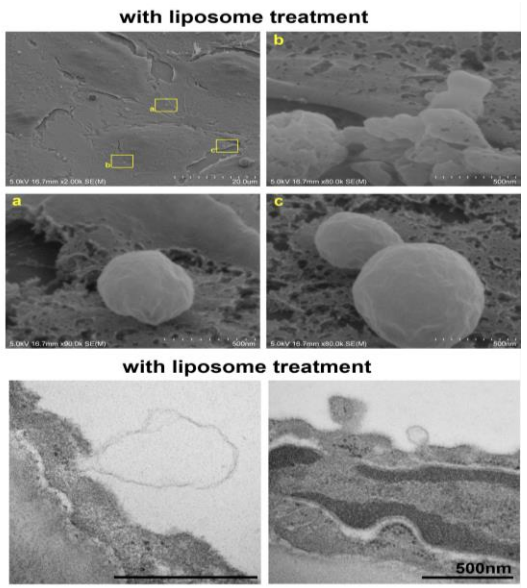
Forskolin/rolipram are well known cAMP-enhancing agents

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

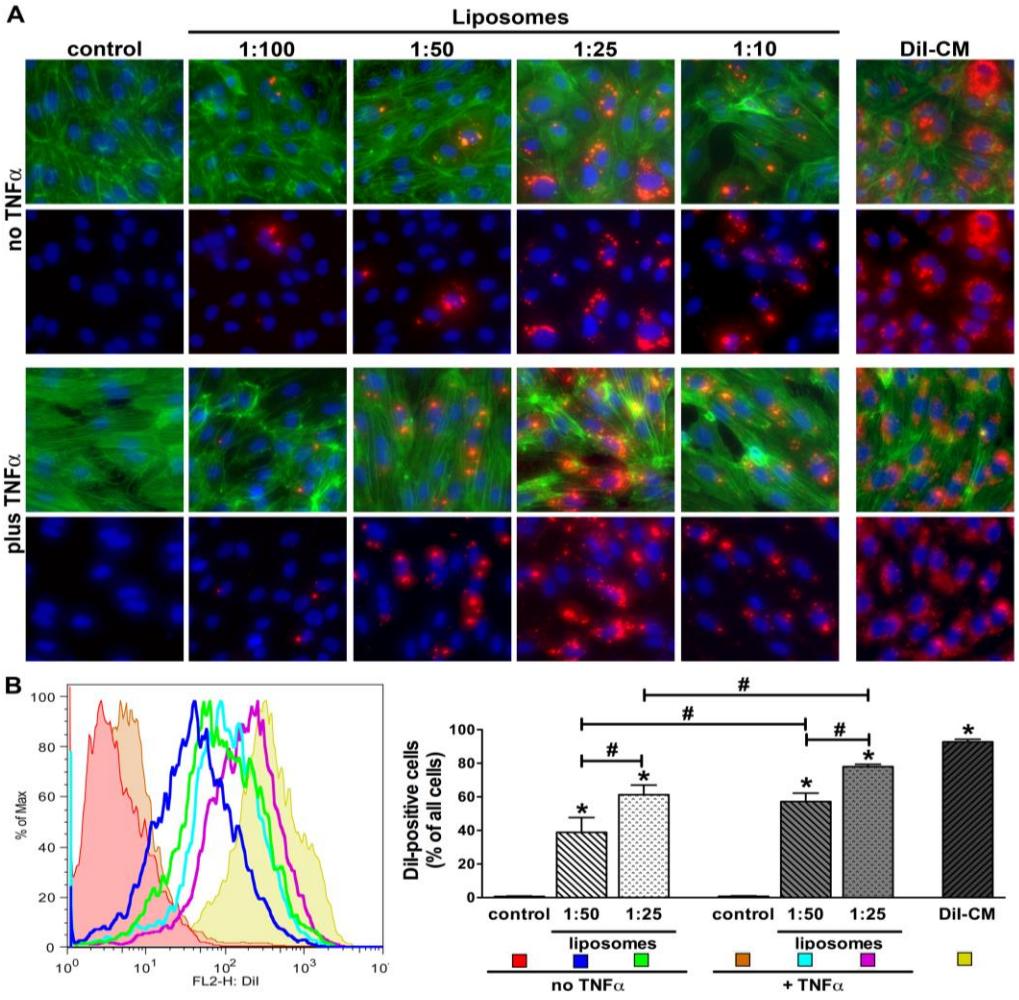


Characterization of F/R-containing liposome

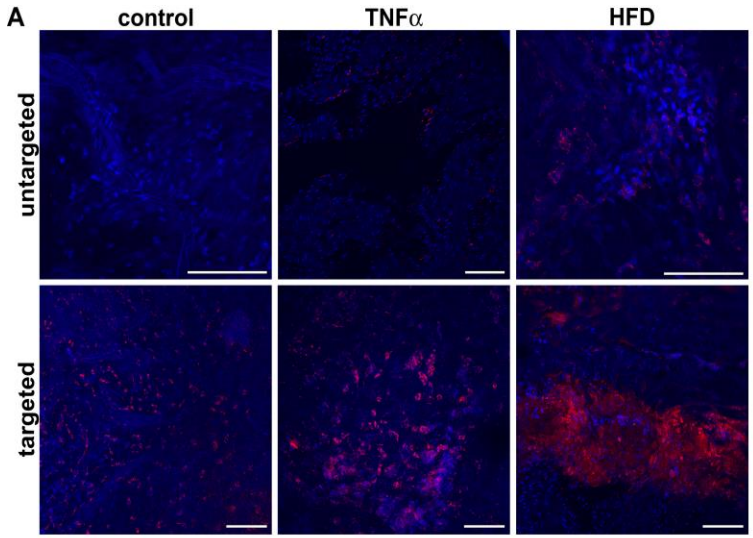


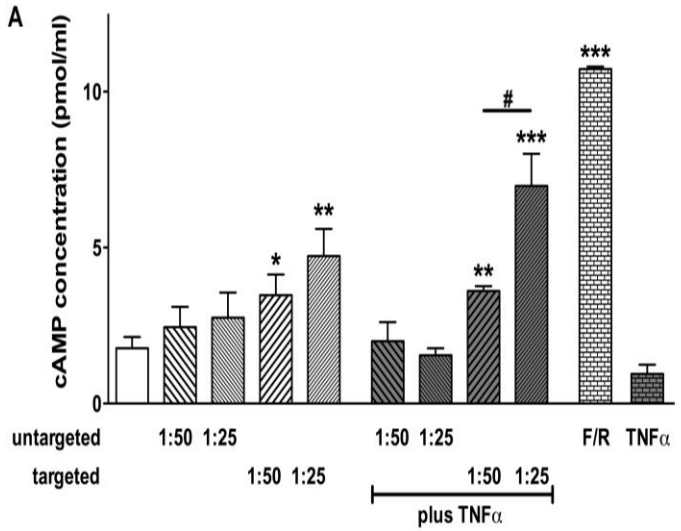


Binding of F/R-liposome to endothelium

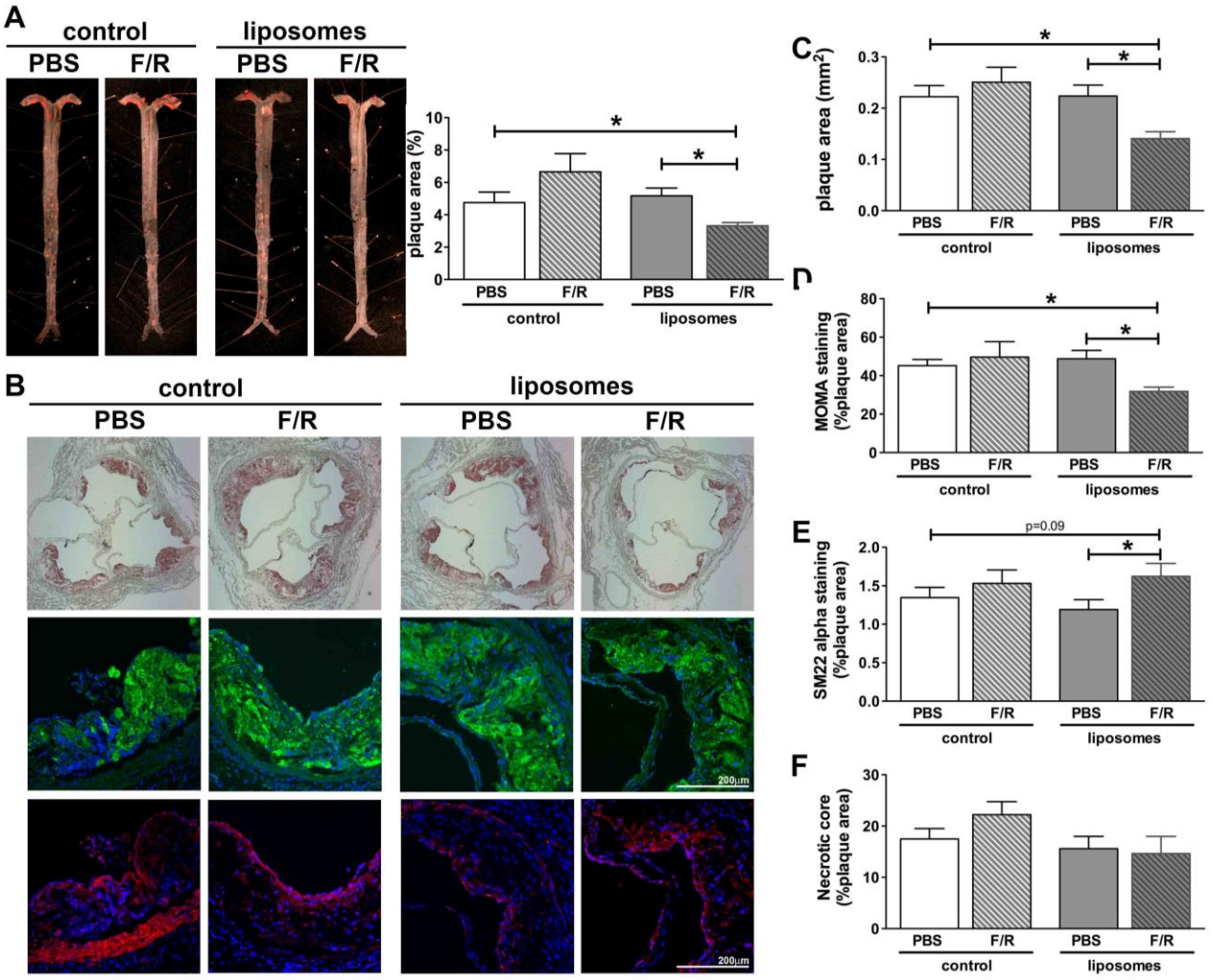


Effects of F/R liposome administration *in vivo*



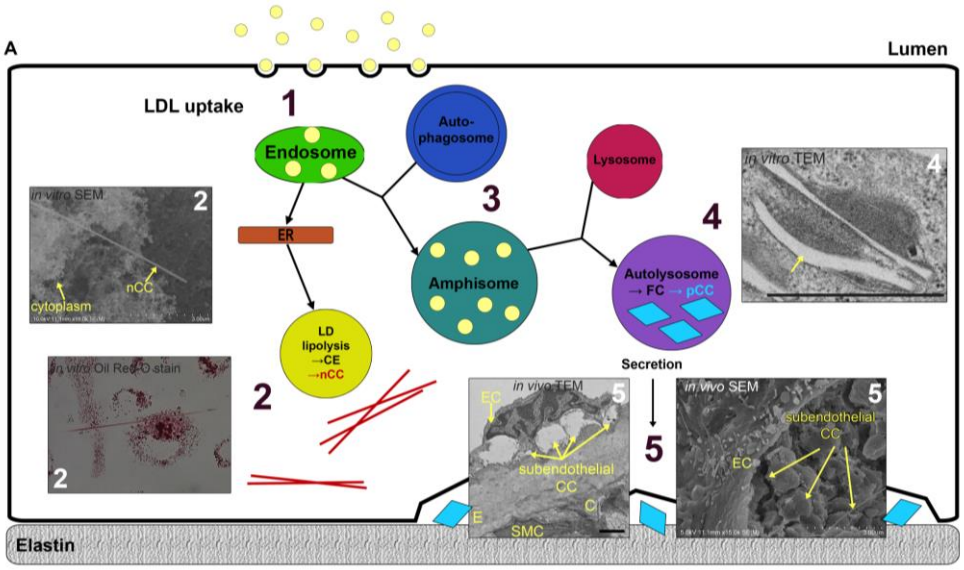


F/R liposome treatment in HFD-fed *apoE-/-* mice



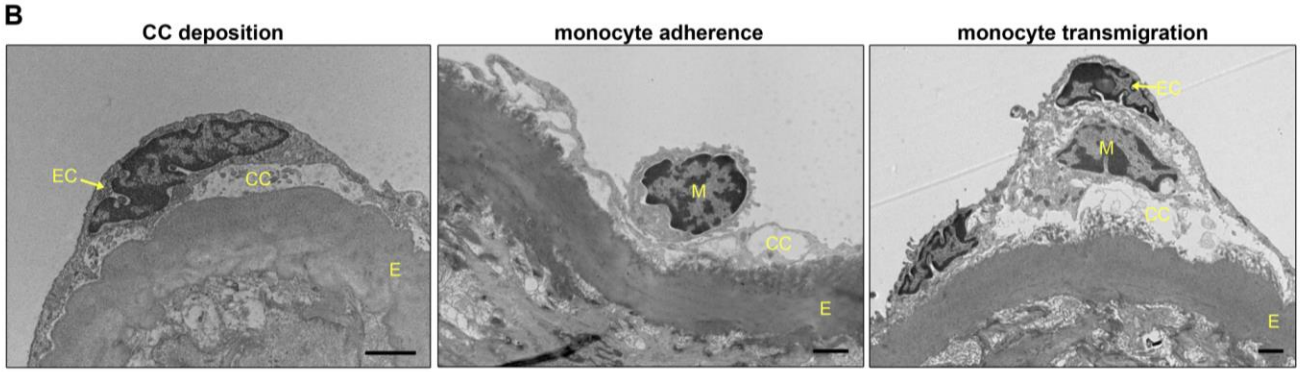
Summary

- EC robustly take up LDL and process the lipid intracellularly
- When the cells are overwhelmed with cholesterol the EC produce cholesterol crystals that get deposited subendothelially



Summary

- CC causes increase in RhoA and reduction in Rac1 and cAMP, all consistent with disturbance of endothelial barrier function
- CC increase the endothelial permeability and allow transmigration of leukocytes



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

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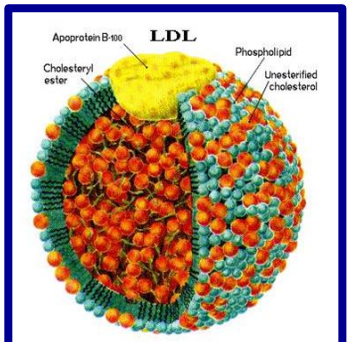
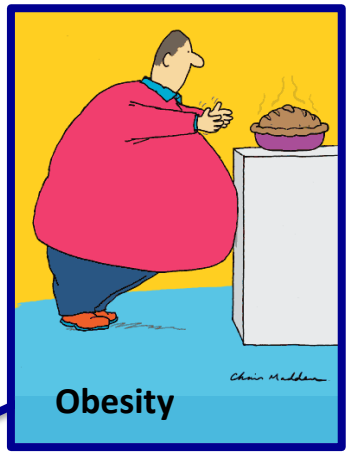
Risk Factors



Dyslipidemia

Hypertension

Sedentary lifestyle

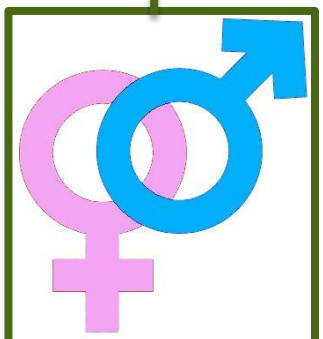


Obesity

Atherosclerosis

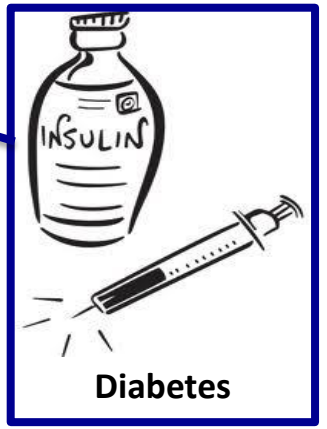
Smoking

Family History



Gender

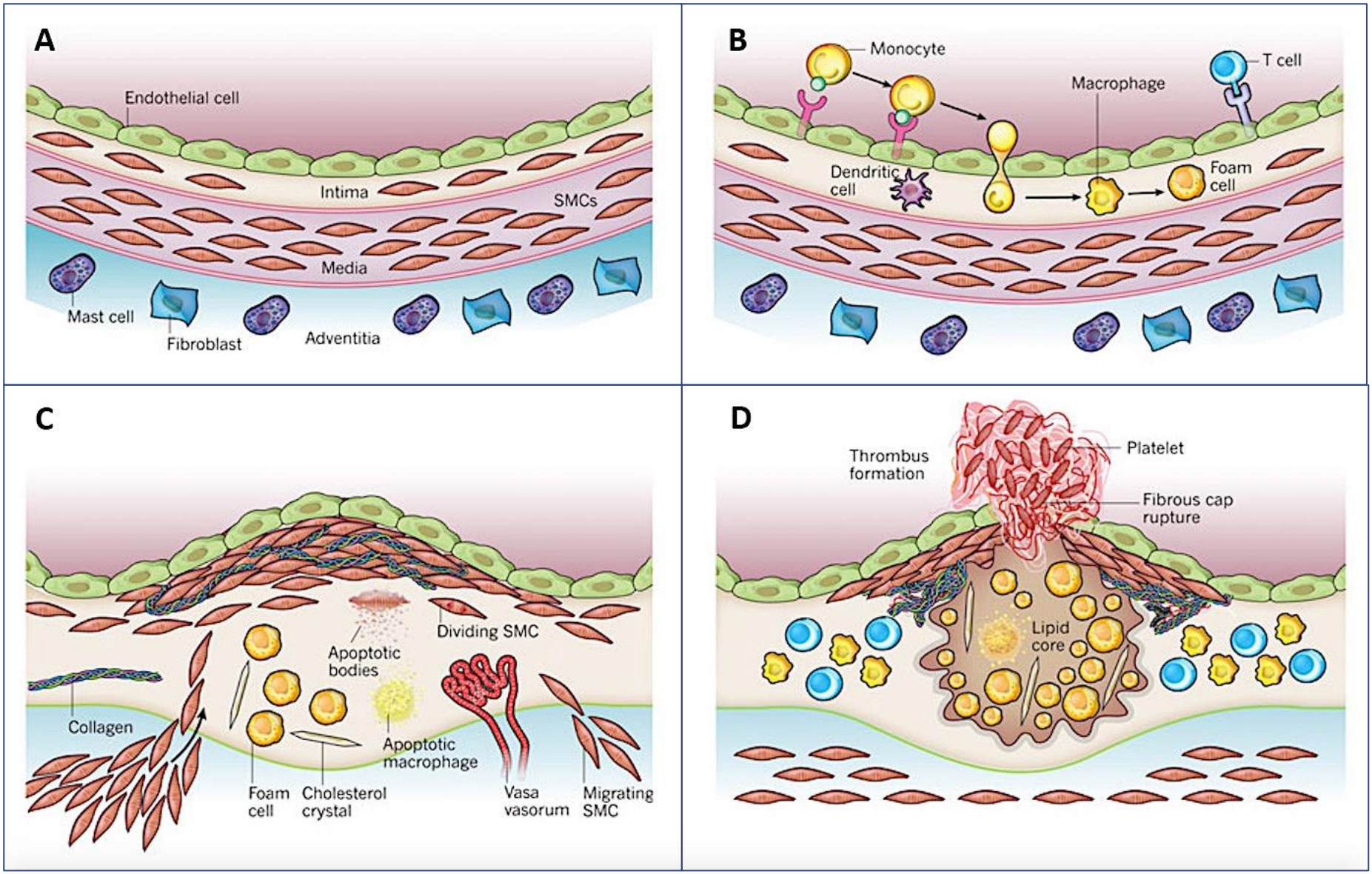
Aging



Diabetes

(*8-13: Image citations)

Stages of Atherosclerosis



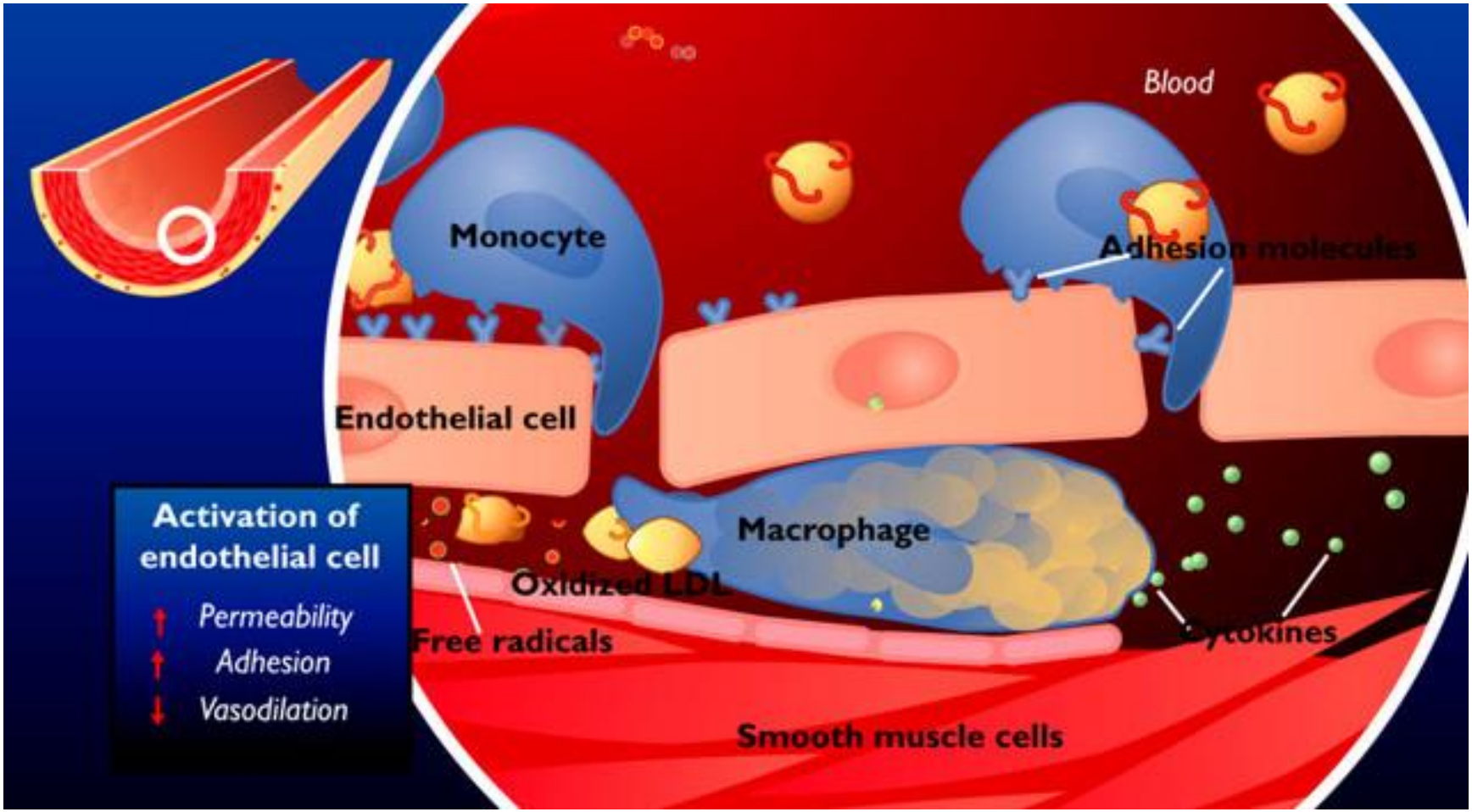
(Adapted from Libby *et al.*, *Nature,* 2011)

Cardiovascular Diseases (CVD)

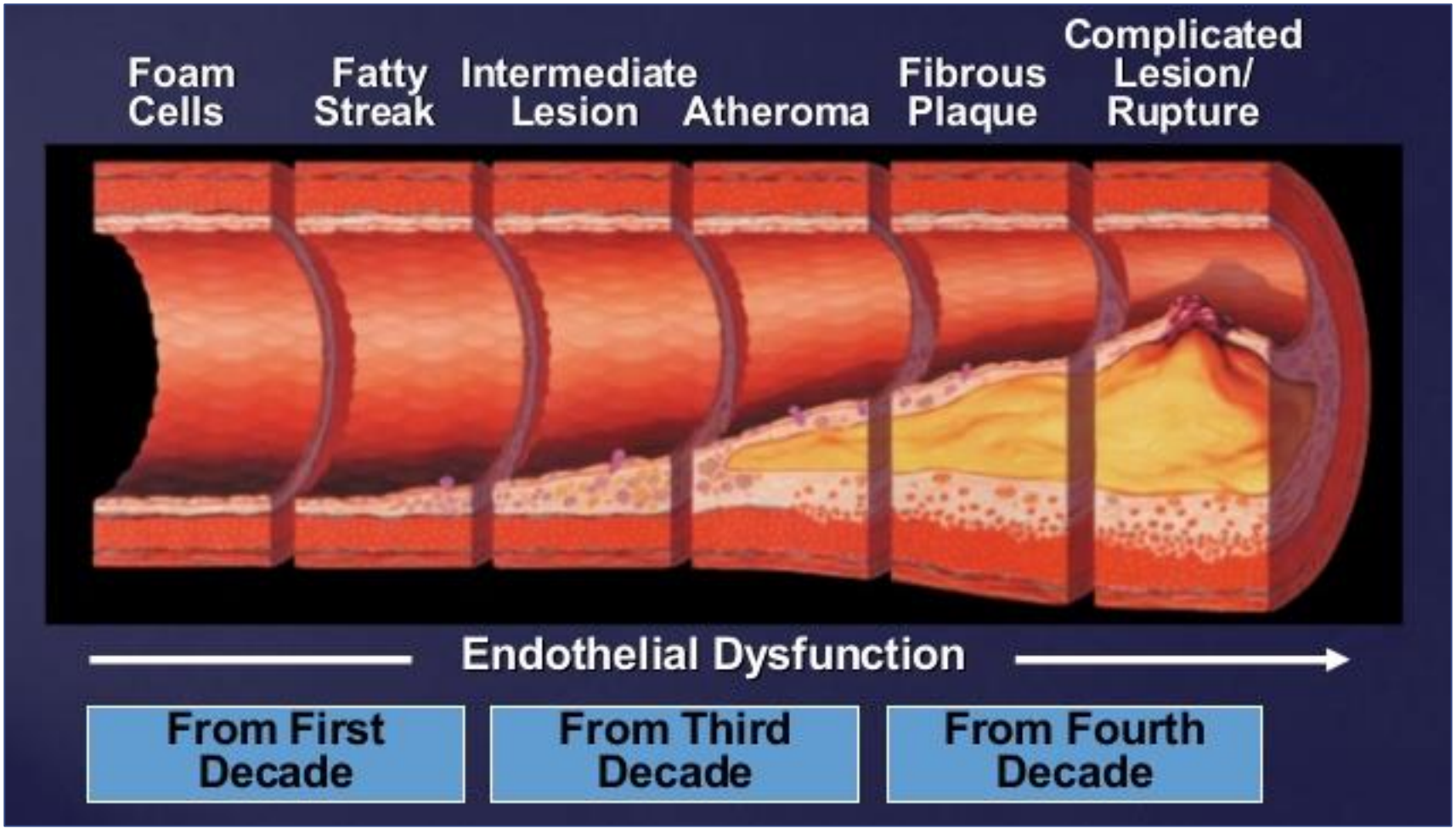
- CVD and stroke are top causes of death in the world – 35% of total
- $863 billion in annual global economic burden
- 17% of US national healthcare costs

http://www.who.int/mediacentre/factsheets/fs310/en/

Vascular endothelium modification in atherosclerosis



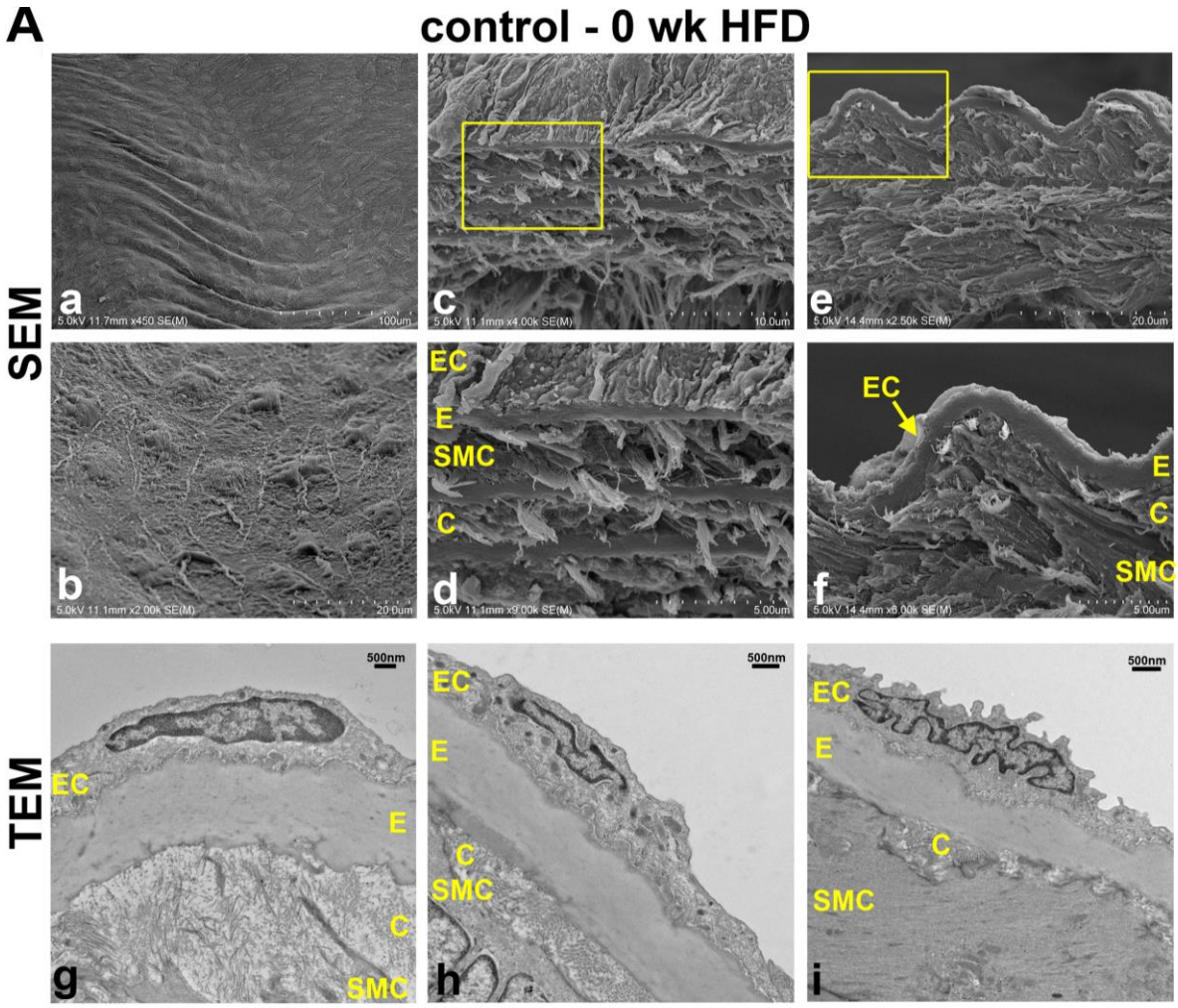
Atherosclerosis Timeline



(Adapted from Pepine CJ. *Am J Cardiol.* 1998)

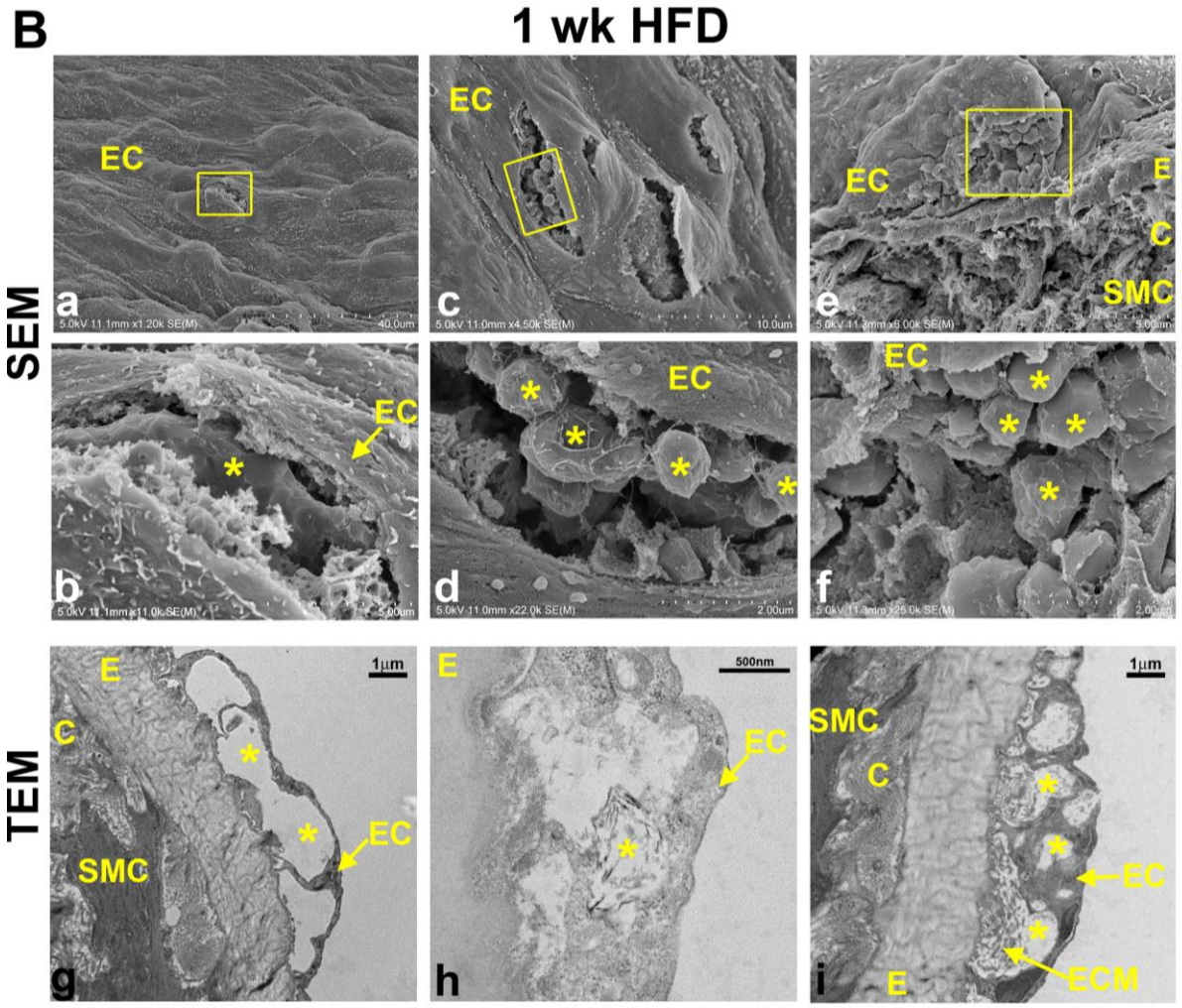
Subendothelial CC deposition in ldlr$^{-/-}$ mouse aorta

No HFD

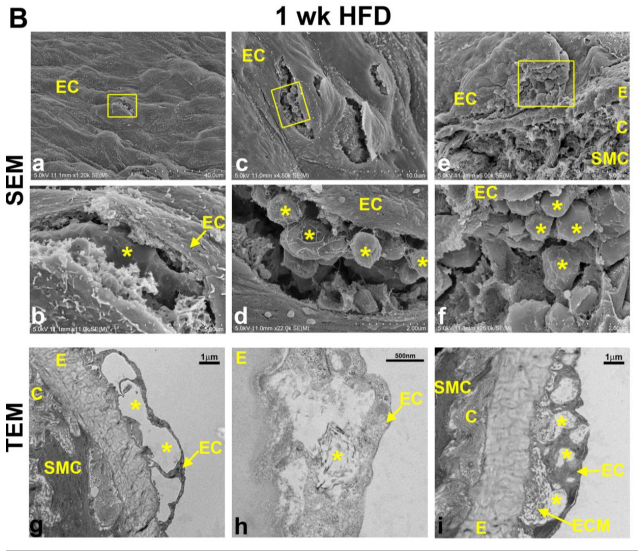


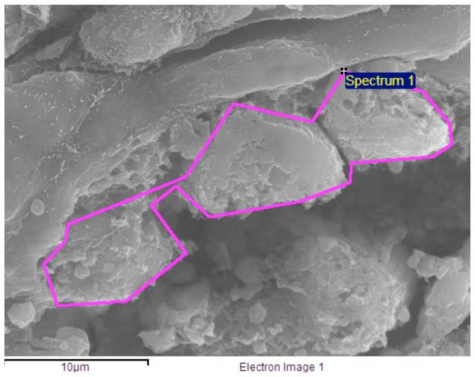
Subendothelial CC deposition in ldlr$^{-/-}$ mouse aorta

1 week HFD

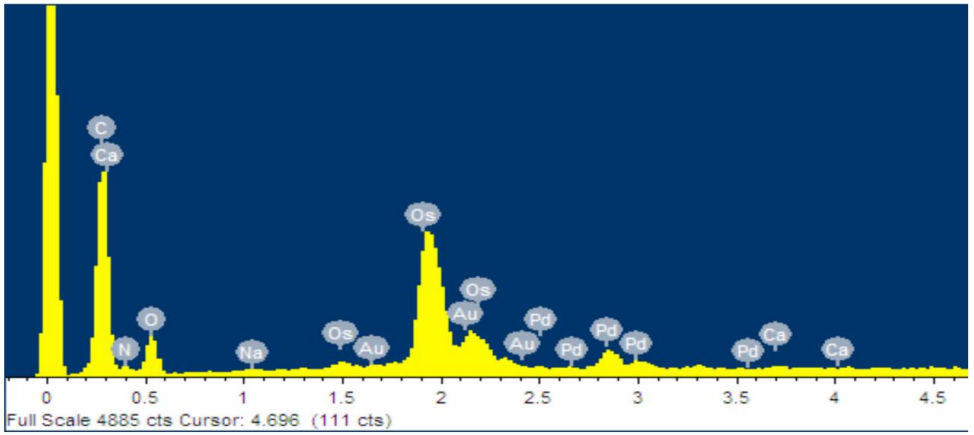


Composition of subendothelial CC





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+}.

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)**