# Autophagy and Metabolic Syndrome Characteristics in the Pathogenesis of Atherosclerosis

Flessa Christina-Maria
B.Sc. In Biology, M.Sc., PhD
Medical School of Athens

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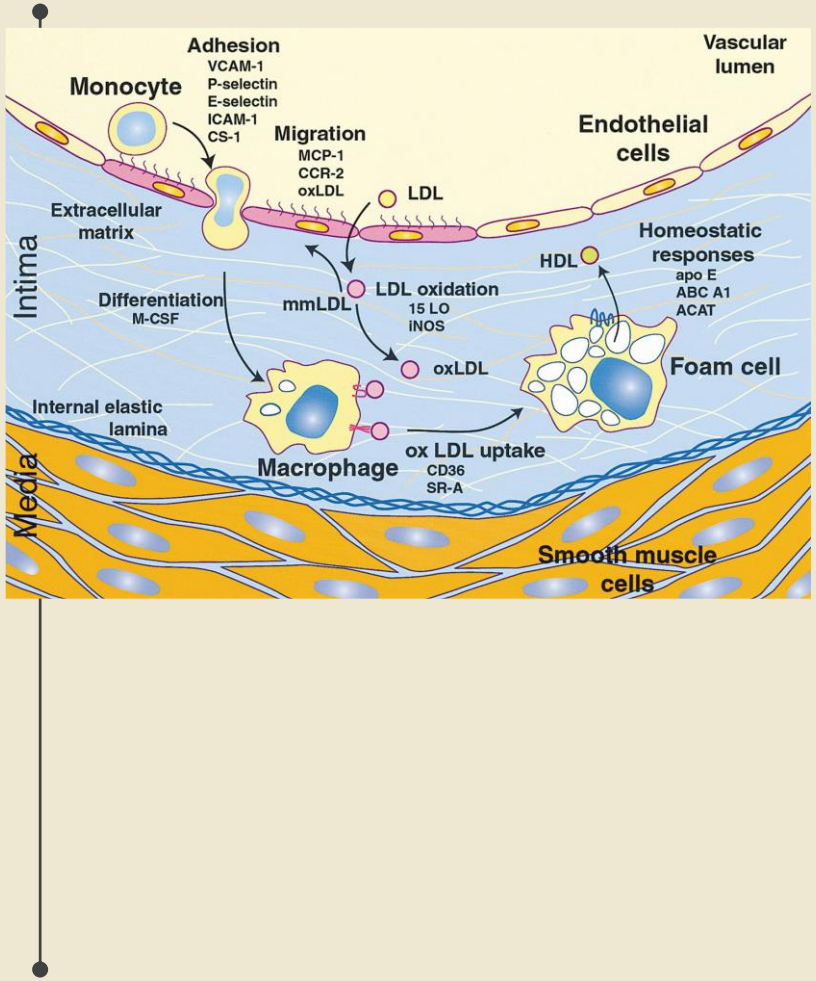
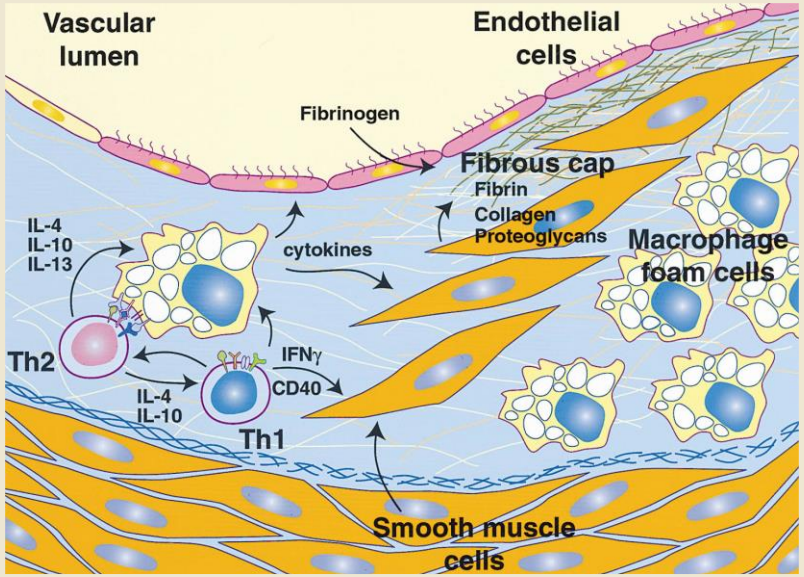
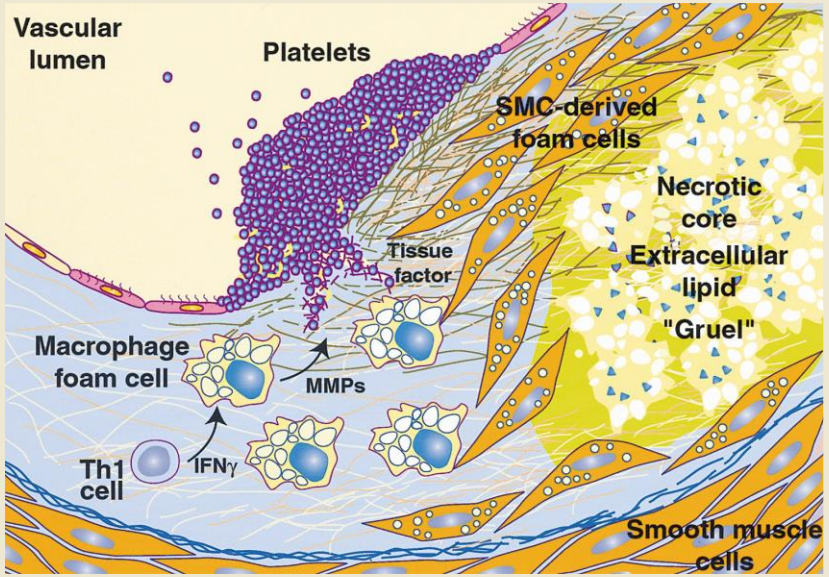
# Plaque development and rupture



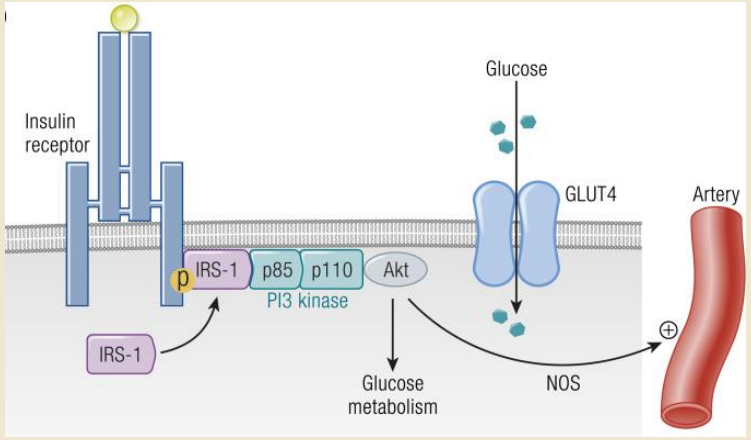




(Glass and Witztum, Cell 2001; 104:503–516)

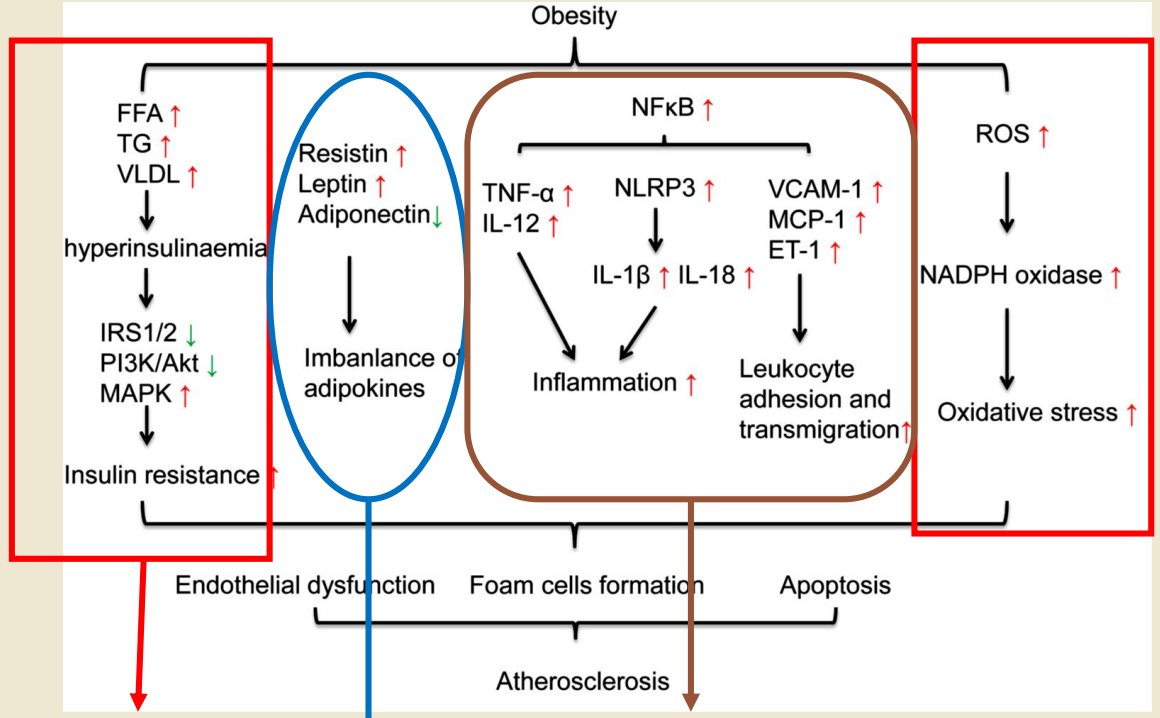
# Metabolic syndrome

# Obesity and atherosclerosis




(Di Pino and DeFronzo, Endocr Rev. 2019; 40(6):1447-1467)

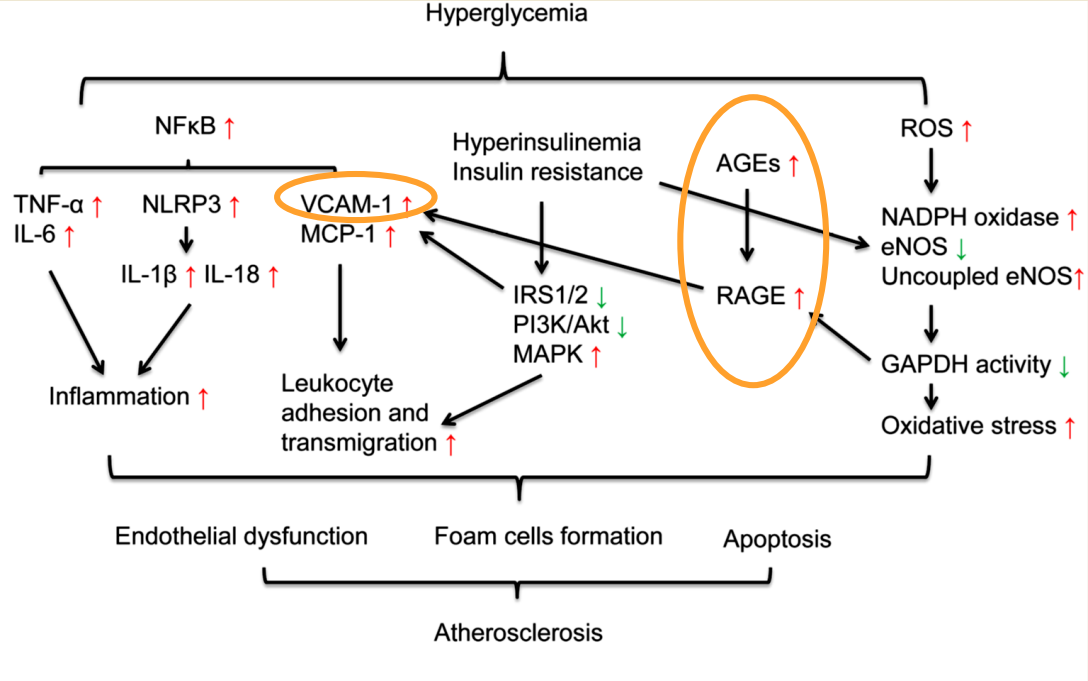
- Normal insulin signaling
  - Insulin signaling → activation of nitric oxide (NO) (vasodilator and antiatherogenic agent) → normal endothelial function
  - Insulin → insulin receptor → tyrosine phosphorylation of insulin receptor substrate-1/2 (IRS-1/IRS-2) and activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) → augmenting glucose transport and other metabolic processes




- **Adipose tissue**
- **Liver**
- **Skeletal muscle**
- **Adipose tissue**
- **Endothelial cells**

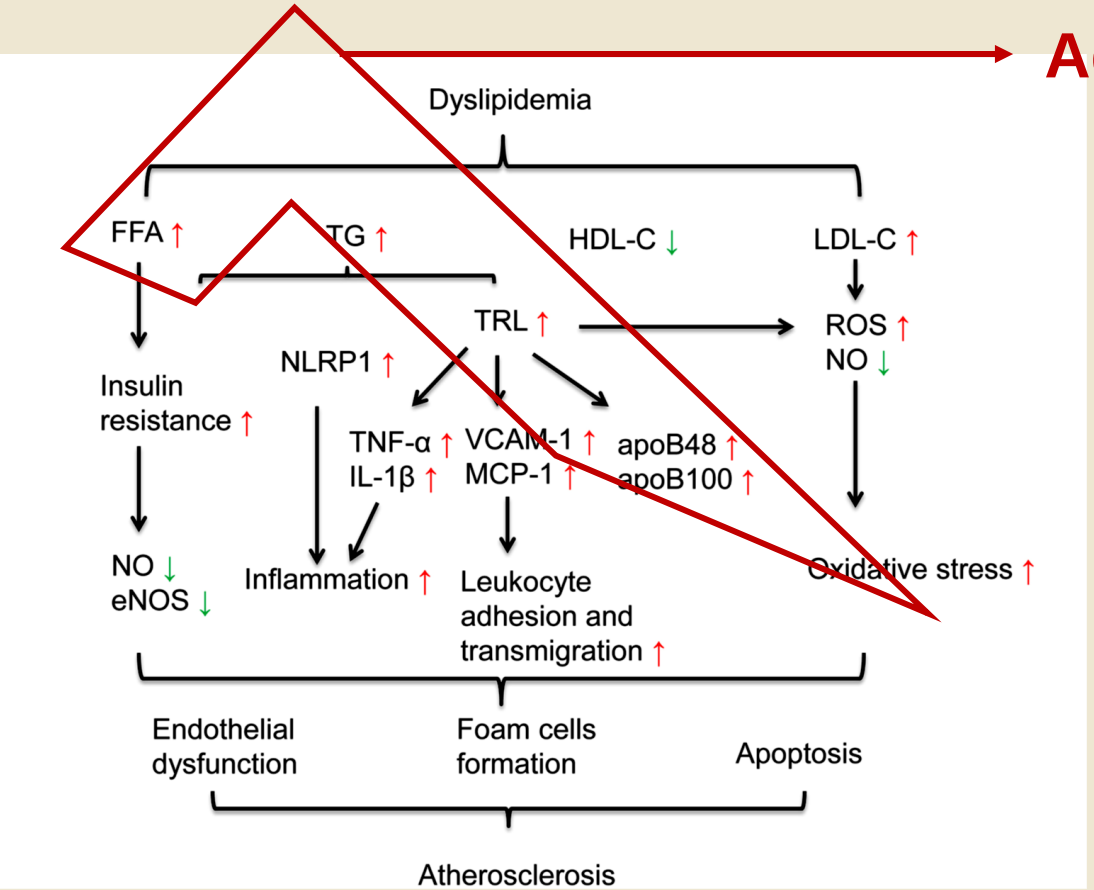
(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Glucose intolerance and atherosclerosis




(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Dyslipidemia and atherosclerosis

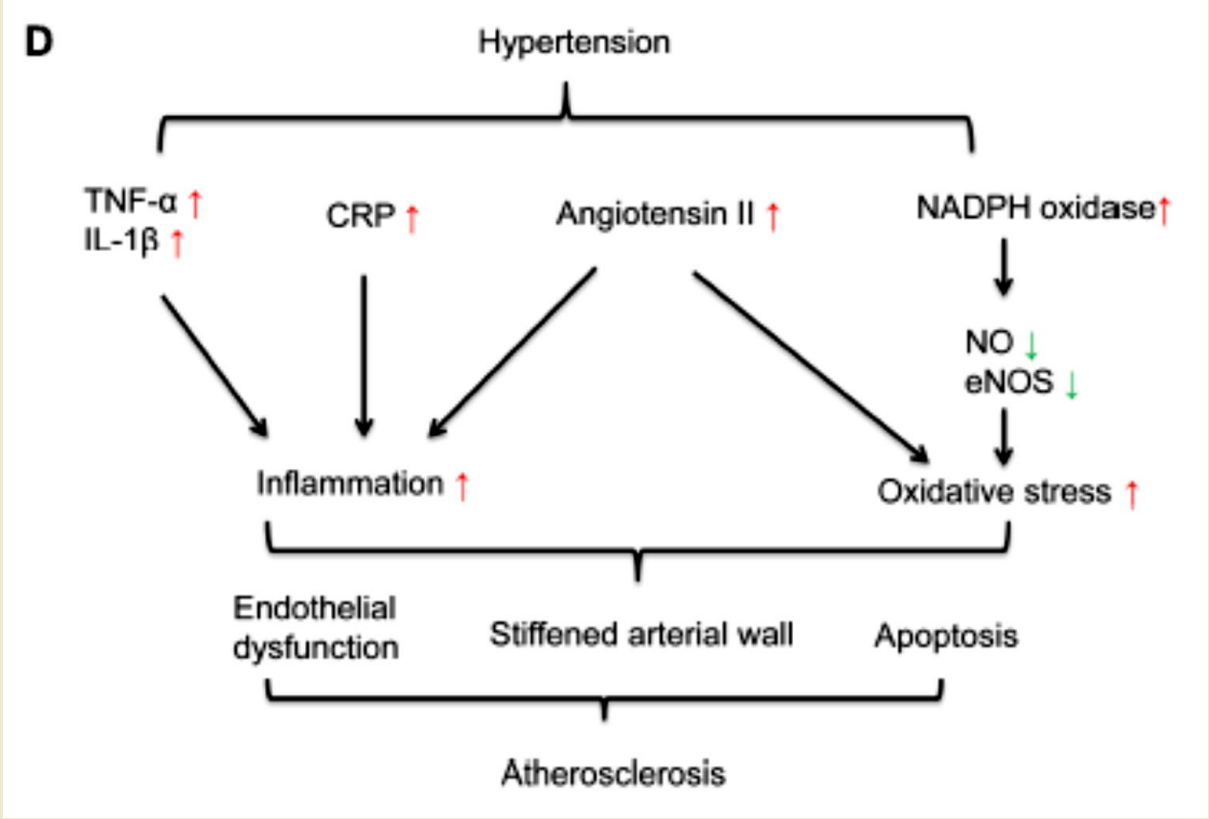



**Adipose tissue → liver**

(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Hypertension and atherosclerosis

- Under insulin resistance/hyperinsulinemia:
  - ET-1 (vasoconstrictor) can suppress insulin-induced Akt activation in VSMCs to exacerbate the development of hypertension and atherosclerosis.
  - Increased systolic BP levels may stiffen the arterial wall and accelerate the progression of atherosclerosis




(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Autophagy and atherosclerosis

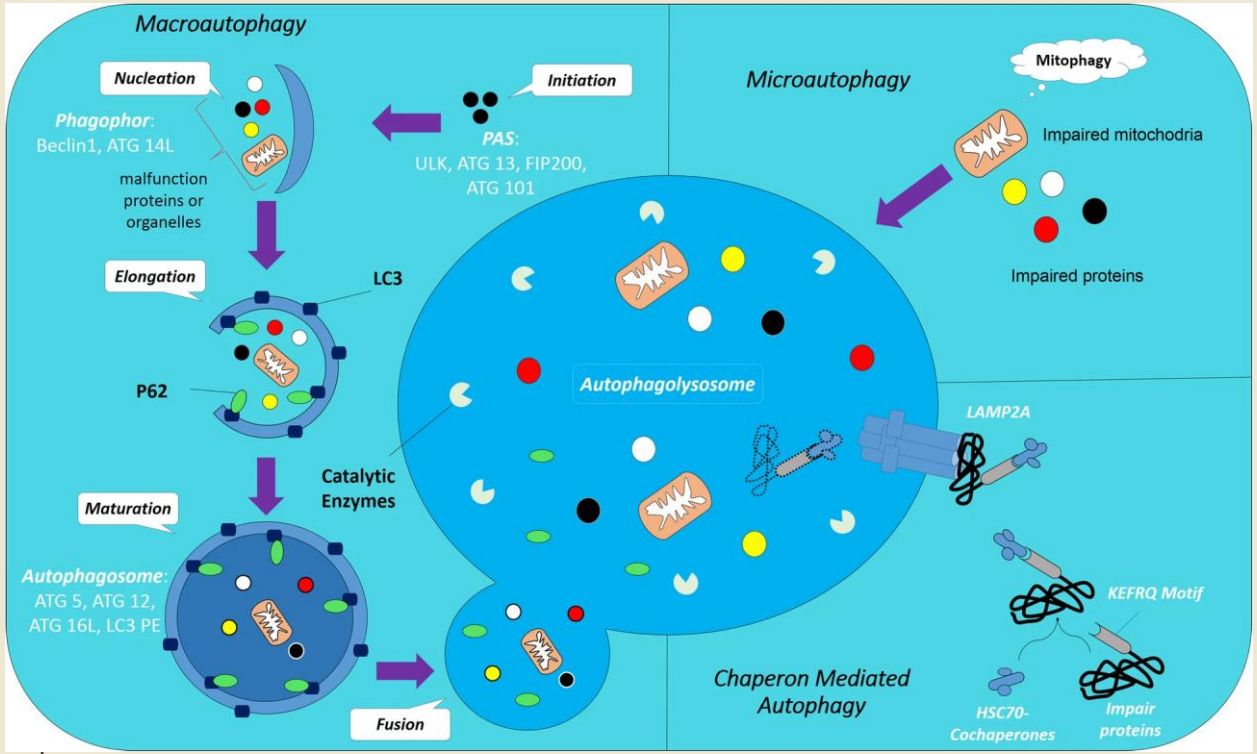
## PROTECTIVE ROLE OF BASAL AUTOPHAGY LEVELS

- MetS →impaired autophagy → accumulation of cytotoxic aggregates, dysfunctional organelles in atherosclerotic plaques
- Drugs repairing impaired autophagy [by targeting mammalian target of rapamycin (mTOR) signaling] showed an effect of stabilizing plaques

## DETRIMENTAL EFFECTS OF EXCESSIVE AUTOPHAGY

- Excessive autophagy may induce autophagy-dependent cell death
- MetS-induced reactive ROS, oxidized lipids and inflammation → related to impaired or excessive autophagy activation → damage of vascular wall and development of atherosclerosis

# Autophagy

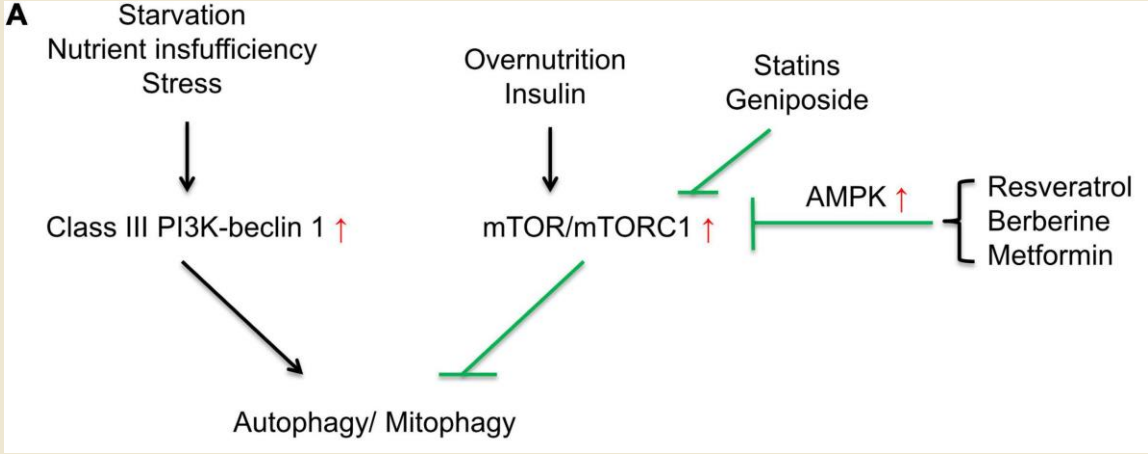



(Hassanpour *et al*., J Inflamm 2019;16:8)

- Autophagy normally occurs at a basal level
- Highly inducible by starvation and other stresses
- Misfolded proteins/damaged organelles → autophagosomes → +lysosomes →autophagolysosomes
- mTOR and AMPK: protein kinases, regulate (inhibit) autophagy through inhibitory phosphorylation of the Unc-51-like kinases ULK1 and ULK2 (mammalian homologues of Atg1)
- Autophagy initiation: Autophagy-related gene 1 (Atg1) and microtubule-associated protein 1A/1B-light chain 3 (LC3)
- Beclin-1: vesicle nucleation
- LC3: vesicle elongation to form autophagosome
- LC3-I ⇄ LC3-II: formation of autophagolysosomes
- Nucleoporin p62 (p62) (LC3-binding protein): docking of cargo to the cell membrane, sequestration of ubiquitinated proteins into autophagosomes

# Autophagy

- Overnutrition/effects of insulin:
  - ⬆Class I PI3K → ⬆mTOR and mTOR complex 1 (mTORC1) → ✖ activation of Atg1 →✖ autophagy
- Nutrient insufficiency:
  - ⬆Class III PI3K-beclin1 complex → assembly of Atg12-Atg5-Atg16L complex and Atg8/LC3 → autophagosome formation
- Pathogenesis of atherosclerosis: caveolin-1 (marker protein for caveolar organelles) is involved in the regulation of autophagy:
  - mTOR and PI3K pathways → phagophore → + Atg5-Atg12-Atg16 → + caveolin-1 → interaction with LC3→ autophagosome formation and caveolin-1 degradation
  - Caveolin-1 deficiency: ⬆ Atg7, beclin1 and LC3-II, which indicated an increasing of autophagic activity and atheroprotection
- Characteristics of MetS including high glucose and dyslipidemia → caveolin-1 activation →✖ autophagosomes

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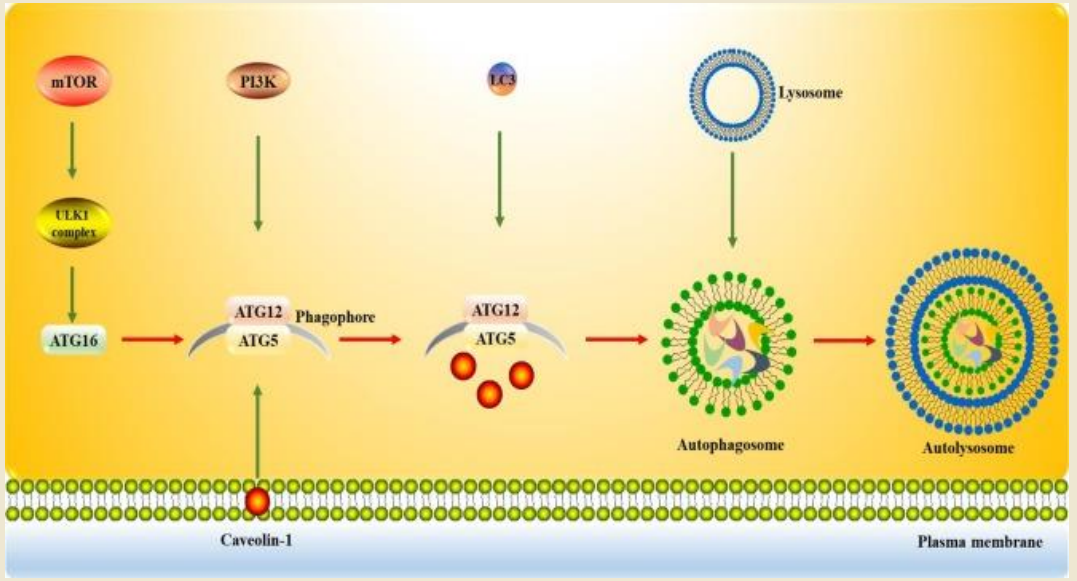
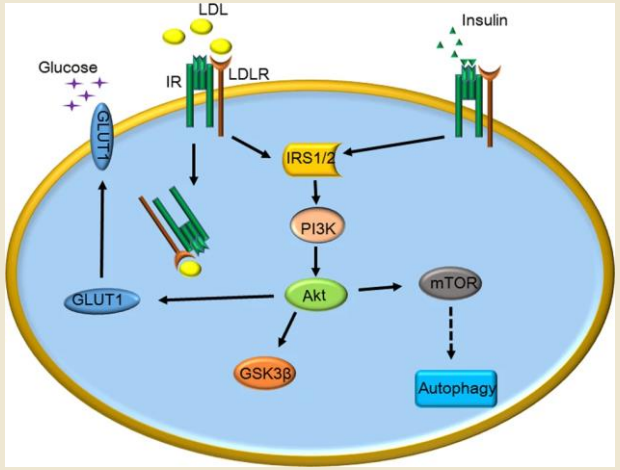



(Hou *et al.*, Clin Chim Acta 2021; 513:25-33)

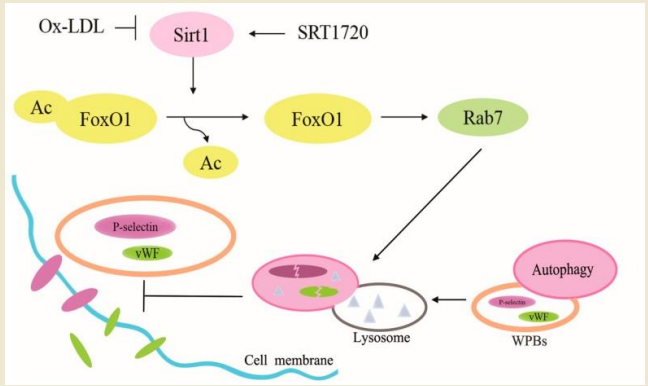
# Autophagy in endothelial cells (ECs)

- LDL: ⬆ PI3K/Akt/mTOR pathway in ECs ⊣ endothelial autophagy
- ox-LDL: ✖ Sirtuin 1 (SIRT1)/forkhead box protein O1 (FoxO1) pathway → ⊣ autophagic flux and promotes apoptosis and adhesion molecule expression




(Zhu *et al*., Sci Rep 2019; 9:3020)

- Balance in autophagy function:
  - **Basal autophagy** ⬆ endothelial eNOS expression and NO bioavailability to maintain endothelial function
  - Autophagy decreases oxidative stress and inhibits the expression of inflammatory cytokines, including MCP-1 and IL-8
- *Basal autophagy is a key regulator of oxidant-antioxidant balance and inflammatory-anti-inflammatory balance in ECs*




(Wu *et al.*, Int J Mol Sci. 2019; 20(17): 4132)

# Autophagy in endothelial cells (ECs)

- Balance in autophagy function
  - **Inefficient/impaired** (via Atg3 siRNA) **autophagy:** ✖ eNOS phosphorylation and NO production, ⬆ ROS accumulation and inflammatory cytokine production, promotes inflammation and apoptosis and contributes to the development of atherosclerotic plaques in ECs
  - Autophagy defects in ECs → IL-6-dependent endothelial-to-mesenchymal transition and organ fibrosis
  - Induced caveolin-1 attenuates autophagic flux, caveolin-1 silencing induces autophagy in ECs

- Balance in autophagy function
  - **Excessive autophagy** may mediate cell death in ECs and lead to plaque instability
  - Ox-LDL → ⬆ ROS → ⬆ autophagy (increased LC3, beclin-1 and Atg5) → ECs apoptosis
  - ⬆ ROS and ⬆ ox-LDL → initiate autophagy in human ECs in an atherosclerotic environment

# Autophagy in vascular smooth muscle cells (VSMCs)

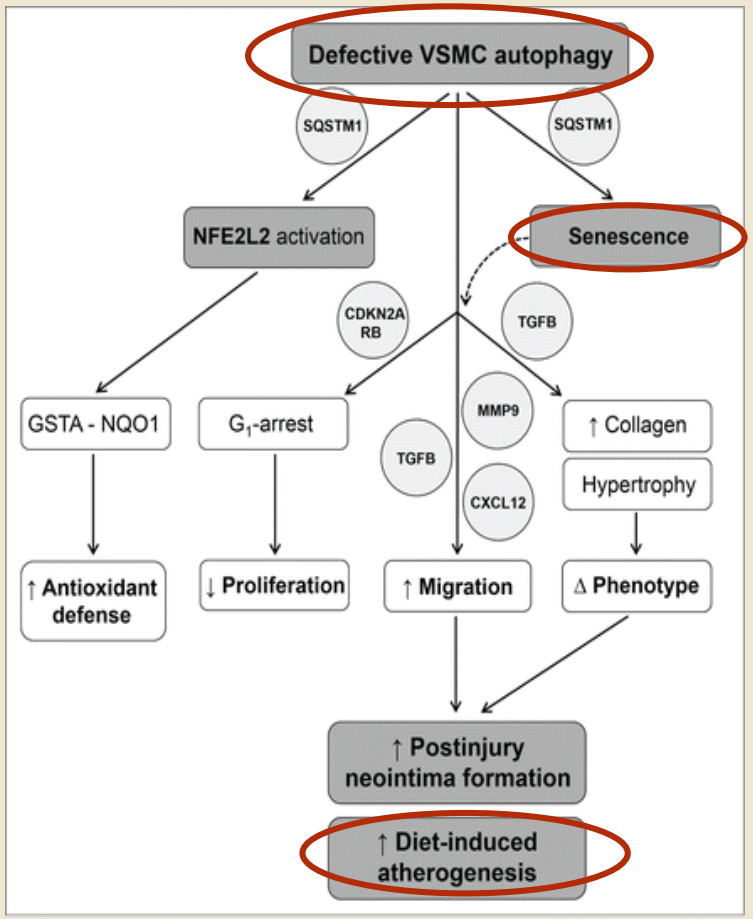
- Abnormalities, death and proliferation in VSMCs participate in the formation and instability of atherosclerotic plaques

- MetS and autophagy in VSMCs:
  - High-fat diet-fed *ApoE*−/− mice (obese) and mice with VSMC-specific Atg7 deletion (impaired autophagy in VSMCs) have ⬆ atherosclerotic lesions compared with *ApoE*−/− control mice
  - Modest ox-LDL concentrations and excess free cholesterol ⬆ autophagy in VSMCs
  - Induced autophagy is considered a cellular survival mechanism to prevent the death of VSMCs

- Balance in autophagy function:
  - **Basal and modest autophagic activity** in VSMCs: protective mechanism against cell death that maintains plaque stability.

# Autophagy in vascular smooth muscle cells (VSMCs)

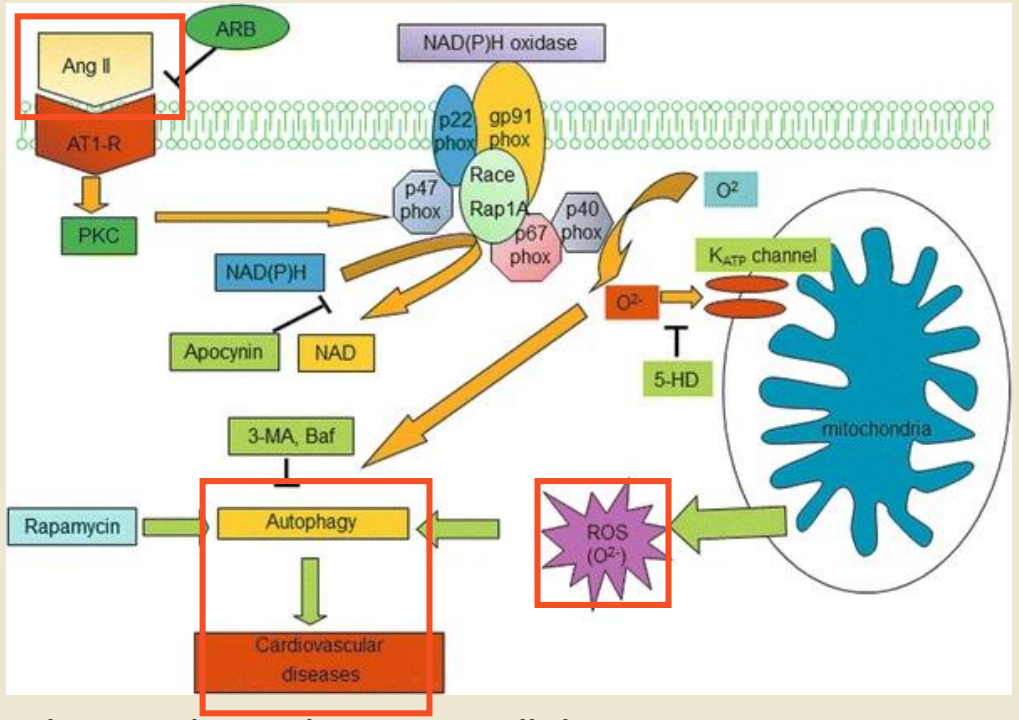
- Balance in autophagy function:
  - **Autophagy deficiency** accelerates cell senescence and promotes diet-induced atherogenesis




(Grootaert *et al.*, Autophagy 2015; 11(11):2014-2032)

# Autophagy in vascular smooth muscle cells (VSMCs)

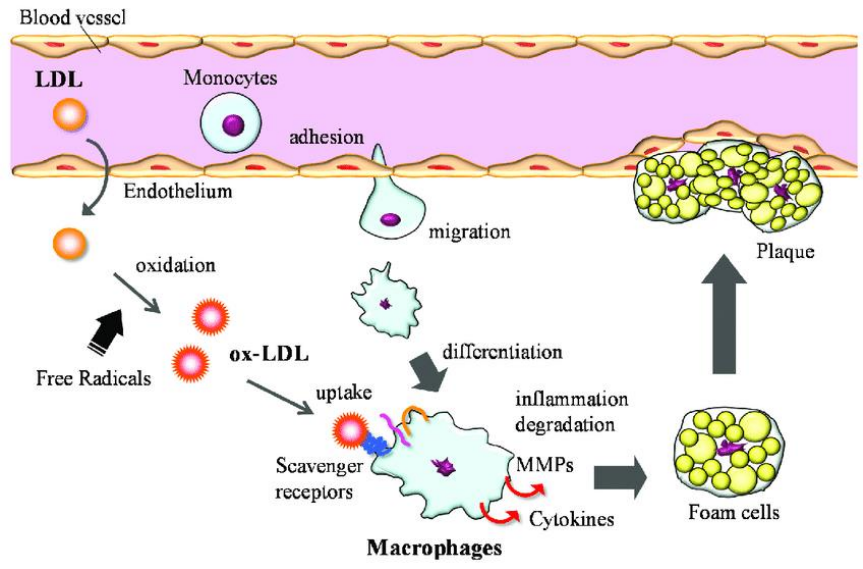
- Balance in autophagy function:
  - **Excess autophagy** may result in VSMC death and plaque destabilization
  - Severe oxidative stress or inflammation (TNF-a) → JNK/Akt pathway → excessive autophagy (increased expression of LC3-II and beclin-1) → VSMC death
  - Ang II ⬆ ROS production and levels of LC3-II and beclin-1 and ⬇ Sequestosome 1 (SQSTM1)/p62 → promotes autophagosome formation (in rat VSMCs) which may also be detrimental




(Yu *et al.*, Basic Res Cardiol 2014; 109:416)

# Autophagy in macrophages

## MACROPHAGES IN ATHEROSCLEROSIS FORMATION



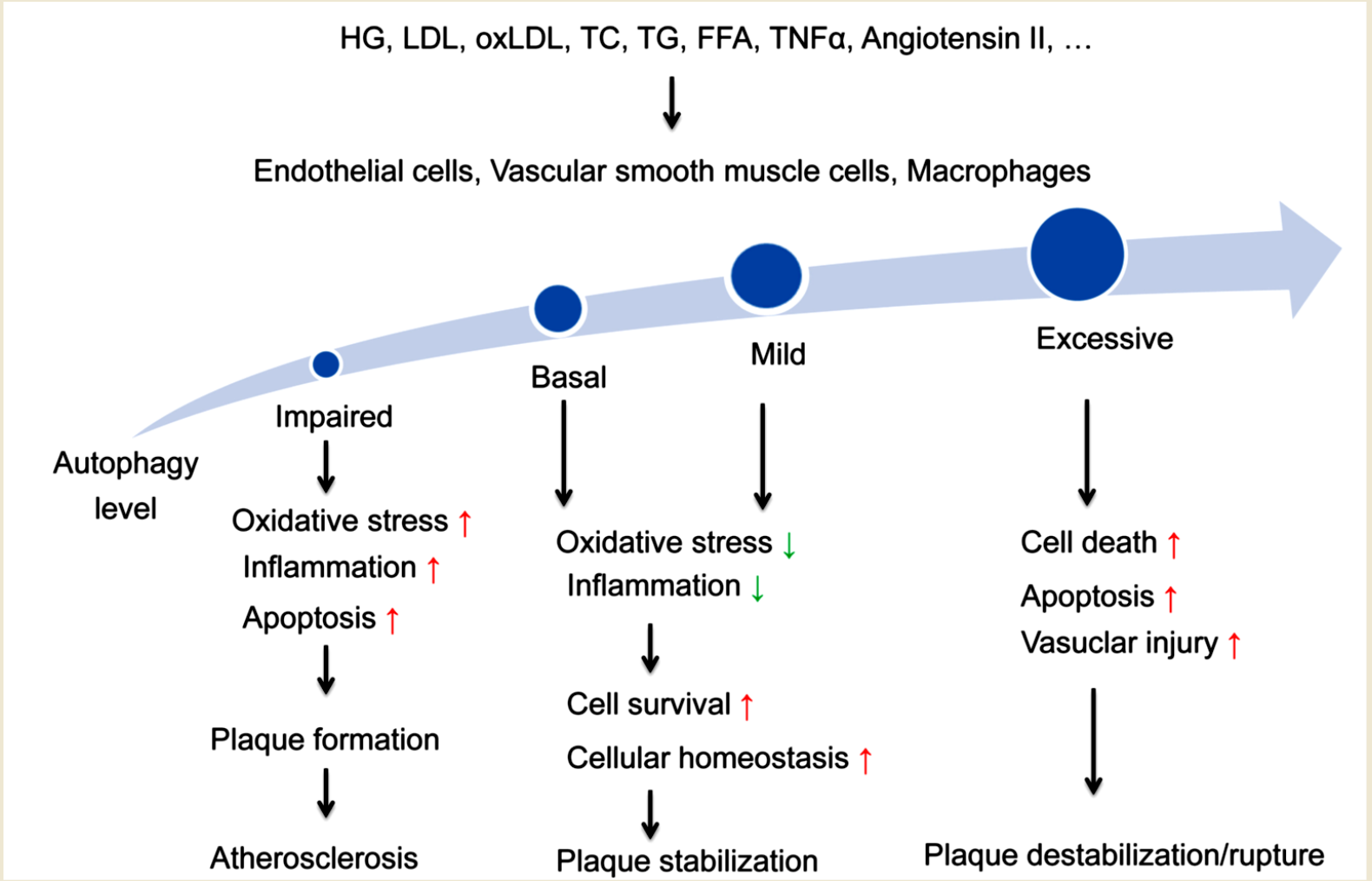

(Kishimoto *et al.*, Mar. Drugs 2016; 14:35)

Monocytes in the bone marrow → stimulated by MetS conditions (elevated TC and LDL) → enter blood circulation → move into subendothelium of vessel walls → differentiate into macrophages, subsequently turning into foam cells that are filled with ox-LDL

## BALANCE IN AUTOPHAGY FUNCTION

- **Basal autophagy** in macrophages → atheroprotective role
- **Suppression of autophagy** →apoptosis and plaque destabilization:
  - Fat-fed LDLR-/- mice: macrophage Atg5 deficiency increases apoptosis and oxidative stress and promotes plaque necrosis
  - Macrophage-specific Atg5-knockout mice: autophagy deficiency (increased p62, decreased LC3 levels)
  - Atg5-null macrophages: IL-1β secretion → inflammasome activation and increased plaques
  - Macrophage autophagy activation via the inhibition of the PI3K/Akt/mTOR pathway can stabilize vulnerable atherosclerotic plaques
- **Excessive autophagy** may also lead to autophagic death in macrophages via poorly understood type II programmed cell death, which further exacerbates the inflammatory response

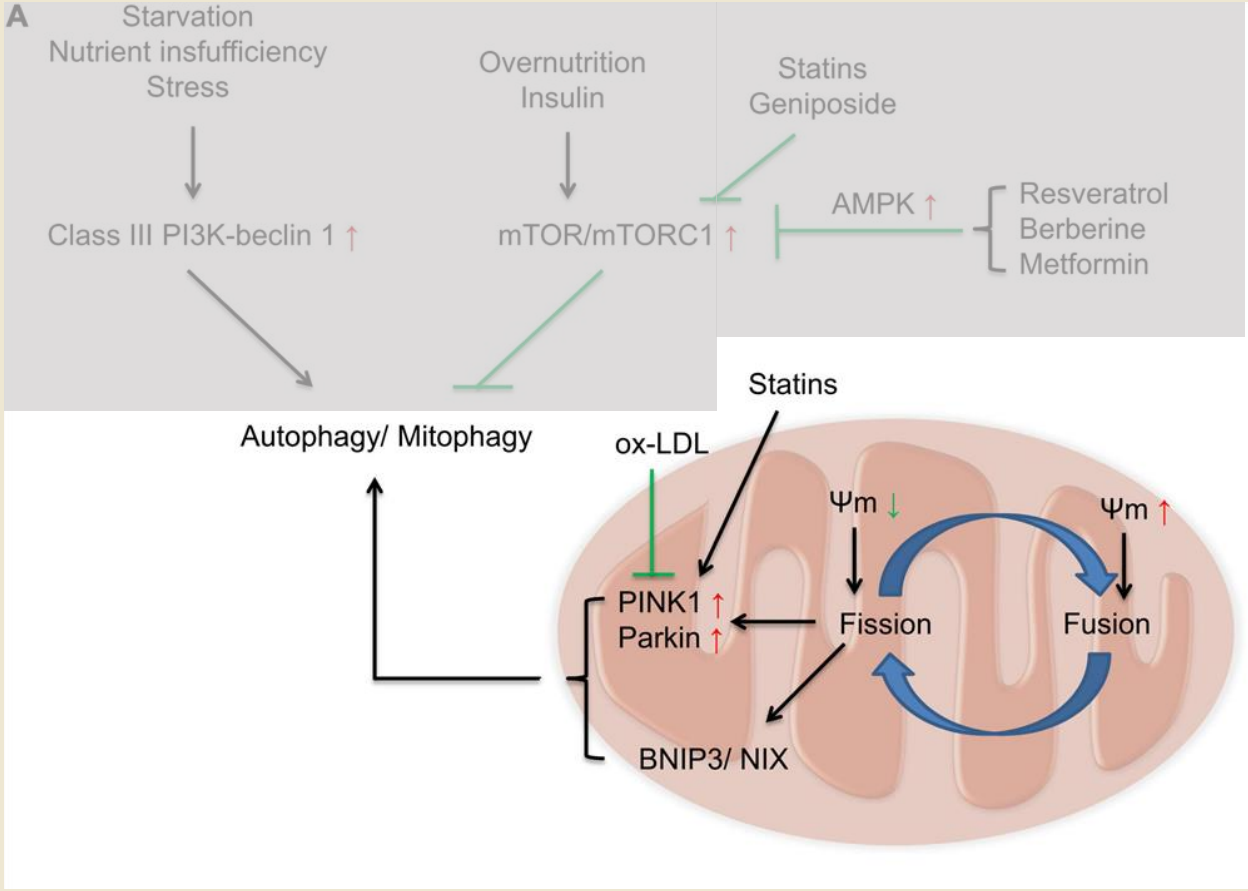
# The importance of autophagy balance




(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Mitophagy

- ↑PTEN-induced kinase 1 (PINK1) and ↑Parkin on the surface of damaged mitochondria →mitochondria elimination
- Changes in mitochondrial membrane potential (Ψm) → mitochondrial fusion and fission process
- Pro-apoptotic BH3-only domain protein (BNIP3) and NIX → selective mitochondrial clearance
- Mitophagy deregulation: ROS overload, ATP depletion → atherosclerosis
- **Dyslipidemia, obesity and mitophagy:**
  - Ox-LDL decreased mitochondrial aldehyde dehydrogenase 2 (ALDH2) via ROS-mediated VSMCs senescence, inhibited fusion, inhibited PINK1 and Parkin → impaired mitophagy → VSMC and endothelial apoptosis
  - SIRT3/FOXO3a/parkin pathway: potential target for suppressing NLRP3 inflammasome activation to attenuate plaque size and vulnerability




(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Pharmacological interventions in the treatment of atherosclerosis

- **Resveratrol:** activator of 5'-adenosine monophosphate-activated protein kinase (AMPK), is a polyphenolic phytoalexin that occurs naturally in many plant parts and products, has antidiabetic and cardiovascular benefits
- **Metformin:** first-line treatment for T2DM, with benefits on MetS and cardiovascular diseases, it can directly activate AMPK and then suppress the mTOR pathway to induce autophagy and inhibit atherosclerosis
- **Metformin and statin (atorvastatin)**: decrease atherosclerotic lesion areas, promote cholesterol efflux to achieve anti-atherosclerotic benefits

| Compounds | Mechanisms of autophagy induction | Primary functions | Antiatherosclerotic effects |
|---|---|---|---|
| Resveratrol | AMPK activation, mTOR inhibition, anti-inflammation, antioxidation, SIRT1 activation | AMPK activation | Decreases the size and density of atherosclerotic plaques, reduces the layer thickness (Wang et al., 2005) |
| Metformin | AMPK activation, mTOR inhibition, anti-inflammation, antioxidation, anti-hyperlipidemia | Anti-hyperglycemia | Reduces monocyte-to-macrophage differentiation (Vasamsetti et al., 2015), promotes cholesterol efflux, attenuates plaque formation, and decreases atherosclerotic lesion areas (Luo et al., 2017) |

(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

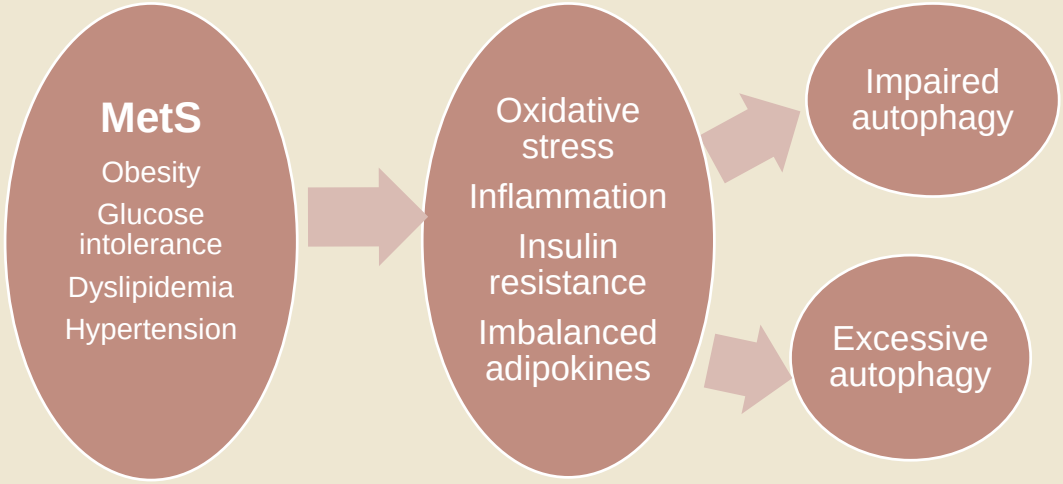
# Pharmacological interventions in the treatment of atherosclerosis

- **Statins:** stabilizing effects on vulnerable atherosclerotic plaques → prevention of atherosclerotic cardiovascular disease
- **Natural products:**
  - **Berberine**, an extract of Coptis, exhibits antioxidant, anti-inflammatory, and antihyperlipidemic effects
  - **Geniposide**, an extract of *Gardenia jasminoides Ellis*, shows antioxidant and anti-inflammatory effects

| Compounds | Mechanisms of autophagy induction | Primary functions | Antiatherosclerotic effects |
|---|---|---|---|
| Statins | mTOR inhibition, anti-inflammation | Anti-hyperlipidemia | Plaques stabilization (Bea et al., 2002; Rodriguez et al., 2017), reduces infarct size (Andres et al., 2014) |
| Berberine | AMPK activation, mTOR inhibition, anti-inflammation, antioxidation, anti-hyperlipidemia | AMPK activation | Inhibition of inflammation in macrophages (Fan et al., 2015) |
| Geniposide | mTOR inhibition | Anti-inflammation | Decreases the size of atherosclerotic plaques (Xu Y. L. et al., 2020) |

(Xu *et al.*, Front. Cell Dev. Biol. 2021; 9:641852)

# Conclusions




- Dysregulation of autophagy induced by MetS → endothelial dysfunction, monocyte/macrophage migration and adhesion → progression of atherosclerosis
- **Basal and mild adaptive autophagy protective** against the progression of atherosclerotic plaques
- **Impaired or excessive** autophagy activation induced by MetS is related to oxidative stress, inflammation, apoptosis, and foam cell formation, contributing to **plaque instability or even plaque rupture**
- Drugs used to treat MetS or CVD symptoms regulate autophagy beyond their fundamental effects
- **Precise control of autophagy** should be considered a potential therapeutic strategy in the prevention and treatment of atherosclerosis

- Main reference:

frontiers in Cell and Developmental Biology

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# Relationship Between Autophagy and Metabolic Syndrome Characteristics in the Pathogenesis of Atherosclerosis

*Jing Xu[1,2], Munehiro Kitada[1,3]*, Yoshio Ogura[1] and Daisuke Koya[1,3]*

*[1] Department of Diabetology and Endocrinology, Kanazawa Medical University, Uchinada, Japan, [2] Department of Endocrinology and Metabolism, The Affiliated Hospital of Guizhou Medical University, Guiyang, China, [3] Division of Anticipatory Molecular Food Science and Technology, Medical Research Institute, Kanazawa Medical University, Uchinada, Japan*

# Thank you!!!