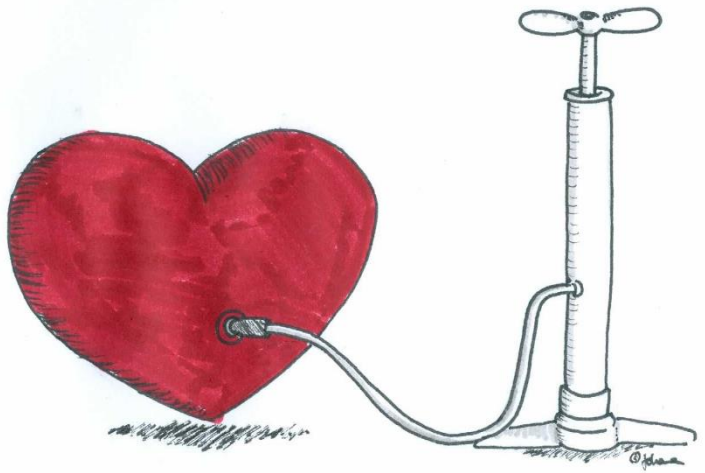
Atherosclerosis (AS) as a pathophysiological process leading to coronary artery disease

Content

- Heart is a pump in need of energy!
- Myocardial blood supply & metabolism
- Coronary blood flow
- Risk factors of AS
- Etiopathogenesis of AS



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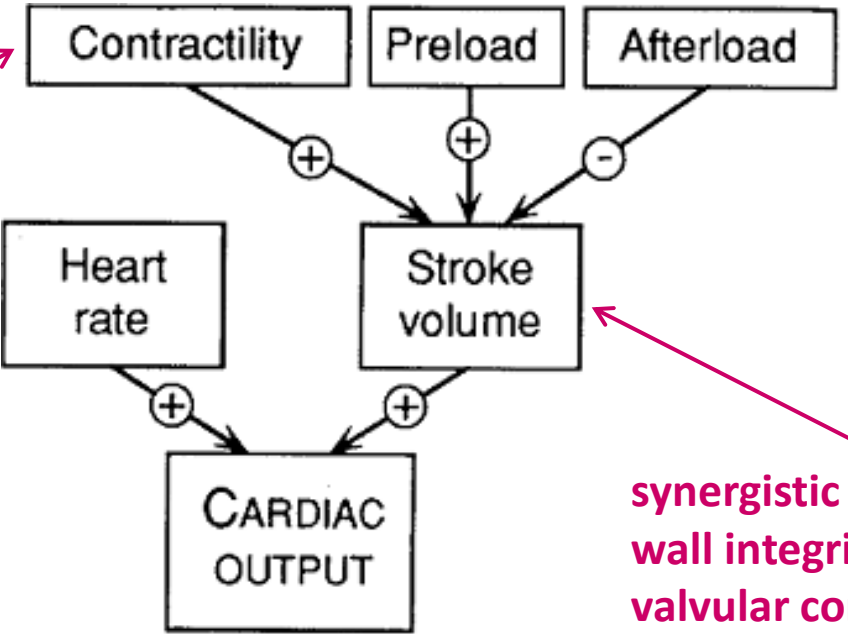
Heart needs a lot of energy (= ATP) to continually perform as a pump (~7,500 L/day, ~ 40 mil beats/year)

venous return (i.e. circulating volume)

systemic vascular resistance (i.e. blood pressure or valve obstruction)



sympathetic nervous system

parasympathetic nervous system

synergistic LV contraction
wall integrity
valvular competence

- quantitatively
  - heart rate ~70/min
  - SV ~70ml
  - CO 70 × 70 = 4,900 ml/min ~ 5 L/min at rest
  - **CO ~ 20 - 25L/min during exercise!!!!**
- influenced by
  - autonomic nervous system activity
  - hormones
  - age
  - gender
  - genetics
  - drugs
  - fitness
  - anatomy/size of the heart

SV × f = CO → 70mL × 70 bpm = ~ 5 L/min

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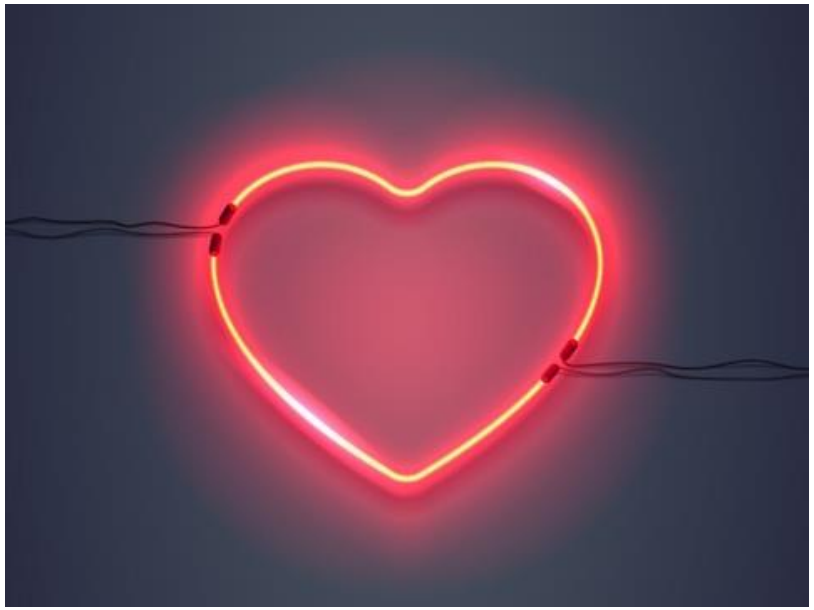

MYOCARDIAL METABOLISM

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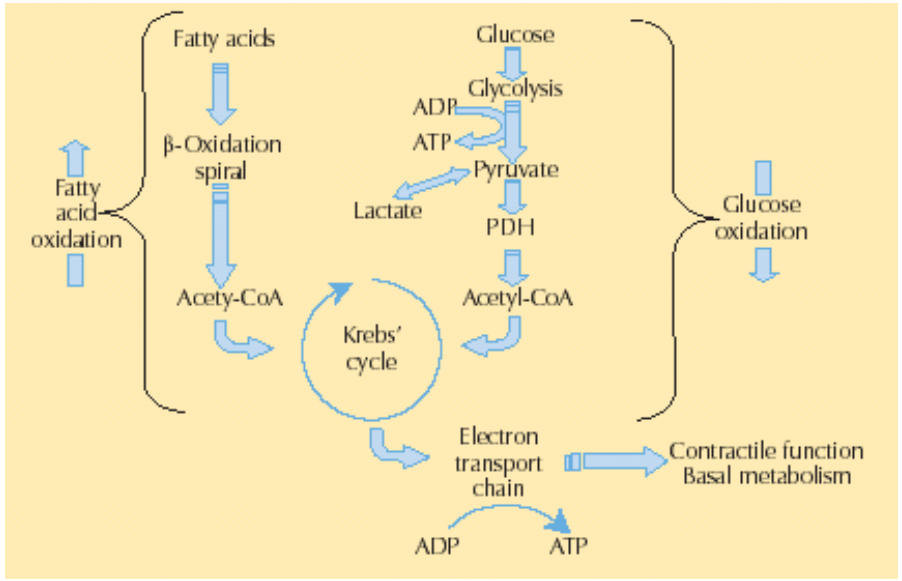
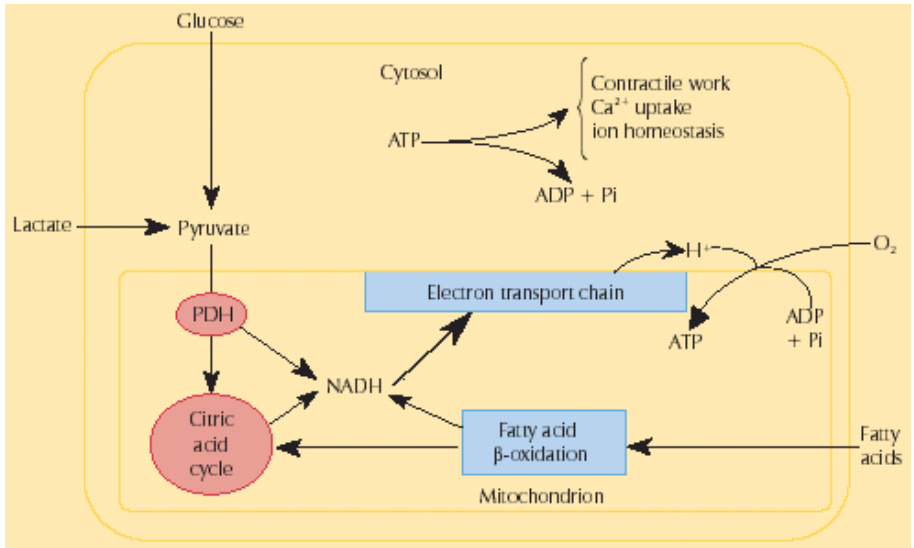
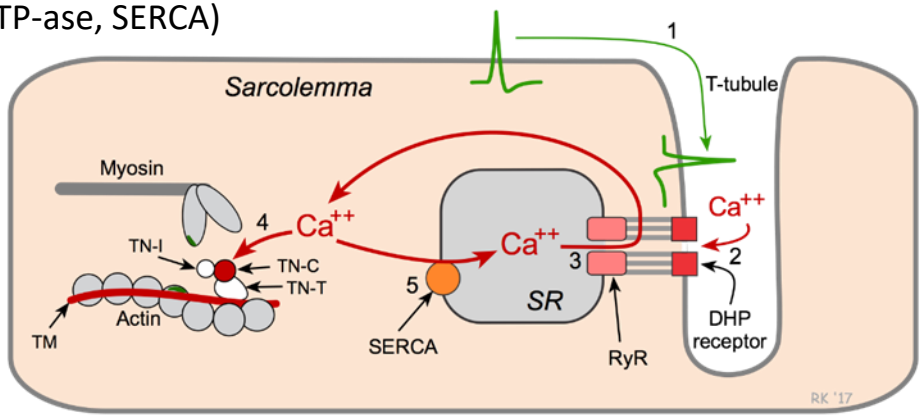
Myocardial metabolism – a lot of ATP is needed





- heart is a **pump** that has to continually perform 2 processes:
  - **(1) automacy** = generation of action potential in order to perform
  - **(2) contraction**
- myocardium thus has a very high demand for **ATP** even in the resting state
  - for contraction
    - actin/myosin – ATP
    - Ca^{2+} handling (Ca^{2+}-ATP-ase, SERCA)
  - for repolarisation
    - Na^{+}/K^{+}-ATP-ase



- ATP is produced by oxidation of substrates
  - FFA - preferentially
  - glucose (glycogen)
  - ketone bodies and lactate
- since myocardium requires **large amounts of O_2** it must be, therefore, well perfused !!

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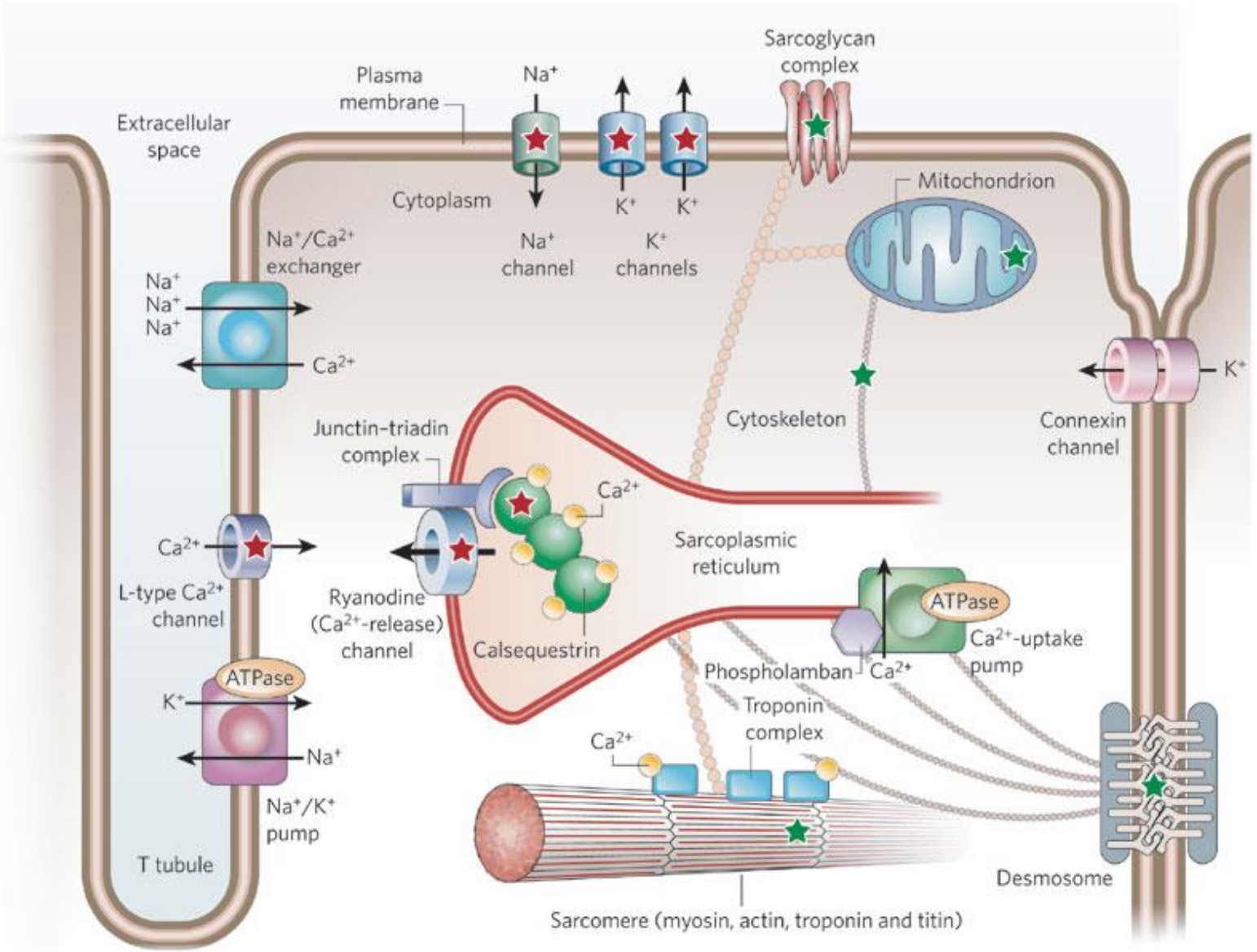
Excitation-contraction coupling in a ventricular cardiomyocyte



- The initial event in the cardiac cycle is membrane depolarization, which occurs with ion entry through connexin channels from a neighbouring cardiomyocyte (right) followed by opening of voltage-gated Na+ channels and Na+ entry (top).
- The resultant rapid depolarization of the membrane inactivates Na+ channels and opens both K+ channels and Ca2+ channels. Entry of Ca2+ into the cell triggers the release of Ca2+ from the sarcoplasmic reticulum through the ryanodine channel.
- Ca2+ then binds to the troponin complex and activates the contractile apparatus (the sarcomere, bottom).
- Cellular relaxation occurs on removal of Ca2+ from the cytosol by the Ca2+-uptake pumps of the sarcoplasmic reticulum and by Na+/Ca2+ exchange with the extracellular fluid.
- Intracellular Na+ homeostasis is achieved by the Na+/K+ pump.
- The molecular components that are required for normal electrophysiological activity, contractile function and cell–cell adhesion (the latter mediated by desmosomes) all need to be positioned correctly within the cell and anchored to each other and the cytoskeleton.
- Some cardiomyocyte components are not shown (for example, stretch-activated channels, and ankyrins that target channels and other proteins to their correct locations within the cell).
- Red stars indicate proteins encoded by genes that are mutated in primary arrhythmia syndromes; many of these proteins form part of macromolecular complexes, so mutations in several genes could be responsible for these syndromes.
- Green stars indicate protein complexes in which mutations in multiple genes cause cardiomyopathies often associated with arrhythmias; these complexes include the sarcomere (in hypertrophic cardiomyopathy), the desmosome (in arrhythmic right ventricular cardiomyopathy), and the cytoskeleton, sarcoglycan complex and mitochondrion (in dilated cardiomyopathy).

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Oxygen extraction by various tissues/organs

Organ	CaO_2 - CvO_2 (vol %)	% extraction
heart	10 – 12	**65 – 70**
skeletal muscle (resting)	2 – 5	13 – 30
kidney	2 – 3	13 – 20
intestine	4 – 6	25 – 40
skin	1 – 2	7 – 13
whole body		20 – 30 %

- Theoretically, the maximal amount of oxygen that can be extracted is **20 vol %** (if CaO_2 = 200 ml O_2/l)
- In reality, however, the maximal oxygen extraction is around **15 - 16 vol %** because of the kinetics of oxygen dissociation from haemoglobin
- Therefore, the heart is extracting one-half to two-thirds of the physiologically available oxygen under normal operating conditions
- Meeting increased demands (during exercise) is only possible by increasing coronary perfusion (= **coronary flow reserve, CFR**)

CaO2 - Arterial oxygen content CvO2 Venous oxygen content

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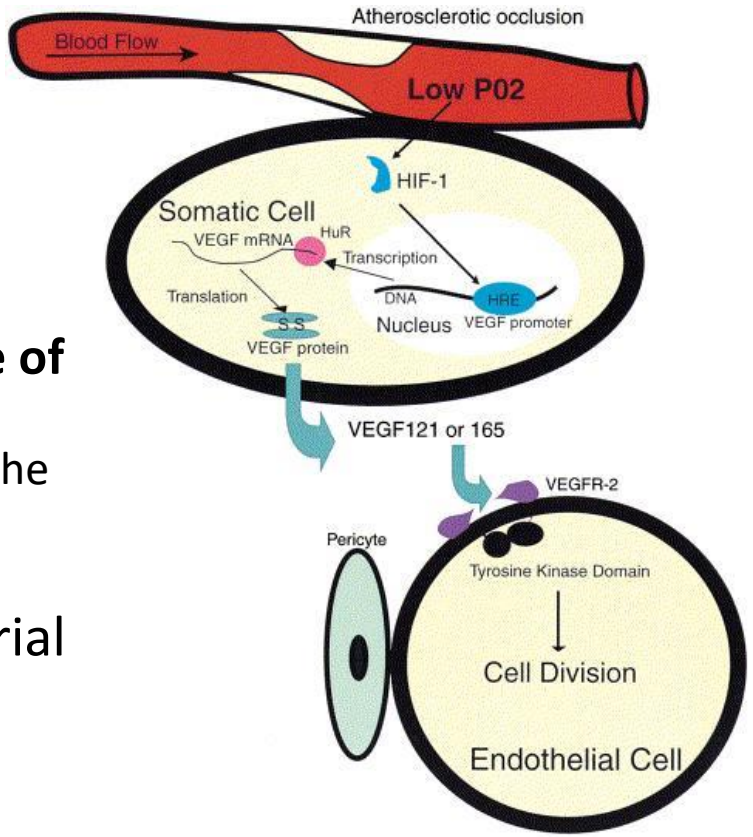
Oxygen consumption – quantitative aspects

- amount of oxygen supplied by the coronary blood (VO_2): ~ 45 ml O_2/min
  - $VO_2 = Q_m \times CaO_2$
    - myocardial perfusion (Q_m) = 210 – 240 ml/min in the resting state
    - but 1000 – 1200 ml/min during the exercise
    - CaO_2 = 200 ml O_2/l
      - for PaO_2 = 13.3 kPa and c[Hb] = 150 g/l
- consumption in the resting state: ~ 30 ml O_2/min (~60 - 80%)
  - very high O_2 extraction (A - V_{O2} difference) compared to other organs
- therefore, the only mechanism increasing the oxygen supply is **an increase of coronary blood flow**
  - because aorta has a constant pressure, it has to be done by **vasodilatation** in the coronary bed = **CFR**
  - small scale neovascularisation is also possible
- majority of oxygen is **consumed by LV** (generating much larger arterial pressure compared to right heart supplying pulmonary circulation)
  - therefore **CBF is a critical determinant of** its function (i.e. **contractility** mainly)



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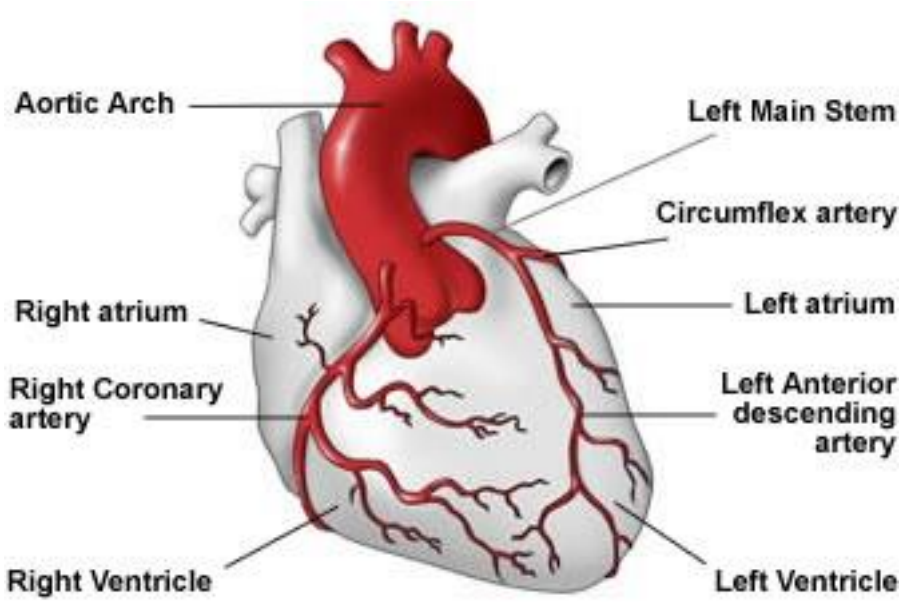
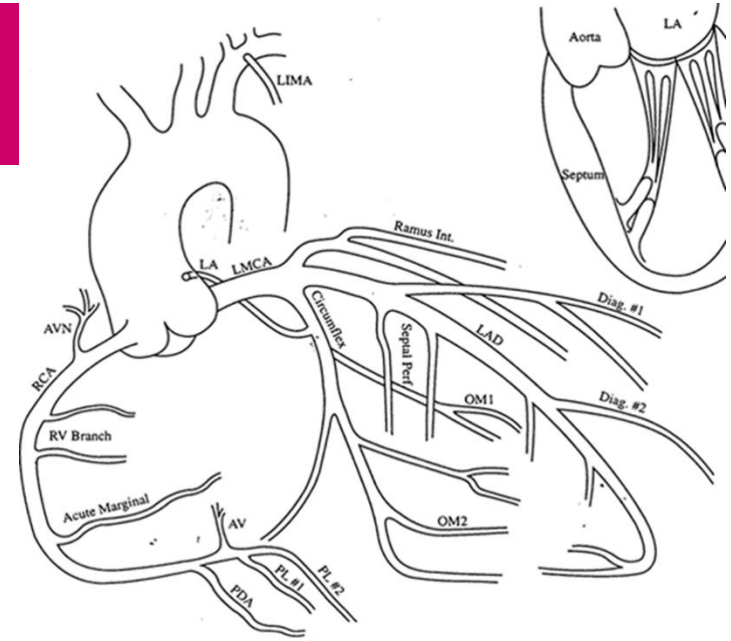
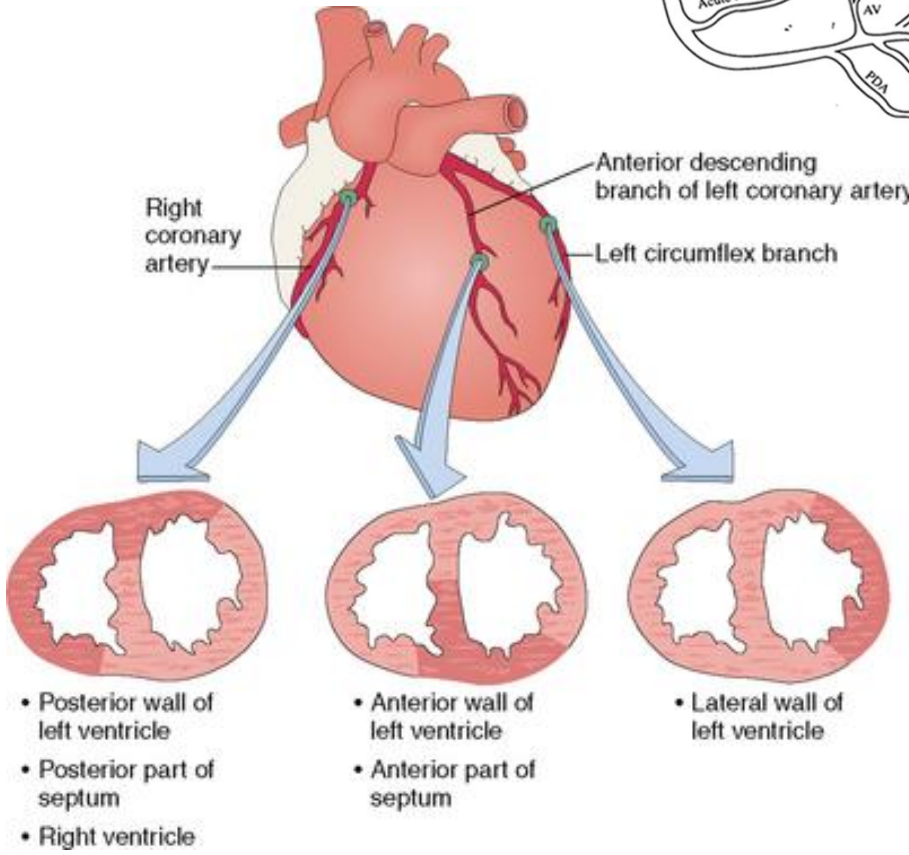
Blood supply of the heart







- demand for O_2 and substrates is met by heart blood vessels - **coronary arteries** - branching from the ascendant aorta
  - (1) left coronary artery
    - (a) left ant. desc. branch
      - supplies front part of the LV and RV and front part of the septum
    - (b) circumflex branch
      - supplies left and back wall of the LV
  - (2) right coronary artery
    - supplies RV

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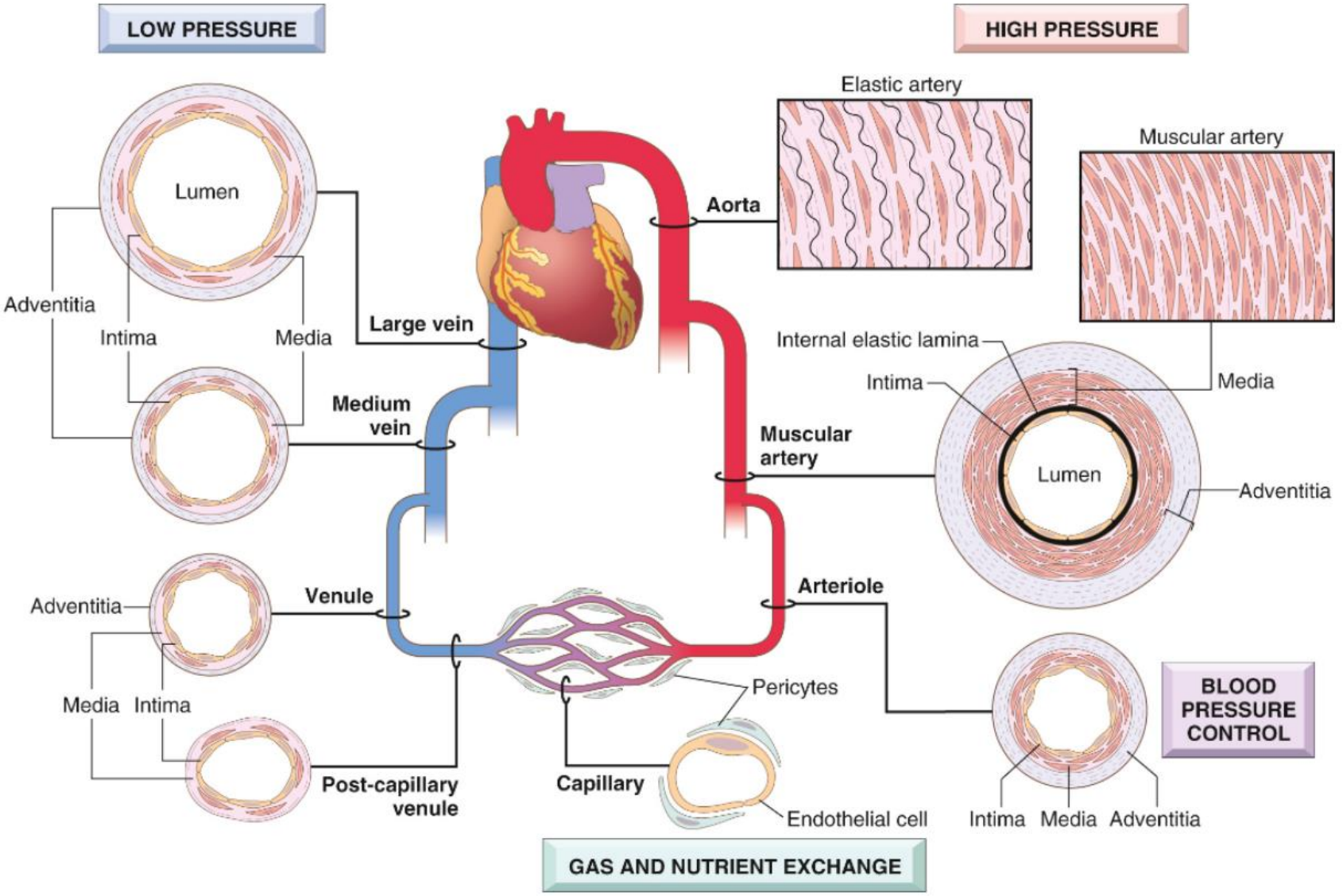
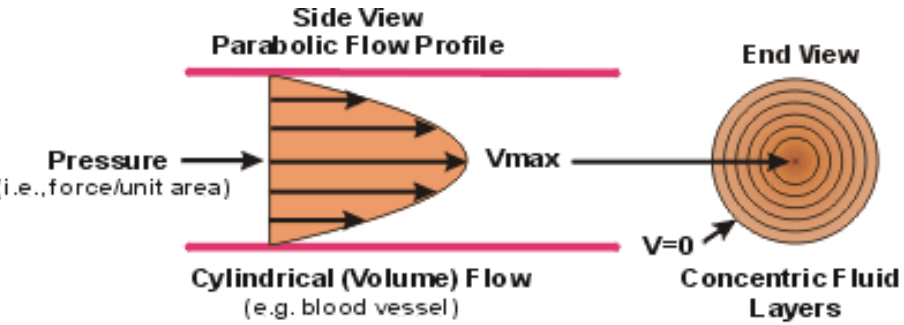
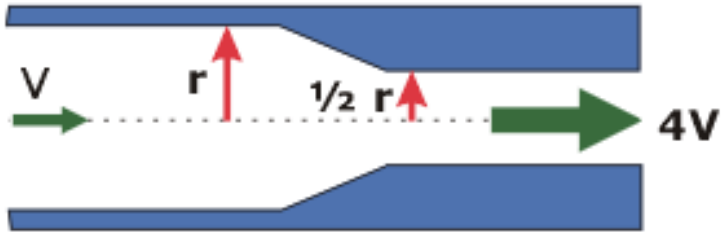
Vessel morphology related to the function



Physical parameters of circulaction

- flow
  - laminar
    - normally in majority of circulation
  - turbulent
    - behind stenosis, bifurcation
- velocity [V]
  - Reynold´s number
- perfusion (Ohm´s law)
  - flow [F] = pressure [P] / resistance [R]
- resistance (Poiseuille law)
  - $R = \eta \times L / \pi \times r4$
- viscosity [η]
  - haematocrit, temperature
- pressure (Laplace law)
  - P = tension [T] / diameter [r]

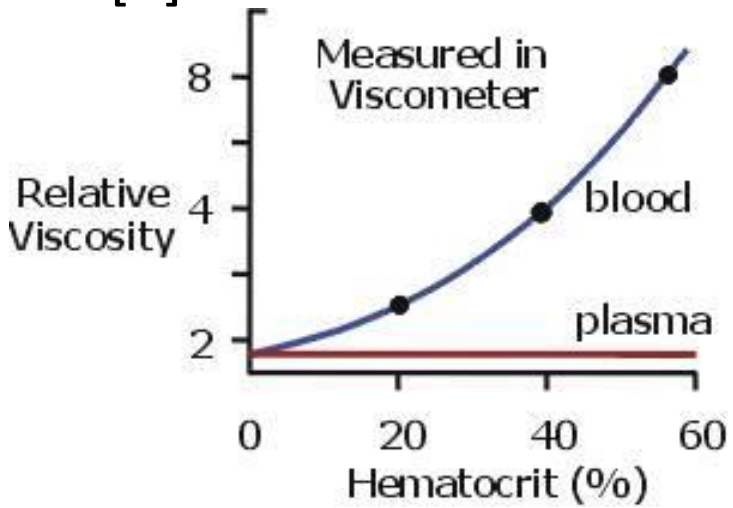




$$F = V \cdot A \quad and \quad A = \pi \cdot r^2$$

therefore,

$$V \propto 1/r^2$$



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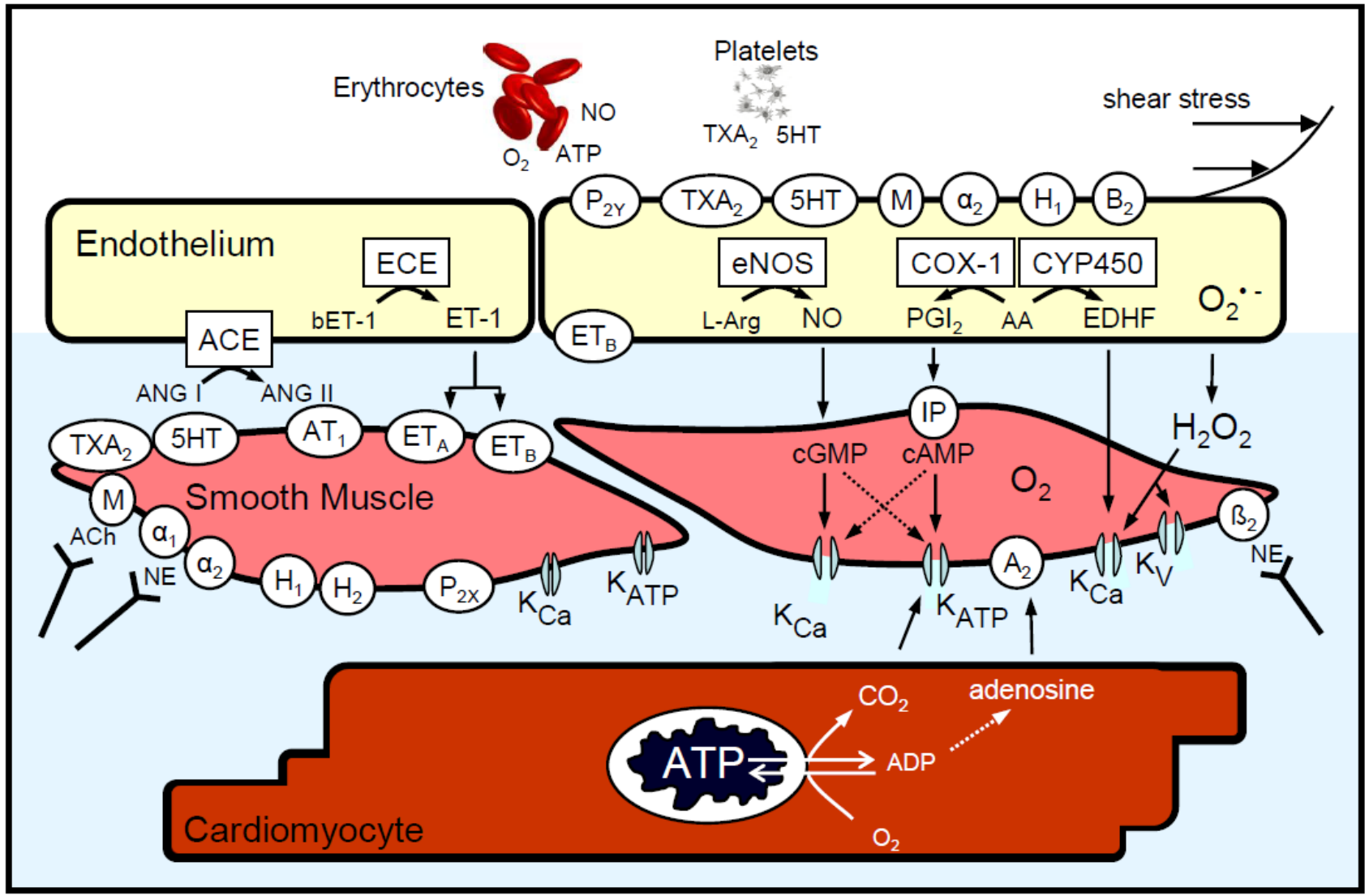
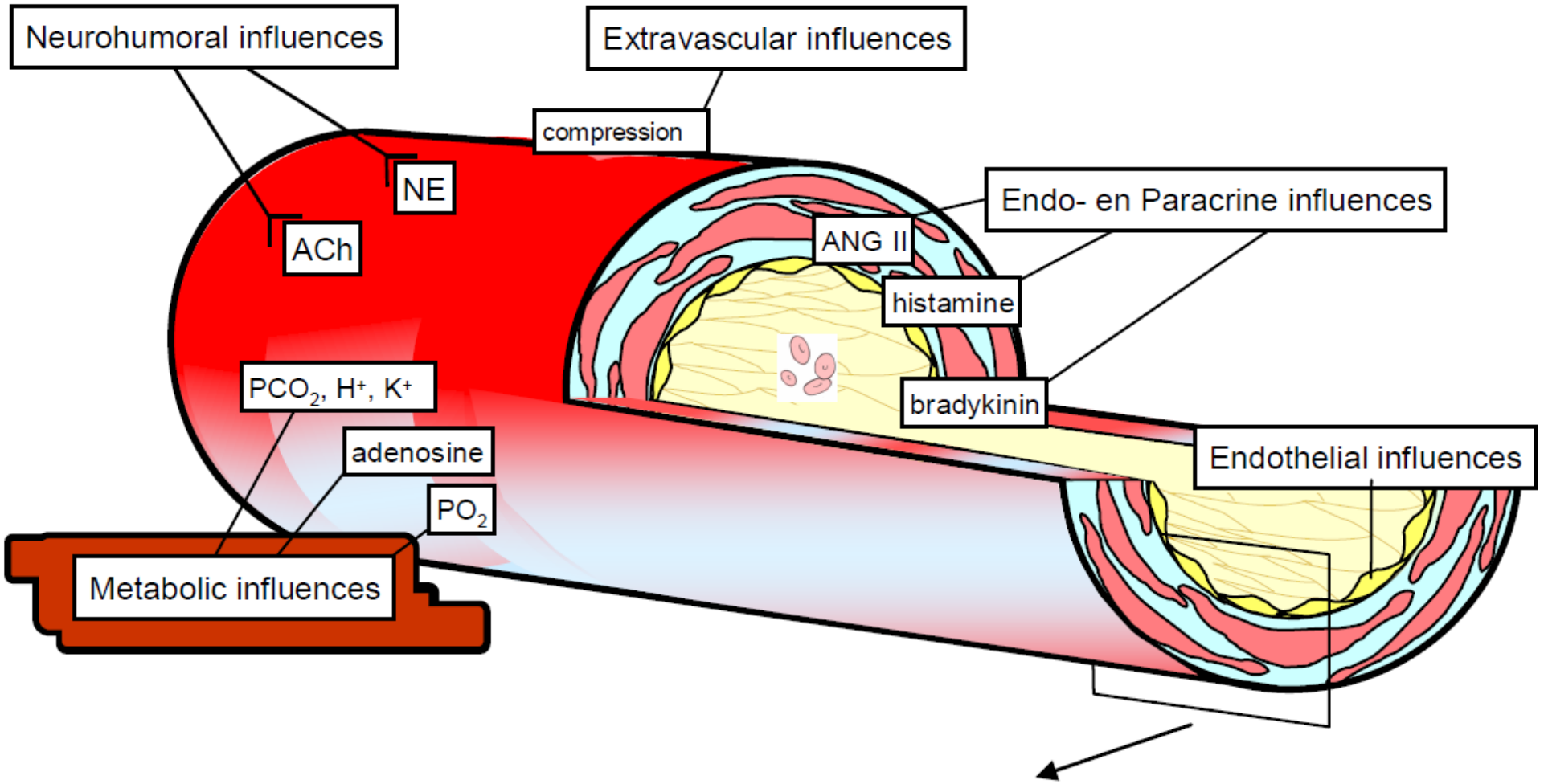
Control of Coronary Microvascular Tone

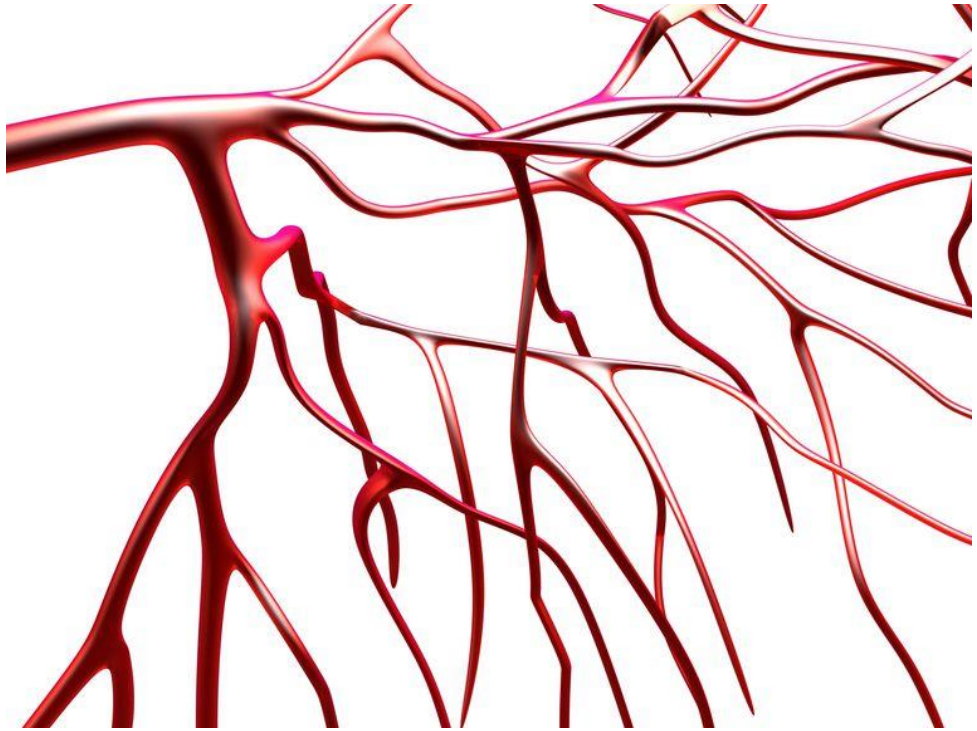


Laughlin et al Compr Physiol 2012

Summary of control mechanisms of coronary microvasculature tone



Duncker & Bache Physiol Reviews 2008



ENDOTHELIAL DYSFUNCTION AS A TRIGGER OF ATHEROSCLEROSIS

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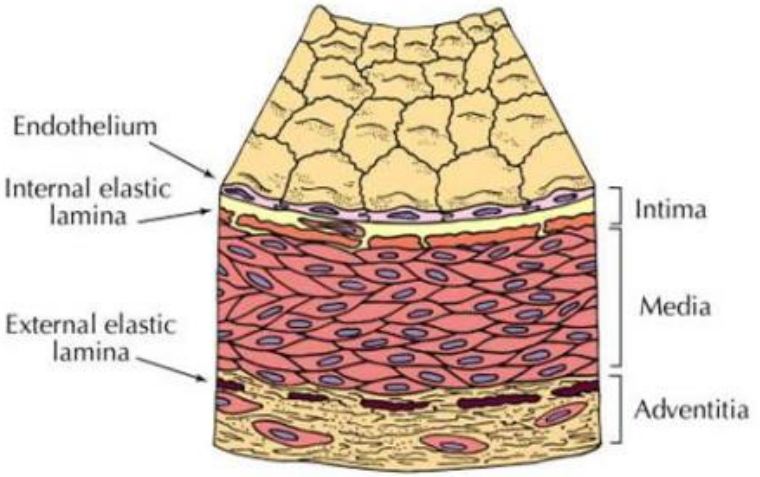
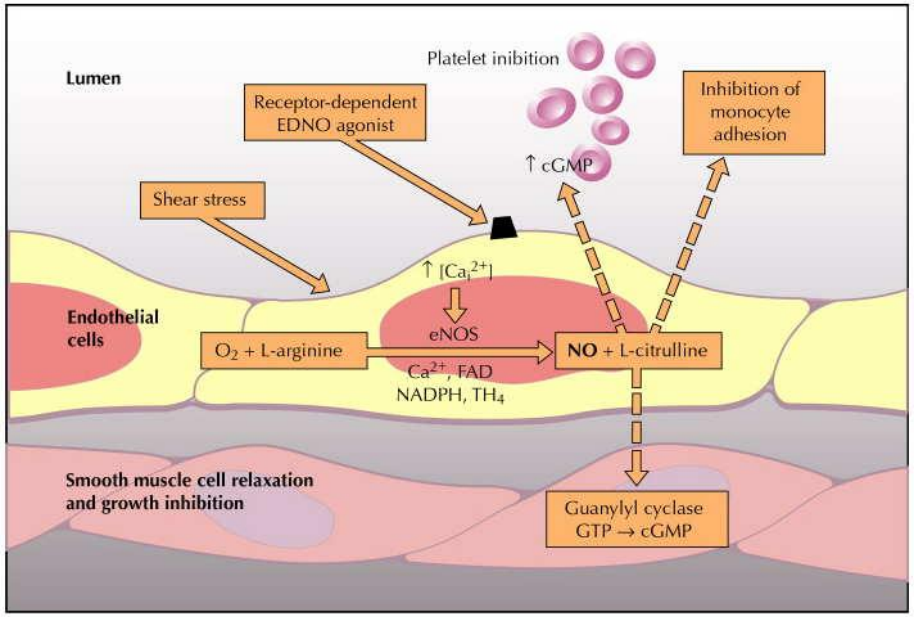
Endothelium - physiological role of ECs

- (1) **vasodilation**
  - smooth muscle cells (SMC) in blood vessels - notably arterioles - work in close association with the overlying ECs
  - action of hormones, neurotransmitters (**ACh**) or deformation of the ECs by flow of blood (shear stress) trigger reactions that influence associated SMC, these effects operates via second messenger systems
    - phospholipase A2 (**PLA2**) which activate cyclooxygenase (**COX**) / prostacyclin synthase (PCS) to produce prostaglandins (**PGI_2**) which diffuse readily through the tissue fluids to act on SMC
    - alternatively, nitric oxide synthase (L-arginase) (**NOS**) produces highly diffusible gaseous "neurotransmitter„ **NO** acting on SMC either through G-protein systems or directly on ion channels
- (2) **antiadhesive /anti-inflammatory action**
  - no VCAM, ICAM, selectins, …
- (3) **antithrombotic, antiagregant** and **fibrinolytic action**
  - heparansulphate
  - thrombomodulin
  - tPA





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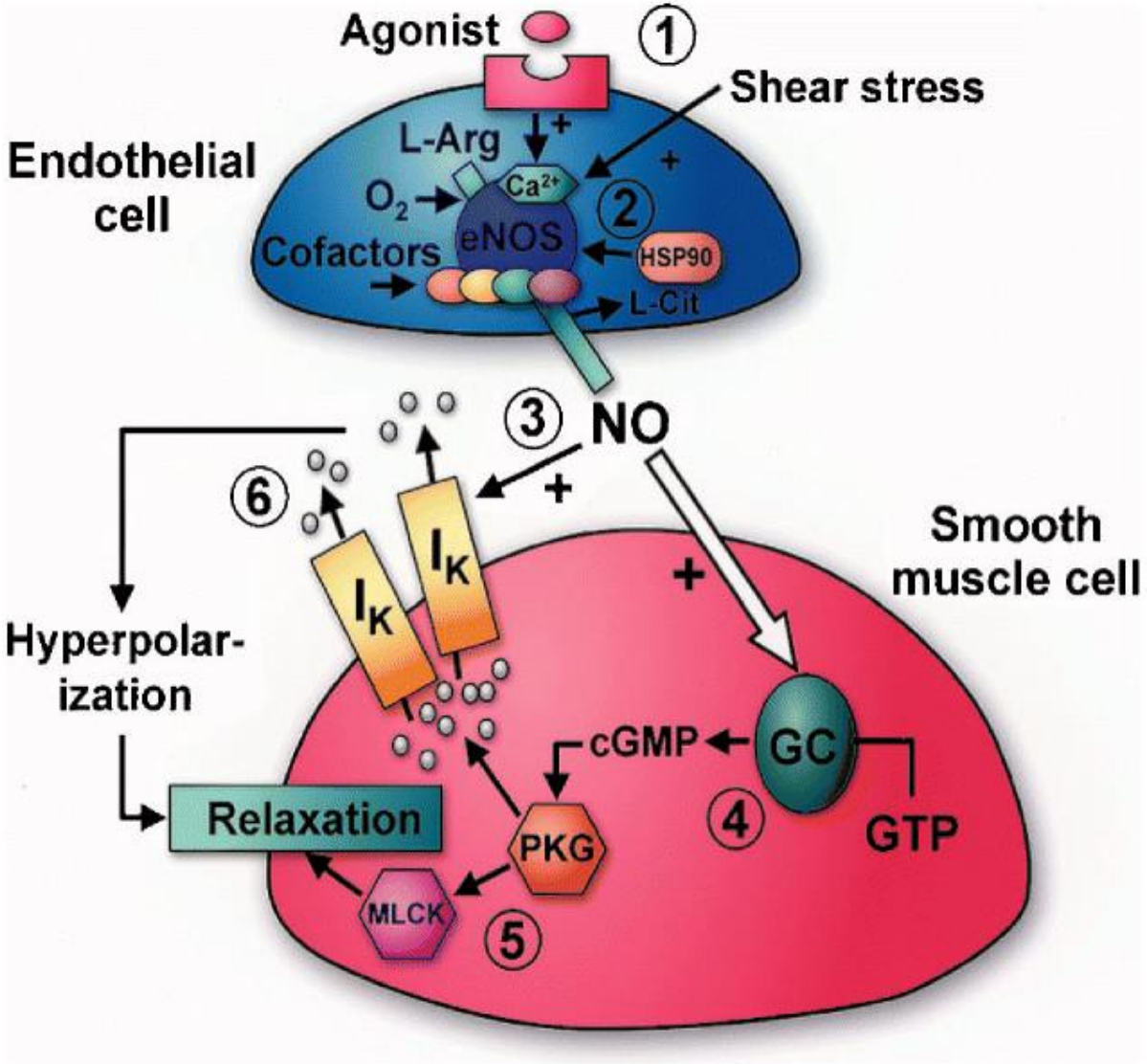
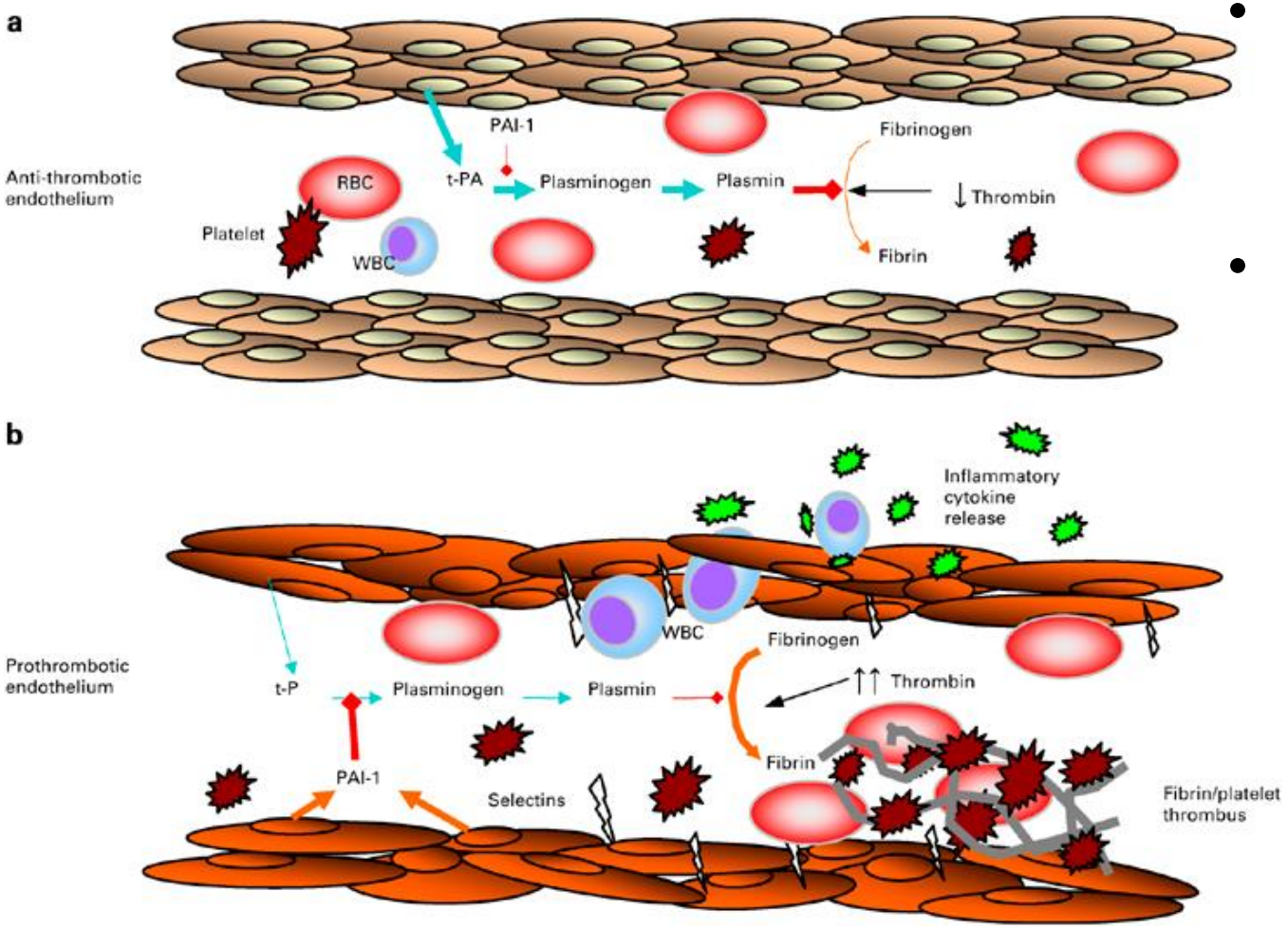
NO-mediated vasodilation

- biosynthesis of the key endogenous vasodilator NO is principally performed by the calcium-dependent endothelial isoform of nitric oxide synthase (eNOS)
- this is triggered by the binding of agonists or by shear stress (1) and facilitated by a variety of cofactors and the molecular chaperone heat-shock protein 90 (HSP90)
- amino acid L-Arg is converted by eNOS into NO (2), with L-citrulline as a by-product. NO diffuses into adjacent smooth muscle cells (3) where it activates its effector enzyme, guanylate cyclase (GC)
- GC (4) converts GTP into the second messenger cyclic guanosine monophosphate (cGMP), which activates protein kinase G (PKG) (5), leading to modulation of myosin light chain kinase and smooth muscle relaxation.
- PKG also modulates the activity of potassium channels (IK; 6), thereby increasing cell membrane hyperpolarization and causing relaxation



Endothelial dysfunction



- given the essential role of endothelial integrity in maintenance of normal vessel morphology endothelial dysfunction act as a pro-atherogenic factor increasing adhesivity, permeability and impairing vasodilatation
- causative factors:
  - increase BP (hypertension)
  - mechanical shear stress
    - turbulent flow
    - bifurcations
  - biochemical abnormalities
    - glucose
    - modified proteins
      - incl. LDL
    - homocysteine
  - oxidative stress
    - oxygen radicals
      - formed by smoking
      - inflammation
  - certain infections
    - Chlamydia pneumoniae?
    - Helicobacter pylori??

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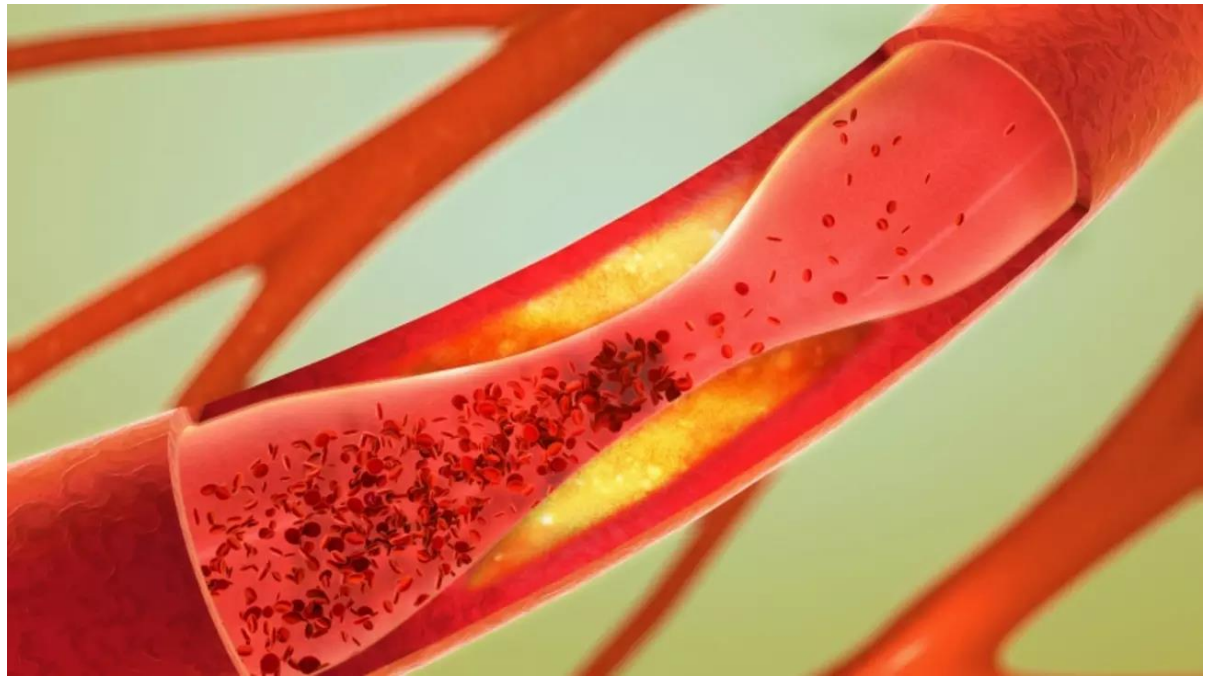
Endothelium - summary

Functional	Dysfunctional
constant vasodilation due to mechanical stimuli (shear stress) and mediators (Ach, bradykinine) mediated by NO, PGI_2 (event. adenosine)	increased sensitivity to paracrine constrictive mediators (epinephrine, norepinephrine, AT II, serotonin) and active formation of vasoconstrictors (ET-1)
anti-adhezive / anti-inflammatory state (NO, PGI_2), inhibition of expression of adhezive proteins	expression of adhesive molecules (ICAM, VCAM, selectins), production of cytokines (e.g. MCP-1) attracting migration of inflammatory cells into subendothelial space
constant local anticoagulant production (heparansulphate, thrombomoduline), antiagregant and thrombolytic state (tPA)	prothrombotic (vWf, TF), anti-fibrinolytic (PAI-1) phenotype



CORONARY ATHEROSCLEROSIS

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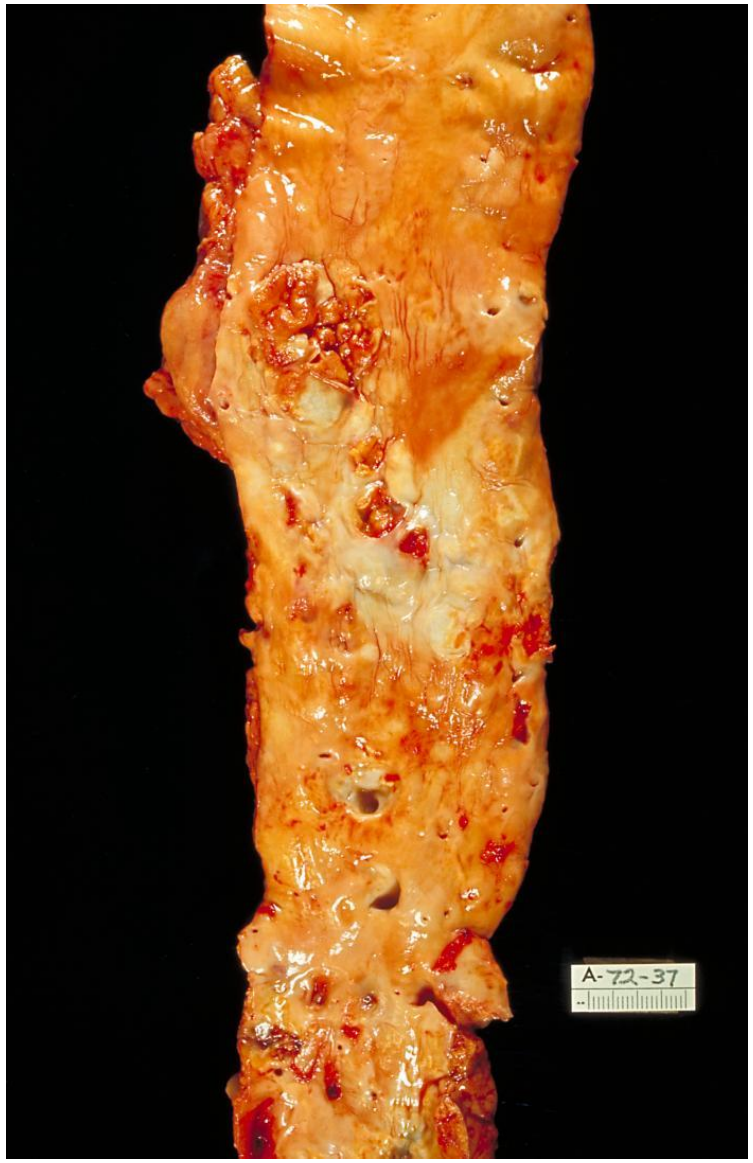
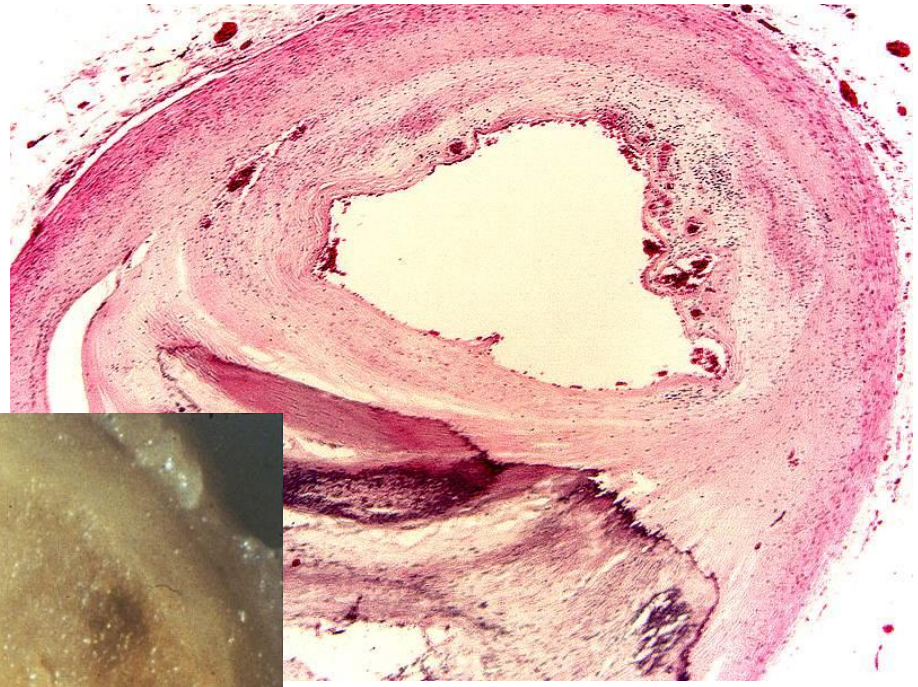
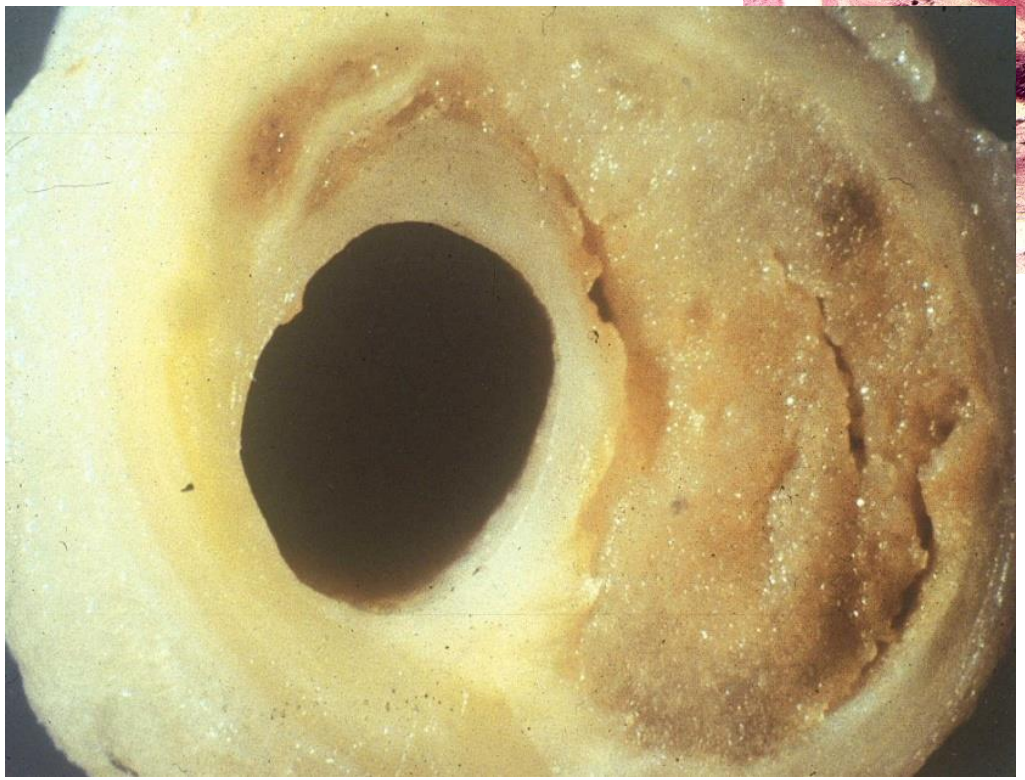
Vessels affected by AS







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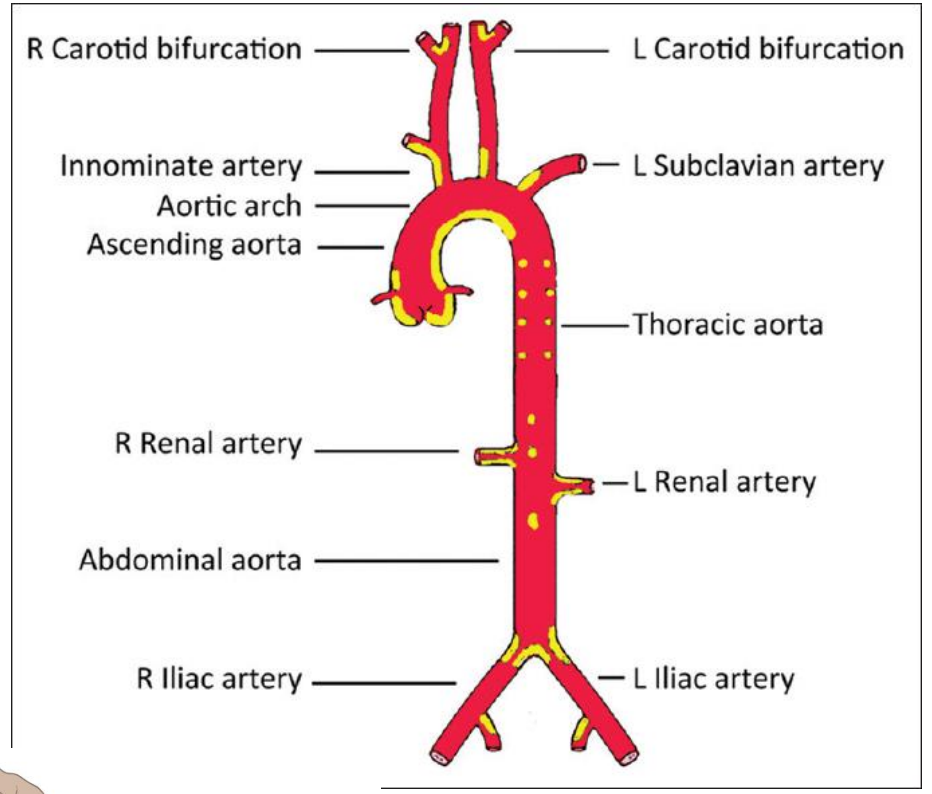
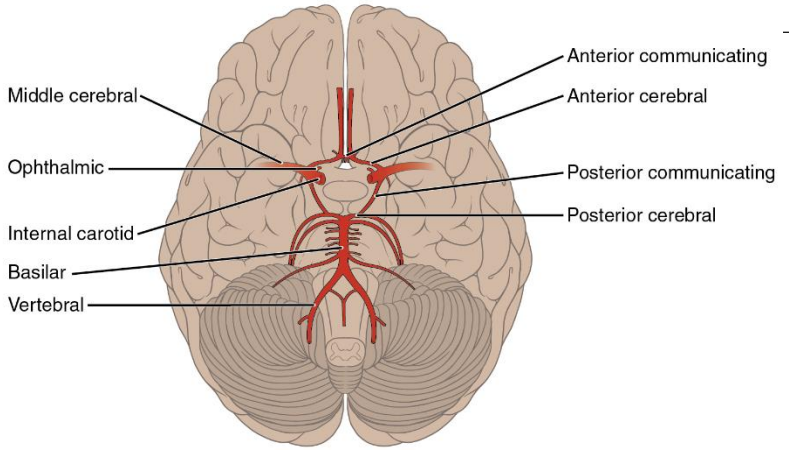
AS – main facts

- AS is the most common form of **arteriosclerosis**
  - i.e. any form of lowering vessel elasticity, e.g. due to calcification, lipid accumulation etc.
  - 3 forms
    - Mönckeberger´s – deposition of calcium in tunica media in elderly
    - hyaline arteriolosclerosis in hypertensive subjects
    - and classical AS with atheromas (athero = porridge + sclerosis = hardening)
- AS is a degenerative process characterized by **chronic inflammation** of the vessel wall
- AS represents **multifactorial disease** due to endogenous (typically with significant genetic component) and environmental factors
- AS can theoretically affect any vessel, in reality AS is limited only to arteries (= arteriosclerosis)
  - due to the role of **blood pressure and hemodynamic factors in general** as a pathogenic factor
  - moreover, not all arteries are equally affected, but most often those in **predilections** (bifurcations, non-laminar flow) and here again in a **segmental** manner
    - coronary aa.
    - cerebral bed
    - carotid aa.
    - renal artery
    - lower extremities artery bifurcations
  - on the contrary, other sites are typically resistant
    - e.g. int. thoracic artery (used as a graft for by-pass surgery)
    - a. hepatica
    - a. radialis





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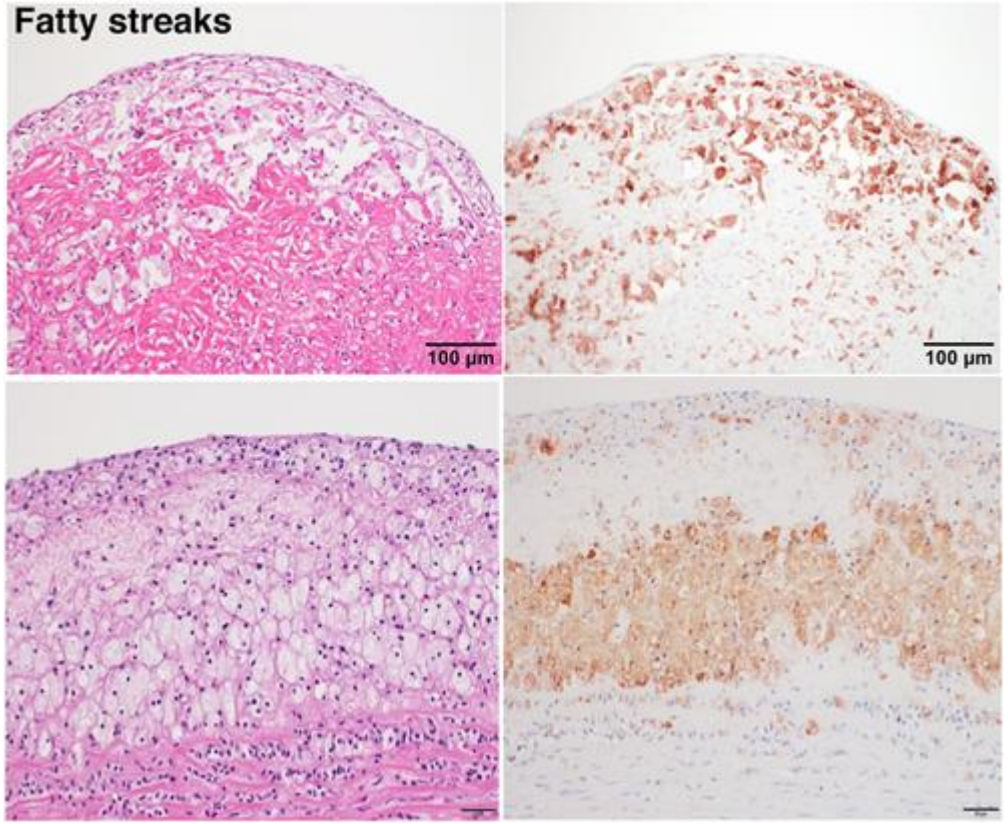
AS – main facts

- main players in the AS etiopathogenesis
  - (1) modified lipoproteins (LDL)
  - (2) endothelial cells changing their properties (namely adhesivity and permeability, secreting chemokines)
  - (3) monocyte-derived macrophages migrating to intima and forming „foam“ cells
  - (4) normal cells of vessel wall (smooth muscle cells)
  - (5) other immune cells (mainly T lymphocytes and mast cells)
- temporal pattern of AS – several **pathogenic phases** (with variable time course) and morphology – **stages** (resp. microscopic findings) during AS process:
  - (1) initiation
    - endothelial dysfunction
    - fatty streak stage – *very early, detected in childhood by section findings*
  - (2) progression
    - diffuse intimal thickening (DIT) due to foam cell accumulation, SMC migration from media and extracellular matrix expansion
    - fibrous plaque (atheroma with fibrous cap)
    - calcification
  - (3) complication
    - vessel remodelling – plaque initially expands towards abluminal diameter (outward), later reduces vessel lumen – stenosis, clinically ischemia symptoms in a given mal-perfused tissue area
    - plaque erosion (superficial thrombus, healing, re-endothelisation)
    - fissure, plaques rupture and subsequent thrombosis, vessel occlusion, critical ischemia and tissue necrosis



*(cit. **Atherosclerosis: Known and unknown.** https://doi.org/10.1111/pin.13202)*

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Cardiovascular/AS risks

- identification of the main CV risks by prospective epidemiologic studies
  - **Framingham study** = ↑ TK, ↑ cholesterol, ↑ triglycerides, ↓ HDL, smoking, obesity, diabetes, physical inactivity, ↑ age, gender (male) and psychosocial factors
    - original cohort (from 1948)
      - 5,209 subjects (aged 32 – 60 yrs) from Framingham, Massachusetts, USA
      - detail examination every 2 years
    - II. cohort (from 1971)
      - 5,124 adult offspring
    - III. cohort
      - 3,500 grandchildren of original participants
  - identified late clinical manifestation of long-term untreated / decompensated hypertension as well:
    - **heart attack, stroke (→ atherosclerosis)**
    - heart failure (→ left ventricular hypertrophy)
    - renal failure (→ hyperfiltration, nephrosclerosis)
    - retinopathy

	Risk factors of AS	
significant genetic contribution	**Major**	
	dyslipidaemia (↑ LDL and VLDL, ↓ HDL)	components of a metabolic syndrome driven by obesity
	hypertension	
	diabetes mellitus	
	male gender	
	Minor (20% of CV events occur in subjects without major risks)	
	↑ plasma homocysteine	
	↑ plasma haemostatic factors (e.g. fibrinogen, PAI, ..)	
	↑ Lipoprotein (a) – Lp(a)	
	chronic inflammation incl. low-grade (hsCRP as a surrogate marker) e.g. in visceral obesity	
	clonal haematopoiesis (CHIP)	
	gut microbiome	
non-genetic	**Environmental**	
	smoking (major risk!) = ROS and chron. inflammation of airways	
	physical inactivity	
	diet	
	certain infections	

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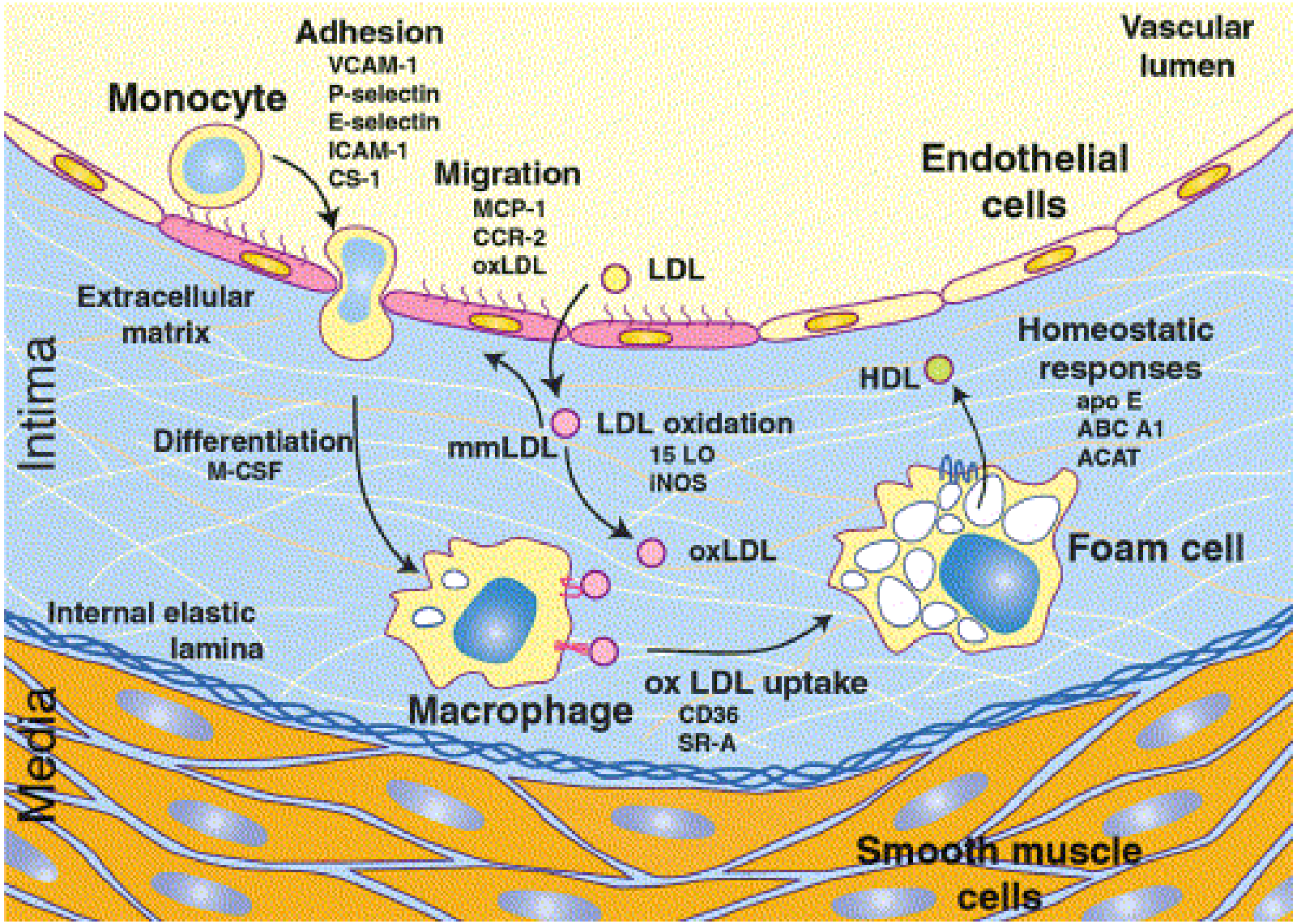
(1) Initiation – formation of fatty streak



- **LDLs** can exist in a native or modified forms
  - native LDL is recognised and bound by LDL-R
  - modified LDL is up-taken by scavenger receptors
- in vivo LDL is modified by oxidation (acetylation or glycation) in circulation and in subendothelial space
  - minimally at first (mmLDL), extensively later (oxLDL)
- mmLDL and oxLDL are cytotoxic and pro-inflammatory, they increase expression of adhesive molecules (VCAM, ICAM, selectins) by EC
- monocytes and T lymphocytes adhere to endothelium and migrates to subendothelial space, here monocytes transform to macrophages
  - interestingly, neutrophils that are constant cell type present in inflammatory lessions are completely absent in AS, finding not entirely understood; it might be because of the particular cytokine spectrum – expression of MCP-1 (monocyte chemotactic protein) by EC
- macrophages ingest oxLDL via their scavenger receptors (SR-A and CD36) and form this was so called **"foam cells"** (= lipid-laden macropghges)
  - macroscopically seen as a yellowish dots or streaks in subendothelium, hence **"fatty streaks"**
- free cholesterol from oxLDL in macrophages is again esterified by ACAT-1 (acyl-CoA cholesterol acyl-transferase) and stored together with lipids, inversely, it can be transform into soluble form by hormone-sensitive lipase, inbuilt into plasma membrane and exported from the cell (by transporter ABCA1 and HDL)
  - reverse CH transport via HDL is a crucial anti-atherogenic mechanism

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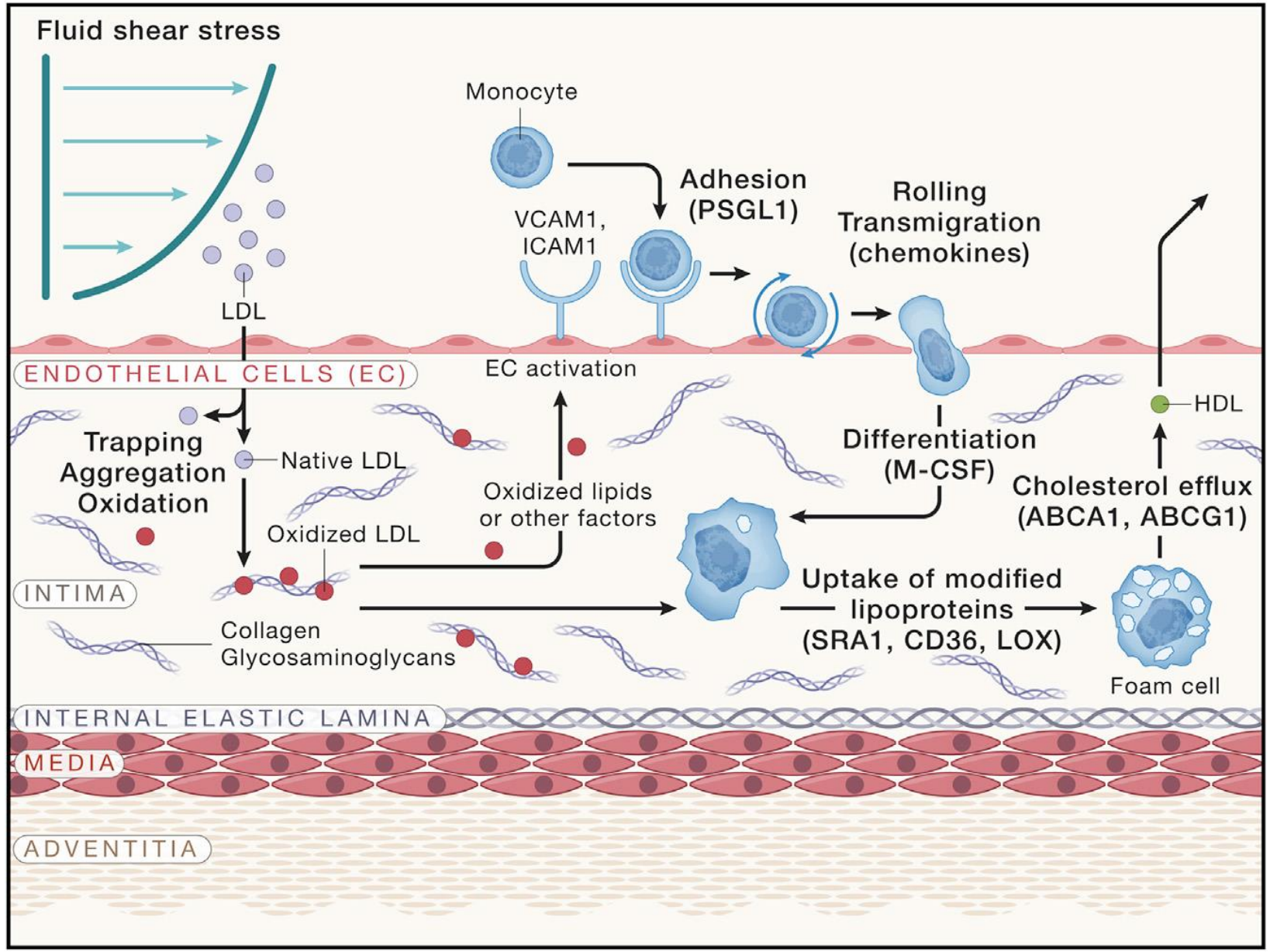
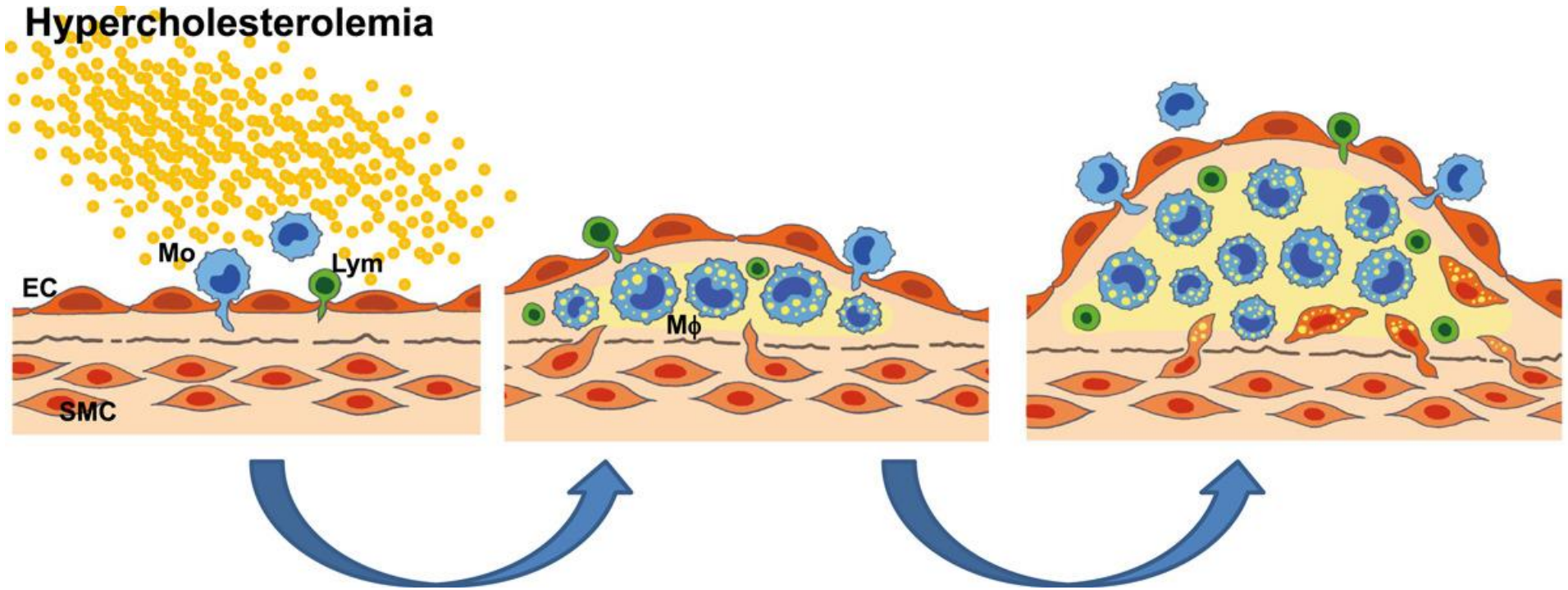

Fluid shear stress
LDL
Monocyte
Adhesion
(PSGL1)
VCAM1,
ICAM1
Rolling
Transmigration
(chemokines)
ENDOTHELIAL CELLS (EC)
EC activation
Trapping
Aggregation
Oxidation
Native LDL
Oxidized LDL
Oxidized lipids
or other factors
Differentiation
(M-CSF)
HDL
Cholesterol efflux
(ABCA1, ABCG1)
INTIMA
Collagen
Glycosaminoglycans
Uptake of modified
lipoproteins
(SRA1, CD36, LOX)
Foam cell
INTERNAL ELASTIC LAMINA
MEDIA
ADVENTITIA

AS pathogenesis – role of risk factors - cholesterol



Risk factors, such as hypercholesterolemia (yellow dots), may upregulate the adhesion molecules of endothelial cells (EC), which leads to the adherence of monocytes (Mon) and T lymphocytes (Lym) on the surface of intima (left). Furthermore, these inflammatory cells migrate into the subintimal space under the induction of chemoattractancts, where monocytes differentiate into macrophages (Mφ) and uptake accumulated lipids and transform into foam cells (middle). As the lesions progress, the medial smooth muscle cells (SMC) migrate into the subintima and proliferate (right). Some smooth muscle cells can take lipids and transfer into foam cells. T lymphocytes may mediate the entire process of the lesion formation

(cit. ***Atherosclerosis: Known and unknown.*** *https://doi.org/10.1111/pin.13202)*

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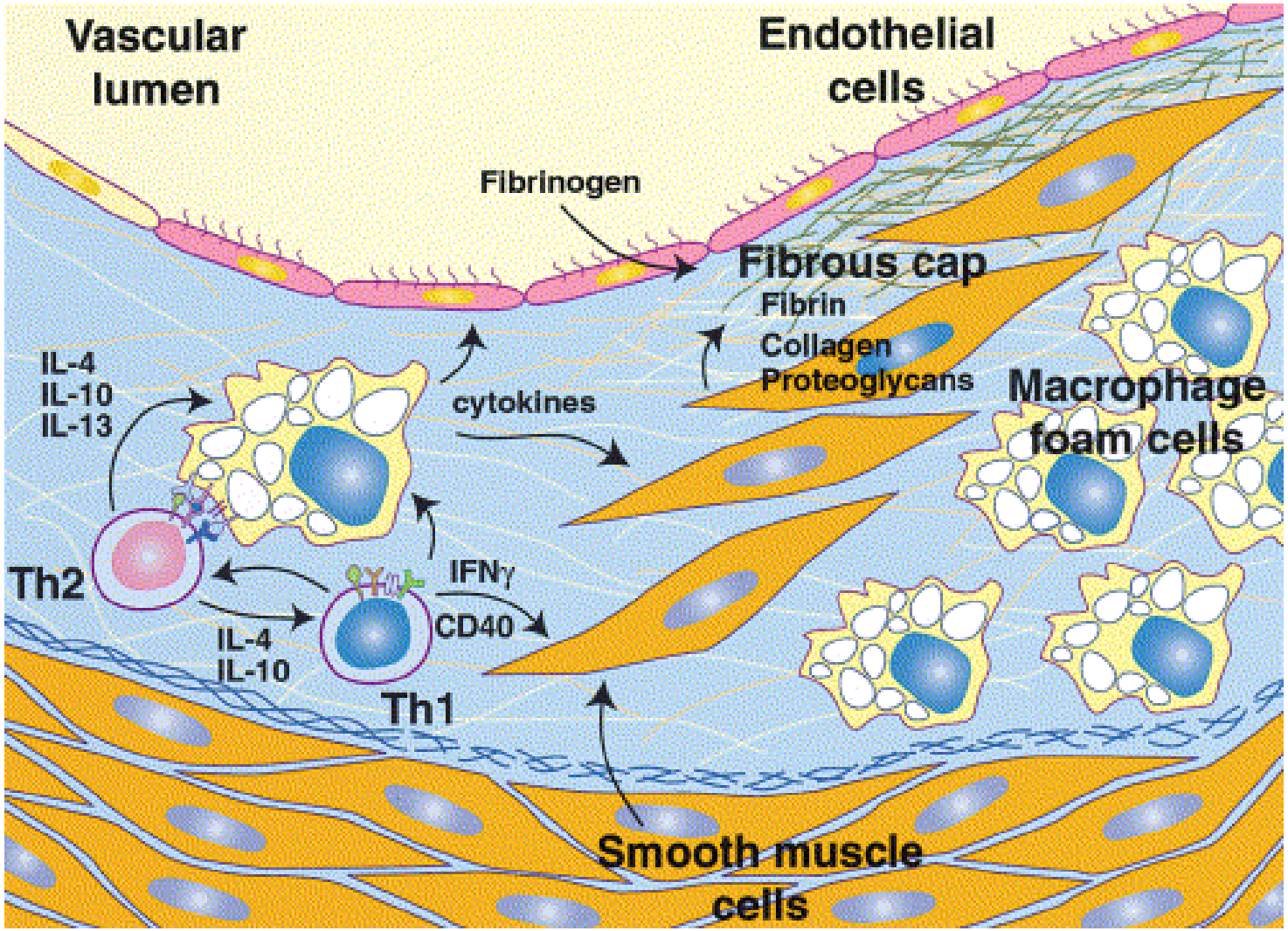
(2) Progression – formation of plaque



- immunologic interaction between macrophages and T lymphocytes (Th1 and Th2 sub-population) locally maintains the chronic inflammation
  - production of both pro-atherogenic Th1 cytokines (MCP-1, IL-6, TNF-α, …) and anti-atherogenic Th2 (IL-4)
  - mutual balance between Th1 and Th2 is topically modified by many factors
- macrophages as antigen-presenting cells help to activate B lymphocytes to wards production of auto-antibodies against oxLDL → formation of immune complexes → inflammation
- cytokines stimulate other cells, mainly SMCs to migrate from media into intima, proliferate (→ intima thickening) and secrete proteins of extracelullular matrix (collagen) → fibrose plaque
- pathologic calcification of atherosclerotic vessel wall is not a passive consequence but result of changed gene expression in macrophages (osteopontin)

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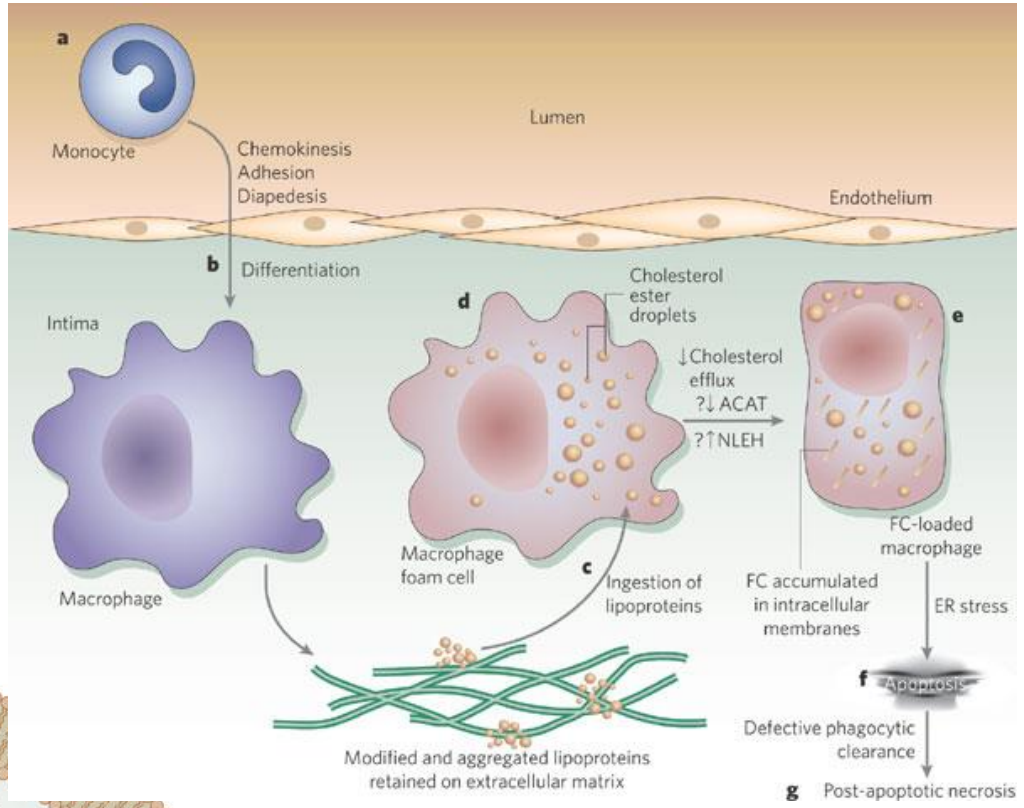
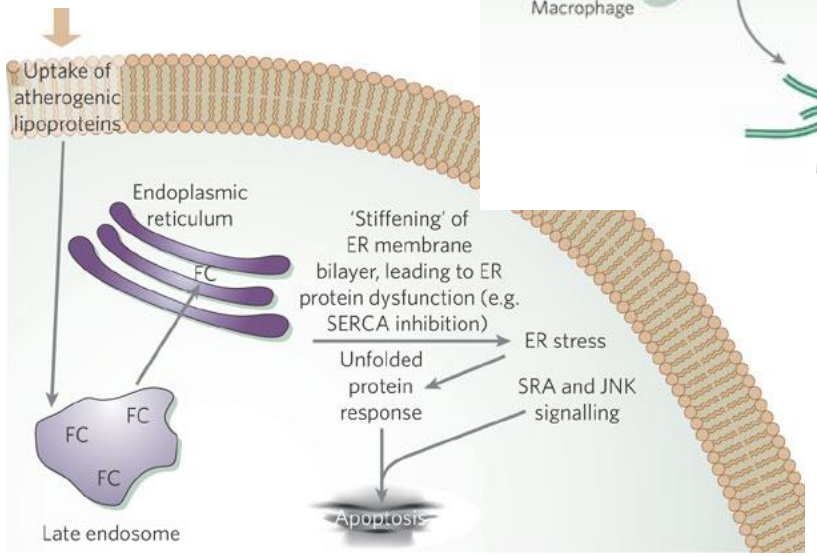
Macrophages in advanced AS – role in AS

- M in early lesions
  - majority of Ch in the form of esters (enzyme ACAT)
    - non-thrombogenic
  - HDL reverse transport works
- M in advanced lesion
  - accumulation of free Ch (FCH)
    - highly thrombogenic
  - FCH in membranes of endoplasmic reticulum changes its permeability and Ca concentration inside → ER stress → apoptosis of macrophages → more of FCH extracellularly → increased thrombogenicity of atheroma
  - production of MMPs





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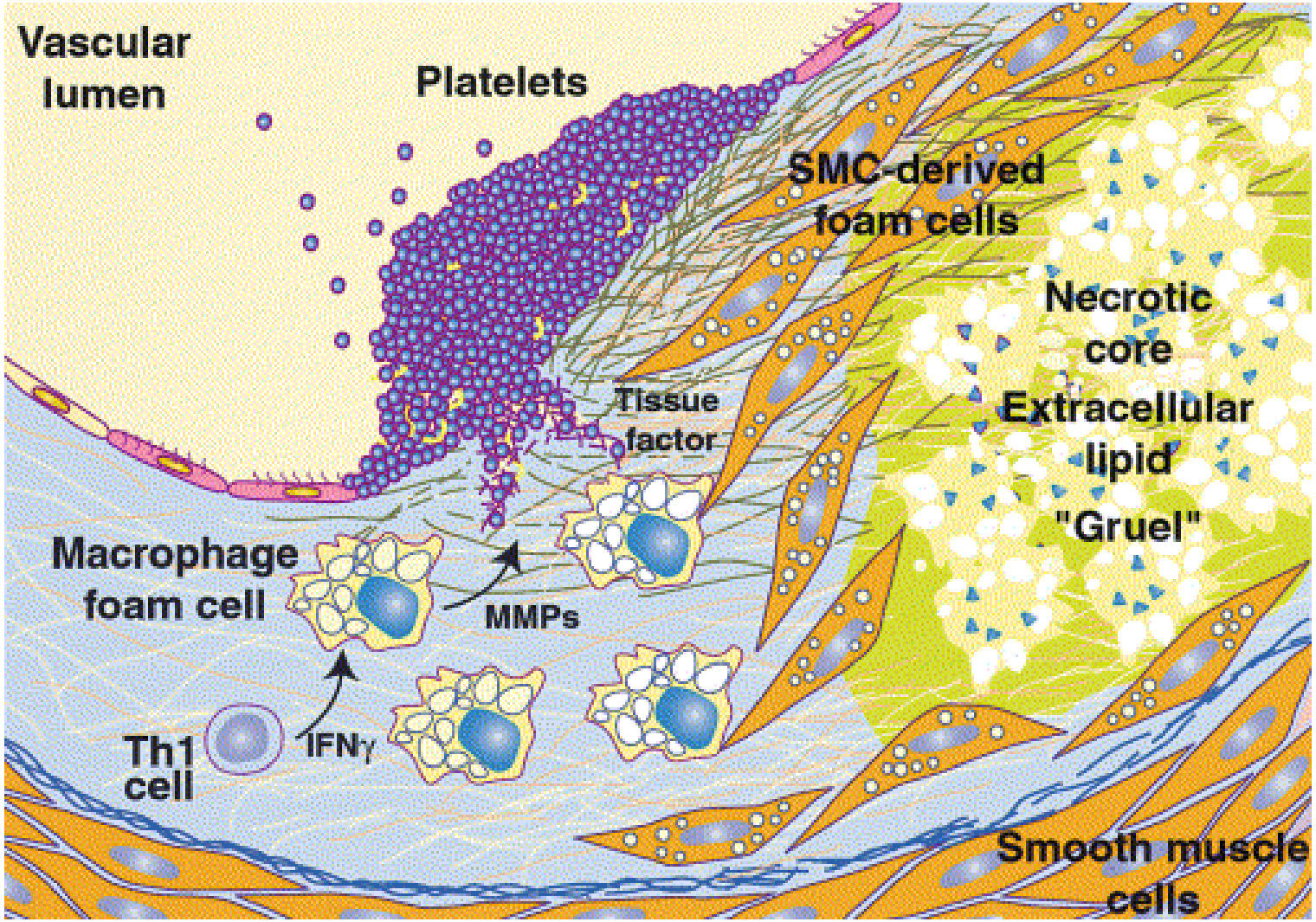
(3) Advanced AS plaque



- plaque can grow and slowly obstruct lumen or it can become unstable and lead to rupture/fissuration and thrombosis and acute complete obstruction → "complicated plaque"
- intimal macrophages and SMC die (necrosis and cytokine-induced apoptosis) and establish necrotic core of the plaque with accumulated extracellular CH
- stimulated and hypoxic macrophages produce proteolytic enzymes degrading extracellular matrix proteins (matrix metaloproteinases, MMPs) which further weaken the plaque
- plaque rupture (often eccentric and CH-rich), typically in the plaque "shoulder" lead to exposure of accumulated lipids and tissue factors to platelets and coagulation factors and cause thrombosis
- this can be manifested as a complete vessel occlusion and thus lead to tissue necrosis (e.g. myocardial infarction or stroke) or incomplete occlusion as a consequence of repeated cycles of rupture → microthrombotisation → fibrinolysis → healing = "unstable plaque" or angina
- vulnerable plaque (i.e. rupture-prone) vs. vulnerable patient
  - see further

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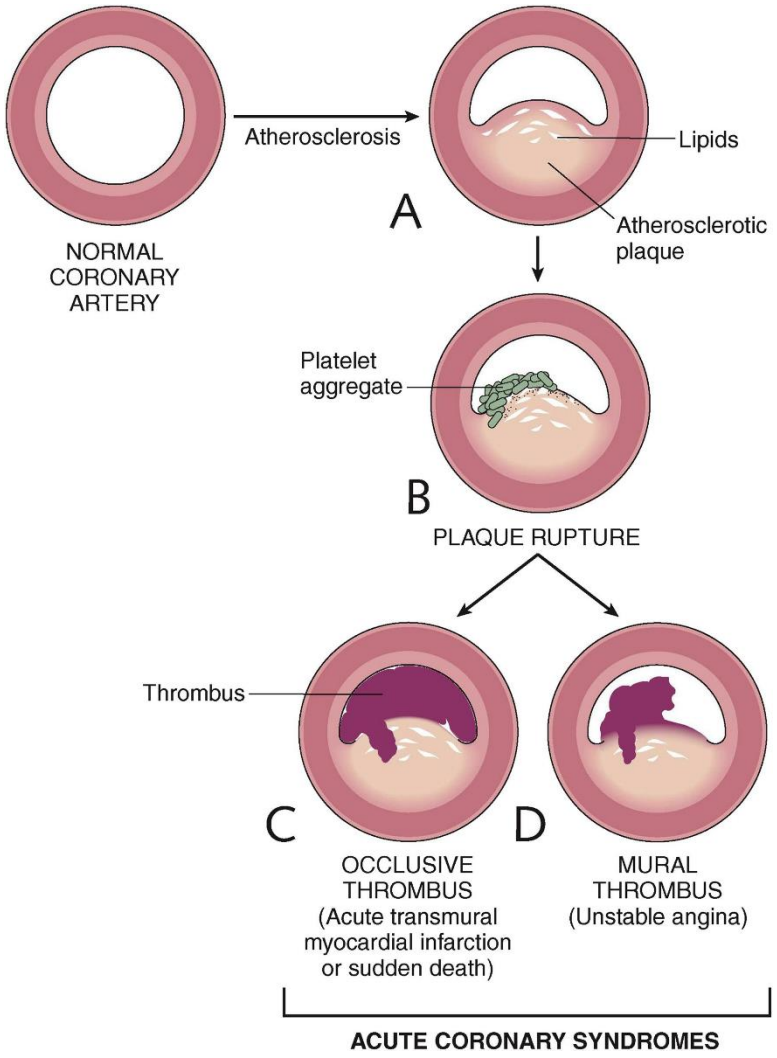
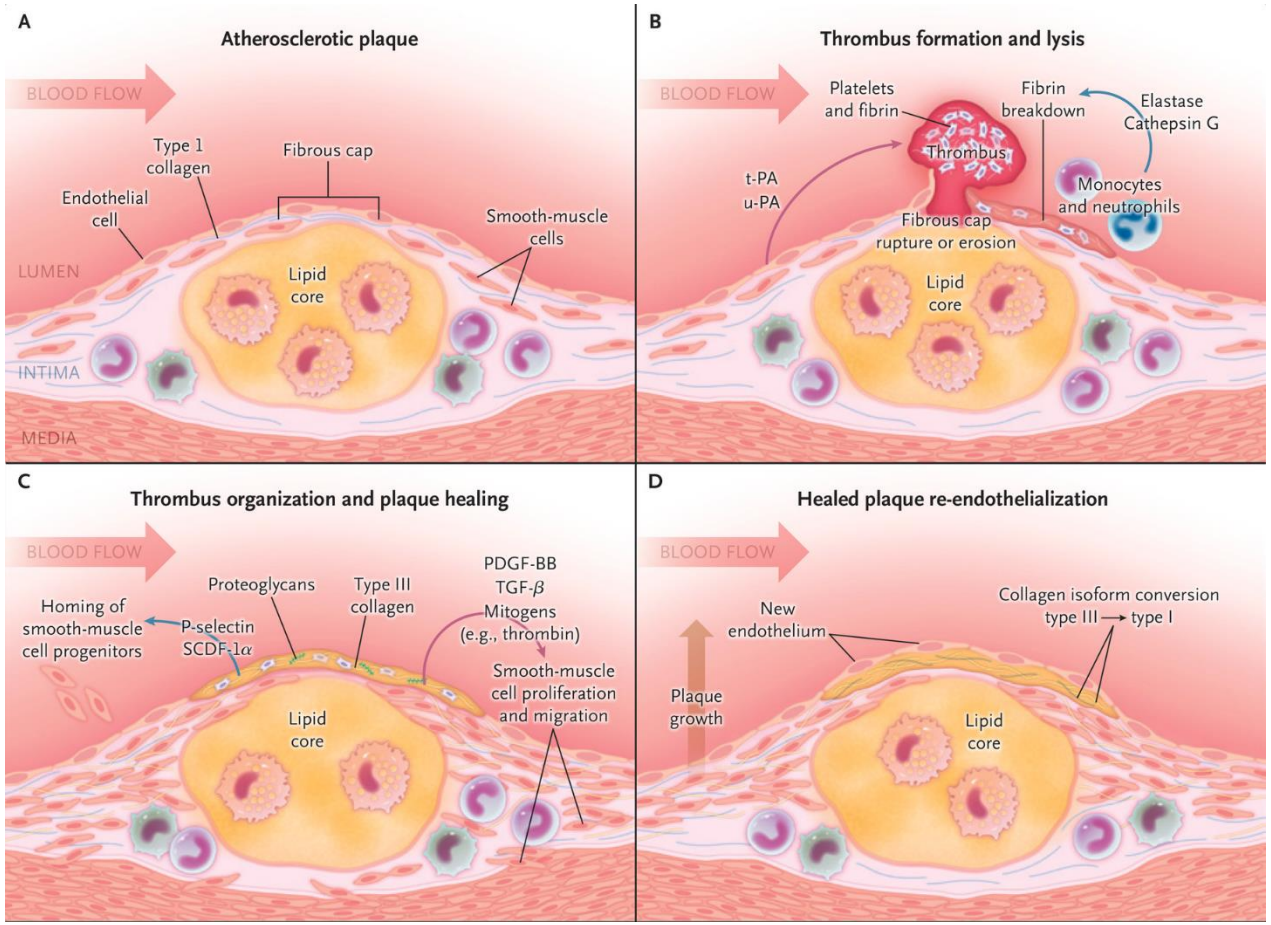
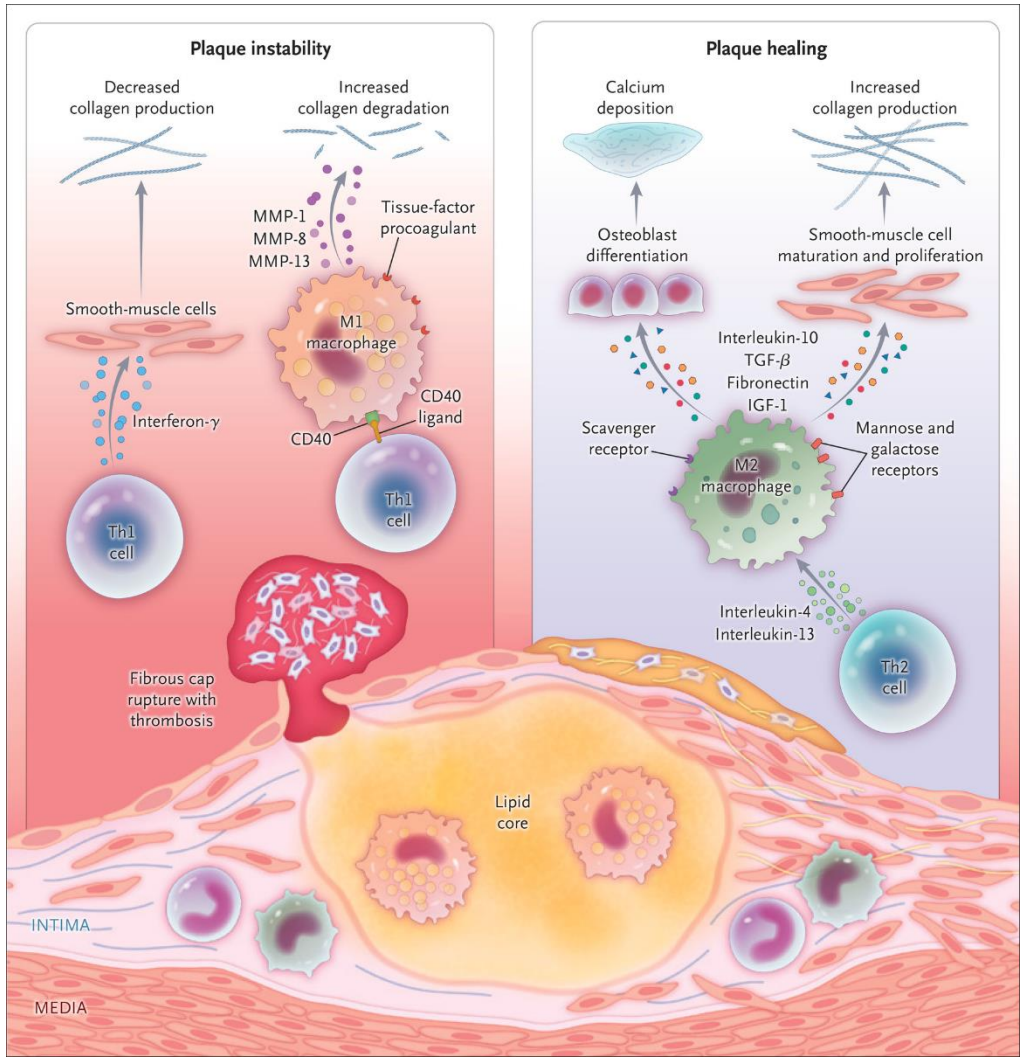
Fate of advanced AS plaque - rupture an thrombosis

- Pathophysiological scenarios
  - (1) progressive growth of the plaque
    - asymptomatic until >50% of diameter reduction (= >75% cross-sectional area reduction)
    - typical cause of **stable angina**
  - (2) superficial erosion
    - denudation/apoptosis od ECs – mural platelet thrombi – healing – further lumen reduction → change in symptom severity = **unstable angina**
  - (3) plaque rupture and thrombosis
    - clinically leads to **acute coronary event** (MI or sudden death) in case of total occlusion of the vessel or unstable angina or no symptoms in case of a healing
    - very often happens in haemodynamically insignificant stenosis
    - plaque composition rather than plaques size matters
    - can happen due to the fracture of the fibrous cap or in the „shoulder“
      - imbalance between forces/mechanical strength



(ad 2) Mechanisms of AS plaque healing





pro zájemce: N Engl J Med 2020; 383:846-857

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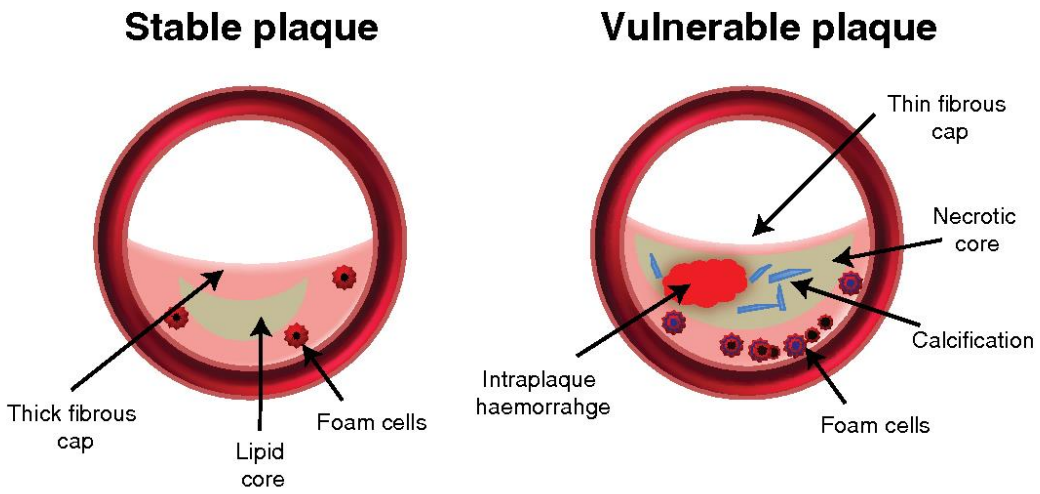
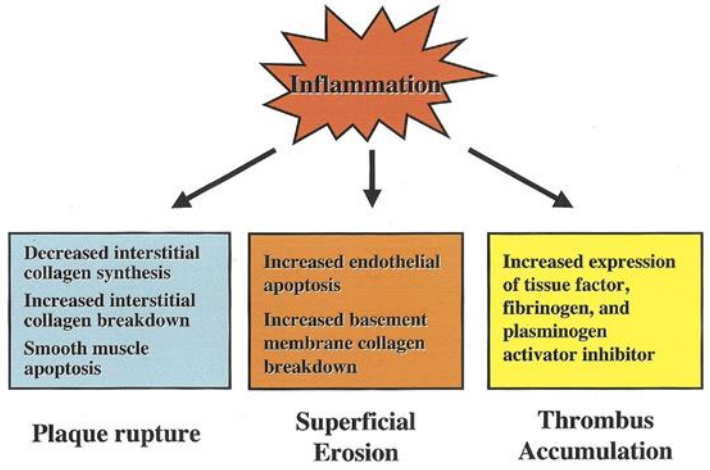
(ad 3) Vulnerable plaque - thin cap fibroatheroma

- Typical features
  - a thin fibrous cap
  - extensive inflammatory infiltration by macrophages and T lymphocytes
  - large lipid core
  - small numbers of SMCs
  - intra-plaque haemorrhage
- inflammation is the most important part of progression and destabilization of an atherosclerotic plaque
  - release of proinflammatory cytokines and matrix metalloproteinases contributes to degradation of collagenous components in the fibrous cap of the atheroma
  - apoptosis of collagen synthesizing SMCs
  - tissue factor produced by intra-plaque inflammatory cells
- identification of vulnerable plaque (= prone to rupture) is clinically extremely important





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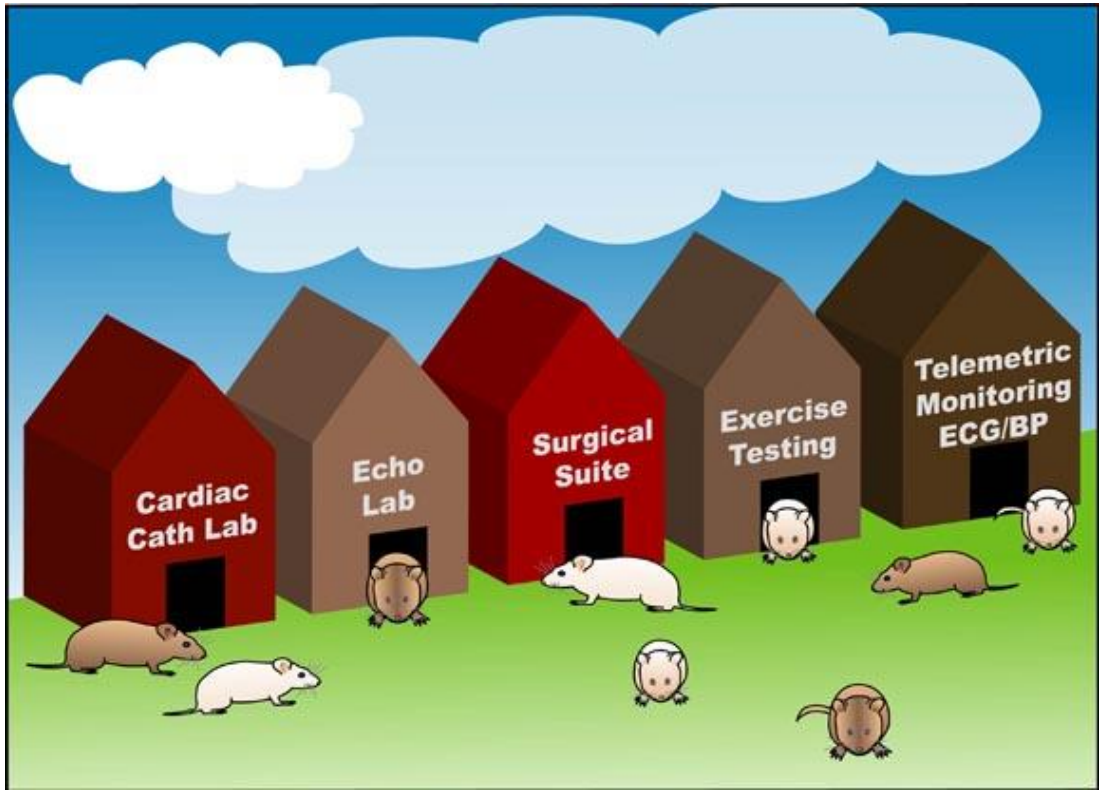
Animal models of AS - mouse

- generally, it is extremely difficult to simulate AS in animals, even those kept in captivity
  - in this aspect Homo sapiens is quite unique in their susceptibility to damage of vessel wall
- although mice is the most studied model, exp. induced AS is not entirely similar to man

- exp. model of AS
  - induced
    - high CH diet + endothelial denudation + hypertension (ligation of a. renalis)
  - spontaneous (knock-out)
    - ApoE -/- mouse
    - LDL-R -/- mouse
- exp. model spontaneous IM
  - induced
  - ligation of coronaries
  - spontaneous
    - comb. apoE/LDL-R -/- + mental stress + hypoxia



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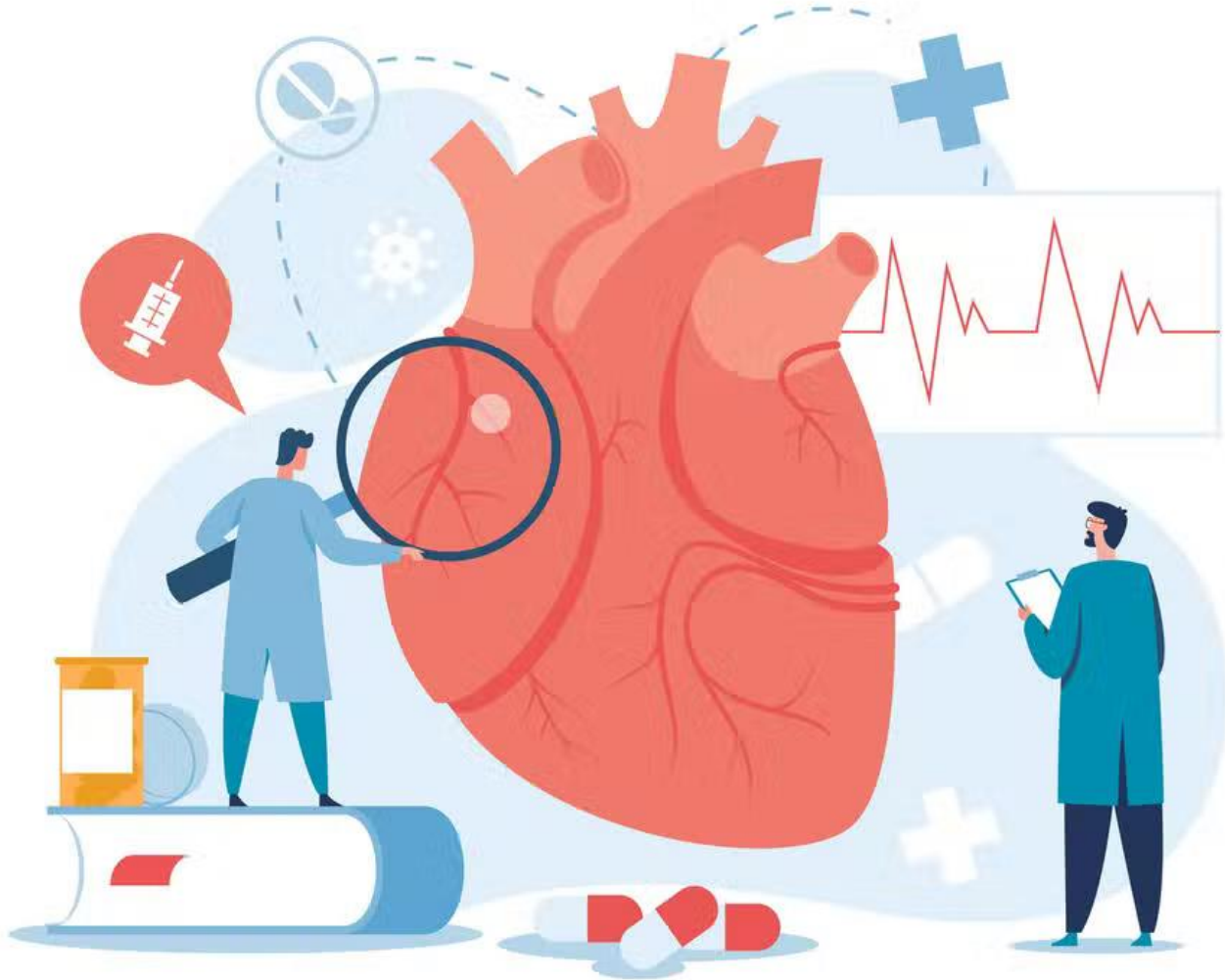

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