

A model of atherosclerosis in guinea pigs induced by atherogenic diet



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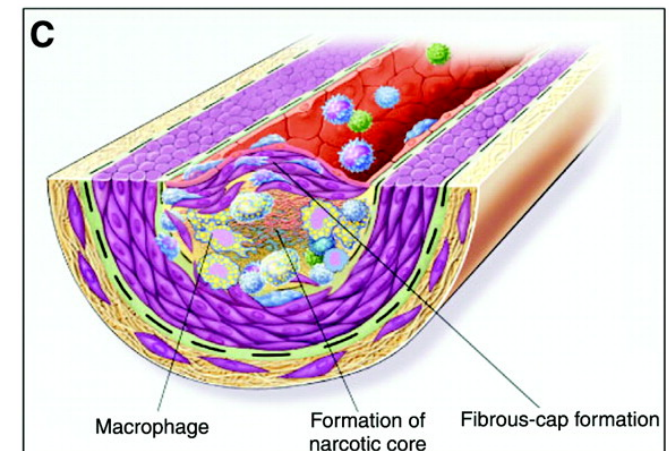
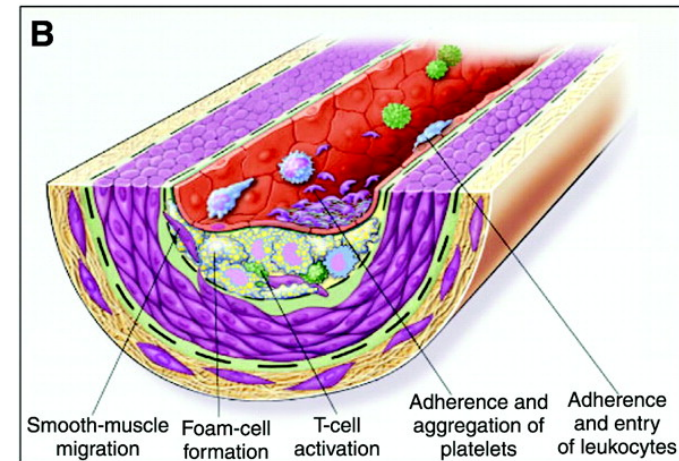
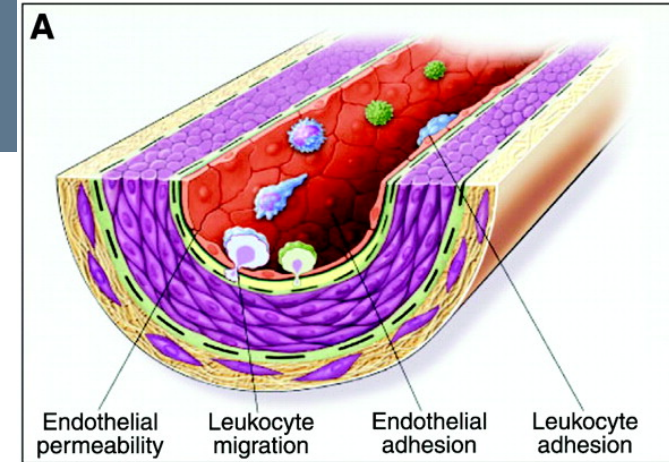
Atherosclerosis vs. arteriosclerosis

- Arteriosclerosis - arteries become thick and **stiff**, lacking flexibility and elasticity, restricting blood flow to organs and tissues.
- Atherosclerosis is a type of arteriosclerosis, buildup of fats, cholesterol and etc **in and on** artery walls (**plaques**), which restrict blood flow.
- If plaques burst, a blood **clot** can be triggered.
- *Atherosclerosis is preventable and is treatable.*

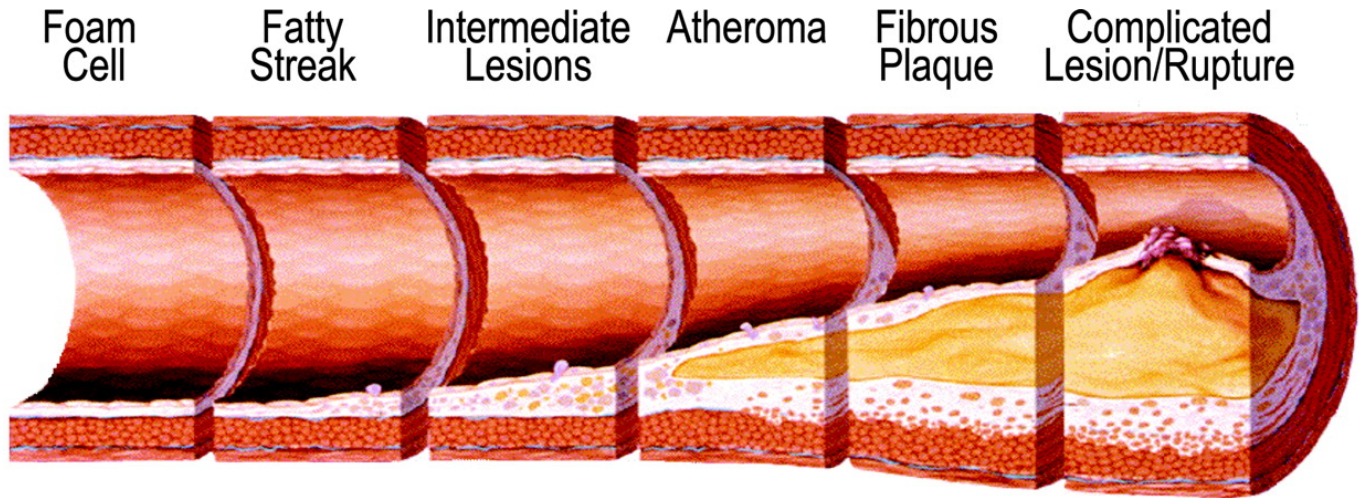
- Two things that can happen where plaque occurs are:
 - 1.) A piece of the plaque may break off.
 - 2.) A blood **clot** (thrombus) may form on the plaque's surface (can be experimentally induced by arterial or venous stasis).
- If either of these occurs and blocks the artery, a heart attack or stroke may result.

Atherosclerosis

- Atherosclerosis - a chronic, multiple processes,
- impairment of functional (endothelial dysfunction) and structural (plaque formation) arterial wall properties,
- progressively leads to cardiovascular events, such as myocardial infarction, critical limb ischemia and stroke.



Markers of inflammation and plaque instability: from foam cell to plaque rupture

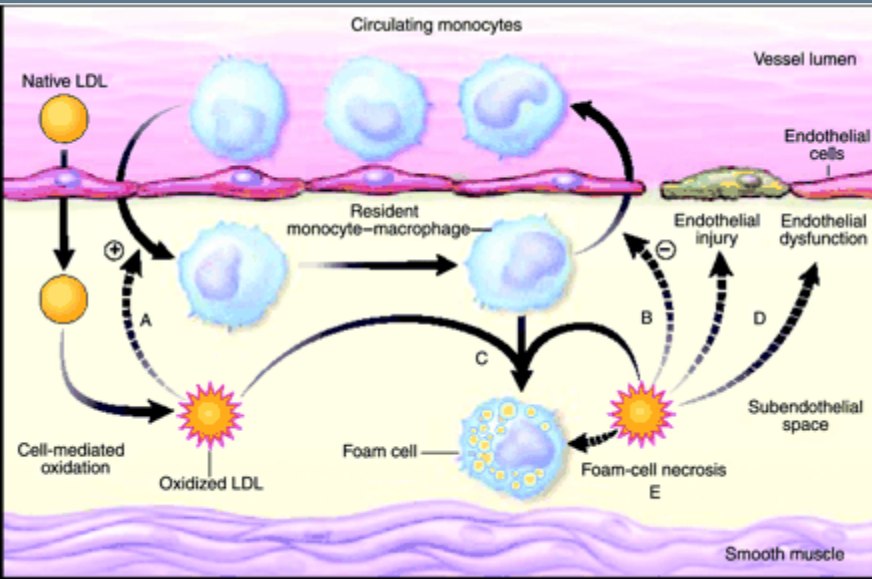


1° & Messenger Inflamm. Cyto/Chemokines		Cellular Adhesion Molecules	Plaque Destabilization		Plaque Rupture
IL-1	IL-6*	sICAM	IL-18*	MPO*	PAPP-A*
TNF- α	IL-18*	sVCAM	oxLDL*	MMPs *	sCD40L*
	MCP-1*	sSelectins	Lp-PLA ₂ *	MCP-1*	
			GPx-1*	PIGF*	

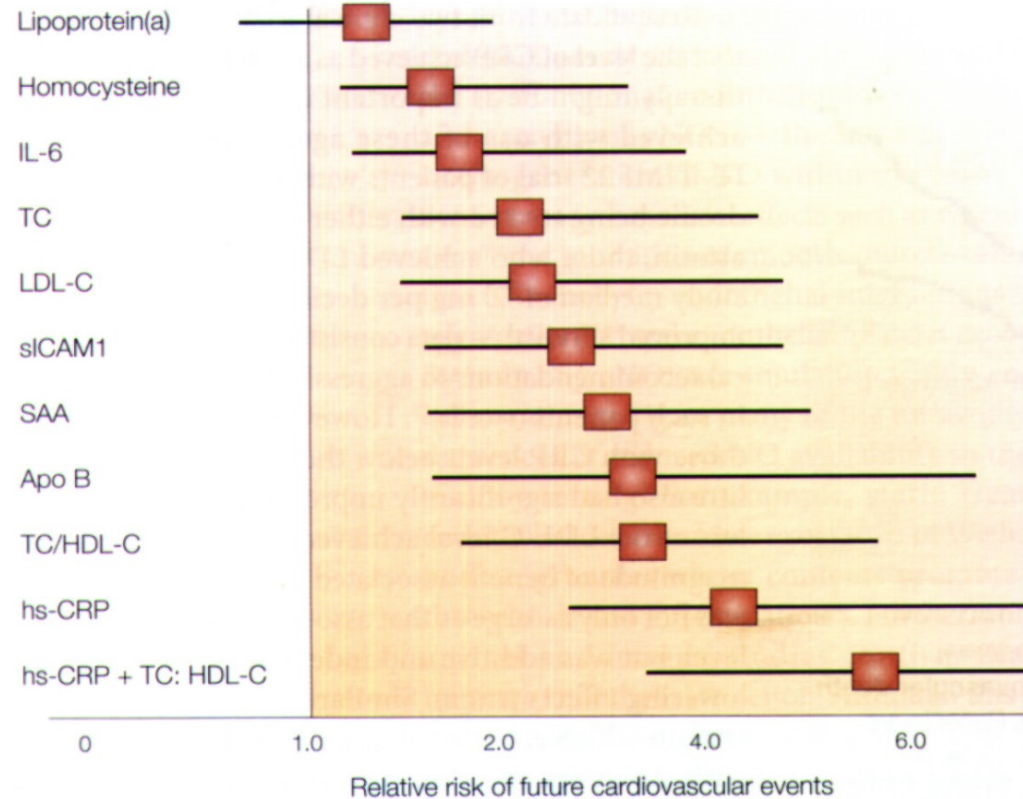


Acute Phase Reactants
 CRP*, sPLA₂*, SAA, Fibrinogen, WBCC

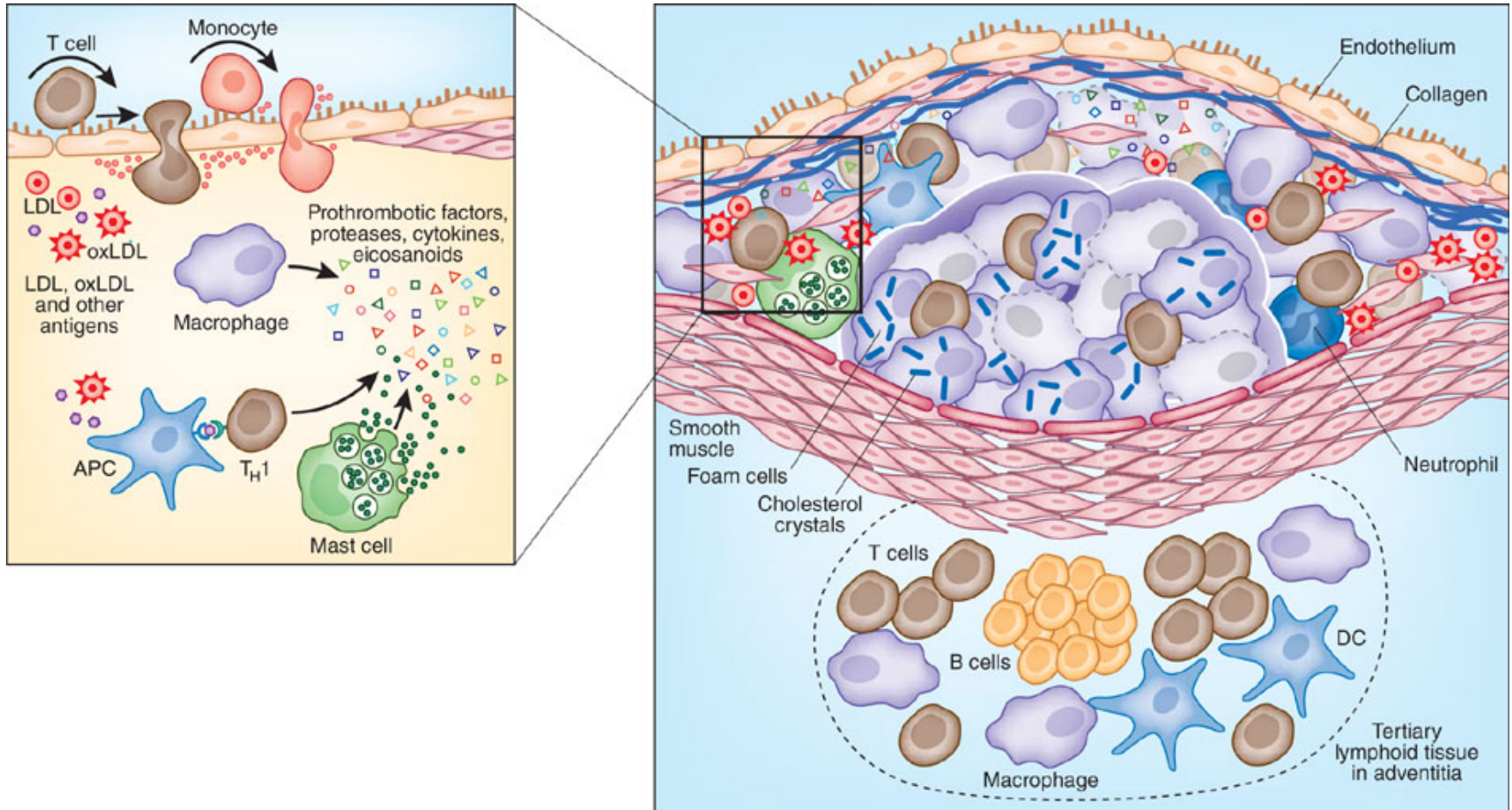
Atherosclerosis as an inflammatory process: from foam cell to plaque rupture



Ridker PM et al. *N Engl J Med* 2000;342:836-843.

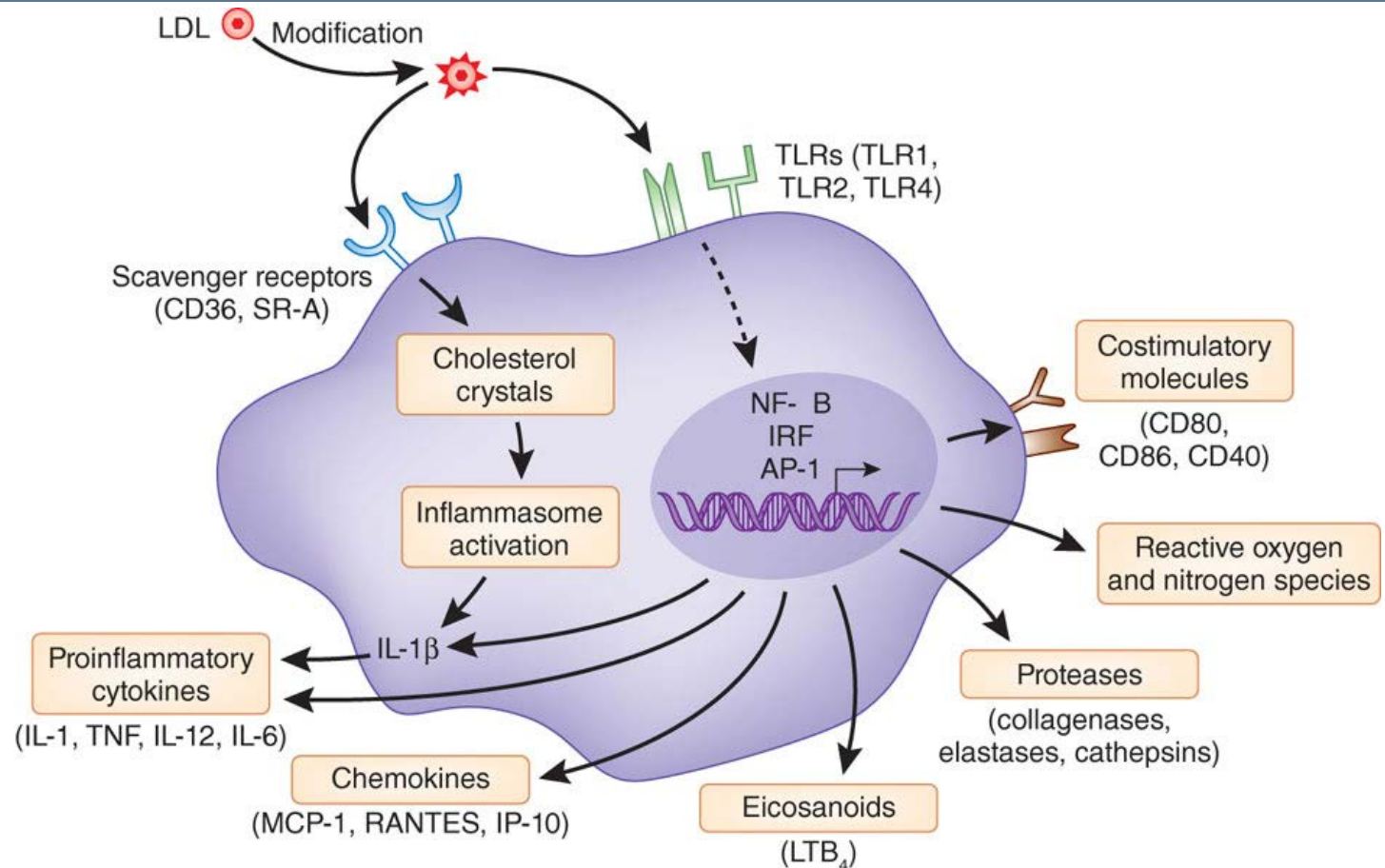


The immune system in atherosclerosis



The atheroma - a core of lipids and a fibrous cap with smooth muscle cells and collagen. Cells of the immune response are macrophages, T cells, mast cells and dendritic cells (DCs). Location: intima, the innermost layer of the artery. Cells of the immune response accumulate outside advanced atheroma and may develop into tertiary lymphoid structures with germinal centers.

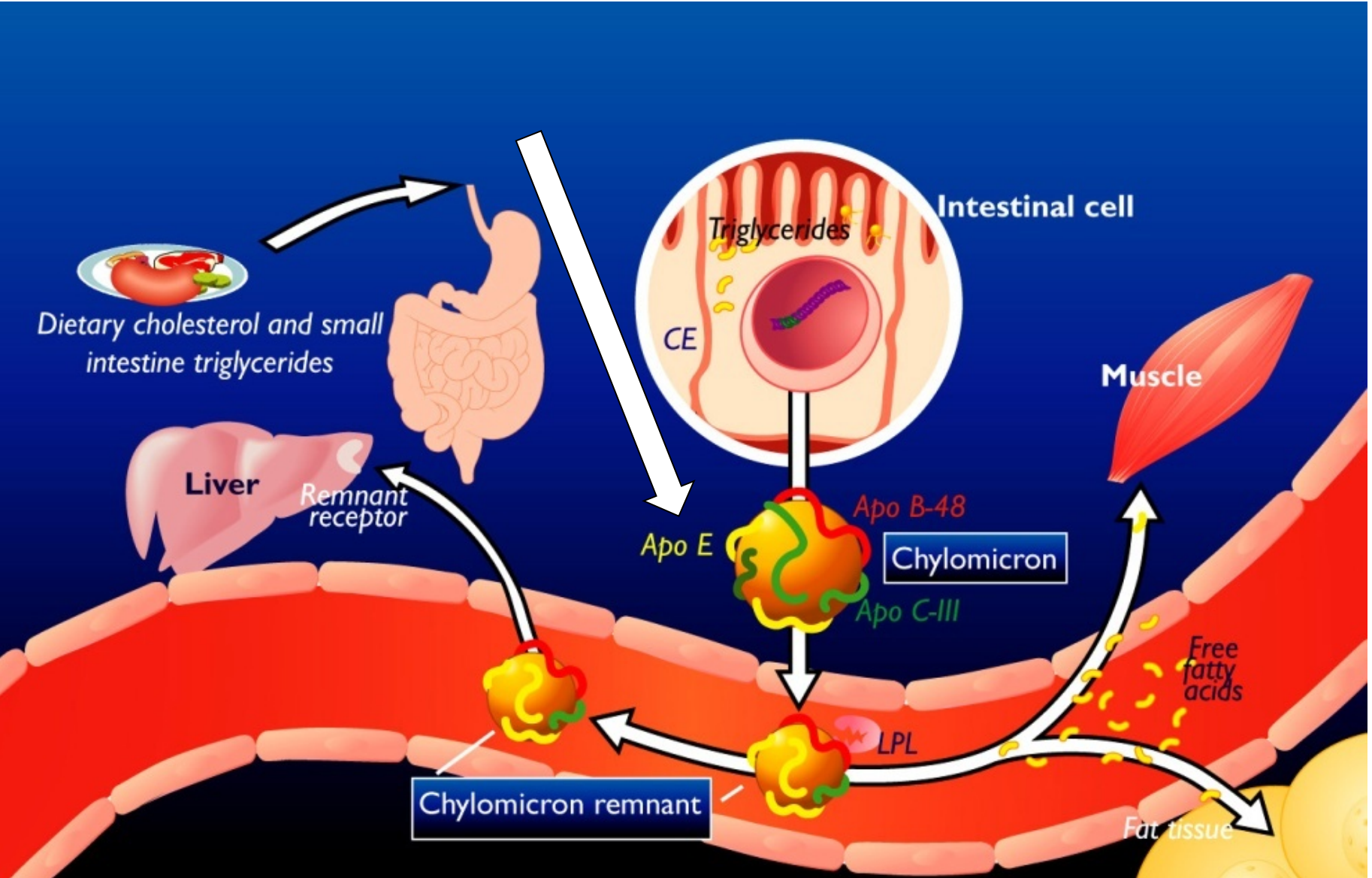
The immune system in plaque ruptures



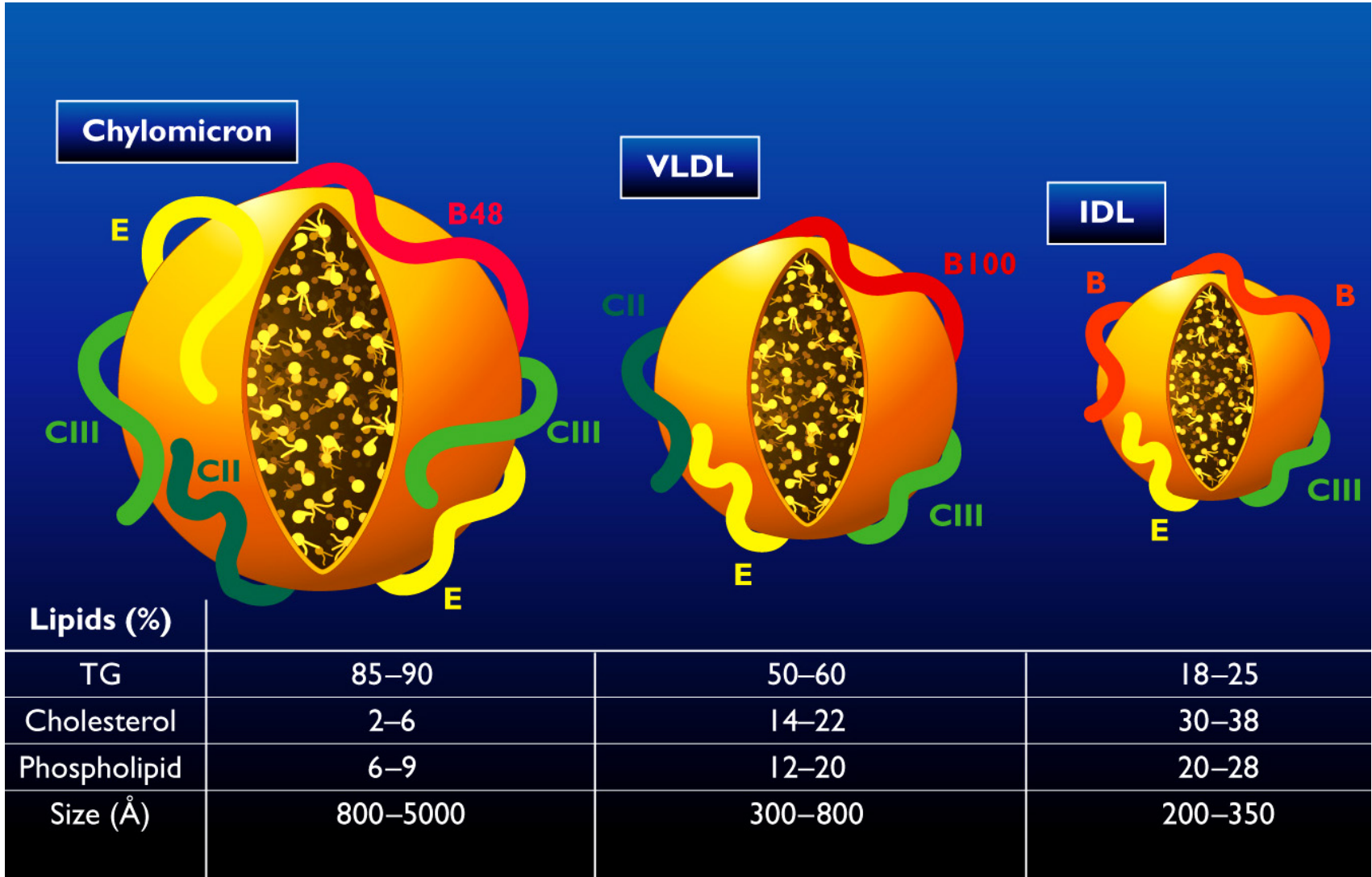
Uptake of oxLDL through scavenger receptors \rightarrow intracellular accumulation of cholesterol \rightarrow activate the inflammasome \rightarrow IL-1 β secretion.

oxLDL \rightarrow TLRs \rightarrow intracellular signaling cascade \rightarrow expression of genes encoding proinflammatory molecules (cytokines, chemokines, eicosanoids, proteinases, oxidases and costimulatory molecules). NF- κ B, IRF, AP-1 - transcription factors.

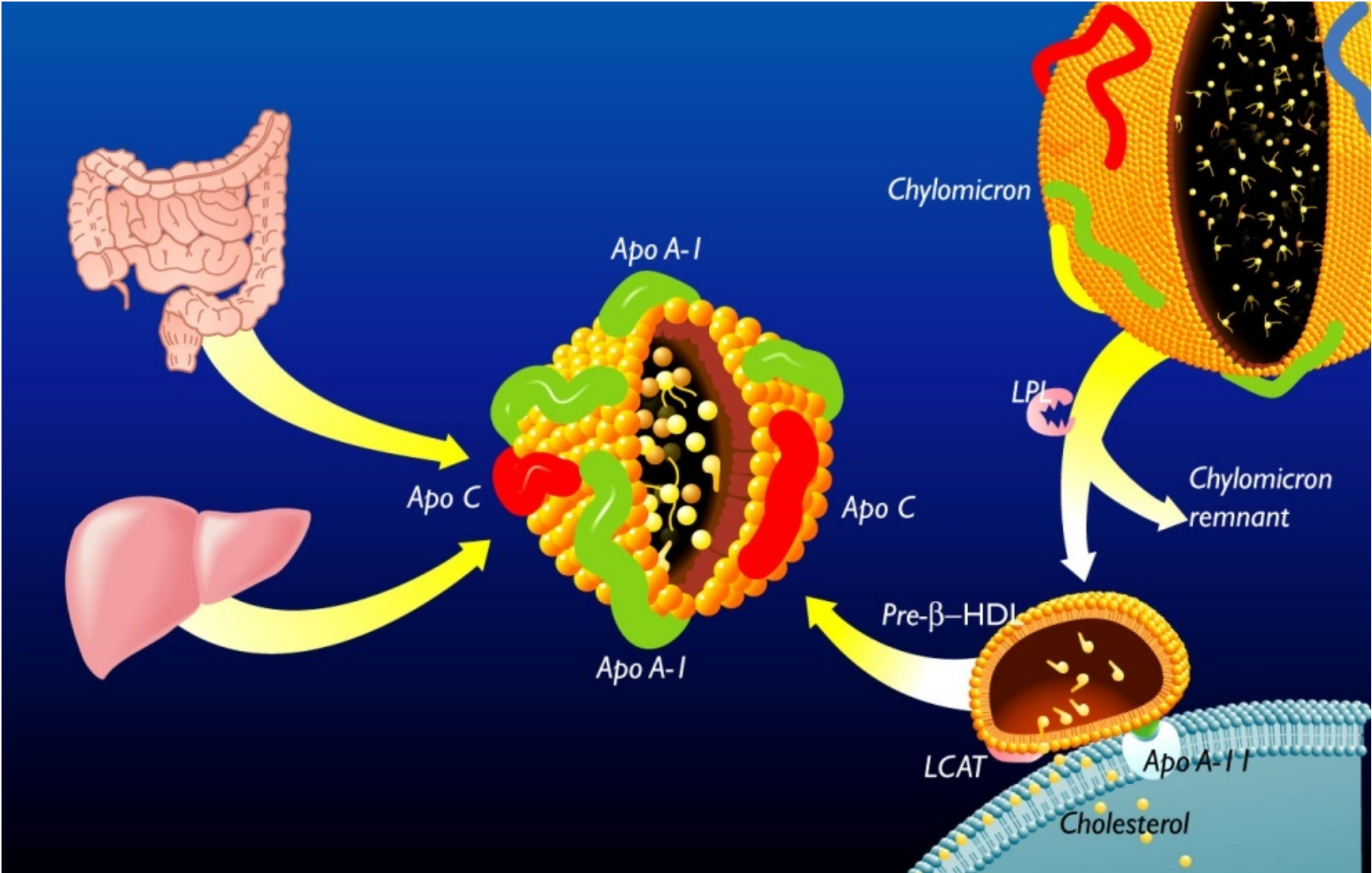
Exogenous vs. endogenous fats: ADM(E)



TG rich lipoproteins: size, structure, composition



HDL: apo...



Guinea pigs as a model for atherosclerosis

A good animal model enable detailed study of the atherogenesis and possible sites of intervention, specificity, reliability.

Present animal models of atherosclerosis

(Medline publications in 1000 = k)

-Mouse (apoE-/-)-4k, **rabbit**-1k, **guinea pig**-0.18k, **mini pig**-0.16k,...

Types of plaque induction:

-chemical lesions (local), topically induced by catheter,

-mechanical lesions (local): laser induced, balloon induced, scratching,, OR combined.

Diets: carbohydrates, lipids, agents,

Present strategies: types of hyperlipidemias

	<u>I</u>	<u>IIa</u>	<u>IIb</u>	<u>III</u>	<u>IV</u>	<u>V</u>
Lipids						
Cholesterol	N- ↑	↑	↑	N- ↑	N- ↑	N- ↑
Triglycerides	↑	N	↑	N- ↑	↑	↑
Lipoproteins						
Chilomicrons	↑	N	N	N	N	↑
VLDL	N- ↑	N- ↓	↑	N- ↑	↑	↑
LDL	↓	↑	↑	↑	N- ↓	↓
HDL	↓	N	N	N	N- ↓	↓

N = normal, ↑ = increased; ↓ = decreased; ↑̂ = moderately inc.; ↓̂ = moderately decreased

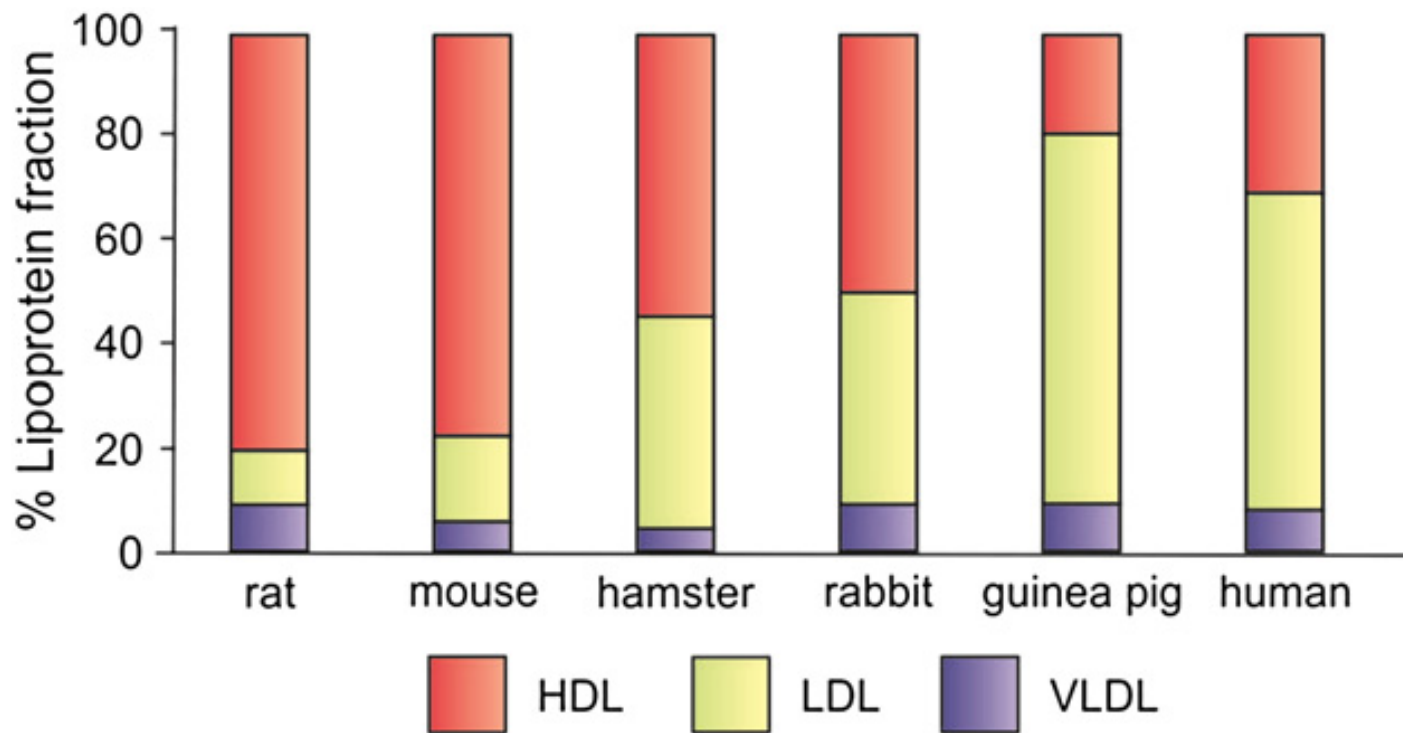
Many different potential targets and therapeutic strategies

Present lipid lowering drugs and decreasing plaque forming processes:

- statins** (▼ oxLDL, ▲ HDL),
- fibrates** (▼ TC),
- resins** (i.e. cholestyramine) (▼ LDL),
- niacin** (▼ LDL, TC, ▲ HDL),
- ezetimibe** (▼ LDL),
- others** (antioxidants, probucol, omega-3 f.acids ▼ VLDL...)

Why guinea pig?

- Guinea pigs are one of the animal models of choice for the study of atherosclerosis:
 - comparable distribution of cholesterol and plasma lipoproteins to humans.



1.) Proatherogenic diet model

- The aim:

To establish a simple and „effective“ model of atherosclerosis in guinea pigs by proatherogenic diet for studying possible anti-atherogenic effects of potential new agents and drugs.

1.) Diet

- **Methods:** Dunkin-Hartley guinea pigs (16)
- control group fed with standard diet-Altromin No. 3123
- atherogenic group fed with atherogenic diet (8 weeks, chow composed of 77 % standard diet, 1 % **cholesterol**, 8 % **yolk powder**, 5 % **lard** and 9 % **fructose**).

1.) Protocol

After 8 weeks the animals were sacrificed:

- blood samples collected,
- abdominal aortas excised (for atherosclerotic plaque area determination),
- thoracic aortas isolated (for endothelial function testing).

1.) Results: area aorta covered by plaques

- Atherogenic diet significantly induced the formation of atherosclerotic plaques in abdominal aorta to **6.95 ± 0.5 %** compared to control group, where the plaque area was **0.19 ± 0.02 %** ($P < 0.001$).

1.) Results: isolated artery response

- Endothelium-dependent relaxation of thoracic aorta was significantly impaired in atherogenic group compared to control group (**48.2 ± 3.6 %** versus **78.8 ± 2.3 %**, respectively; $P < 0.001$).
- Atherogenic diet significantly increased the concentration of total cholesterol, LDL cholesterol and triglycerides.

1.) Conclusions

-We established a simple and effective guinea pig model of atherosclerosis by feeding the animals with atherogenic diet.

-optimal time duration of feeding lasted eight weeks.

1.) Conclusions

-functional (aortic endothelium dysfunction) and structural (plaques in abdominal aorta) arterial wall changes were significantly increased.

-model is appropriate for pharmacological studies and for studying the atherosclerotic process itself.

2.) Atherosclerosis, diet and test of drugs

- Atherogenic diet-induced impairment of the arterial wall in guinea pigs.
- gene expression of constricting peptides increased and relaxing ones decreased within arterial wall.
- short-term, low-dose treatment with the **statin** and **sartan** combination improving arterial wall properties.

2.) Protocol

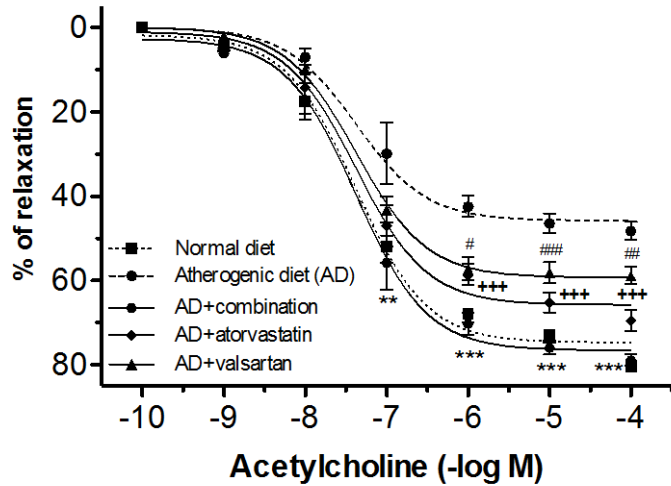
Dunkin-Hartley guinea pigs (25) randomly assigned to five experimental groups:

- 1) normal diet;
- 2) atherogenic diet (AD);
- 3) AD + a low-dose atorvastatin and valsartan combination (5 mg/kg/day and 2.4 mg/kg/day);
- 4) AD + low-dose atorvastatin (5 mg/kg/day);
- 5) 5) AD + low-dose valsartan (2.4 mg/kg/day).

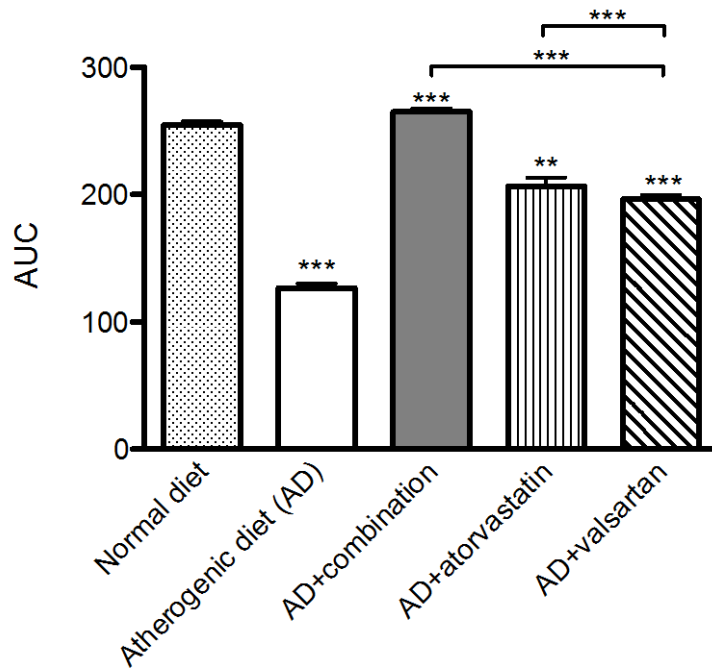
8 weeks of treatment, animals sacrificed, blood samples collected and thoracic and abdominal aortas isolated.

2.) Results

a)



b)



- a.) 8 weeks treatment:

-normal diet,

-atherogenic diet (AD),

-AD with low-dose combination of atorvastatin and valsartan,

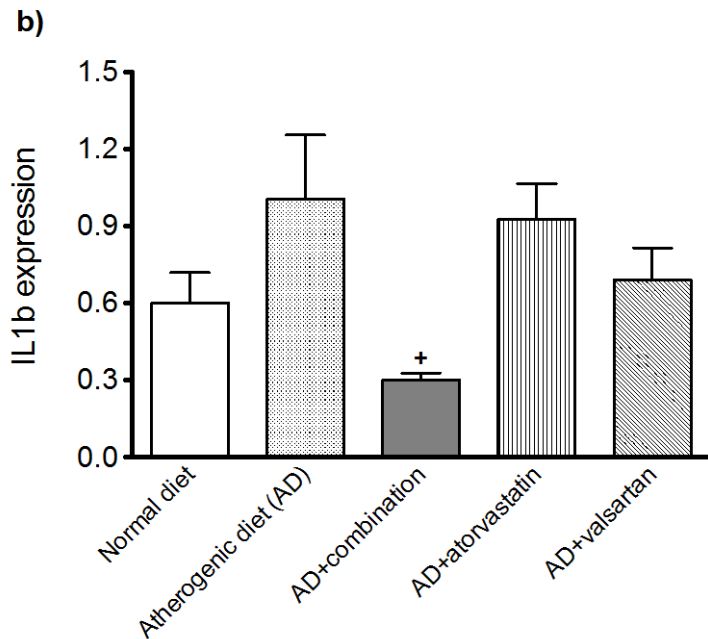
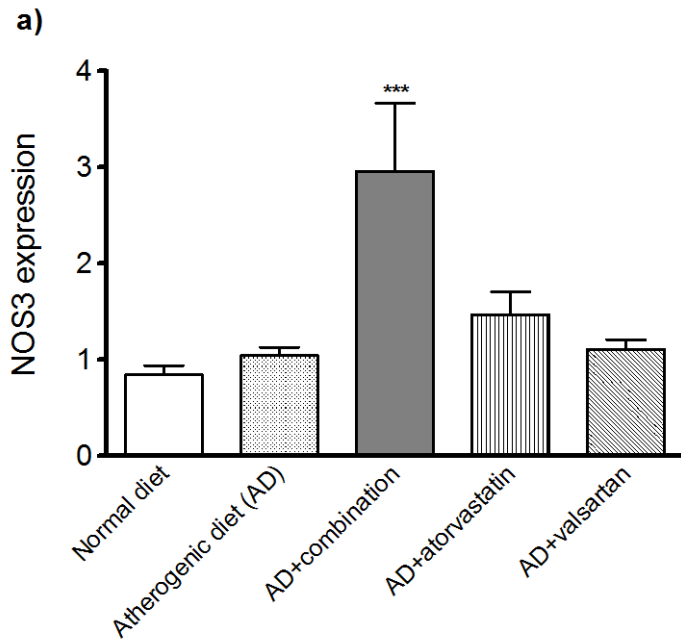
-AD + acetylcholine-induced endothelium-dependent relaxation of the thoracic aorta.

(mean \pm SEM; n=5; #-P<0.05; ###-P<0.001 AD + valsartan vs AD; +++-P<0.001 AD + atorvastatin vs AD; **-P<0.01; ***-P<0.001 AD + combination vs AD).

- b) AUC for acetylcholine-induced endothelium-dependent relaxation of thoracic aorta.

(mean \pm SEM. All values vs. AD or linked by bars. **-P<0.01; ***-P<0.001).

2.) Gene expression



- a) endothelial **nitric oxide synthase** gene (NOS3)

- b) **IL-1** gene (IL1b)

- guinea pigs receiving

- normal diet,

- atherogenic diet (AD),

- AD with a low-dose combination of atorvastatin and valsartan,

- AD with separate drugs.

N=5 (means \pm SEM. ***-P<0.001; +-P<0.05 vs. atherogenic diet (AD) group.

2.) Serum analysis

Normal diet, atherogenic diet (AD) or AD with low-dose combination of atorvastatin and valsartan combination or AD with separate drugs.

(*-P<0.05; **-P<0.01; ***-P<0.001 vs. normal diet; +-P<0.05 vs. AD.

	Cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	Triglycerides (mmol/l)
Normal diet	1.3 ± 0.02	1.23 ± 0.40	2.15 ± 0.04	0.17 ± 0.04
AD	7.6 ± 1.30 ***	4.64 ± 0.44 ***	1.44 ± 0.39	1.25 ± 0.24 *
AD + combination	6.0 ± 0.40 ***	3.64 ± 0.24 *	3.23 ± 0.29 +	0.41 ± 0.05
AD + atorvastatin	6.9 ± 0.08 ***	3.90 ± 0.63 **	2.40 ± 0.19	0.72 ± 0.10
AD + valsartan	7.3 ± 0.37 ***	4.33 ± 0.29 **	1.76 ± 0.50	0.74 ± 0.38

2.) Conclusion

- The atherogenic diet impaired maximal thoracic aorta endothelium-dependent relaxation by 40.1 %.
- The low-dose drug combination preserved thoracic aorta endothelium-dependent relaxation to the level of group receiving normal diet.
- This effect is partially explained by significantly increased gene expression of NOS3 and decreased expression of IL1b.
- Treatment with either low-dose combination or the separate drugs prevented the formation of atherosclerotic plaques in the abdominal aorta.

3.) Atherosclerosis and natural flavonoid extract

- Silver fir (*Abies alba*) bark extract protects function and morphology of arterial wall of guinea pigs against damage caused by atherogenic diet,
- polyphenols as the main constituents in the white fir bark (*Abies alba*, cortex) extract,

3.) Methods

-guinea pigs (18) fed for 8 weeks:

1) Standard chow,

2) Atherogenic,

3) atherogenic + bark extract.

-relaxation-contraction ability of aorta
(contraction with 0.1 mM phenylephrine,
relaxation by acetylcholine).

-fatty plaques in aorta walls (Oil Red O dying).

3.) Plaques in abdominal aorta

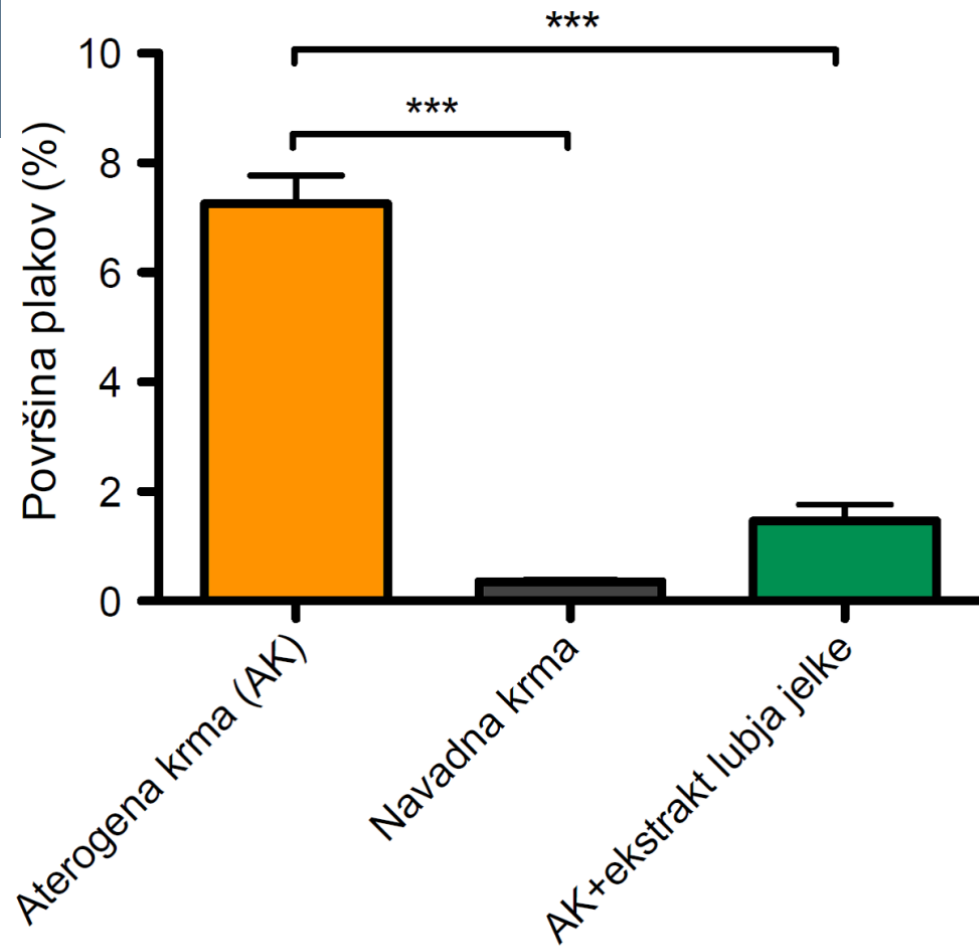
Plaques area: Oil Red O dyeing of abdominal aorta.

A: atherogenic diet,

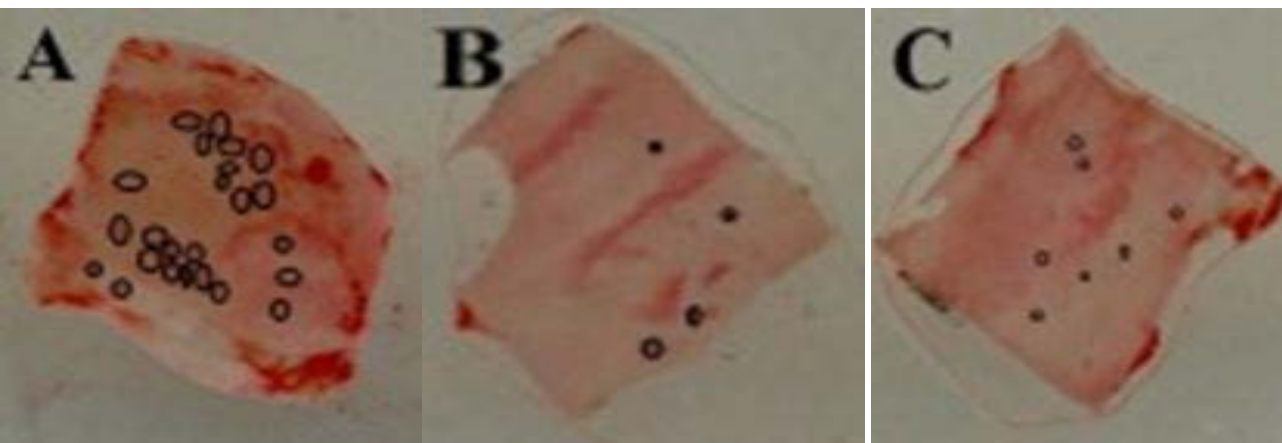
B: standard diet,

C: atherogenic diet + fir bark extract.

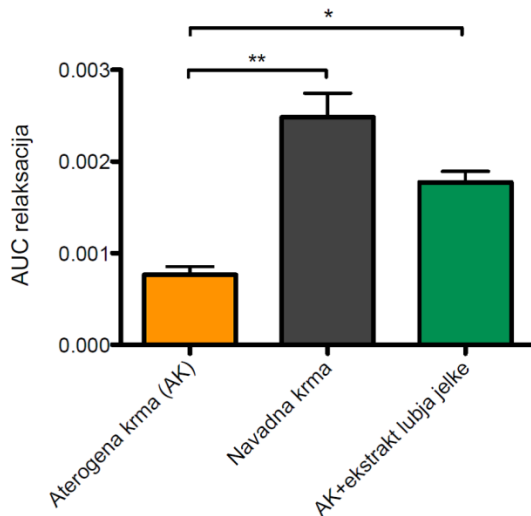
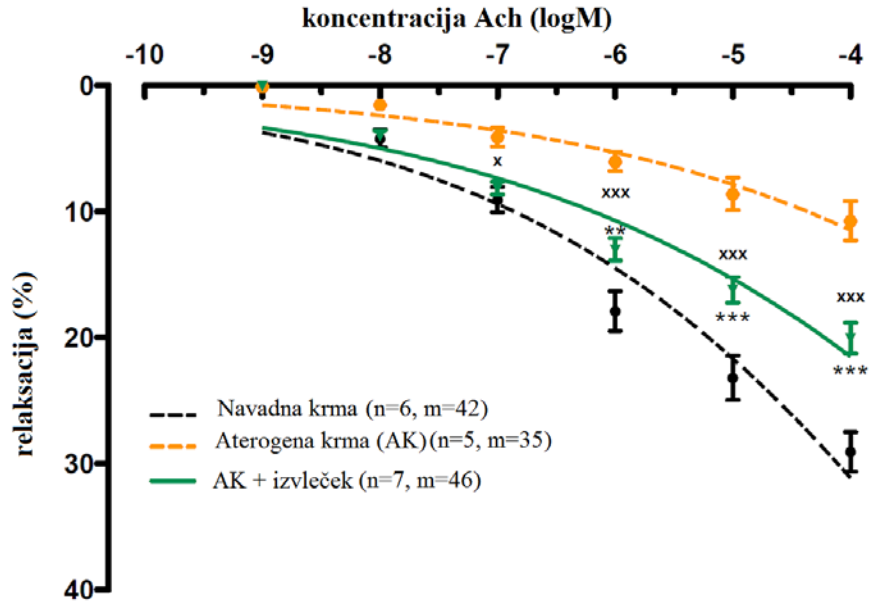
Fatty plaques in aortic lumen are in dark circles.



(*** $p < 0,001$).



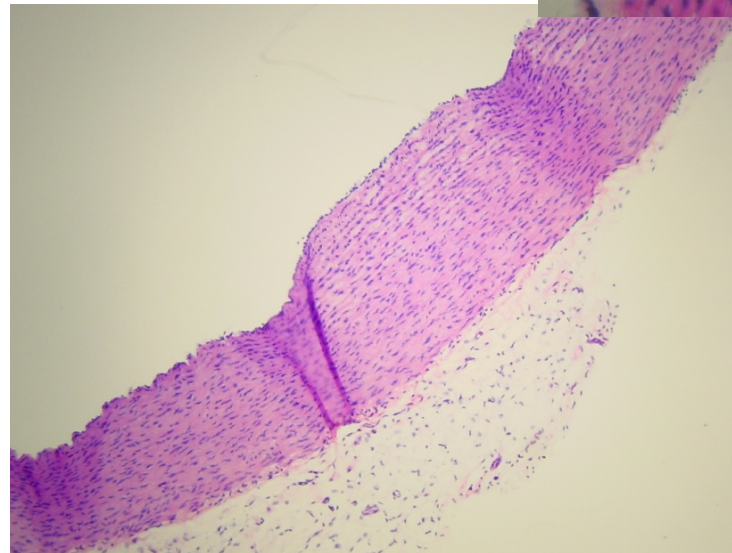
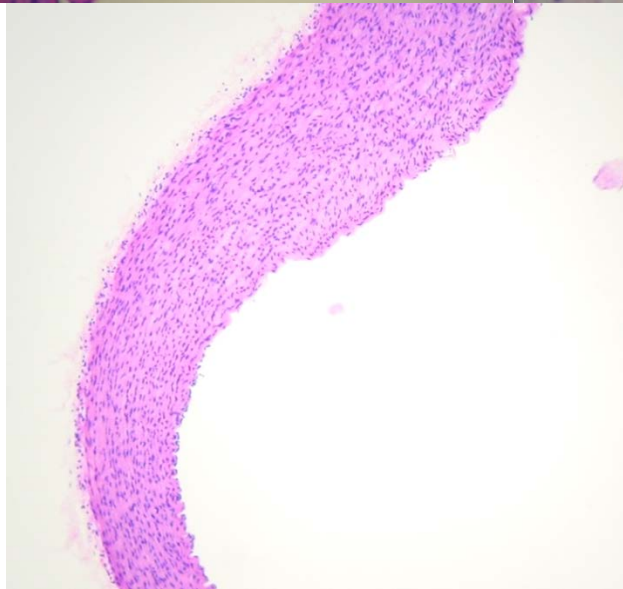
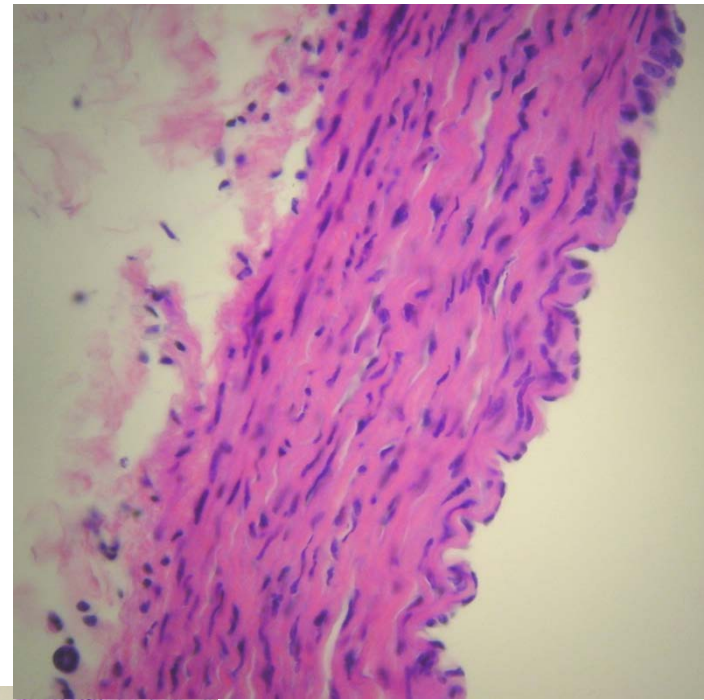
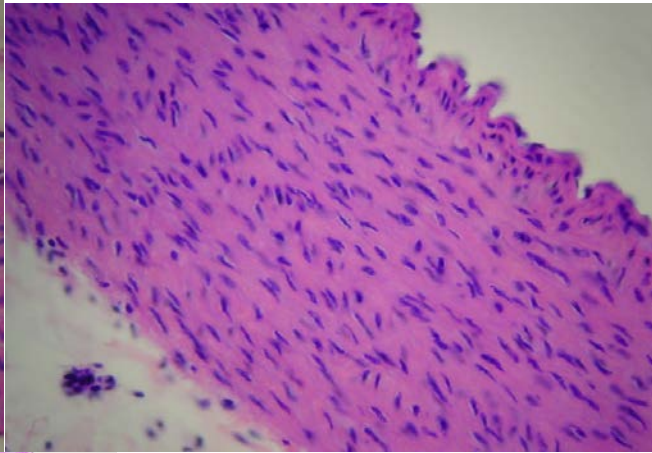
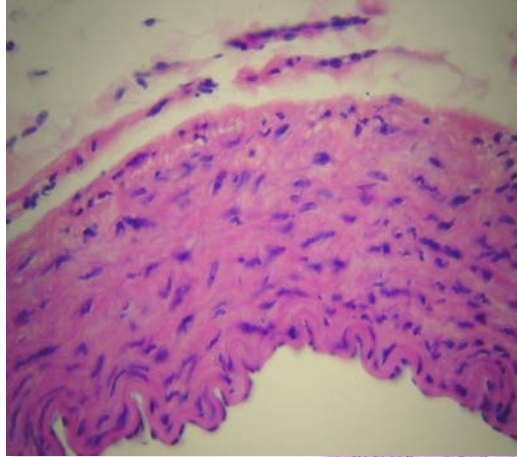
3.) Arteries response



Relaxation-contraction ability of aorta (contraction with 0.1 mM phenylephrine, relaxation by acetylcholine)

- 1.) AD vs. Bark extract (xxx-p < 0,001; xx-p < 0,01; x-p < 0,05).
- 2.) Bark extract vs. Standard diet (***-p < 0,001; **-p < 0,01; *-p < 0,05).

3.) Histology (preliminary)



Can JUPITER influence VENUS?

- hsCRP, biomarker of inflammation, is an independent predictor of future cardiovascular events. Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (**JUPITER**) was a landmark primary prevention trial (LDL <130 mg/dL, hsCRP >2 mg/L).
- VenUS - venous leg ulcers (pain, antibiotics, ...) represent less dangerous but more life quality influencing disease.
- Therapy? Animal models? Biomarkers?

- Psychopharmacology:

Sharman et al. (2013) The Guinea Pig as a Model for Sporadic Alzheimer's Disease (AD): The Impact of Cholesterol Intake on Expression of AD-Related Genes. PLoS ONE 8(6): e66235.

-Precursor amino acids?

-Immunology?

Drug discovery still by accident?

