ATHEROSCLEROSIS
pathogenesis
risk factors

Marek Vecka
Cause of death and burden of disease

worldwide mortality

non-communicable diseases

- cardiovascular diseases: 46%
- cancers: 22%
- diabetes: 4%
- respiratory diseases: 10%
- other: 18%

other causes: 32%

non-communicable diseases: 68%
Atherosclerosis

Cardiovascular diseases

atherosclerosis is most important cause

cholesterol plays a crucial role in the pathogenesis of atherosclerosis
Seven countries study: cholesterolemia and mortality

Cholesterol and CHD: Seven Countries Study

- Northern Europe
- United States
- Southern Europe, inland
- Southern Europe, Mediterranean
- Siberia
- Japan

CHD mortality rates (%) vs TC (mmol/l)
Atherosclerosis

**Definition**

*Former approach*: combination of changes in arterial intima

focal accumulation of lipids, complex glycides, blood and blood products, fibrous tissue and calcium, in connection with the changes in media

*New definition*: signals of various etiology

proliferative response of endothelium and intima

lipid/matrix accumulation

the key role – oxidized lipoproteins
Phases of atherosclerosis

**early phase** – accumulation of lipids

**late phase** – intimal proliferation and adjacent thrombosis
**Initial lesion**
- histologically "normal"
- macrophage infiltration
- isolated foam cells

**Fatty streak**
- mainly intracellular lipid accumulation

**Intermediate lesion**
- intracellular lipid accumulation
- small extracellular lipid pools

**Atheroma**
- intracellular lipid accumulation
- core of extracellular lipid

**Fibroatheroma**
- single or multiple lipid cores
- fibrotic/calcific layers

**Complicated lesion**
- surface defect
- hematoma-hemorrhage
- thrombosis

**Earliest Onset**
- from first decade
- from third decade
- from fourth decade

**Main Growth Mechanism**
- growth mainly by lipid addition
- increased smooth muscle and collagen increase
- thrombosis and/or hematoma

**Clinical Collateral**
- clinically silent
- clinincally silent or overt

**Endothelial Dysfunction**
Early phase of atherosclerosis

1\(^{st}\) type of lesion – isolated foam cells derived from macrophages

2\(^{nd}\) type of lesion (fatty streak) – accumulation of foam cells

3\(^{rd}\) type of lesion (intermediary lesion) – small amounts of extracellularly deposited lipids (debris from foam cells)

4\(^{th}\) type of lesion (atheroma) – lipid core localised in the basis of the lesion (almost only extracellularly accumulated lipids)
DEVELOPMENT OF FATTY STREAK

1. transendothelial transport of LP
2. retention of LP
3. oxidative modification of LP
4. adherence of monocytes
5. monocyte chemotaxis
6. monocyte differentiation
7. foam cells formation
Late phase of atherosclerosis

5th type of lesion (*fibroatheroma*) – proliferation and expression of secretional phenotype of SMC, ↑ synthesis of extracellular matrix (collagen and elastic fibres), the cover = thin layer of smooth muscle cells forming fibrous crust (“cap”) over the lipid core

6th type of lesion (*complicated lesion*) - exulceration, hemorrhage into plaque, calcification of necrotic material and artery wall thrombosis

unstable plaque – see further
ATHEROSCLEROTIC PLAQUE

VEssel Lumen

NORMAL ARTERY

endothelial cell
monocyte
macrophage
smooth muscle cell
dendritic cell
foam cell
mast cell

T lymphocyte

cellular debris
cholesterol
PLAQUE THROMBOSIS

VEssel Lumen

- Platelet
- Erythrocyte
- Fibrin
- Rupture

Cellular Debris

- Smooth muscle cell
- Macrophage
- Endothelial cell
- Monocyte
- Elastic lamina
- T lymphocyte

Cholesterol

Dendritic cell

Foam cell

Mast cell
Atherothrombosis

sudden/impredictable rupture of atherosclerotic plate → platelet activation and thrombus formation
## Characteristics of unstable plate in coronary artery

<table>
<thead>
<tr>
<th></th>
<th>Unstable plate</th>
<th>Stable plate</th>
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<tbody>
<tr>
<td><strong>Size</strong></td>
<td>30 - 40% stenosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>eccentric</td>
<td></td>
</tr>
<tr>
<td><strong>Core lipids</strong></td>
<td>cca 40% (FC cryst.)</td>
<td>cca 10%</td>
</tr>
<tr>
<td><strong>Monocytes/macrophages/foam cells</strong></td>
<td>30% (v/v)</td>
<td>10% (v/v)</td>
</tr>
<tr>
<td><strong>Vascular SMC</strong></td>
<td>3 – 5%</td>
<td>10 – 15%</td>
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</table>
Schematic Time Course of Human Atherogenesis

Lesion initiation

- No symptoms
- ± Symptoms
- Symptoms

Time (y)

Ischemic Heart Disease
Cerebrovascular Disease
Peripheral Vascular Disease
Obviously, we wouldn´t like to end like this....
Risk factors of atherosclerosis and coronary heart disease

Risk factor

= abnormality found in individual without manifestation of ATS during clinical/laboratory examination

present risk factor $\uparrow$ relative risk of future manifestation of ATS

not causally connected neither denies one another

manifestation of atherosclerosis

$\uparrow$ incidence raises with $\uparrow$ incidence of risk factor association with risk factor should be independent, gradual and continual risk factors act synergically and/or additively

this must be supported by intervention studies
Categories of cardiovascular risk factors

1st category risk
- intake of exogenous CH/saturated fat
- thrombogenic factors
- cigarette smoking
- high LDL-C
- hypertension

2nd category risk
- high TAG
- small dense LDL
- low socio-economic status
- menopause
- low HDL-C
- type 2 DM
- lack of physical exercise

3rd category risk
- folic acid deficiency
- EtOH abstinence
- oxidative stress
- Lp(a)
- psychosocial factors (A type behaviour)

4th category risk
- family history of premature CVD
- age
- male gender

**Proven increase in cardiovascular risk**

**Probable increase in cardiovascular risk**

**Possible increase in cardiovascular risk**

**Not influenceable increase in cardiovascular risk**
Risk factors of CAD

✓ Unmodifiable risk factors
  • gender
  • positive family history (genetic background)
  • age
  • ethnicity

✓ Modifiable risk factors
  • smoking
  • hypertension (LVH, ECG, ECHO)
  • hyper LDL-C
  • hypo HDL-C
  • hyper TG
  • diabetes mellitus
  • sedentary life
  • obesity
  • inflammation
  • social factors (socio-economic status, type A/B of behaviour)
  • exogenous estrogens
A. UNMODIFIABLE RISK FACTORS
A. Unmodifiable risk factors

I. Age and gender
age: > 45 years in men,
  > 55 years in women

II. Family history of early CHD
< 55 years in male first-stage relatives
< 65 years in female first-stage relatives

National Cholesterol Education Program (NCEP), ATP III, 2004
Lipid change with age and gender
CHD incidence – effect of age and gender
Risk factors of CVD for stratification of risk in primary prevention of CHD

II. Family history of early CHD

*Candidate genes:*

- **Apolipoproteins** (A-I+CIII+AIV, AII, B, CI, CII, E, Lp(a))
- **Receptors** (LDL-R, Ins-R, ILGF1-R, SCR-1, SCR-2, AGTR1, PPARG1)
- **Enzymes** (CETP, LCAT, HL, LPL, CBS, renin, ACE, PON1, NOS, MTHFR)
- **Endothelium function** (ELAM, MMP3)
- **Coagulation factors** (thrombine, vWf, f.VII, fibrinogen, PAI-1, t-PA, f.XII)
- **Growth and inflammatory factors** (ILGF-1, IL-6, insulin, PDGF-β, TGFβ-1)
- **Membrane Transporters** (ABCA1)
- **Gender** (ESR1)
- **Other** (CRP, ADIPOQ)
B. MODIFIABLE RISK FACTORS
Intake of fatty acids

The graph illustrates the percentage of energy from fatty acids (%)

- **Hunter-gatherers**
- **Agricultural society**
- **Industrial society**

**Total Fat**

**Fatty acids**:
- Saturated FA
- PUFA n-6
- Trans FA
- PUFA n-3

Time periods:
- 4 My BC
- 10,000 BC
- 1800
- 1900
- 2000
Excessive intake of saturated fats potentiates the rise in plasma TC:

\[ \Delta TC = 2.74 \Delta \text{SFA} - 1.31 \Delta \text{PUFA} + 1.5 C^{-1/2} \]

but not all SFA are similar:

C12:0 - C14:0 - C16:0 > C18:0
Intake of EtOH

Ethanol abuse = more than 40 g EtOH daily
(high E substrate $\rightarrow$ ↑ NADH in liver $\rightarrow$ ↓ FA oxidation $\rightarrow$ fat=TAG excess)

induction of HTAG $\rightarrow$ ↑ VLDL (↑ synthesis in hepatocytes)

$\uparrow$ HDL-C (↑ apo A-I synthesis in enterocytes)

Zieve syndrome

- can be a result of chronic EtOH abuse
- hyperlipoproteinemia with high CH/VLDL-C and low HDL-C
- secondary deficiency of LCAT
- jaundice and reversible hemolytic anemia

Healthy liver $\rightarrow$ EtOH $\rightarrow$ steatosis $\rightarrow$ EtOH $\rightarrow$ cirrhosis

Cancer
Intake of sugar

Sugar sweeteners consumption in U.S.A.

- refined sugar
- high fructose corn syrup
- glucose syrup
- dextrose
- total corn sweeteners
- honey
- edible syrups
- total sweeteners
Intake of fructose

fructose → fructose-1-P

fructose-1-P → dihydroxyacetone-P → glyceraldehyde → glyceraldehyde-3-P → 1,3-PP-glycerate → pyruvate → acetyl CoA → fatty acids

glucose-6-P → glucose-6-P → fructose-6-P → fructose-1,6-PP

INSULIN LEPTIN

control

without control

ectopic accumulation of fat in liver

increased assembly and secretion of VLDL

glycerol → glycerol-P

glycerol-P → triacylglycerols
Overweight and obesity

Gynoid obesity - only increased TAG and VLDL

Android obesity - often with ALP
(oxidative stress, ↑ coagulability, chronic inflammation)

atherogenic lipid phenotype (ALP)
↑ TAG (VLDL) + ↓ HDL-C + ↑ sdLDL
(↑ NEFA, ↑ LDL- apoC-III+)
Effect of higher concentrations of particles rich in triacylglycerols on LDL and HDL metabolism

- Liver
- Adipose tissue
- HL: hepatic lipase
- TAG: triacylglycerols
- LPL: lipoprotein lipase
- CE: cholesteryl esters
- CETP: CE transfer protein
- LDL: low-density lipoprotein
- HDL: high-density lipoprotein
Phenotypes of LDL size

prevalence of large LDL particles

prevalence of small LDL particles

Phenotype A

Phenotype I

Phenotype B

I, II, III, IV - designation of LDL subfractions
number below x-axis indicates the size of LDL particle in nanometers
Generation of oxidatively modified LDLs

LDL

large LDLs

small LDLs

oxidative stress
sites of inflammation

more resistant
to oxidation

prone to oxidation

increased (prolonged) oxidation

native LDL

minimally modified LDL

oxidized LDL

cytotoxic LDL
Properties of oxidatively modified LDL

- Immunogenicity
- Enhance retention of LP
- Oxidative modification of LP
- Endothelial cytotoxicity
- Monocyte chemoattraction
- Macrophage differentiation
- Catabolism via SR-BI
Mechanisms of antiatherogenic effect of HDLs

I. Direct effects on lipoprotein metabolism
- reverse transport of CH to liver \((CH \text{ acceptor from cells})\)
- ↑ catabolism \((VLDL \rightarrow IDL \rightarrow LDL)\) \((TAG \text{ acceptor via CETP})\)
- block transendothelial LDL transport \((closure \ of \ junctions)\)
- VLDL,LDL protection to oxidation \((PON-1, \ PAFAH \ carrier)\)
- oxLDL cytotoxicity inhibitor \((PON-1, \ PAFAH \ carrier)\)

II. Other effects
- anti- and dysaggregative effects on thrombocytes
- antiarrhythmic effects
- restauration of endothelial dysfunction
- inhibition of expression of cytoadhesive molecules
Reverse cholesterol transport
sterol transport from macrophages
Risk factors of CVD for stratification of risk in primary prevention of CHD

B. Modifiable risk factors

Diabetes mellitus

considered as an equivalent of CHD, the presence of DM classifies the patient to the same risk group as those with already manifested ATS

National Cholesterol Education Program (NCEP), ATP III, 2004
Incidence of myocardial infarction in diabetics

- No DM/No MI
- No DM/MI
- DM/No MI
- DM + MI

7-year incidence rate of myocardial infarction

- p < 0.001

Graph showing increased incidence of MI in individuals with diabetes compared to non-diabetic individuals.
Risk factors of CVD for stratification of risk in primary prevention of CHD

B. Modifiable risk factors
Cigarette smoking
cigarette smoking in the last month
Hypertension
BP > 140/90 mmHg, or antihypertensive medication
Total plasma cholesterol
< 5.2 mmol/l – 5.2-6.1 mmol/l – > 6.2 mmol/l
appropriate  borderline  high

National Cholesterol Education Program (NCEP), ATP III, 2004
Risk factors of CVD for stratification of risk in primary prevention of CHD

B. Modifiable risk factors

**LDL-cholesterol**

- **< 2.6 mM**  optimal
- **2.6 – 3.3 mM**  borderline high
- **3.4 – 4.0 mM**  elevated
- **4.1 – 4.8 mM**  considerably increased
- **> 4.9 mM**

**HDL-cholesterol**

- **< 1.0 mmol/l**  low
- **1.0 – 1.6 mmol/l**  normal
- **> 1.6 mmol/l**  „negative risk factor“

Subtracts 1 RF from risk calculation

*National Cholesterol Education Program (NCEP), ATP III, 2004*
Cholesterolemia and mortality

![Graph showing the relationship between deciles of serum cholesterol and 6-year CHD mortality per 1000 (age adjusted). The mortality rate increases significantly in the highest deciles.]
Additive properties of risk factors

10-year probability (%) for probands aged 42-43 yrs

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure 150-160 mmHg</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HDL 0.83-0.90 mmol/l</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TC 6.20-6.77 mmol/l</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
multiplicative effect of risk factors
Other supposed risk factors for CHD

Lp[a]
chronic inflammation
   (CRP, SAA) → HDL remodelation
mild hyperhomocysteinemia
states with hypercoagulation
chronic infection
   *Chlamydia pneumoniae*
CMV
HSV-1
Lipoprotein particle resembling LDL
apo (a) attached to apo B-100 with S-S bond
- similar to plasminogen

**function of Lp(a)**
not fully resolved
Lp(a) competes with plasminogen for fibrin binding sites
inhibits fibrinolysis *in vitro*
*carrier for oxPL in plasma?*

high Lp(a)  high risk for cardiovascular disease
SAA and HDL remodelation

- Functionally defective HDL particles
- Acute phase response/inflammation

HDL particles lacking antiatherogenic functions

Decreased capacity for RCT
 Decreased antioxidant capacity of HDL
Homocysteine

1. Hcy is noncoding amino acid - has SH group redox balance connection with oxidative stress

2. Hcy is linked to methylation events possible DNA methylation (gene expression)

Mild hyperhomocysteinemia suggested as a risk factor for atherosclerosis - controversial results of interventional studies - important factor in those with DM + nephropathy, thrombosis
Hypercoagulable states

= states with venous/arterial thrombosis

predisposition for thrombosis  easy thrombus formation

  damaged vessel wall

a) mutations in factor V (Leiden), prothrombin
  - linked to higher risk of MI/CAD (in younger?)
b) hyperhomocysteinemia (see previous slide)
c) antiphospholipid syndrome/SLE
  - associated with CAD, stroke
Cell types involved in atherogenesis:

- endothelial cells (EC)
- thrombocytes
- blood monocytes, macrophages (mo/ma)
- smooth muscle cells (SMC)
- T-lymphocytes
**Cell types involved in atherogenesis**

**Endothelial cells (EC):**
- Transendothelial transport of apoB LP: LDL, IDL, Lp(a) *(inhibited by HDL)*

**Contraction of EC**
- Vascular homeostasis

**Relaxation of EC**
- Endothelium Derived Relaxing Factor
  - PGI$_2$  NO  histamin

**Dyslipidemia + oxidative stress** *(ox-LDL, high Hcy, …smoking)*
- Physical factors: shear stress, hypertension

**Endothelial dysfunction**
- Impaired vasodilatation *(↓NO availability)*

**Activated EC**
- ↑ synthesis of local mediators:
  - Cell adhesion molecules: CAM by IL-1, TNF$_{α, β}$ *(T-cells, Mf)*
  - Differentiating factors: MCP-1, MCSF-1 *(oxLDL)*
  - Adhesion molecules: ELAM *(ox-LDL)*
Thrombocytes:

- hypercholesterolemia → megakaryocyte ABCG4 → platelets

monocyte/macrophage

platelet activation by PAF, 12-HETE → secretion of TxA₂, 5-HT

aggregation and degranulation

releasing of growth factors for SMC – PDGF

many chemokines affecting monocytes/macrophages/T cells
Cell types involved in atherogenesis

**Monocytes/macrophages:**

I. *monocytes*
- can differentiate in macrophages (*via* MCP-1 etc.)

II. *macrophages*
- express receptors for LP: β-VLDL receptor, Ac-LDL-receptor, B/E-receptor Fc-receptor (for complex Ab-LDL)
- synthesize PAF, II-1, II-6, TNFγ, MDGF
- ox-LDL causes expression of genes and synthesis of 15-LO, MCSF, MCP-1

III. *foam cells*
- not able to migrate from the cell wall
- if the capacity for FC is exceeded → disruption of lysosomes → apoptosis → cellular debris
Cell types involved in atherogenesis

Smooth muscle cells (SMC): phenotype switch:
contractive → synthetic (active) type of SMC

atherogenic stimuli

migration from media → intima
proliferation and production of
glycosaminoglycans, collagen,
elastin, growth factors, cytokines

proliferation of SMC

Stimulation
- PDGF
- 12-HETE
- IGF-1

Inhibition
- heparin
- NO
- PGI₂
- INFγ
Mobilisation and activation of immune cells in atherosclerotic plate

- Migration of monocytes and T cells into arterial tissue is supported by locally produced chemokines.

- Proinflammatory cytokines, procoagulants, proteases, and proapoptotic factors are involved in the process.

- Local production of oxidised LDL and other stimuli activates immune cells like monocytes, macrophages, and dendritic cells.

- Endothelial cells and scavenger receptors play a role in the mobilisation and activation of immune cells.
Mobilisation and activation of immune cells in atherosclerotic plate

IFNγ and TNFα,β induce expression of CAM (cell adhesion molecule) in endothelial cells.

IFNγ inhibits proliferation of SMC.

IL-10 and TGFβ attenuate inflammation.
Gender specificity of risk factors

**women vs. men:**
estrogen rec $\alpha/\beta$ → vessel vasodilatation
same spectrum of risk factors for CAD, but worse for women in:

**smoking:** ($\downarrow E_{dep\ vasodilatation}$)
central obesity: metabolic syndrome is more often in women
diabetes: 50% higher risk in women for CHD
dyslipidemia: hyperTAG/low HDL-C - more detrimental for women
physical inactivity: relative risk for CHD higher 4.7% (vs. 3.4% in M)

**Specific risk factors:**
1. menopause → overall worsening of risk profile
2. PCOS → obesity/metabolic syndrome
3. HRT → ??effect on CHD??
4. Preeclampsia → impaired endothelial function/vasodilatation
5. Oral contraceptives(low-dose/3rd generation) → $\downarrow$CHD risk
Further reading
Textbooks, monographs

Articles

Web sources
http://themedicalbiochemistrypage.org - the Medical Biochemistry Page
http://www.bioguo.org/CADgene/index.php
http://www.who.int/healthinfo/en - WHO reports
http://www.trialresultscenter.org - resource for trials (e.g. for cardiology trials)