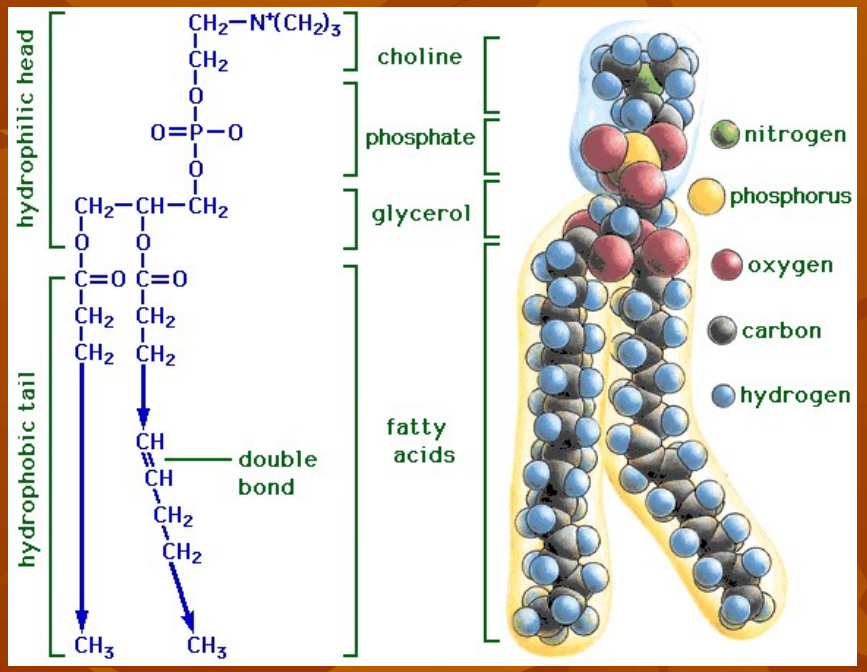
Lipid spectrum disorders

Lipids

- Esters of fatty acids and alcohols (e.g. glycerol, cholesterol, sfingosin)
- Sometimes, they also contain other chemical groups – e.g. Phosphate, choline, inositol (phospholipids), monosaccharide (glycolipids)
- In wider sense lipid involve generally small hydrophobic or amphiphilic molecule with hydrocarbon chain (which involves free cholesterol, free fatty acids, icosanoids, retinoids)

Molecule of phosphatidylcholine



Physiological functions of lipids

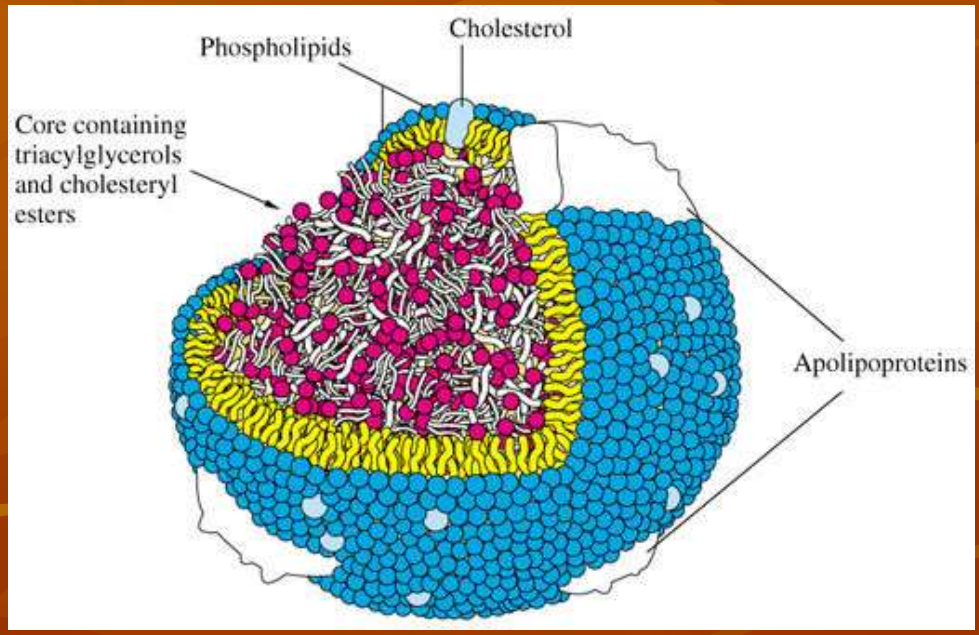
- Energy storage – 1 gram of triacylglycerol can produce 39 kJ, double compared to saccharides and proteins
- Structural – amphiphilic lipids (especially phospholipids, cholesterol) makes most of cellular membranes and intracellular membrane compartments, myelin in nervous system (esp. sphingolipids, cholesterol)
- signal – lipids and their derivates are responsible for endocrine (steroids), paracrine (icosanoids) and intracellular signalization (phosphatidylinositol phosphates)
- Other – role in embryogenesis, vision (retinoids), antioxidants (vitamins A, E)

Transport of blood lipids

- Lipids are not soluble in water
- Part is transformed into soluble metabolites (ketone bodies)
- Free fatty acids (FFA) are bound to albumin in blood
- Most lipids in circulation form compounds of lipoprotein particles

Lipoproteins

- Specific particles present in blood plasma
- They consist of lipid and protein compounds



Lipid compound

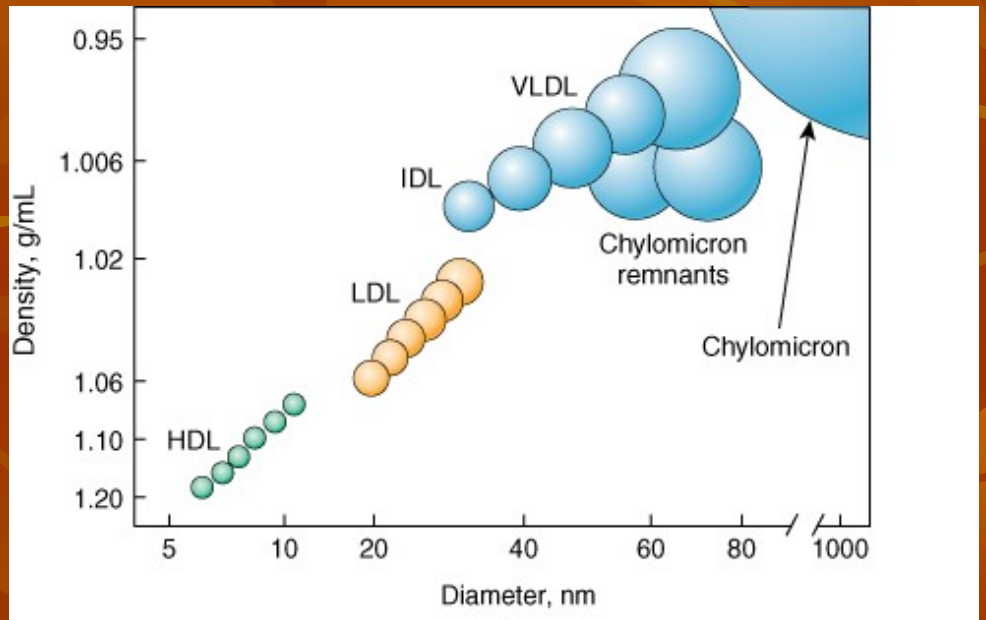
Phospholipids
Cholesterol
Triacylglyceroles (TAG)

Protein compound

Apolipoproteins (Apo) A-M

Lipoprotein classes

- A particle is formed out of amphiphilic coat (apolipoproteins, phospholipids, cholesterol) and hydrophobic core (cholesteryl esters, triacylglycerols)
- In increasing diameter, surface increases with the power of two, volume with the power of three
- That means, the greater the diameter, the bigger is the core compared to the coat
- With the diameter, the ratio of TAG to proteins increases and the density decreases
- Acording to increasing density (and decreasing diameter), lipoproteins can be divided into 5 basic classes– chylomicrons, VLDL, IDL, LDL and HDL

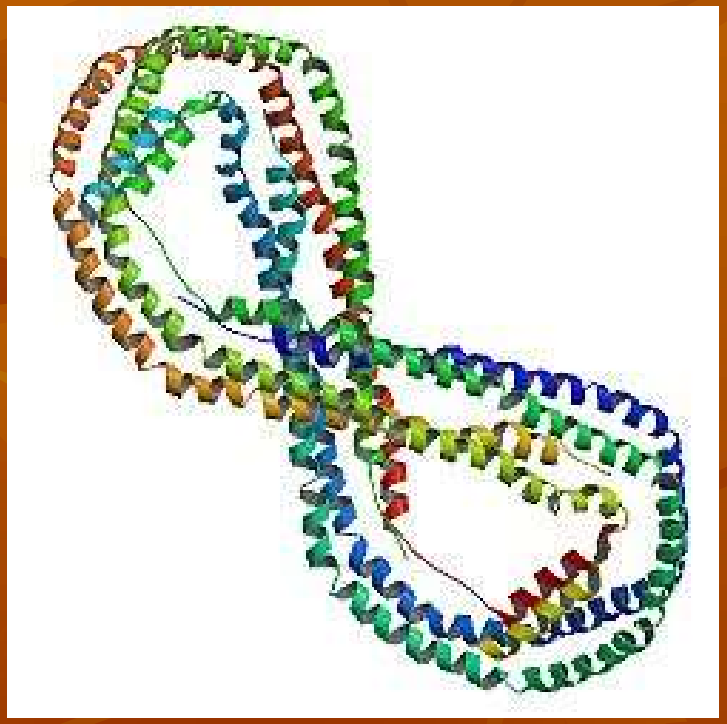


Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Apolipoproteins

- Are situated on the surface of lipoproteins
- Everything what is done with lipoprotein particles is dependent on Apos (i.e.binding specific receptors, induction/inhibition of enzymes and transport proteins)
- They are distinguished by letters A-M
- Some apolipoproteins (A, C and E) can be exchanged between different particles

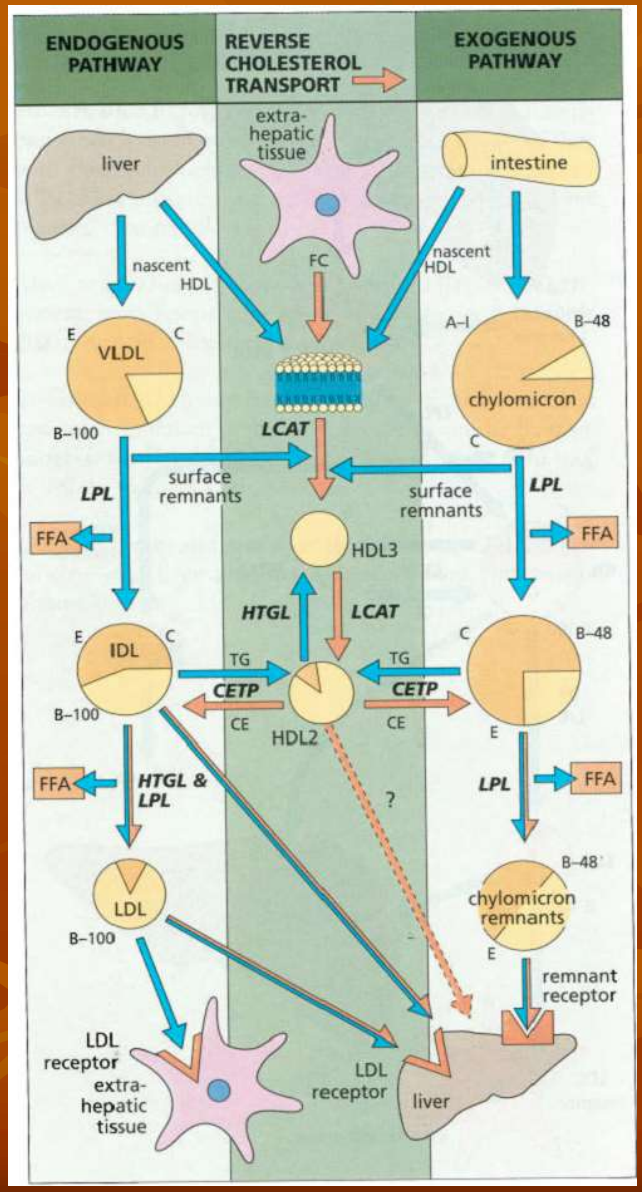
Apolipoprotein A-I



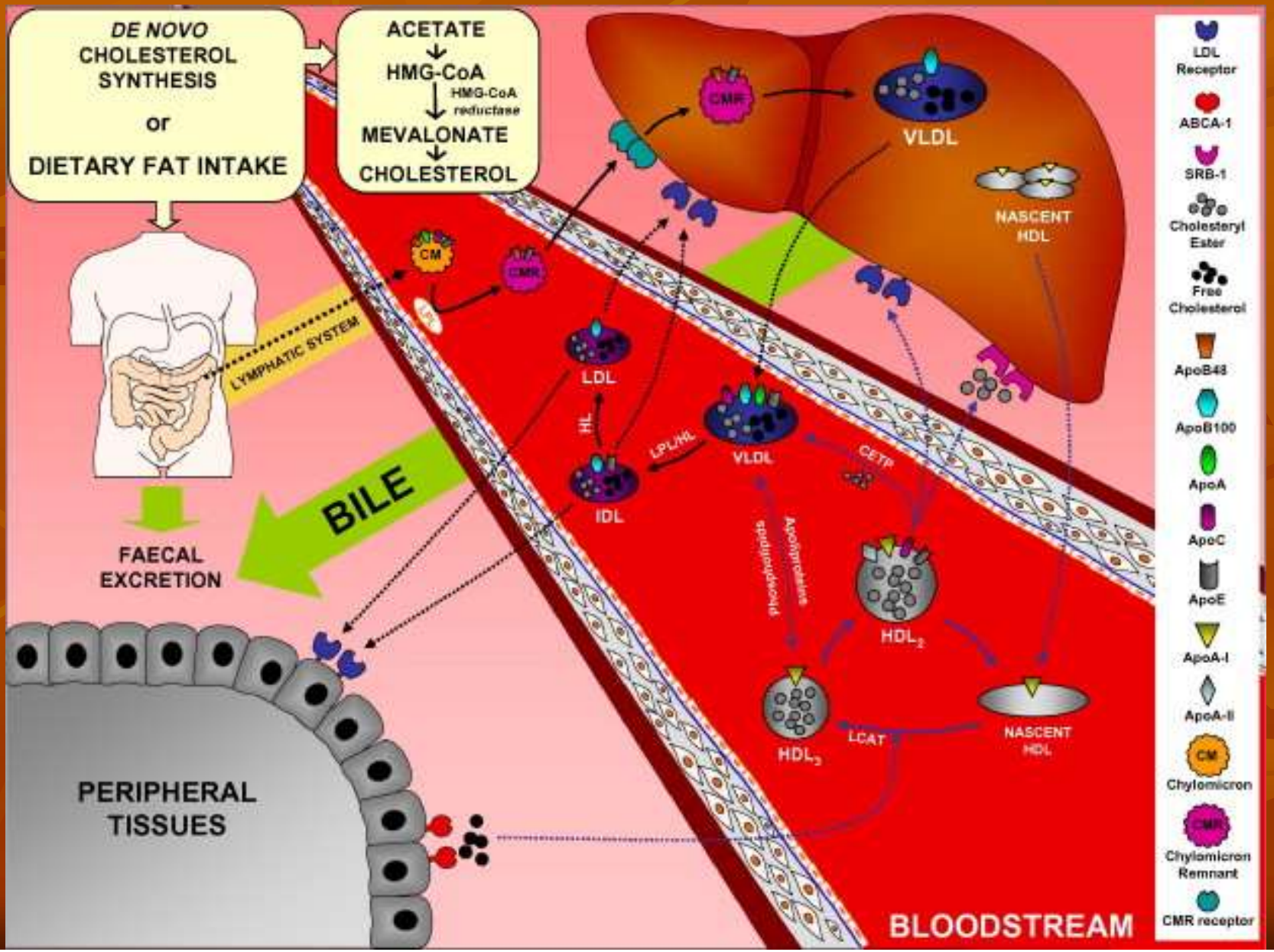
ApoA and ApoC are in fact groups of proteins with similar structure, distinguished by Roman numbers. They, together with ApoE, form a structural family. ApoB occurs in two forms, ApoB-48 and ApoB-100, which are products of the same gene (by mRNA editing, stop-codon can be made, which leads into mRNA translation into shorter ApoB-48).

Metabolism of lipoproteins

- Different lipoprotein classes can exchange both apolipoproteins and lipid compound
- Depending on the composition of protein compound, lipoprotein ensures a specific lipid transport between tissues.
- Lipoprotein metabolism can be divided into three main pathways:
  - Exogennous pathway
  - Endogenous pathway
  - Reverse transport

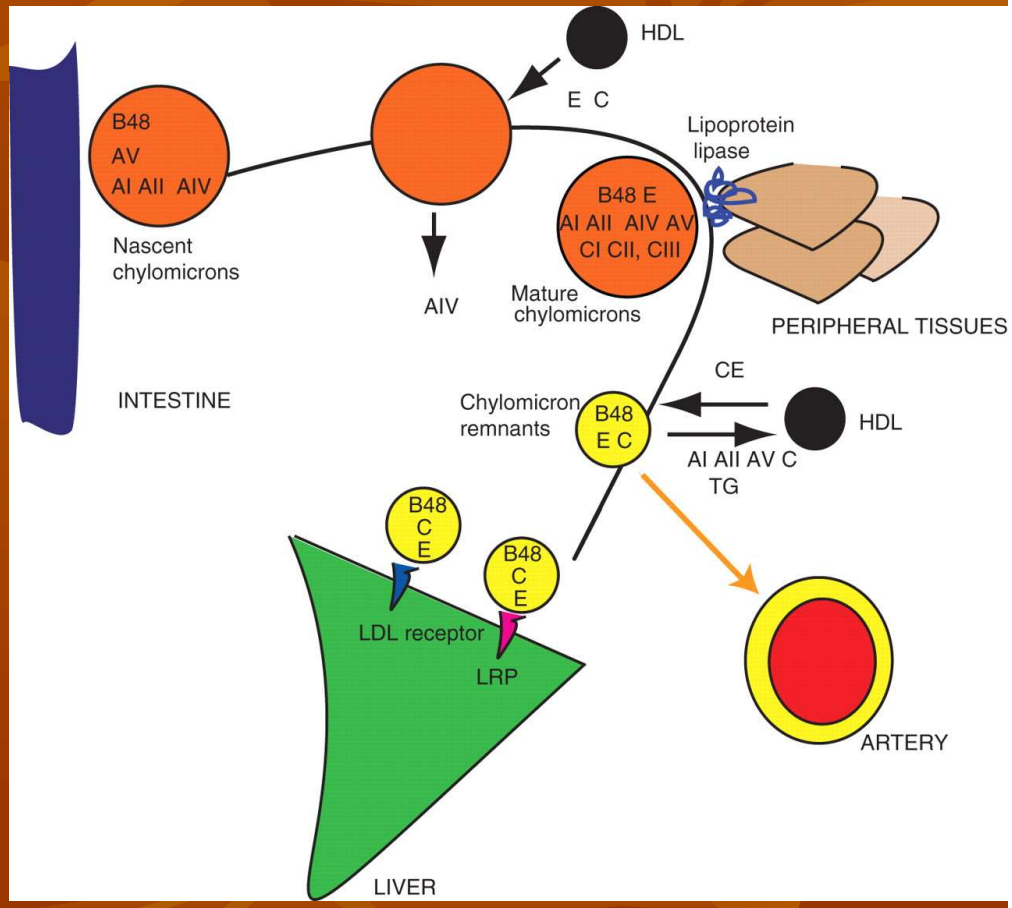


Lipid transport between tissues



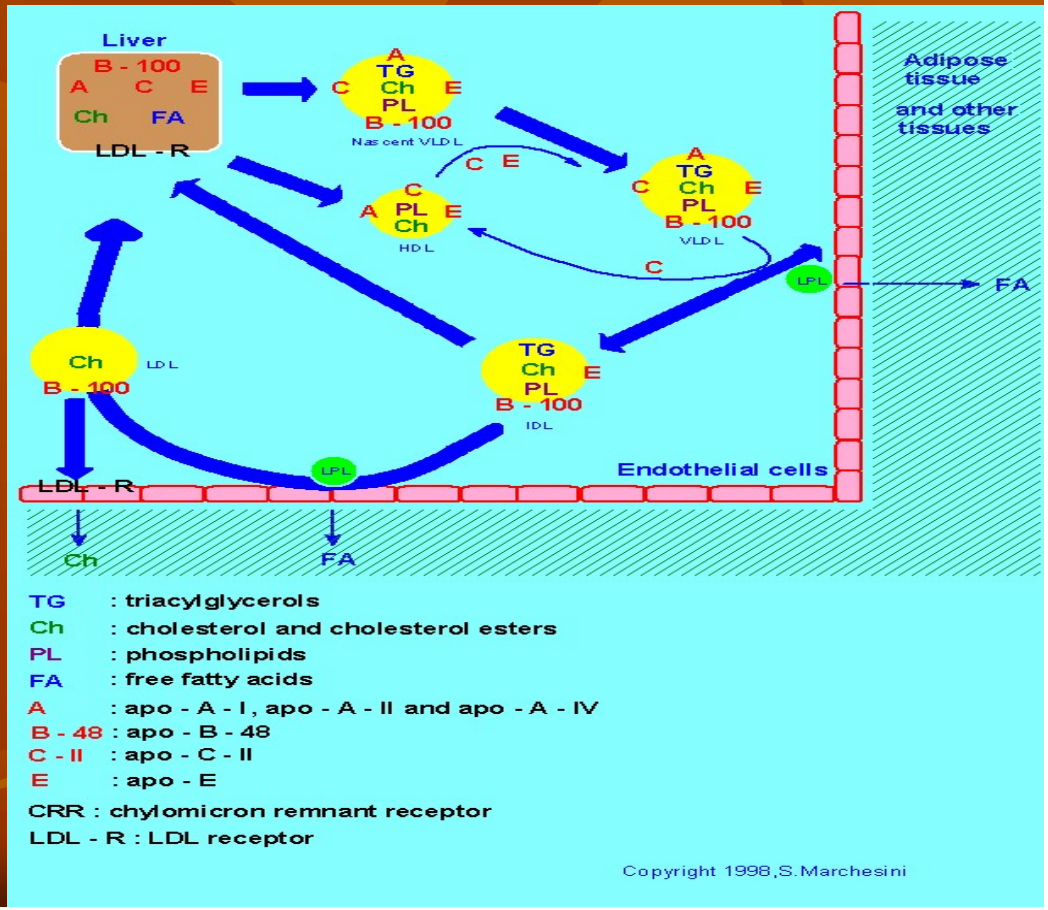
Lipoproteins – exogenous pathway

- Chylomicrons (CMs) are big particles formed in the small intenstine
- They contain all main types of apolipoproteins (A, B, C, E), ApoB-48 is a specific apolipoprotein
- Through lipoprotein lipase (LPL) on capilary endothelium, induced by ApoC-II and inhibited by ApoC-III, CMs get rid of TAG, newly formed FFA get out of capillaries into tissues.
- Most apolipoproteins are, together with TAG, transfered to HDL
- Thus, chylomicrone remnants are formed. Through their ApoE, they bind to LDL or LRP receptors in the liver, where they are internalized



Lipoproteins – endogenous pathway

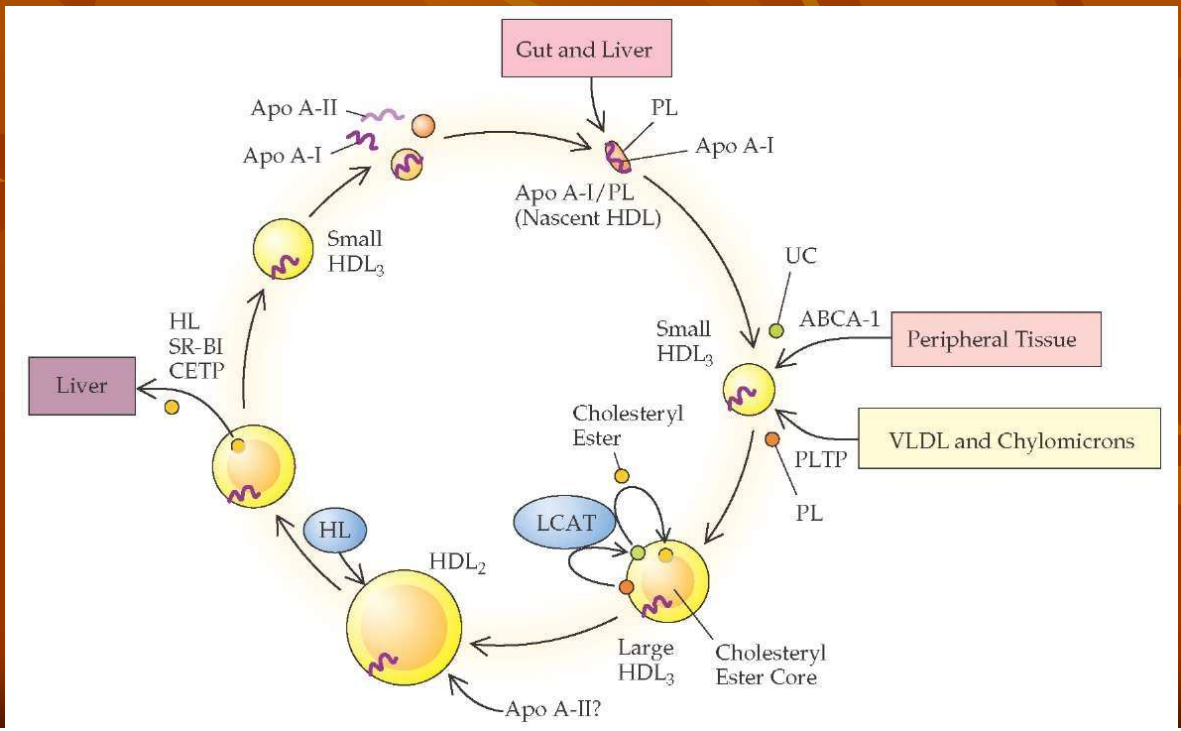
- VLDL are similar to chylomicrons, but they are smaller and contain ApoB-100 instead of ApoB-48
- In peripheral capillaries, they undergo similar modification as chylomicrons. Their remants are called IDL
- Through LPL and hepatic lipase (on the endothelium of hepatic capillaries), they get rid of the rest of lipids (with the exception of cholesterol) and of ApoE
- As a result, LDL particles are formed. They contain only one apolipoprotein, ApoB-100, and dominating lipid compound is cholesterol and its esters
- ApoB-100 binds only to LDL receptor, which is frequent both in liver and peripheral tissues. The process leads to the transport of cholesterol into periphery
- Clearance of LDL is relatively slow. In a consequence, they are prone to oxidation and other modifications
- LDL-receptor is degraded with the help of chaperon PCSK-9



Lipoproteins – reverse transport

- HDL are formed as nascent particles in the liver (and intestine), protein compound - ApoA-I – is dominant
- Using ABCA-I transporter, ApoA-I is capable of reverse transport of cholesterol out of peripheral tissues (by other mechanisms, also ApoA-II a ApoE).
- Apolipoproteins (except of Apo-B), TAG (in exchange for cholesterol esters – CETP) and phospholipids are transferred from other lipoproteins
- Larger, lipid-enriched forms of HDL are formed, using LCAT, cholesterol is esterified.
- Thanks to binding of ApoA-I to SR-BI receptor in liver (and steroidogenic issues), HDL „unloads“ cholesterol and gets back into .circulation. TAG and phospholipides are degraded by hepatic lipase

- If modified HDL contains ApoE, it can be internalized by binding its receptors
- ApoA-I and ApoA-II bind to their receptor in kidney and can be excreted, they can return into circulation by binding protein cubilin

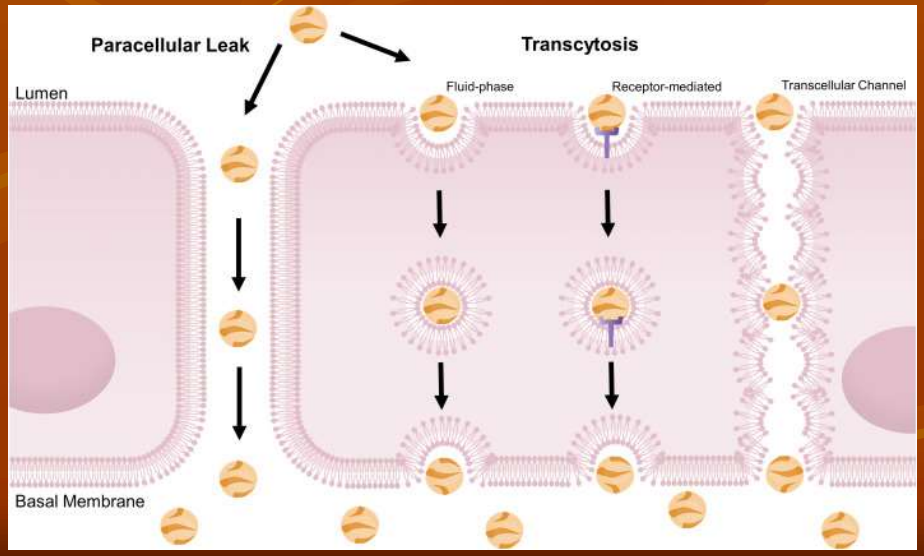


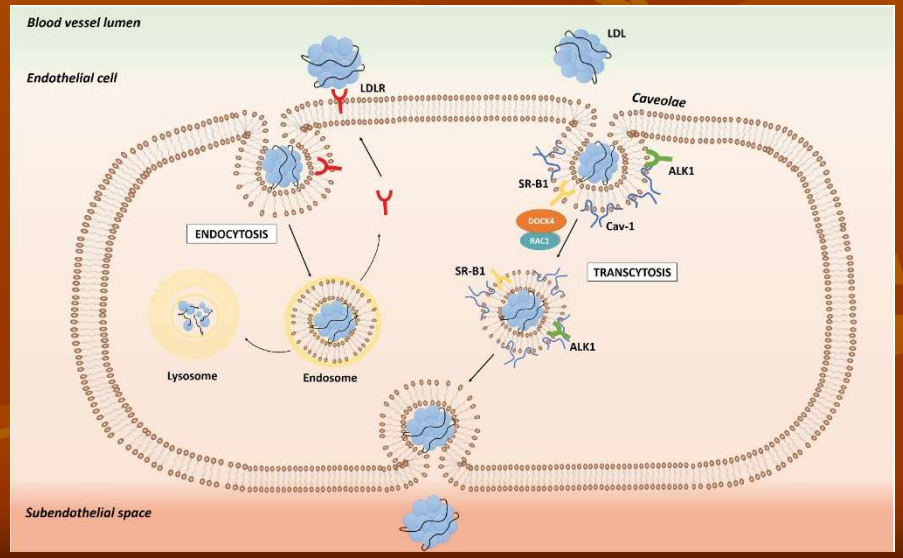
Atherogenic a antiatherogenic lipoproteins

- Antiatherogenic
  - HDL (especially nascent)
- Atherogenic
  - LDL – in subendothelial space and other tissues (gingiva) they undergo oxidative modification, oxLDL are not recognized by LDL-R, but by macrophage scavenger receptors. Formation of oxLDL is easier, when the diet is rich for oxidated lipids. Subgroup of „small dense LDL“ is especially atherogenic
  - Chylomicron remnants and IDL – they bind scavenger receptors without modifications
  - Other atherogenic modifications
    - glycation, glucooxidation, carbamylation (urea), aggregation
  - Lipoprotein (a)

Aterogenic lipoprotein penetration

- They must be sufficiently small (i.e. not chylomicrons and nascent VLDL)
- Endothelium: transcellular transport (vesicles) and paracellular transport („leaky junctions“)
- Scavenger receptors SR-B participate in transcellular transport (on the other hand, the binding to LDL-receptor supports lipoprotein internalization – role of previous atherogenic modifications)





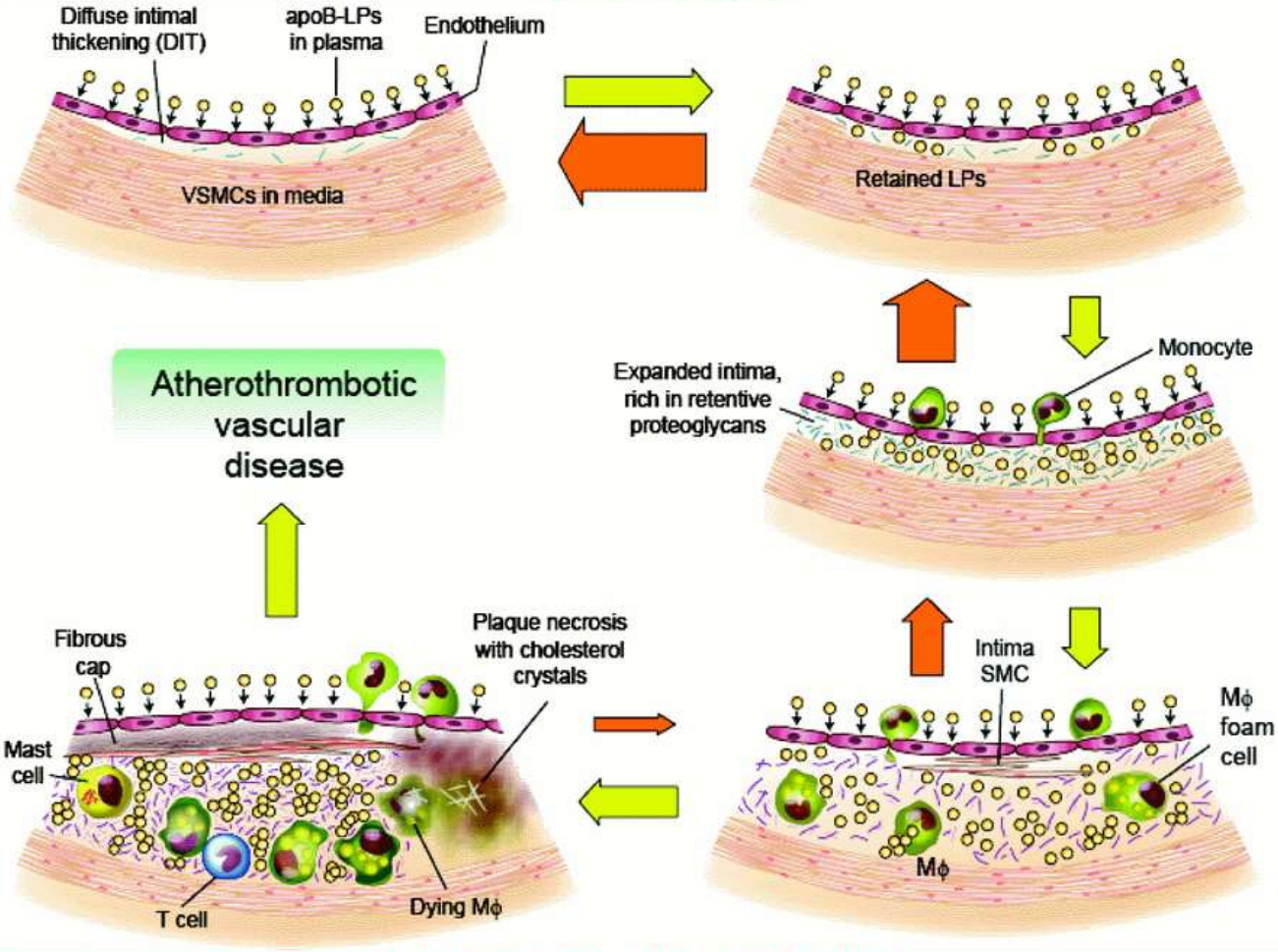
Retention in subendothelial space

- Vesicular transport through the endothelium goes both ways
  - i.e. lipoproteins are rapidly removed from the subendothelial space
- Binding to subendothelial glycosaminoglycans → retention
- Further modification (oxidation / glycation / aggregation…) → binding to macrophage scavenger receptors (“toxic lipoproteins“)

- Pre-lesional susceptible area of the arterial wall with diffuse intimal thickening (DIT)
- Lowering plasma apoB LPs and decreasing risk factors will prevent future vascular disease

- Early lipoprotein retention
- Lowering plasma apoB LPs and decreasing risk factors will readily promote removal of atherogenic components and prevent maladaptive responses and future disease

PRE-TEENS



TEENS

- Early responses to LP retention, *e.g.*, monocyte entry
- Lowering plasma apoB LPs and decreasing risk factors will readily promote removal of atherogenic components and prevent further responses and future disease
- Future strategies to prevent LP retention are likely to be most feasible up to this stage

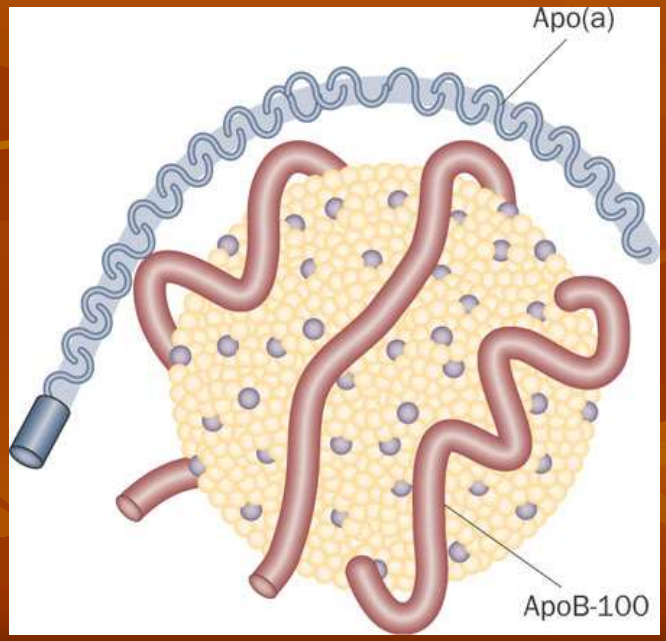
TWENTIES AND BEYOND

- Advanced responses to LP retention, including maladaptive inflammation, Mϕ death, and plaque necrosis
- LP retention continues to accelerates
- Lowering plasma apoB LPs and reducing risk factors can promote removal of atherogenic components and promote regression, but reversal is more difficult and prolonged, and vascular disease may still develop

- Continued responses to LP retention, e.g., Mϕ foam cell formation and SMC migration
- LP retention starts to accelerate
- Lowering plasma apoB LPs and other risk factors can still promote removal of atherogenic components, promote regression, and prevent further responses and future disease

Lipoprotein (a)

- Small particle containing ApoB-100 and Apo(a)
- Its elevated concentration is usually inherited (different genetic substrate)
- It is one of most frequent causes of infarctions in young age (<20 years)
- Its physiological function is unclear, Apo(a) is similar to plasminogene and tPA and binds fibrin. Probably, it is used in a repair of damaged vessel wall.



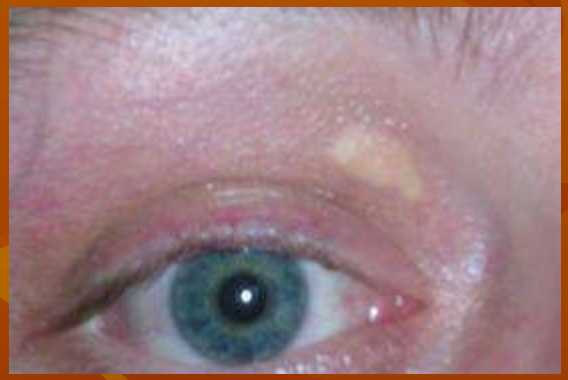
Dyslipidemias

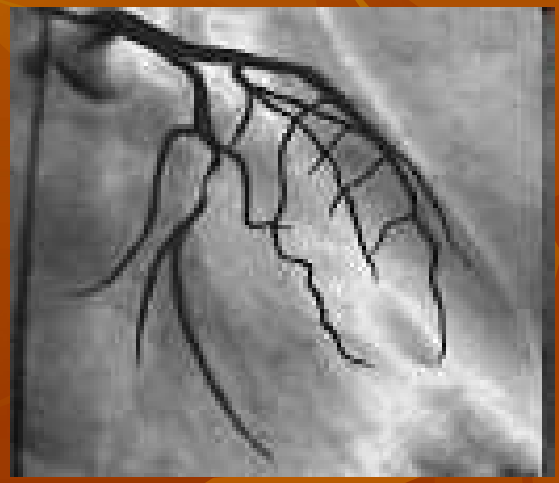
- Disorders of lipid metabolism
- They are not necessarily connected with obesity (but often they are)
- Typically ↑total cholesterol, ↑LDL-cholesterol, ↓HDL-cholesterol and ↑TAG
- Sometimes, only some components are present (isolated hypertriacylglycerolemia, isolated hypercholesterolemia)
- Hyper-TAG is in 90% connected with ↓HDL-C (phospholipid and TAG transfer to HDL leads to rapid degradation). Isolated ↓HDL-C (hypoalfalipoproteinemia) is rare
- LDL concentration is sometimes not measured directly, but is estimated using Friewald formula:

  LDL-C = total chol. – HDL-C – (TAG/2,2)

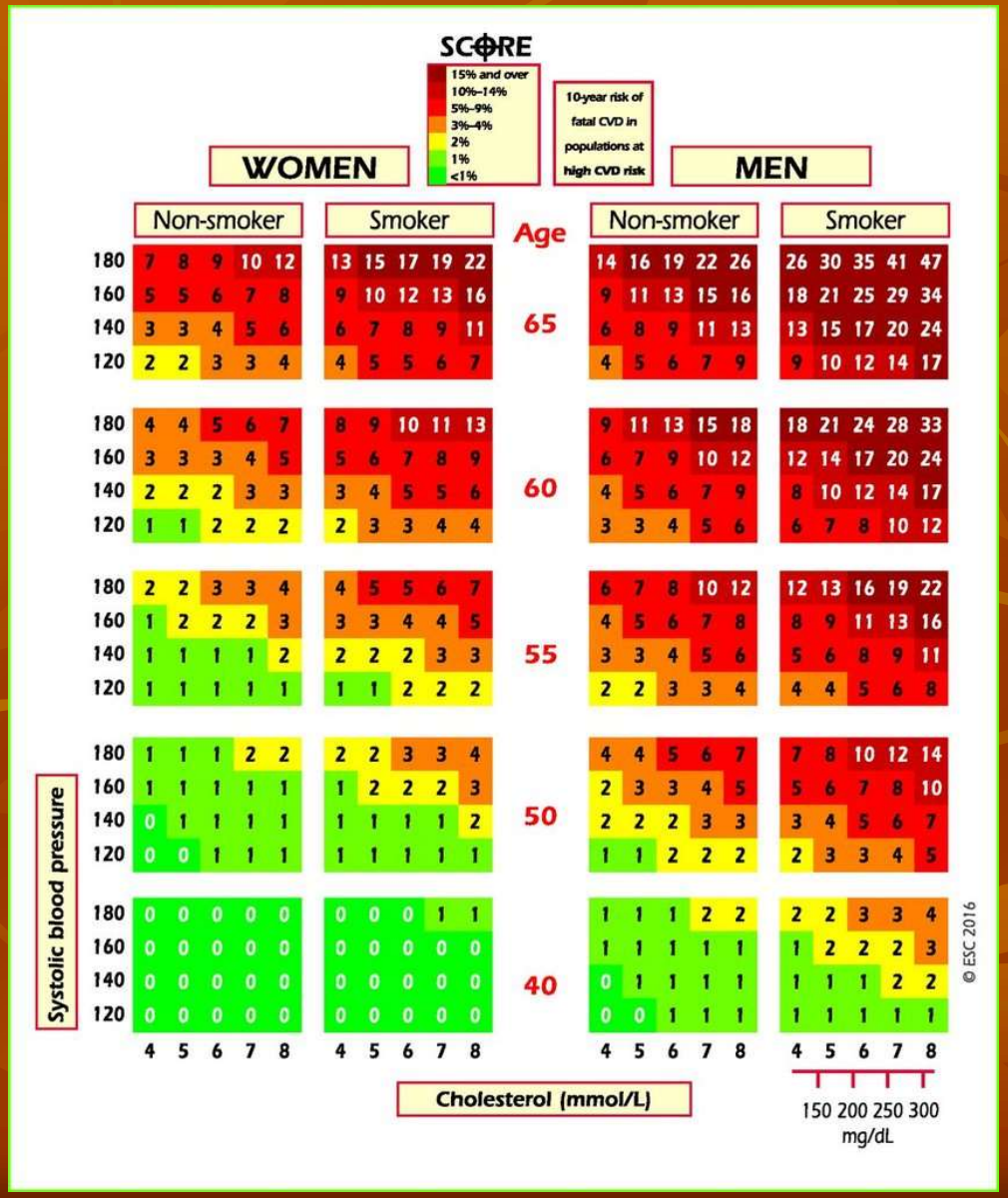
Clinical manifestation of severe hyperchlesterolemia

- Xanthelasmas, xanthomas of tendons
- Arcus corneae
- Polyarthritis, tendinitis
- Accelerated aterosclerosis



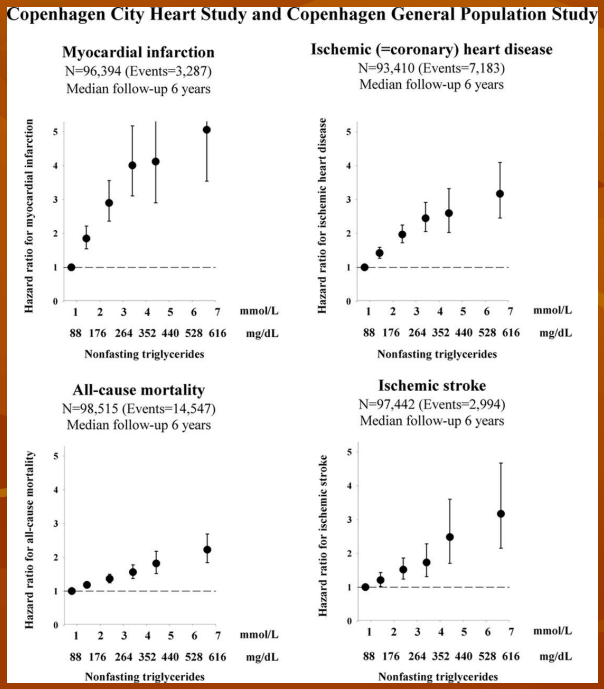


Cholesterol and cardiovascular risk (SCORE)



Consequences of elevated TAG

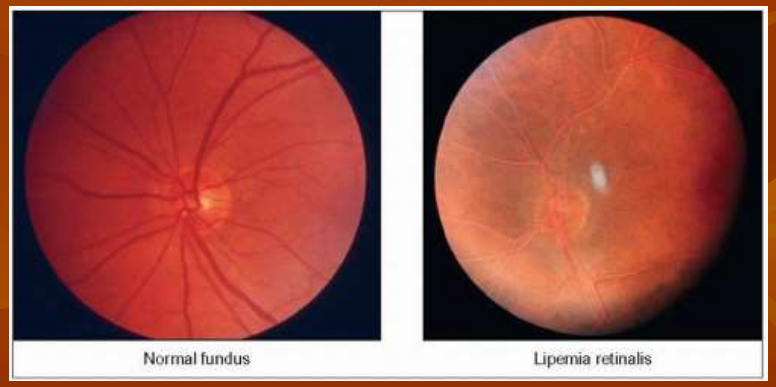
- Cardiovascular risk sharply increases up to approx. 4 mmol/l, but does not substantially change further (contrary to overall mortality)



- In high levels of TAG the TAG-rich lipoprotein particles increases in size, but not in number
  - Large lipoproteins do not pass into vascular intima, but may obstruct the microcirculation – see further

Other complications of hyperTAGemia

- Acute pancreatitis
  - During pancreatitis development in hyperTAGemia, cytotoxic damage of acinar cells by unesterified FFA takes place
- Lipemia retinalis, retinal vein thrombosis
- Xantelasmas



Desired values of blood lipids

Czech atherosclerosis society recommendations, 2007

Patients	Without complications	Risk factors (e.g. DM2, DM1 with mikroalbuminuria)	Presence of atherosclerosis
Lipid	mmol/l	mmol/l	mmol/l
Cholesterol	<5,0	<4,5	<4,0
LDL-C	<3,0	<2,5	<2,0
HDL-C	>1,0 (men), >1,2 (women)		
TAG	<1,7		

Highly above the optimal values, but realistically achievable

Primary and secondary dyslipidemias

- Primary
  - More frequent
  - Usually multifactorial, polygenic heritability, usually as a component of „metabolic syndrome“ (syndrom X, Reaven syndrom)
  - Rare monogenic forms – usually mutations of apolipoproteins or their receptors
- Secondary
  - They are a consequence of other disease
  - E.g. diabetic dyslipidemia, nephrotic syndrome
  - They also may be a component of metabolic syndrome (the boundary between primary and secondary dyslipidemia is not sharp)

Frederickson classification of primary dyslipidemia

- Based on dominating fraction
- Type I - ↑ chylomicrons
- Type IIa - ↑ LDL
- Type IIb - ↑ LDL and VLDL
- Type III - ↑ chylomicron remnants and IDL
- Type IV - ↑ VLDL
- Type V - ↑ VLDL and chylomicrons

- Simple phenotypic classification: cholesterol predominance vs. mixed vs. TAG predominance

Familial hyperlipoproteinemia type I

- Very rare (1/1000000), endemic in Québec
- Hypertriacylglycerolemia with high concentration of circulating chylomicrons
- Defect of LPL (LPLD) or deficiency of ApoC-II
- TAG up to 50mmol/l, manifestation in the childhood, often through acute pancreatitis or retinal thrombosis
- In serious cases, there is a necessity of plasma transfusion

Familial hypercholesterolemia (FH)

- Frequent, prevalence 1:500
- It is caused by defects of LDL-receptor, more rarely ApoB-100 (different sites of genes)
- Phenotype IIa, TAG are not very much elevated (lipoproteins rich by TAG contain also ApoE, so they can use alternative ways of degradation, while LDL clearance is dependent on ApoB-100 and LDL receptor)
- More serious homozygous, less serious heterozygous form

FH - complications

- In heterozygotes MI in 3rd -5th decade, in homozygotes before 20 years of age
- Treatment: plasmapheresis (extracorporal precipitation of LDL by heparin), in serious case, it is an indication for liver transplantation

Polygenic hypercholesterolemia (IIa)

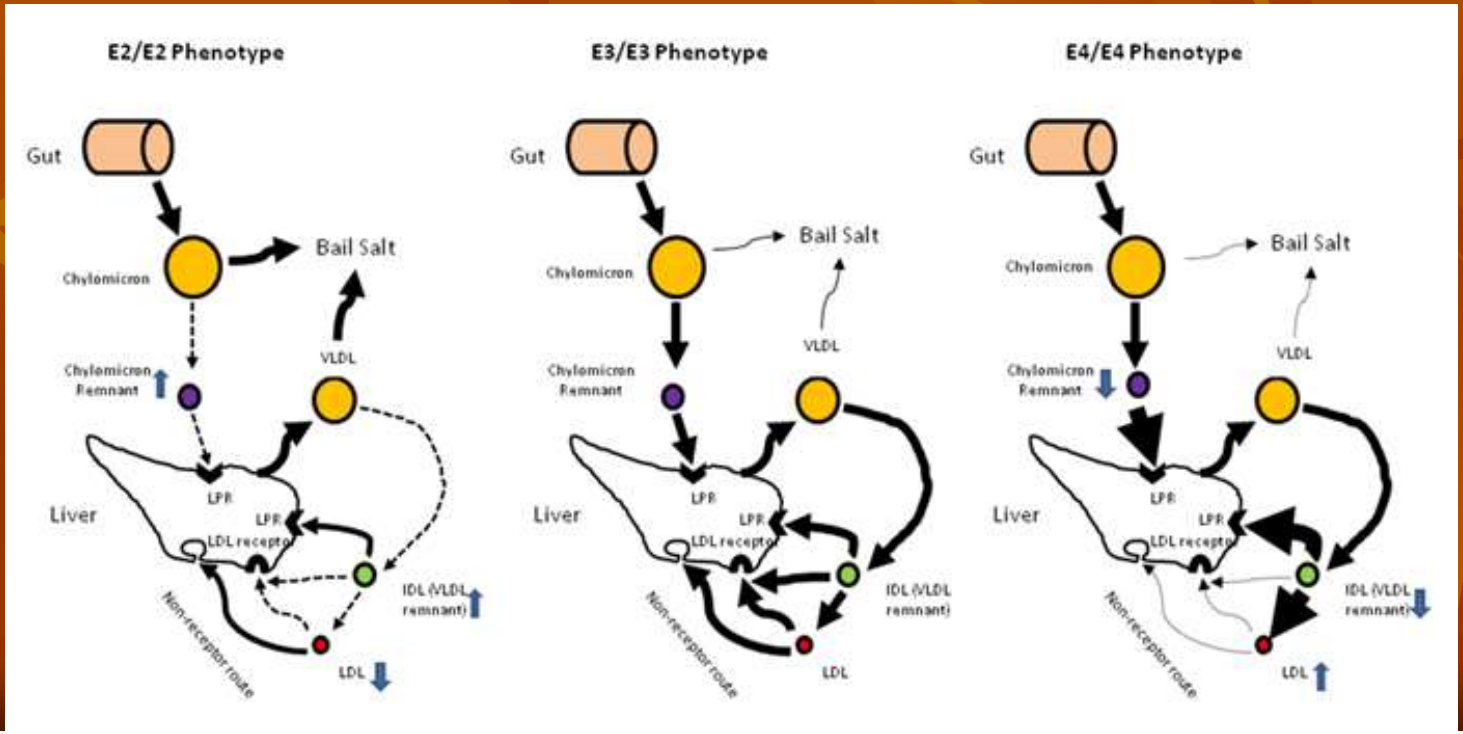
- Combination of „disadvantageous“, cholesterol-raising common polymorfisms in genes for ApoB, ApoE, PCSK9, LCAT, CETP and other proteins together with environmental factors
- Out of environmental factors, namely high caloric intake, high amounts of saturated fats and cholesterol in diet, little physical activity
- Role of fetal programing and early postnatal development
- Clinically, there is also higher susceptibility for gallstones formation

Combined hyperlipidemia (IIb)

- It is usually caused by ApoB overproduction in the liver, often elevated ApoC-III
- ApoB/ApoA-I ratio is one of the most important risk factors for heart and brain atherosclerosis
- Variable fenotype, usually together with insulin resistance
- Monogenic forms are usually caused by variants of the genes for ApoC-II, Apo-C-III or CETP
- More frequent polygenic form is usually part of the metabolic syndrome, heritability cca 20-30% (which is quite low - „**acquired combined hyperlipidemia**“), enviromental risk factors are basically the same as in polygennic hypercholesterolemia

Familial hyperlipoproteinemia type III (familiar dysbetalipoproteinemia, FDBL)

- ApoE occurs in 3 functionally different isoforms, E2, E3, E4, which are coded by three common alleles ε2, ε3 a ε4 (in most European populations, their frequency is ~5-10%, 70-80%, 10-20%)
- Izoform E2 binds badly to LDL-receptor, however ApoE2-containing lipoproteins can be degraded by alternative pathways
- In cca 5% of ε2/ε2 homozygotes, the degradation is impaired as a result of their independent genetic defect and/or metabolic disease (e.g. DM2)



ApoE and FDBL

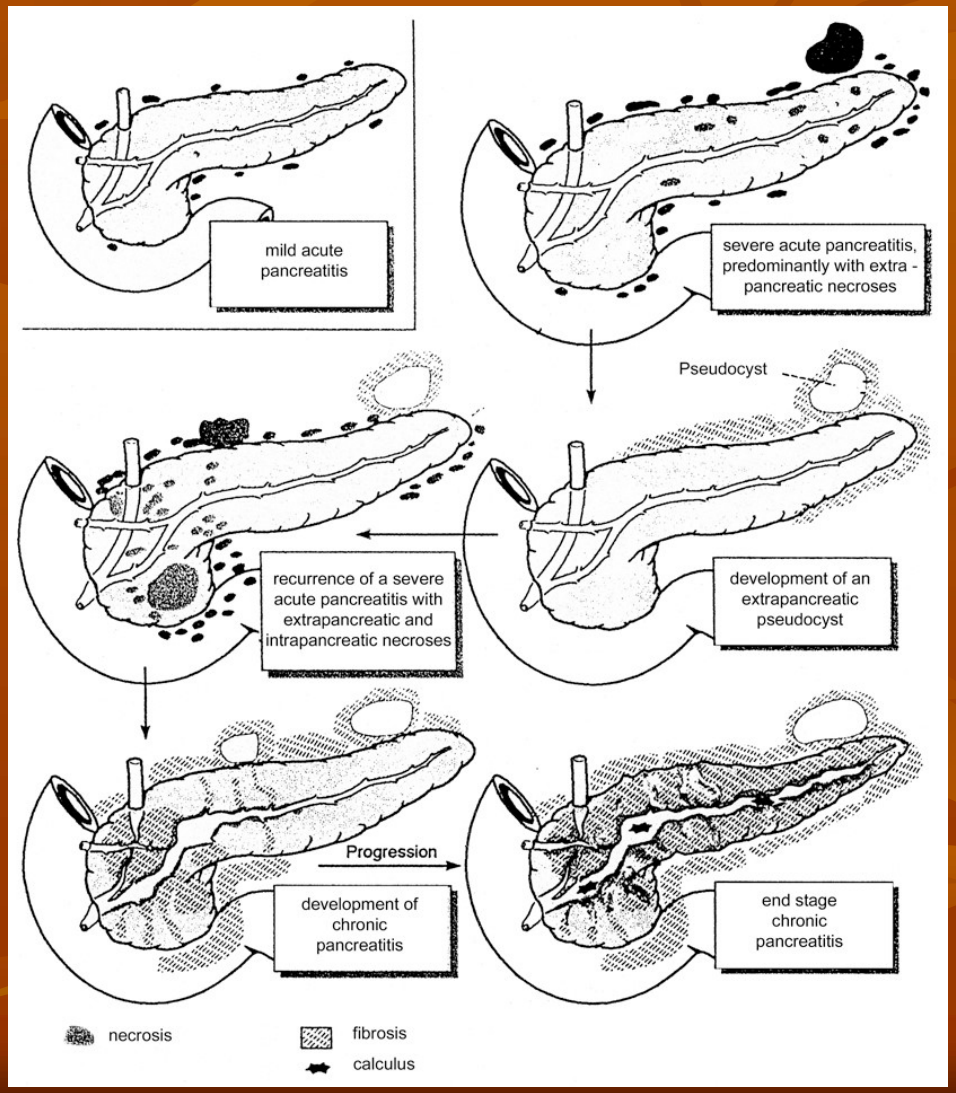
- This leads into the disease known as familial dysbetalipoproteinemia (FDBL, FH III)
- FDBL can be caused also by rare mutations of ApoE, in these cases, it is inherited in dominant fashion with high penetrancy
- Both TAG (more) and cholesterol (less) is present, clinically xanthomas and precocious atherosclerosis
- Most ε2/ε2 homozygotes are normo- to hypolipidemic, in its heterozygous form, the allele is protective against the onset of atherosclerosis and its development
- Allele ε4 mildly increases the risk (and it markedly increases the risk of late-onset neurodegenerative diseases; because of its preferential binding to large lipoproteins it is insufficient for transferring lipids into neurons during their repair. The transport of lipids in the nervous system uses small, HDL-like particles).

Polygennic hypertriacylglycerolemia

- Common, phenotype IV
- Genetically heterogeneous disease
- Polygennic, causes include LPL deficiency, overproduction of VLDL, deficiency of ApoA-V (inhibits chylomicron and VLDL production)
- It often occurs together with diabetes and obesity, but it has probably different genetic background – however the manifestation of hyperTAGemia is much more serious in a coincidence with diabetes
- The onset is usually provoked by alcoholic or nutritional excess
- Clinically often manifestated by serious forms of pancreatitis

Familial hyperlipoproteinemia type V

- Basically intermediate type between 1 and 4
- As well as in all hyperTAGemias, there is a susceptibility to acute pancreatitis (esp. in TAG>10 mmol/l). Sometimes, chronic pancreatitis can occur



Secondary dyslipidemia

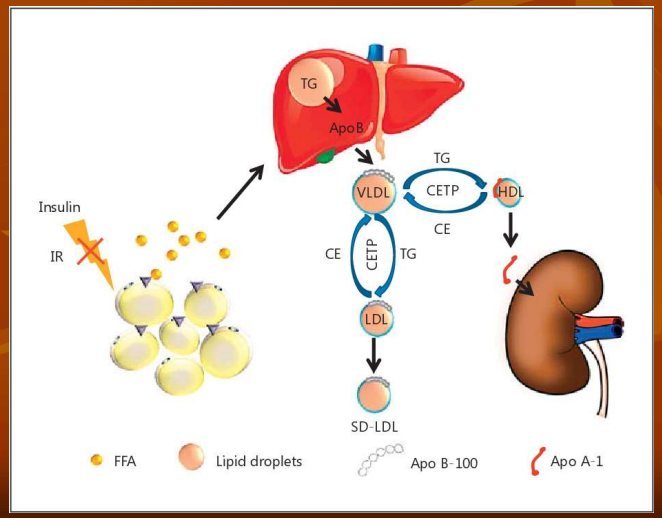
- ↑ cholesterol
  - cholestasis
- mixed
  - Kidney disease
  - Hypothyreosis
  - Obesity (TAG predominance)
- ↑ TAG
  - diabetes mellitus
  - Alcohol abuse

Diabetic hypertriacylglycerolemia

- Lack of insulin and insulin resistance leads into enhanced lipolysis in adipocytes and FFA formation
- In the liver, FFA can be used for TAG synthesis. TAG become part of VLDL.
- Moreover, insulin directly stimulates the production of LPL (and maybe also hepatic lipase). Activity of these enzymes is then lower in DM and that helps ↑VLDL (secondarily also ↓HDL)
- Non-esterified FFA also induce cytolysis of pancreatic β-cells

Kidney diseases and dyslipidemia

- Nephrotic syndrome
  - Loss of LPL activators (↓ ratio ApoC-II / ApoC-III) → ↑TAG
  - ↓ HDL-cholesterol / total cholesterol
  - LCAT loss → impaired transport of cholesterol into HDL
  - ↑ PCSK-9 hepatic expression → ↓LDL-R → decreased clearance of LDL (mediated possibly by increased TNF-α from damaged podocytes)
- CHRI
  - ↑Apo-CIII
  - replacement of ApoA-I in HDL for serum amyloid A
  - ↑PCSK-9 → ↑ small dense LDL
  - CHRI often follows diabetes – see above



Strategies of the treatment

- Lifestyle adjustment, physical aktivity (HDL)
- Lowering of caloric intake, low-lipid (in ↑cholesterol) and low-saccharide (in ↑TAG) diet – more efficient in secondary dyslipidemia
- Pharmacotherapy (clinical efficiency in a range of years!)
  - statins (they inhibit cholesterol synthesis)
  - fibrates, niacin (they lower VLDL synthesis)
  - resins, ezetimib (they lower intestinal absorption of lipids)
  - PCSK- inhibitors (they prevent internalization of hepatic LDL-R)
- In serious case aphaeresis, transfusion of blood plasma, exceptionally liver transplantation

Most expensive cure of history

- Alipogene tiparvorec (Glybera)
- Adenoviral vector with a gene for LPL
- Indication: familial hyperlipoproteinemia type I (LPLD)
- EMA approval in r. 2012 after approx. 10 years of testing – historically first gene therapy
- Controversial expressions of EMA commitees (weak evidence about clinical efficiency with low power of a test in a rare disease)
- 60 i.m. injections per a therapy – total price 1 mil. USD
- First doses came to market in 2015
- Several tens of patients during a period of testing, 1 following the approval (2015 – 2017)
- 2017 the request for prolongation of EMA registration was withdrawn by a company

Thank you for your attention