LIPOPROTEINS metabolism and pathophysiology

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Function of lipids

energy substrate
lipid microenvironment
insulation
membrane component
substrates for further metabolization
modifications of proteins/saccharides

Lipid transport

postprandial phase *– digestion of lipids from the diet*

fasting state *– delivery of lipids to the tissues in need*

Lipid digestion

gastro-salivary phase

Lingual lipase (pH optimum 3.5-6)

secreted by von Ebner´s glands, acts also in stomach

TAG → 1,2-DAG, 2,3-DAG + FFA

Gastric lipase (pH optimum 3.5-5.4)

TAG → DAG + FFA/glycerol + FFA

significant contribution

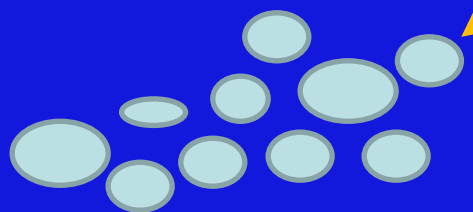
to the digestion (10-30 % of TAG)

gastric movements

peristaltic movements

grinding of the antrum

1. emulsification of lipids



Lipid digestion

intestinal phase - pancreatic lipases I

Pancreatic lipase (pH optimum 6.5-9)

at the interface of lipid droplets

(facilitated by BA micellarization of products)

TAG → 2-MAG + FFA

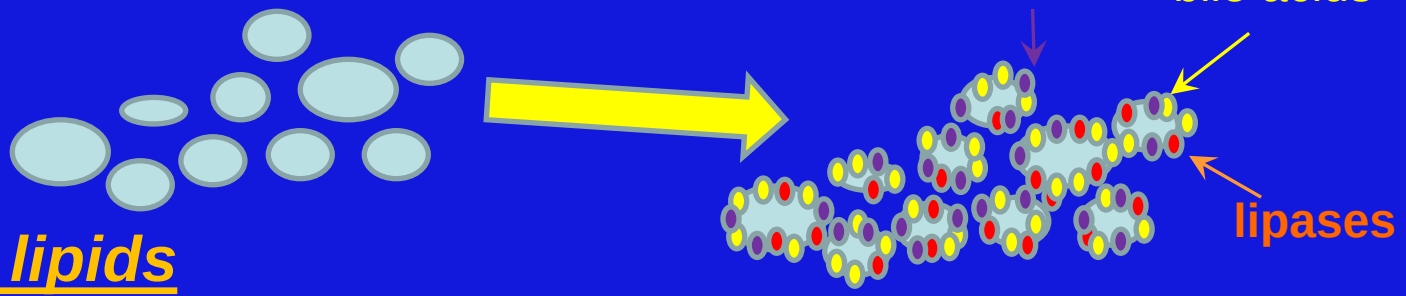
Colipase

exposes the active site of pancreatic lipase

Pancreatic phospholipases PLA_1, PLA_2

activated by trypsin

PL → 2-lysoPL, 1-lysoPL + FFA



2. lipolysis of lipids

Lipid digestion

intestinal phase - pancreatic lipases II

Cholesteryl ester hydrolase (BA activated lipase)

CE → FC + FFA

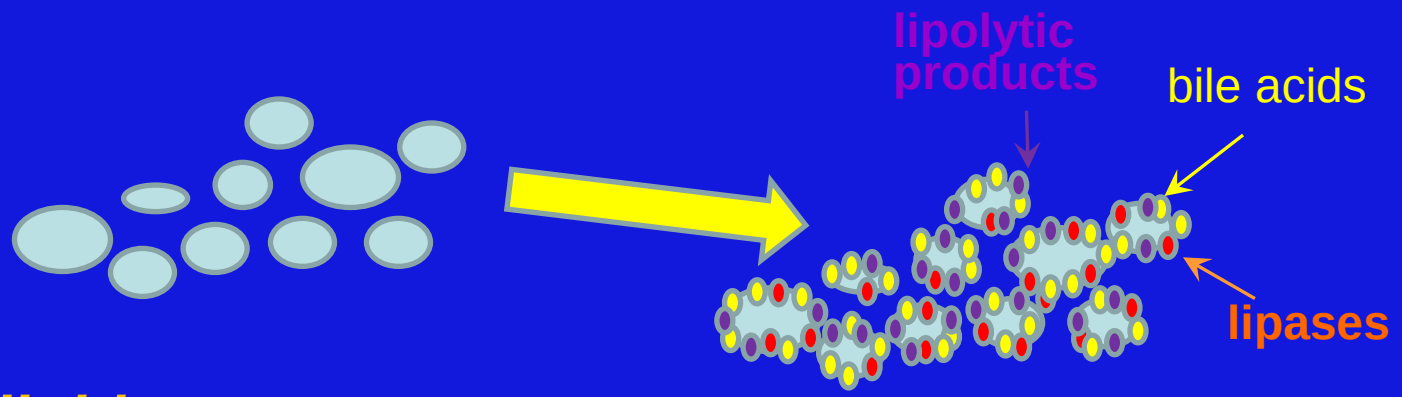
other substrates: retinyl esters, TAG, PL, Cer

alkaline sphingomyelinase

SPH → Cer + P-choline

neutral ceramidase

Cer → sphingosine + FFA

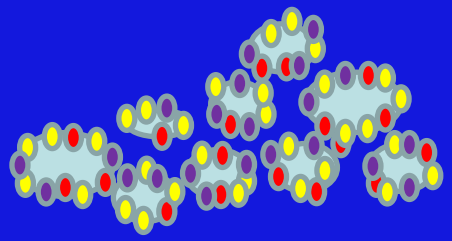


2. lipolysis of lipids

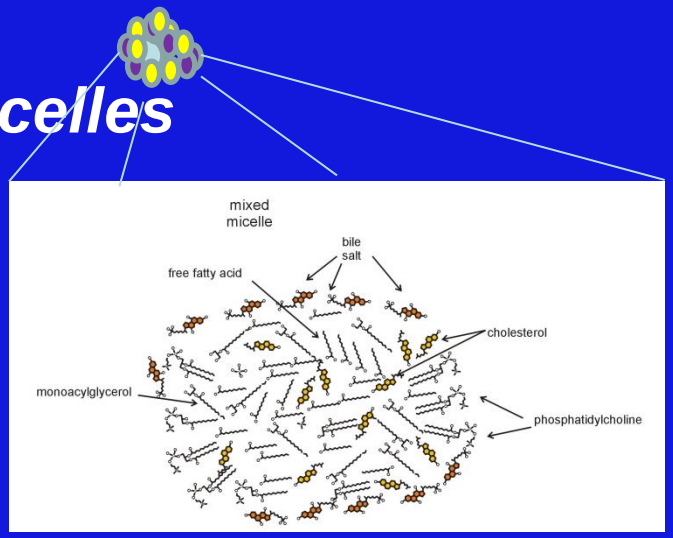
Lipid digestion

intestinal phase - formation of micelles

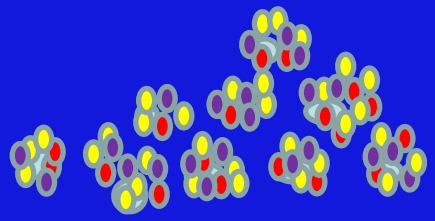
BA and PL displace lipolysis products from the water-oil interface



mixed micelles



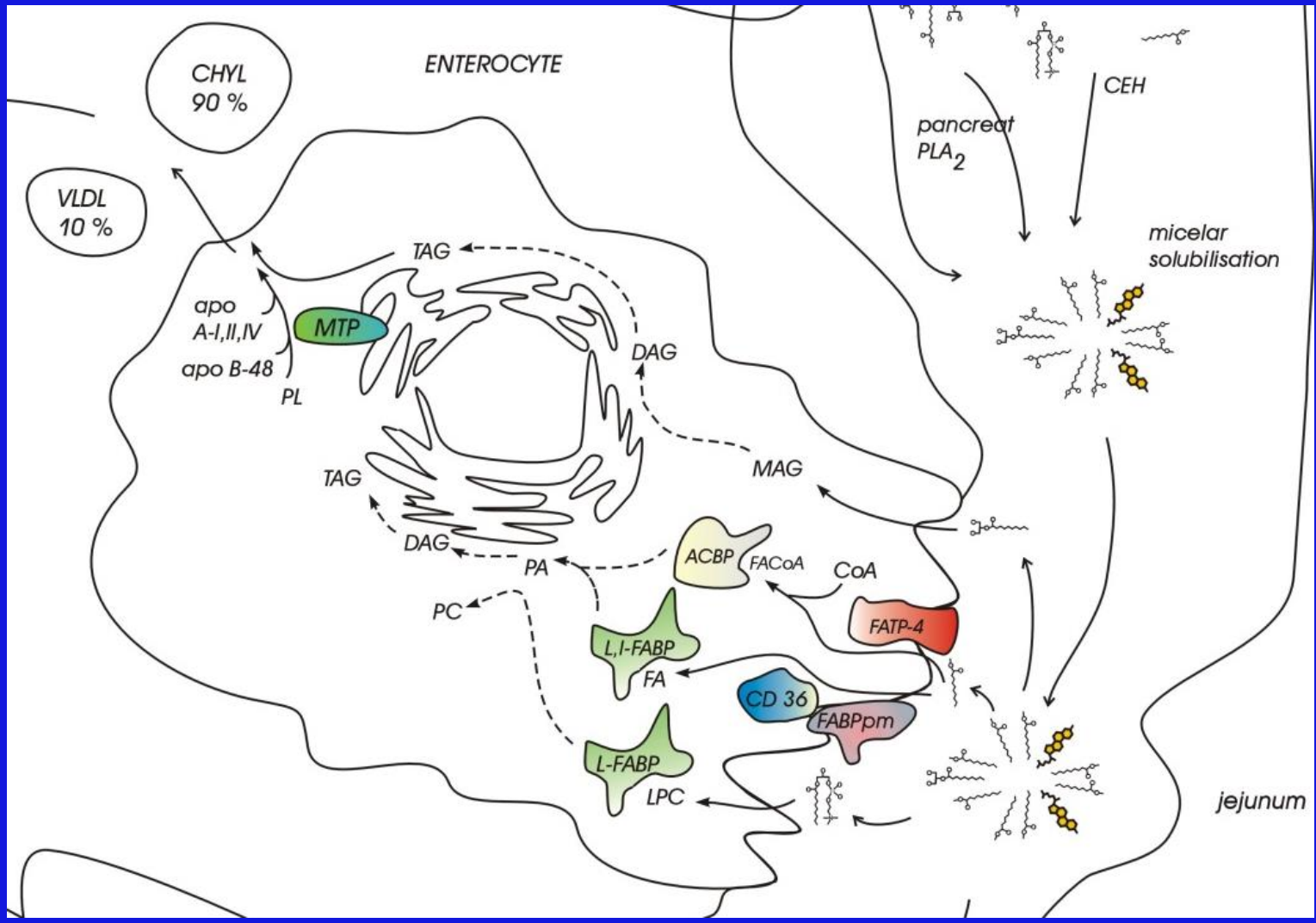
further lipolysis by lipases



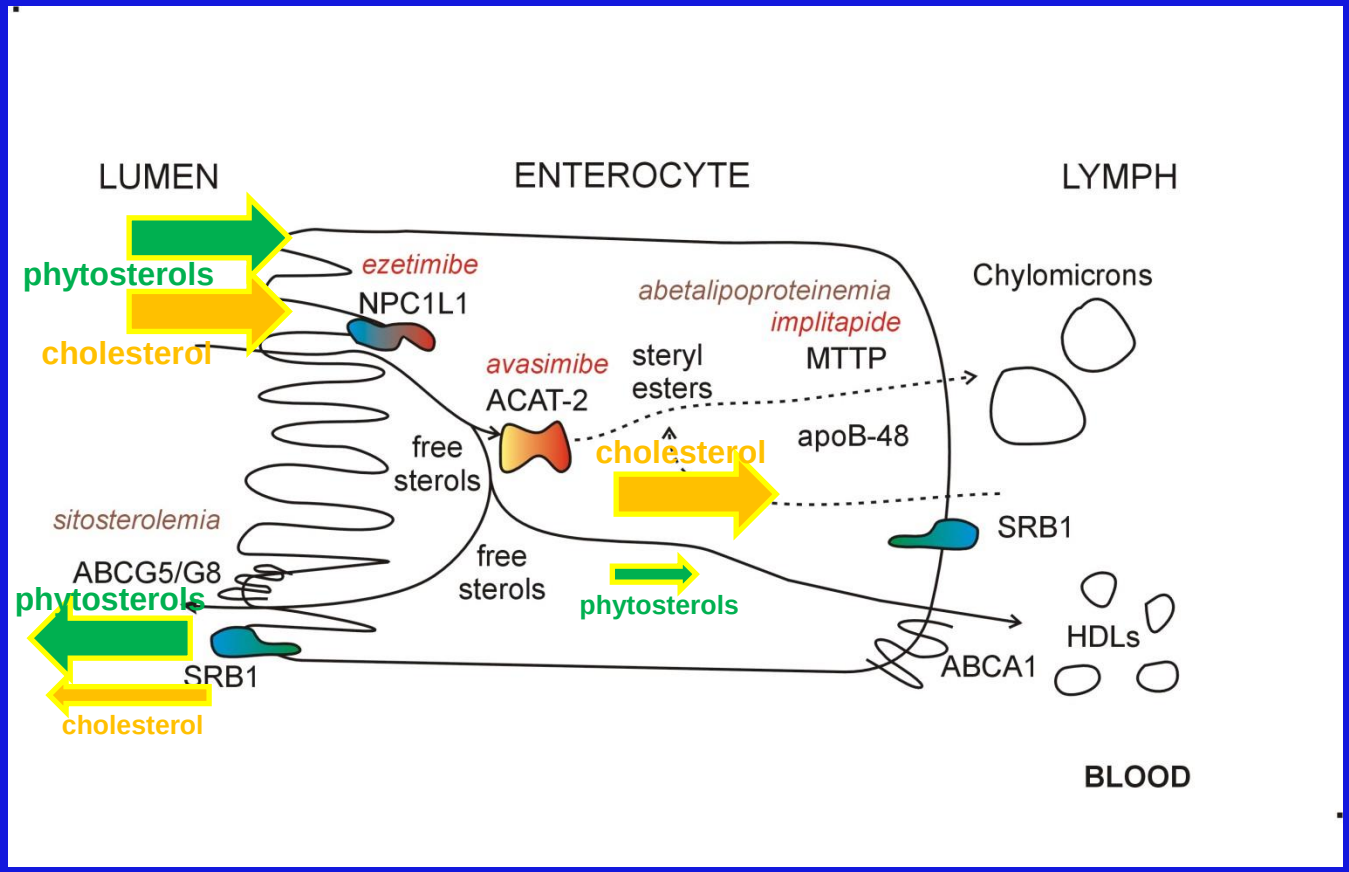
<u>3. solubilization of lipids</u>

4+5. translocation and intracellular metabolism of lipids

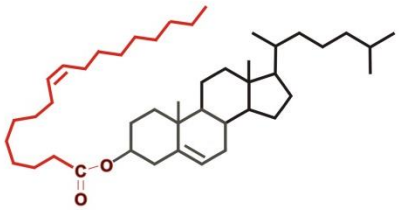
Lipid absorption – fatty acids, PL



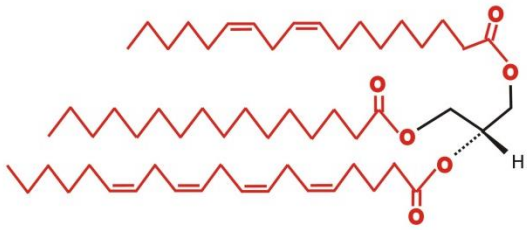
Lipid absorption – sterols



Important lipid classes

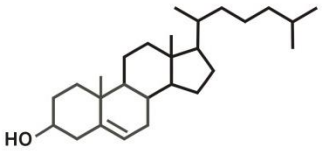


cholesteryl ester, CE

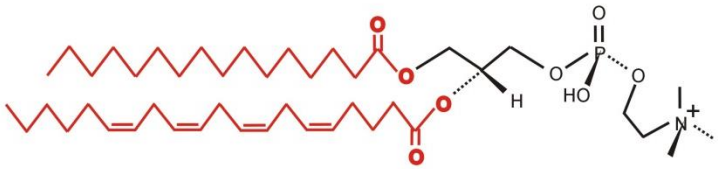


triacylglycerol, TAG (TG)

neutral =
hydrophobic

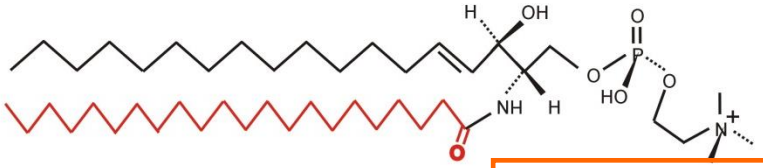


cholesterol, FC

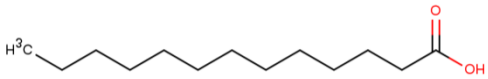


phosphatidylcholine, PC

polar =
amphiphilic



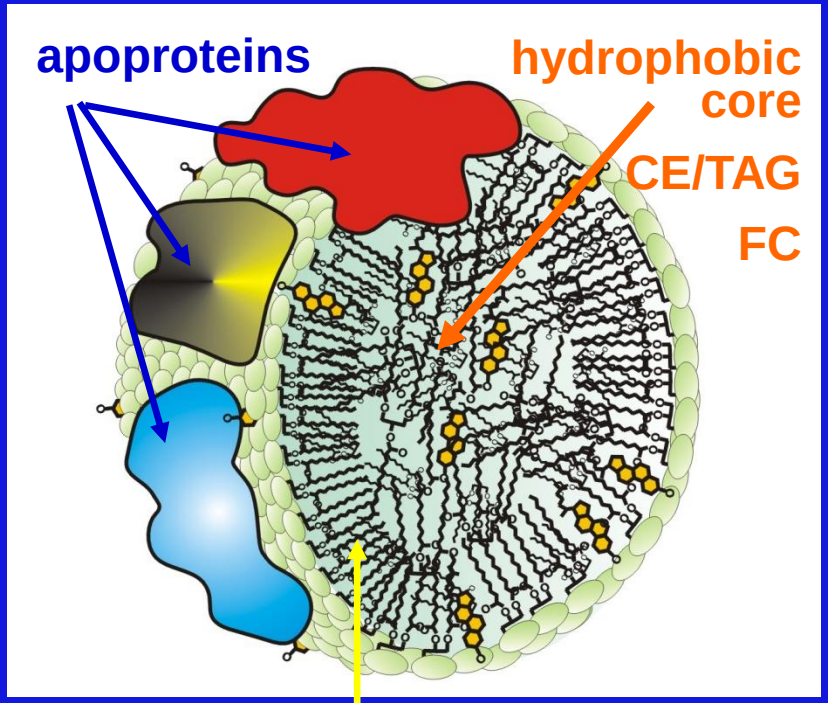
sphingomyeline,SPH



NEFA
very polar

Structure of lipoprotein

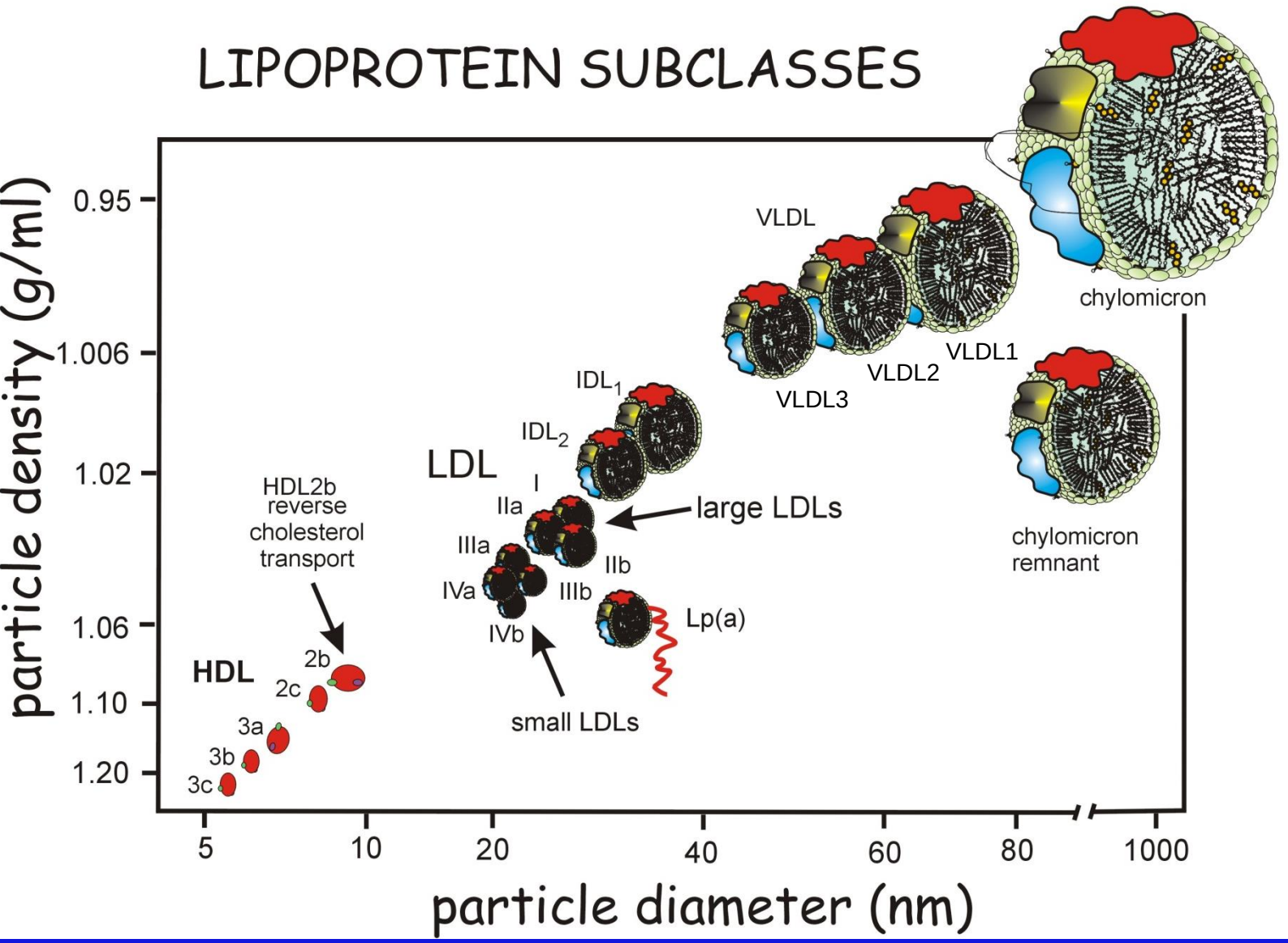
- cca spherical
- micellar
- noncovalent interaction between lipids and proteins
- lipid transporting system
- possible interchange of apoproteins, lipids between lipoproteins



Plasma lipoproteins

Lipoprotein class	Major Lipid class	Apolipoproteins	Source
CM (chylomicrons)	TAG	A-I, A-II, A-IV, C-II, -III, B-48, E	intestine
remnant CM	TAG, CE	B-48, E	catabolism of CM
VLDL (very low density Lp)	TAG	B-100, C-II,-III, E	liver (intestine)
IDL (intermediate density Lp)	CE	B-100, C-II,-III, E	catabolism of VLDL
LDL (low density Lp)	CE	B-100	catabolism of IDL
HDL_2 (high density Lp) *subclass 2*	CE, PL	A-I, A-II	liver, intestine catabolism of CM and VLDL
HDL_3 (high density Lp) *subclass 3*	CE	A-I, A-II, minor apolipoproteins	HDL_2
lipoprotein [a]	CE	B-100 & apo [a]	liver

Lipoprotein size



Plasma apolipoproteins

apolipoprotein = protein part of lipoprotein particle

many functions (intracellular ≠ extracellular)

Non-exchangeable apolipoproteins

structural function: apo B-48, apo B-100

receptor ligands: apo B-48, apo B-100

Exchangeable apolipoproteins

receptor ligands: apo E, apo A-I

structural function: apo A-I

modulation of enzyme activity: apo A-I, apo A-II, apo C-I, apo C-II, apo C-III

enzyme activity: apo K (PON)

acute phase reactant: apo I (SAA)

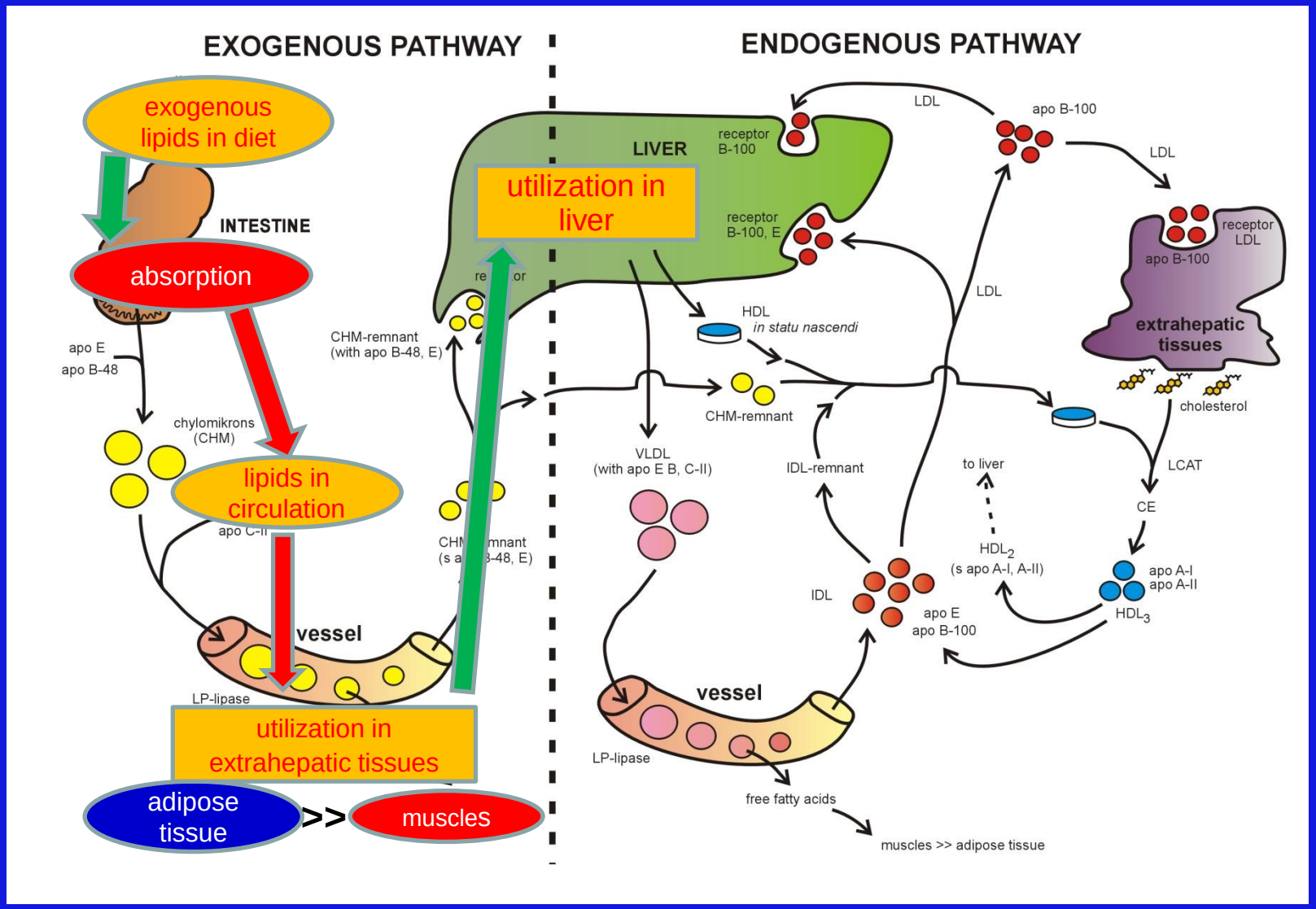
inhibition of metabolic cascades: apo (a) (thrombolysis?) apo J (inhibitor of terminal complement complex)

Important plasma apolipoproteins

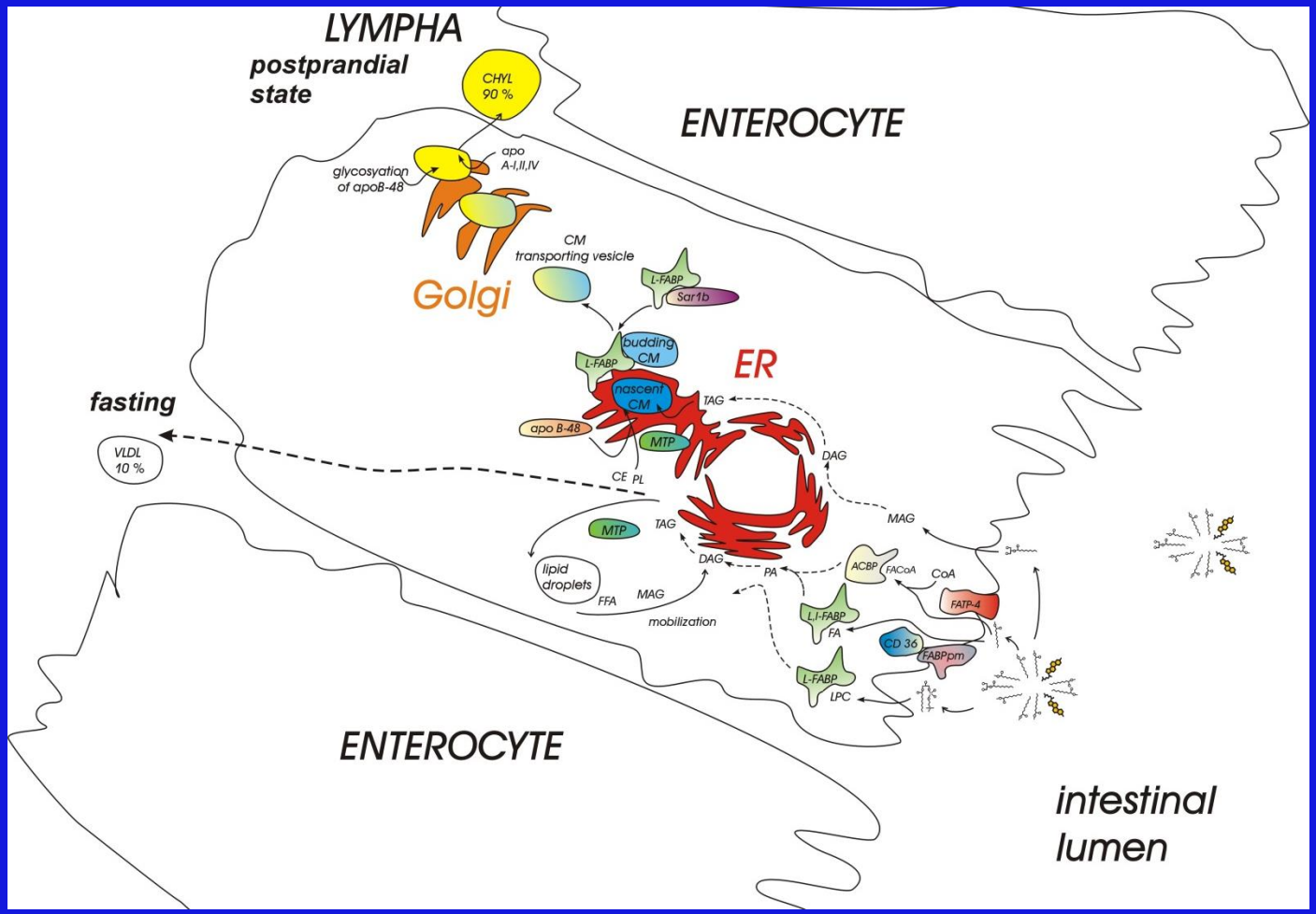
apolipoprotein	major LP class	concentration (g/l)	function
A-I	$HDL_{2,3}$	1.20 - 1.40	LCAT activation HDL-receptor ligand, transport (HDL)
A-II	HDL_3	0.35 - 0.50	activation of hepatic lipase, transport (HDL)
A-IV	CM, $HDL_{2,3}$	< 0.05	RCT(cofactor for LCAT?), abs.of exogenous TAG
B-100	VLDL, IDL, LDL	0.60 - 1.20	transport (VLDL, IDL, LDL), LDL (apo B/E)-receptor ligand
B-48	CM, β-VLDL	< 0.05	absorption of lipids, apoB-48 receptor ligand transport (CM, remnant CM)
C-I	CM, VLDL	0.05 - 0.08	*inhibition of CETP*, LCAT activation
C-II	CM, VLDL	0.03 – 0.07	activation of LPL
C-III $_{0-3}$	CM, VLDL	0.10 - 0.12	catabolism of CM_R, *inhibition of LPL*
D	HDL_3	0.08 - 0.10	free cholesterol esterification?
E	CM, VLDL, HDL-E	0.03 - 0.05	LDL-receptor ligand, VLDL-receptor ligand, RCT LRP-receptor ligand, apoER2-receptor ligand
Apo(a)	Lp(a)	0.05-0.30	homologous to plasminogen; prothrombotic

RCT - reverse cholesterol transport, LCAT - lecithin:cholesterol acyltransferase, LPL - lipoprotein lipase, CE - cholesterylester, TAG - triacyglycerol, CM_R - remnant CM, β-VLDL – remnant VLDL staying in plasma

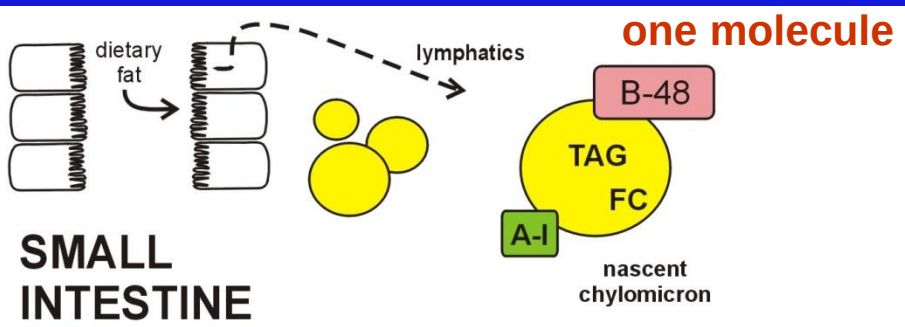
Metabolic lipoprotein pathway



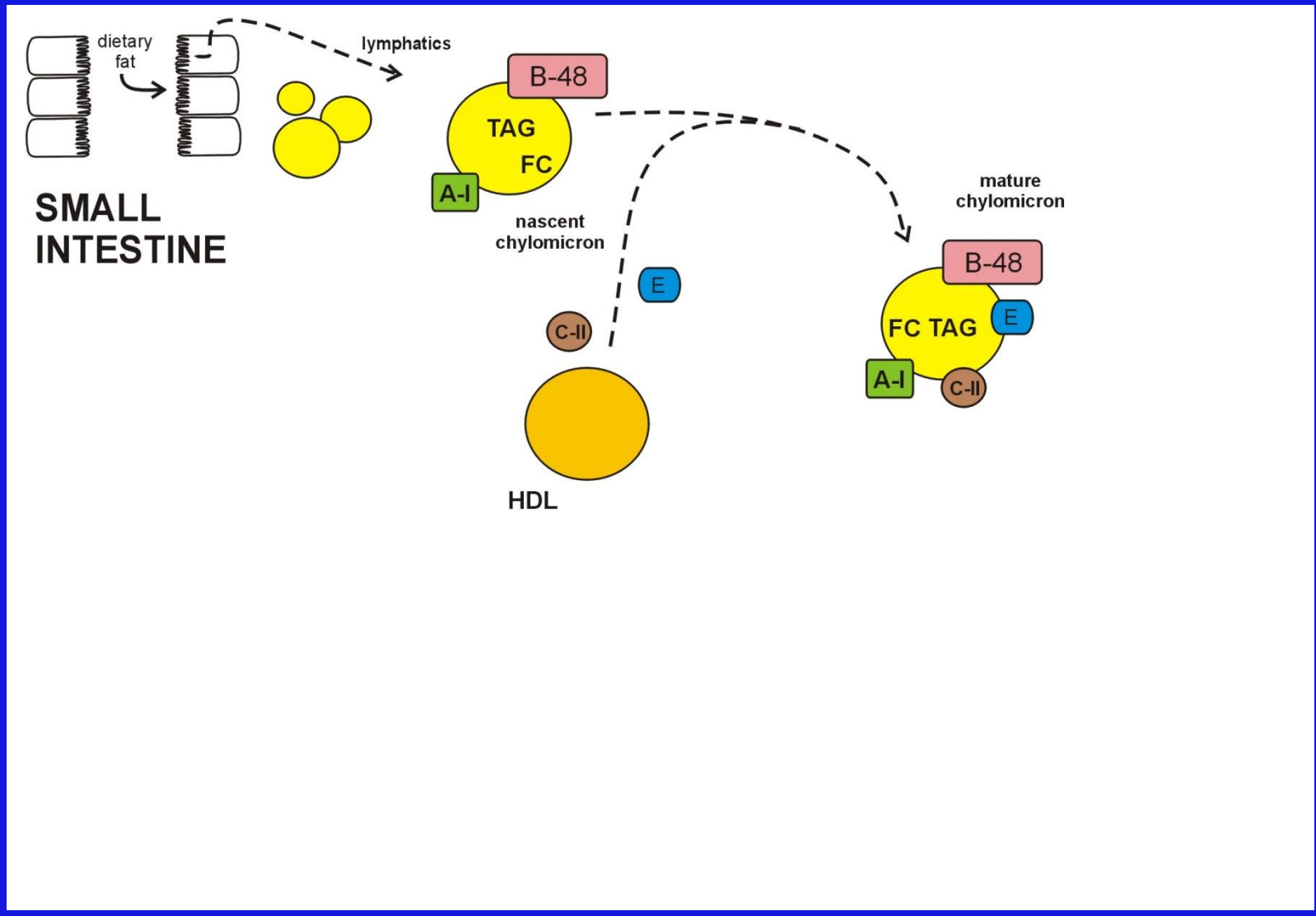
Assembly of chylomicrons



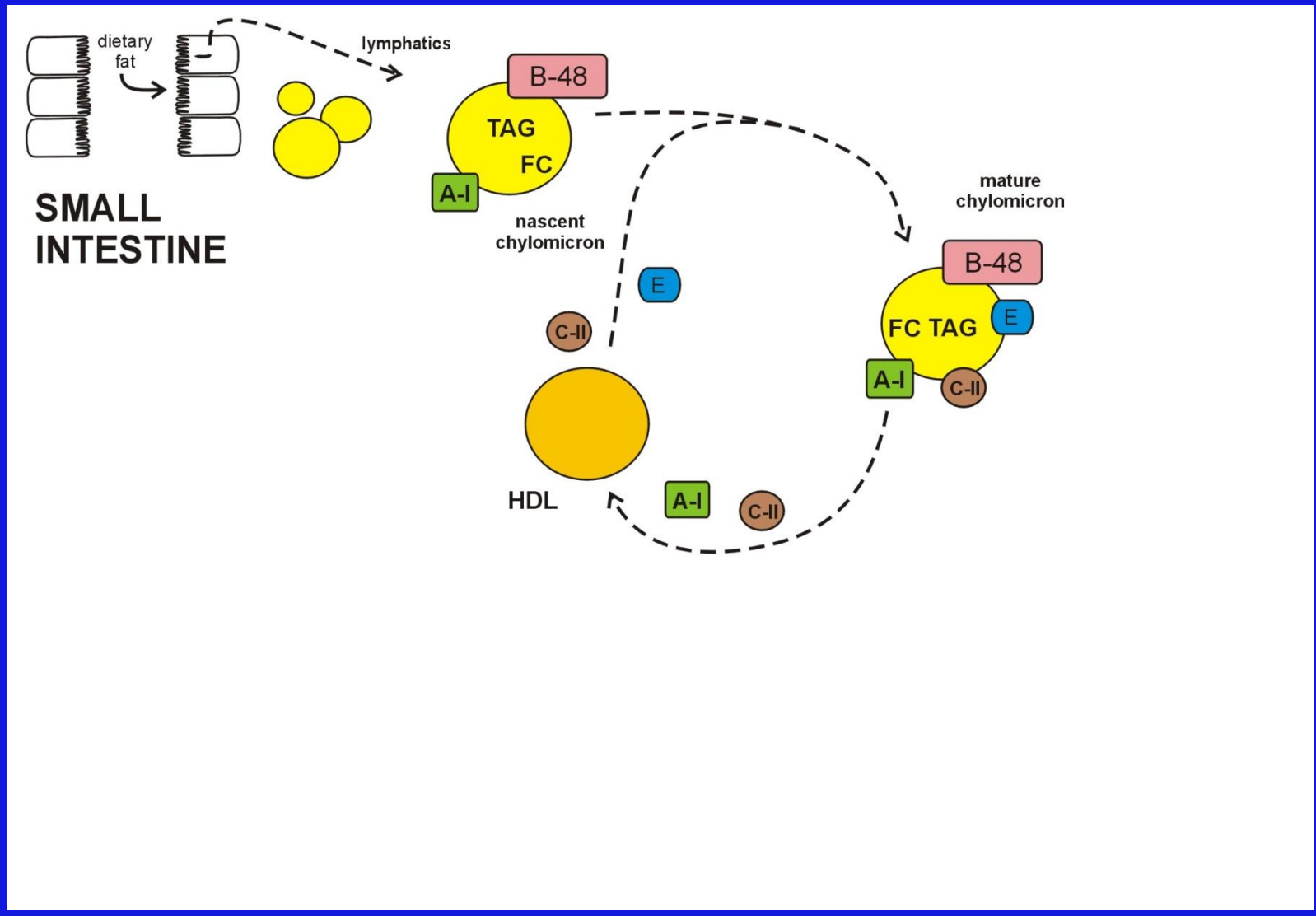
Chylomicrons



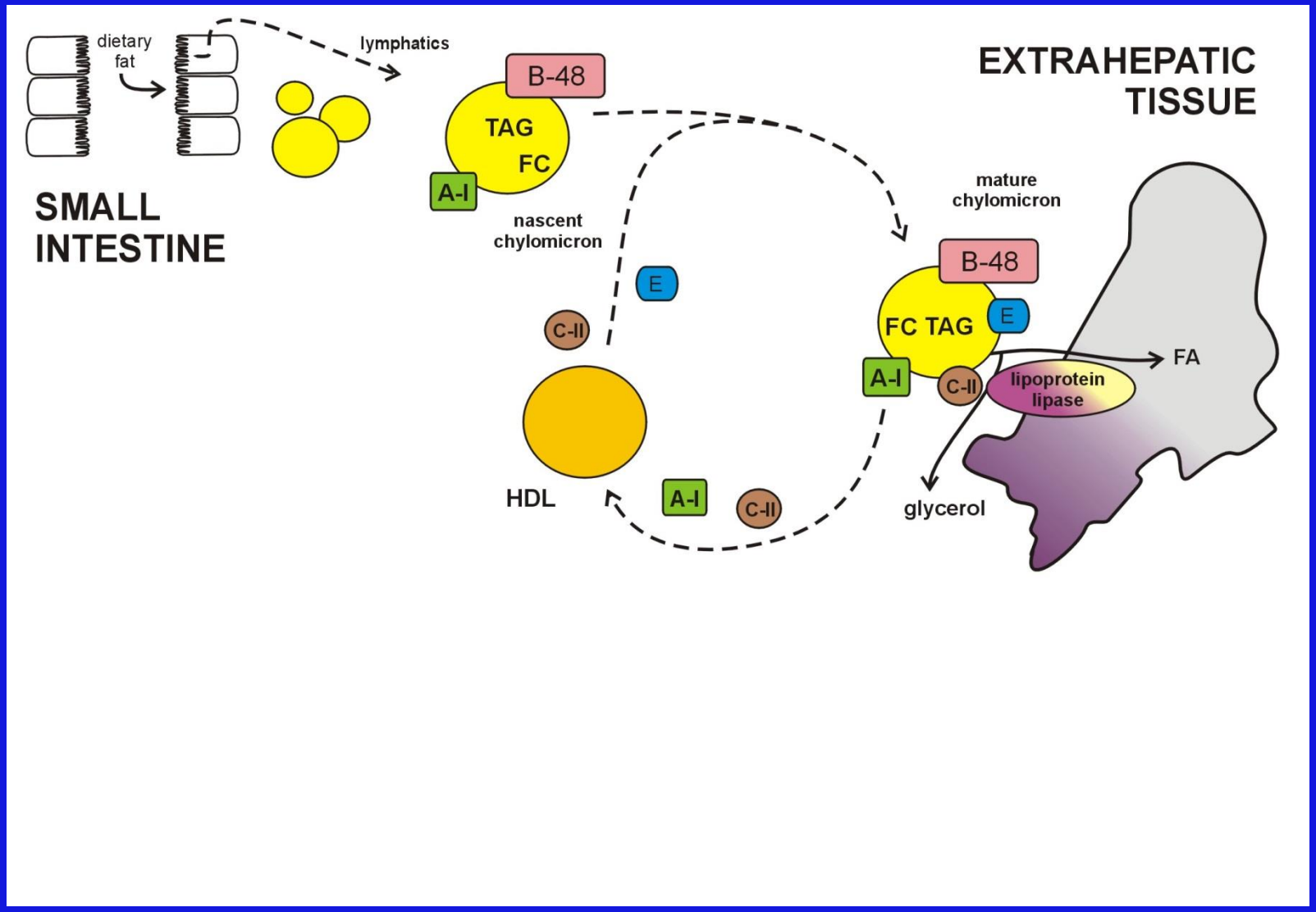
Chylomicrons



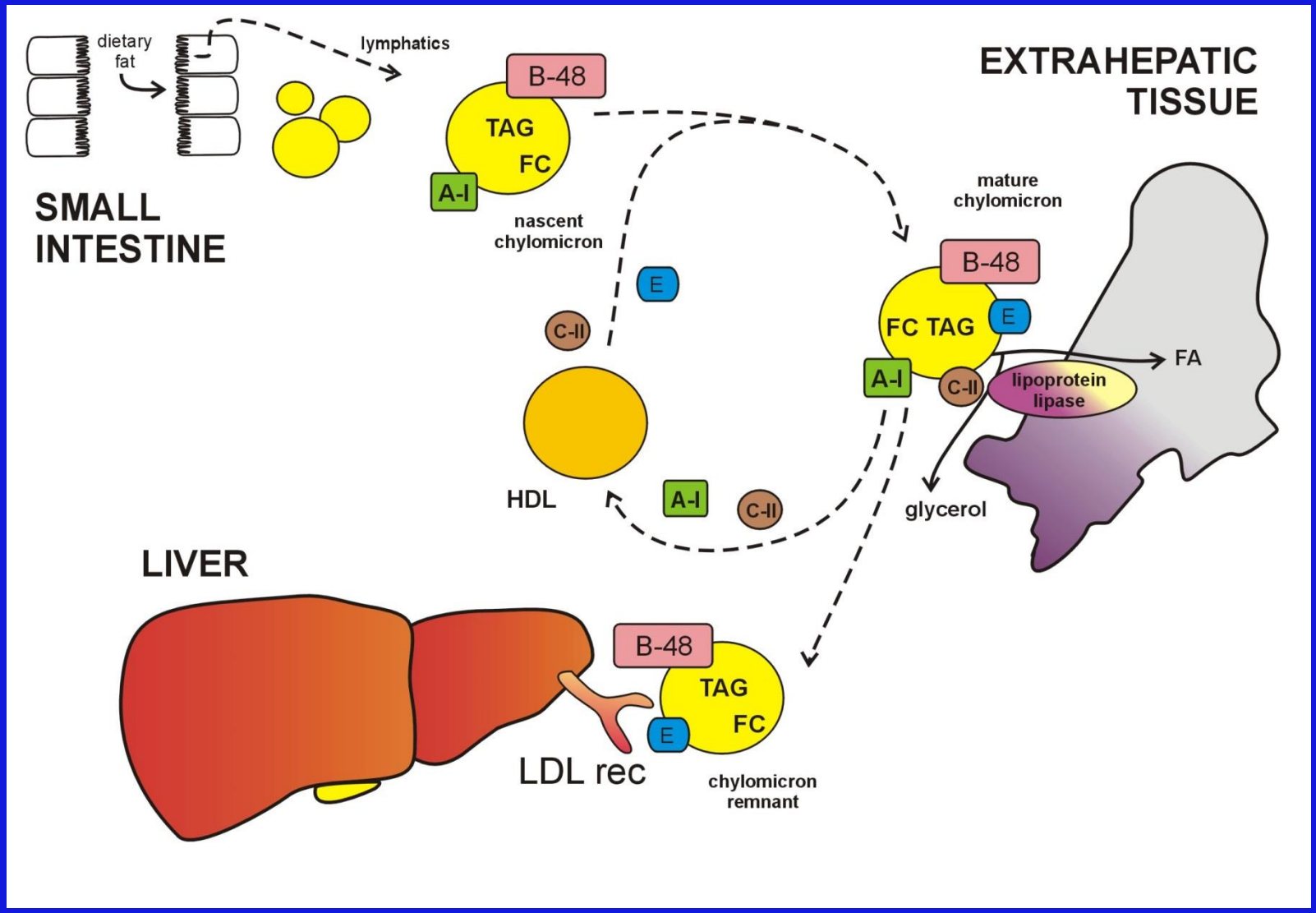
Chylomicrons



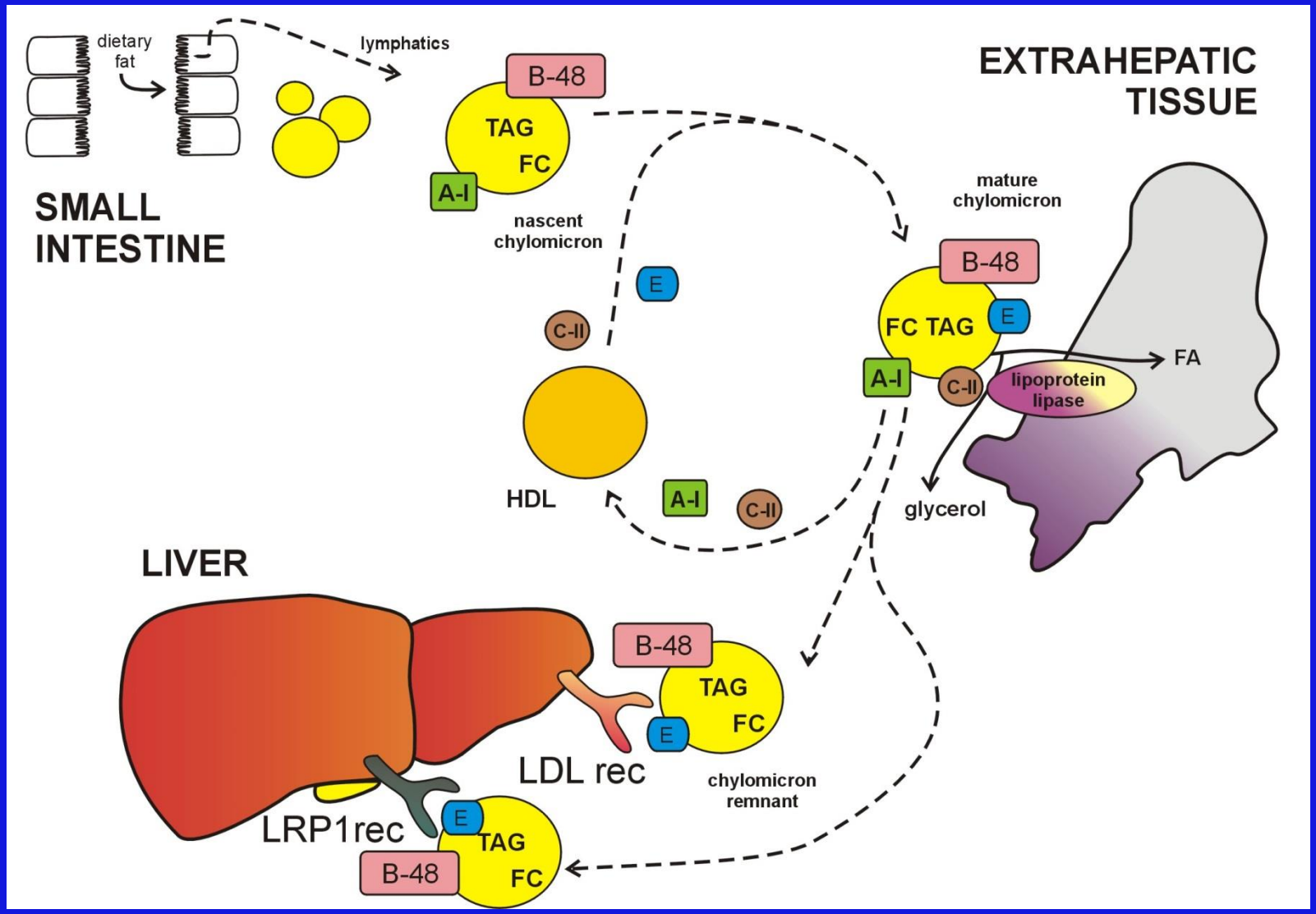
Chylomicrons



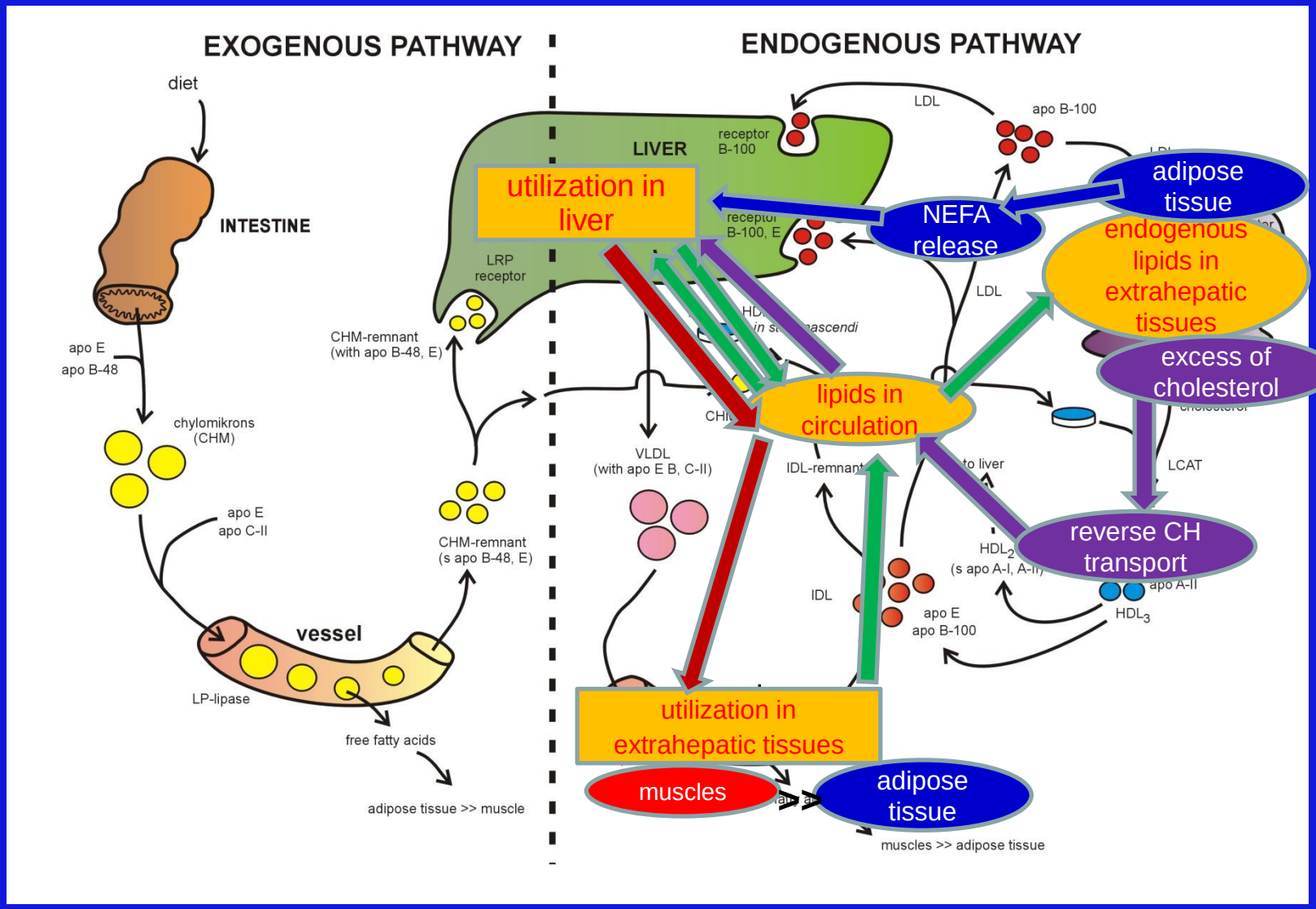
Chylomicrons



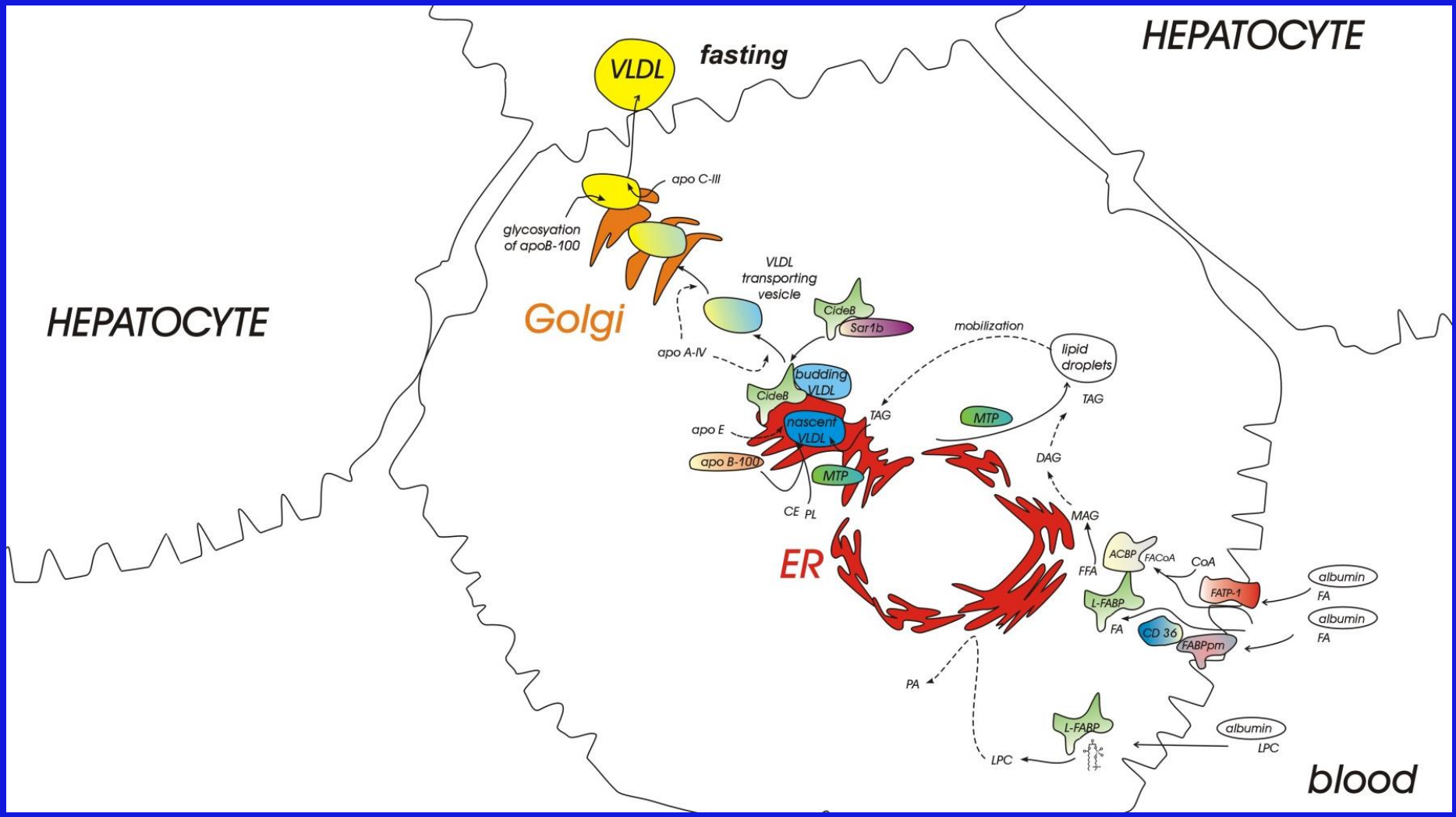
Chylomicrons



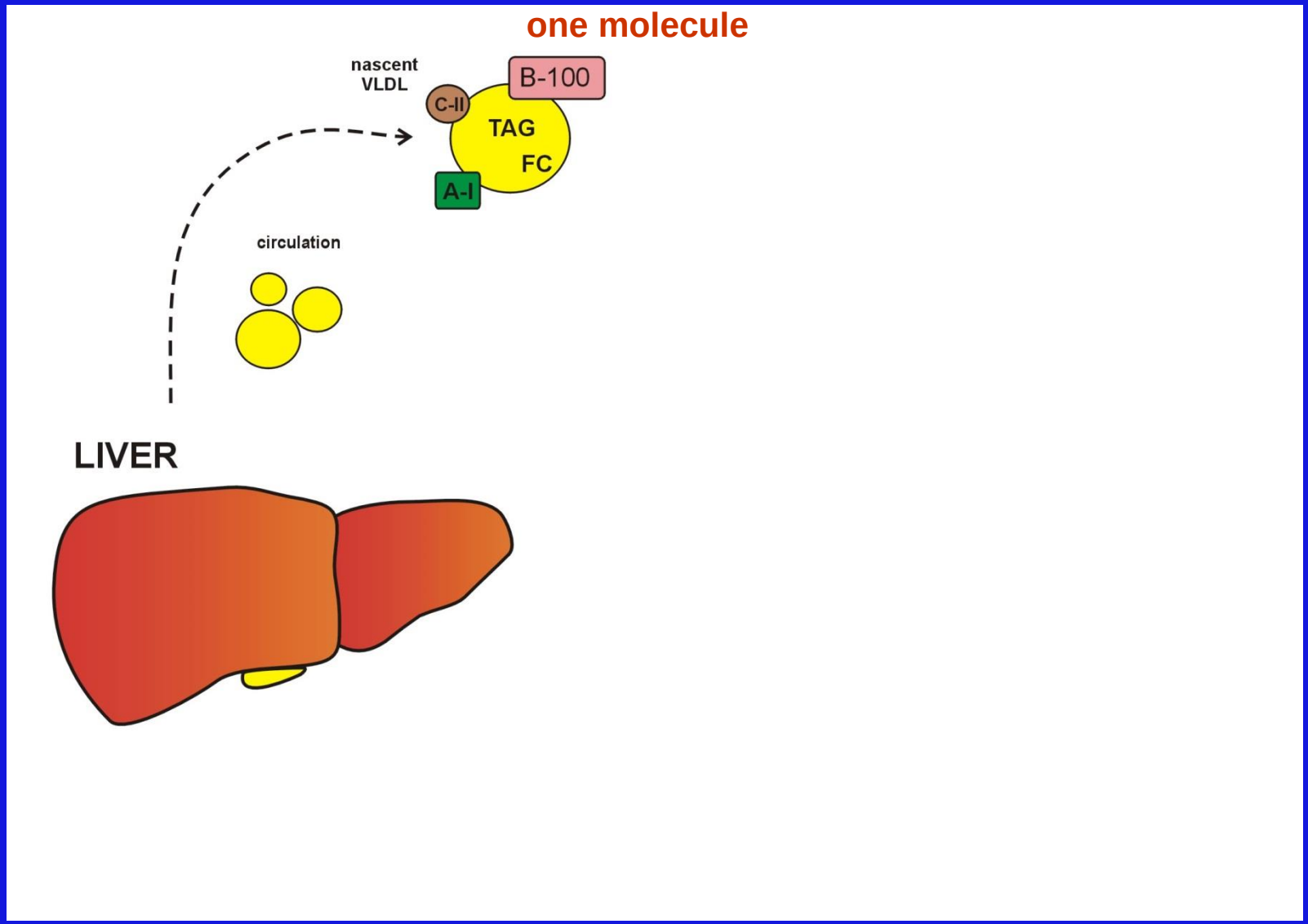
Metabolic lipoprotein pathway



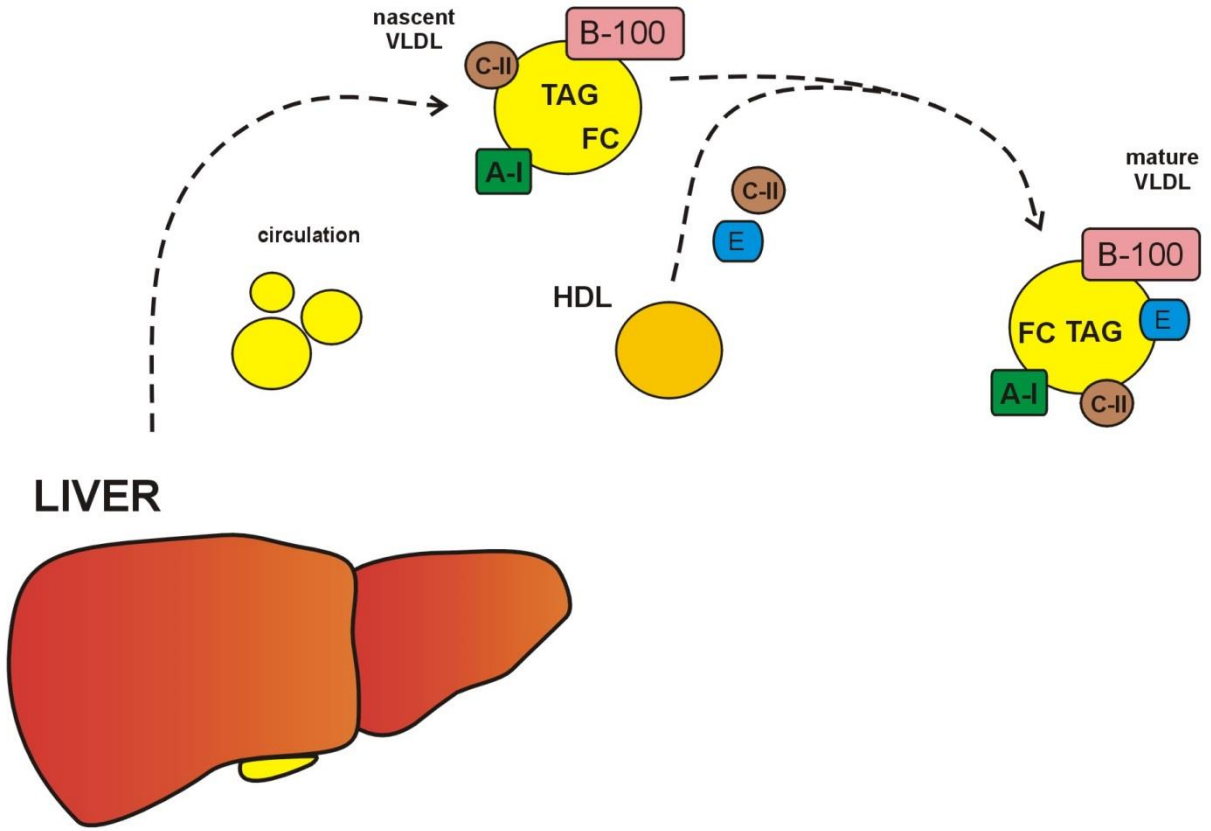
Assembly of VLDL



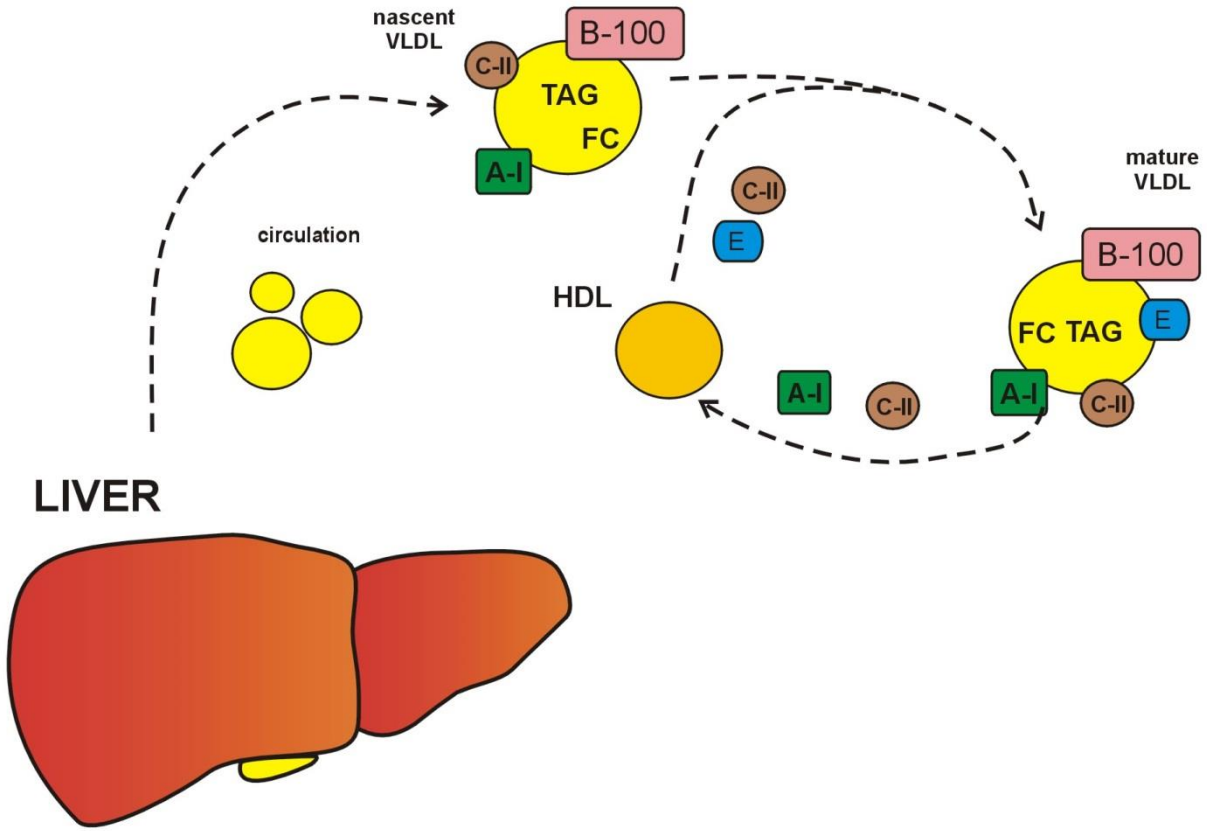
Fate of VLDLs



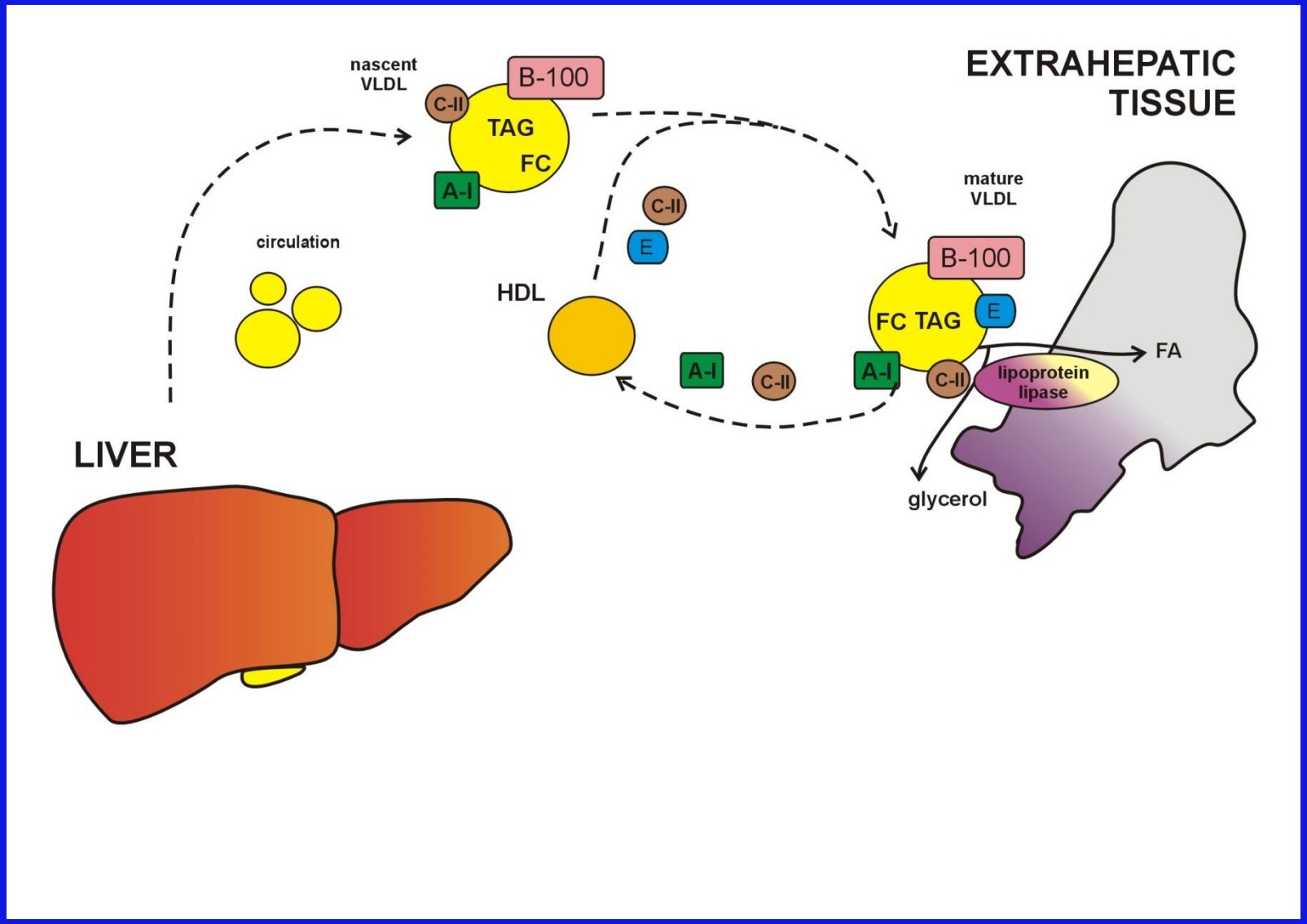
Fate of VLDLs



Fate of VLDLs



Fate of VLDLs



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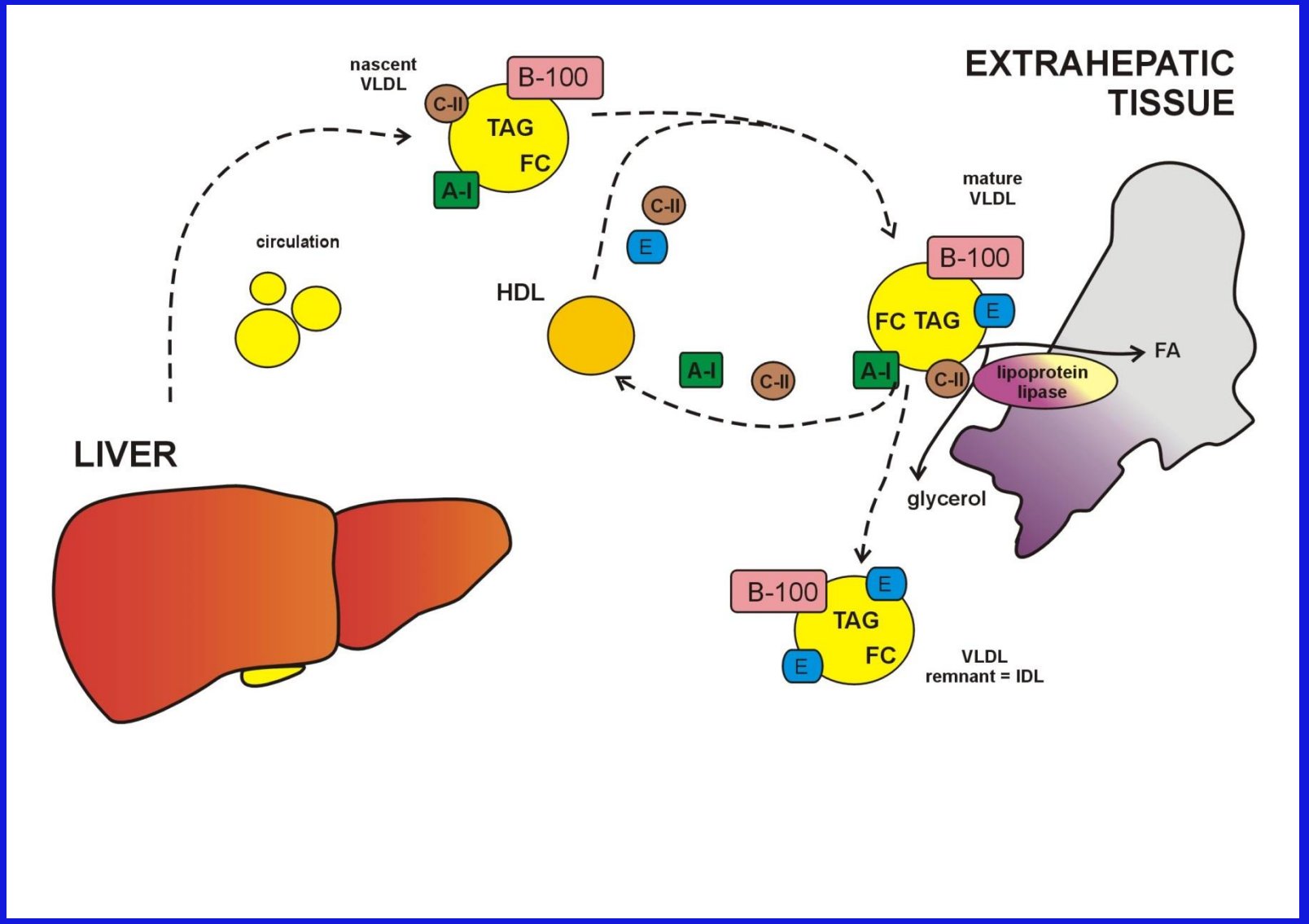

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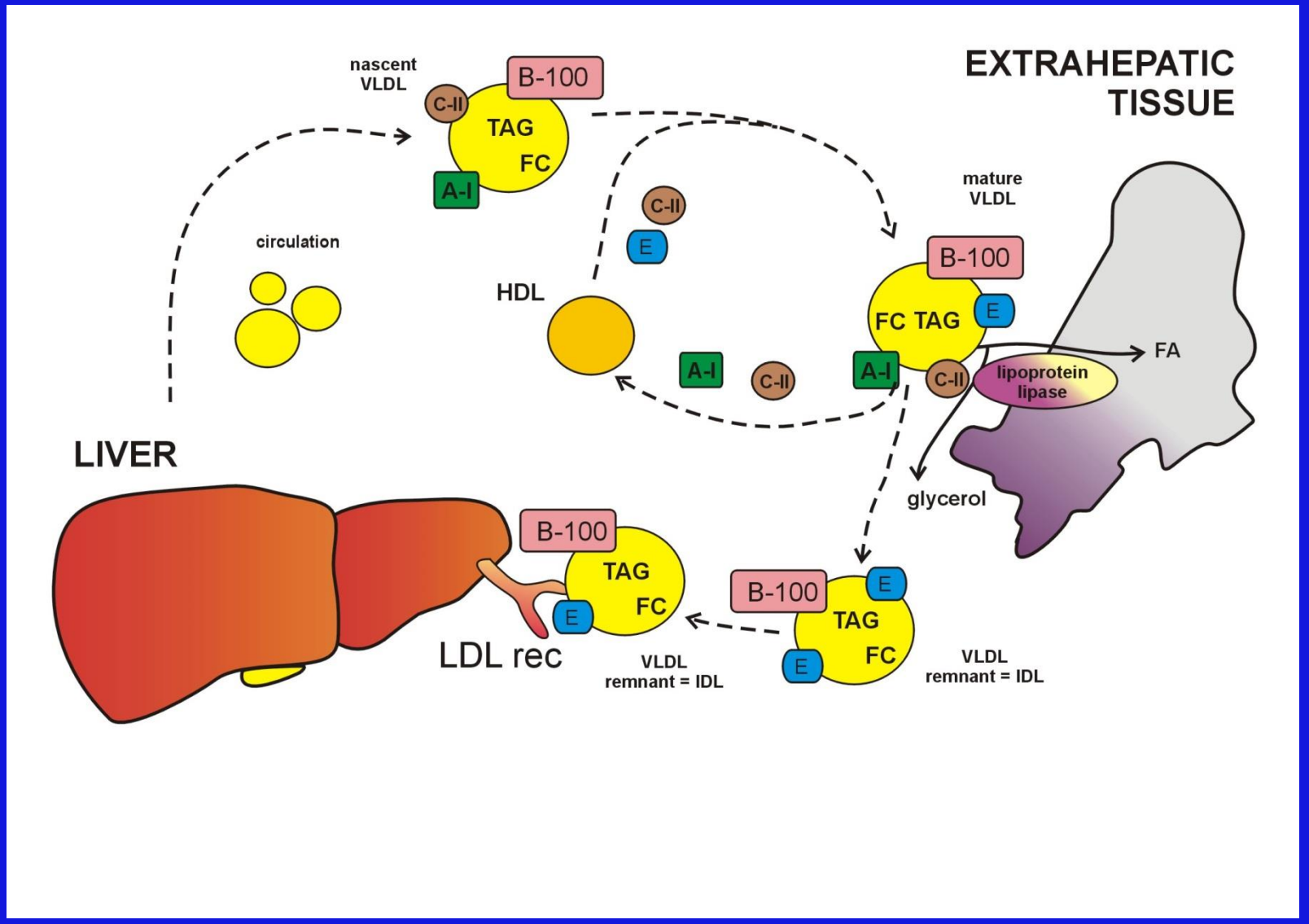
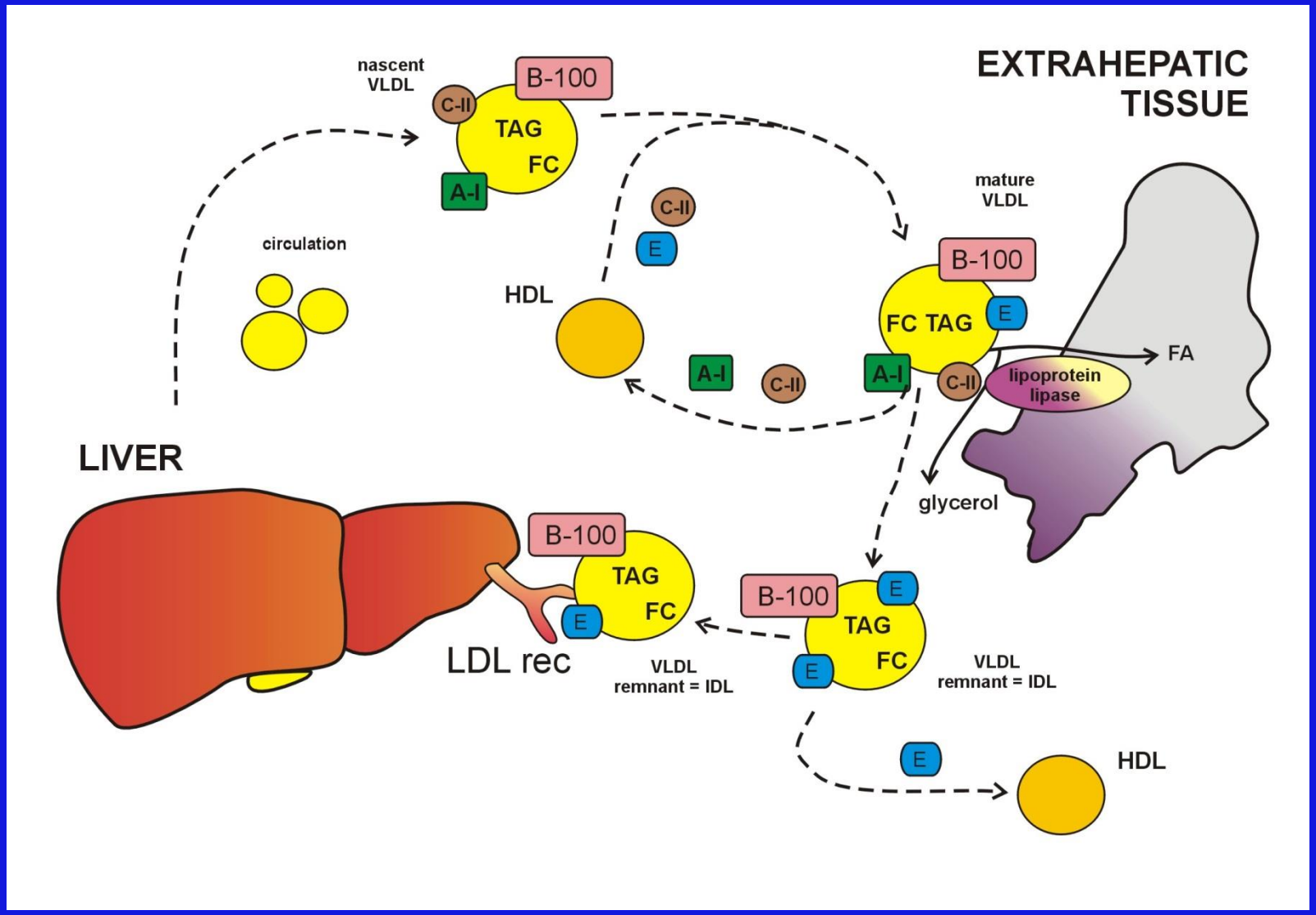
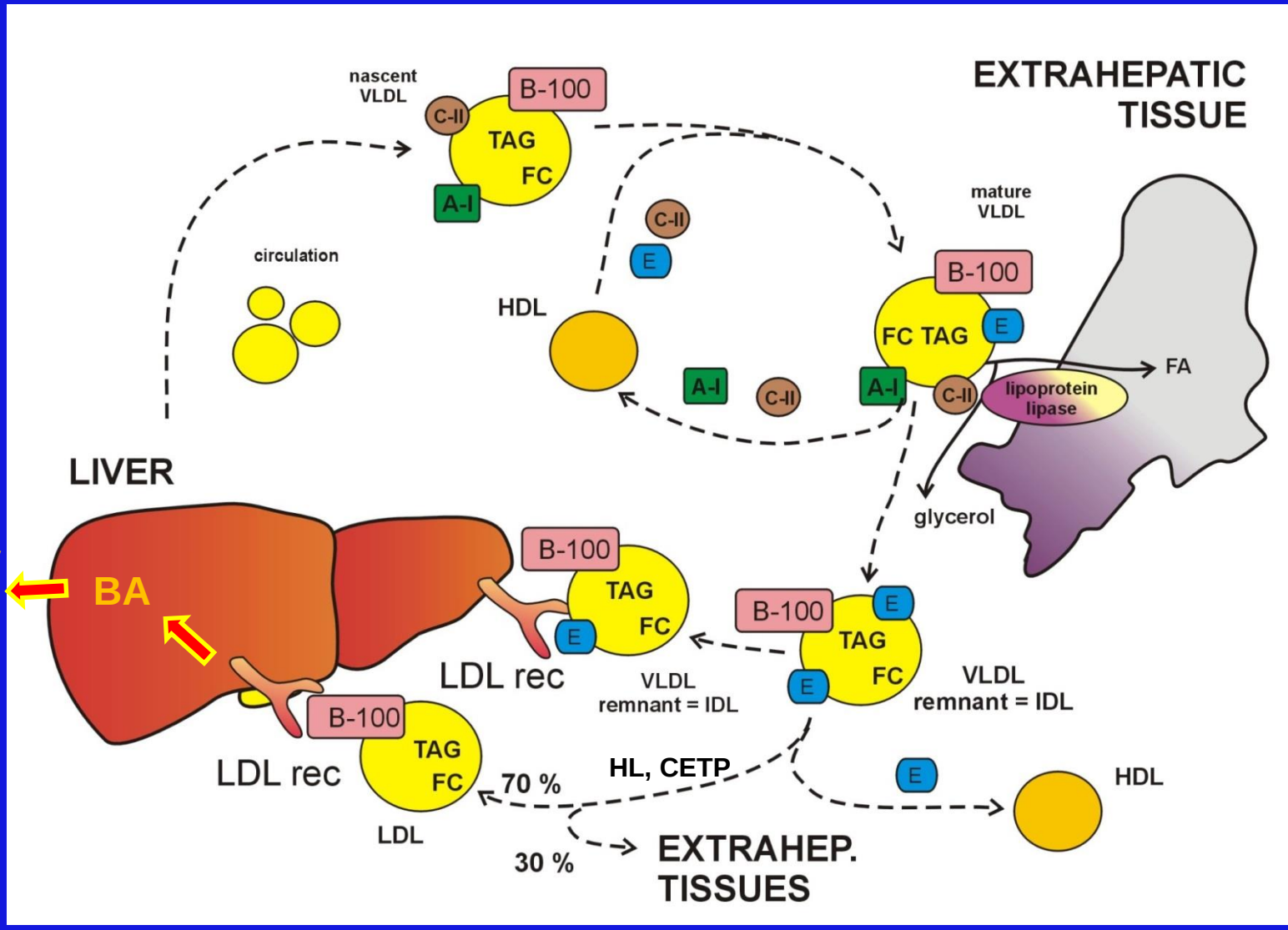


Fate of VLDLs



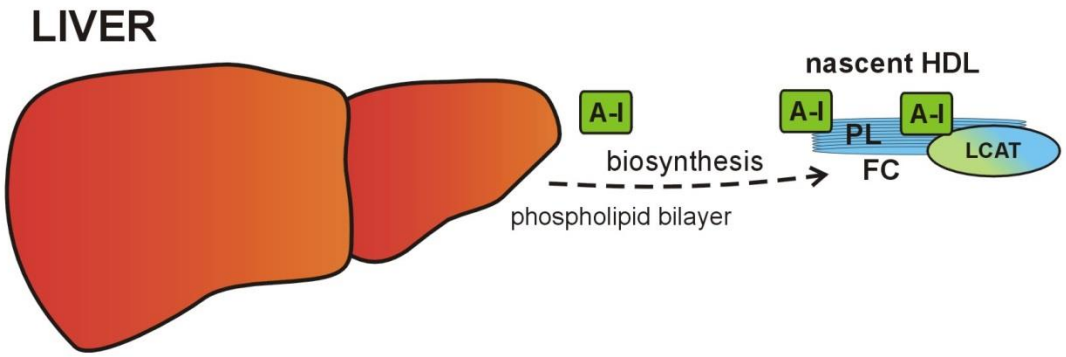
Fate of VLDLs



VLDL and chylomicrons

VLDL		CM
mainly hepatocytes	**source**	enterocytes
apoB-100	**apoB**	apoB-48 *alternative splicing of the APOB gene*
30-80 nm	**size**	100 - 500 nm
MTTP, CideB, ARFRP1	**assembly**	MTTP, CideB, ARFRP1; Sar1b, PCTV, apoA-IV
high TAG ($VLDL_1$) large less TAG ($VLDL_2$) small	**types**	variable TAG content
fasting: →IDL→ LDL→ clearance	**metabolism**	postprandial: →CM_R→ clearance
TAG-$VLDL_1$ ~ hrs	**turnover**	TAG-CM ~ 5 mins
VLDL-rec, LDL-rec	**receptors**	LRP1 (CM_R) *apoB-48 lacks LDLR binding domian*

HDL and reverse cholesterol transport



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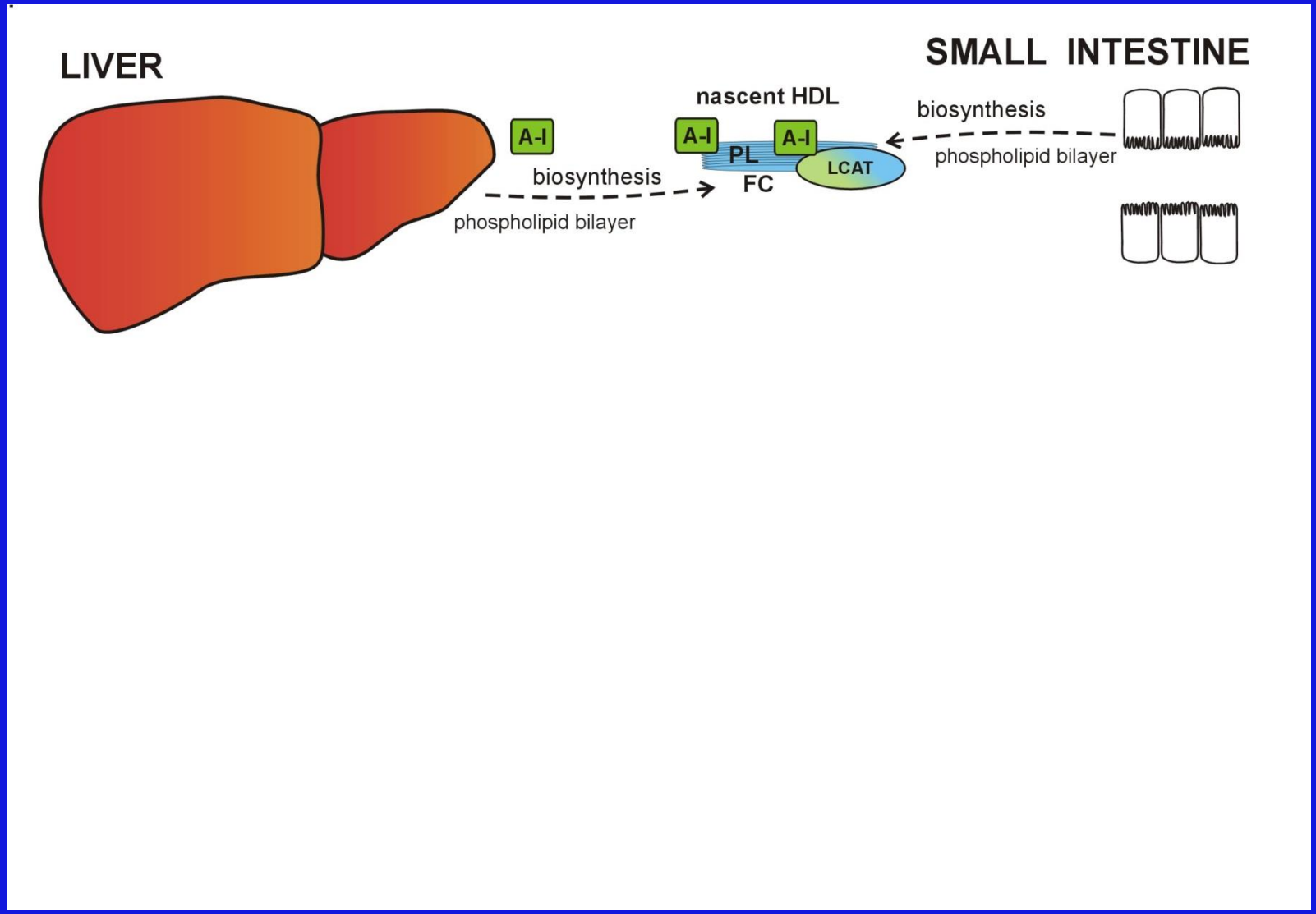

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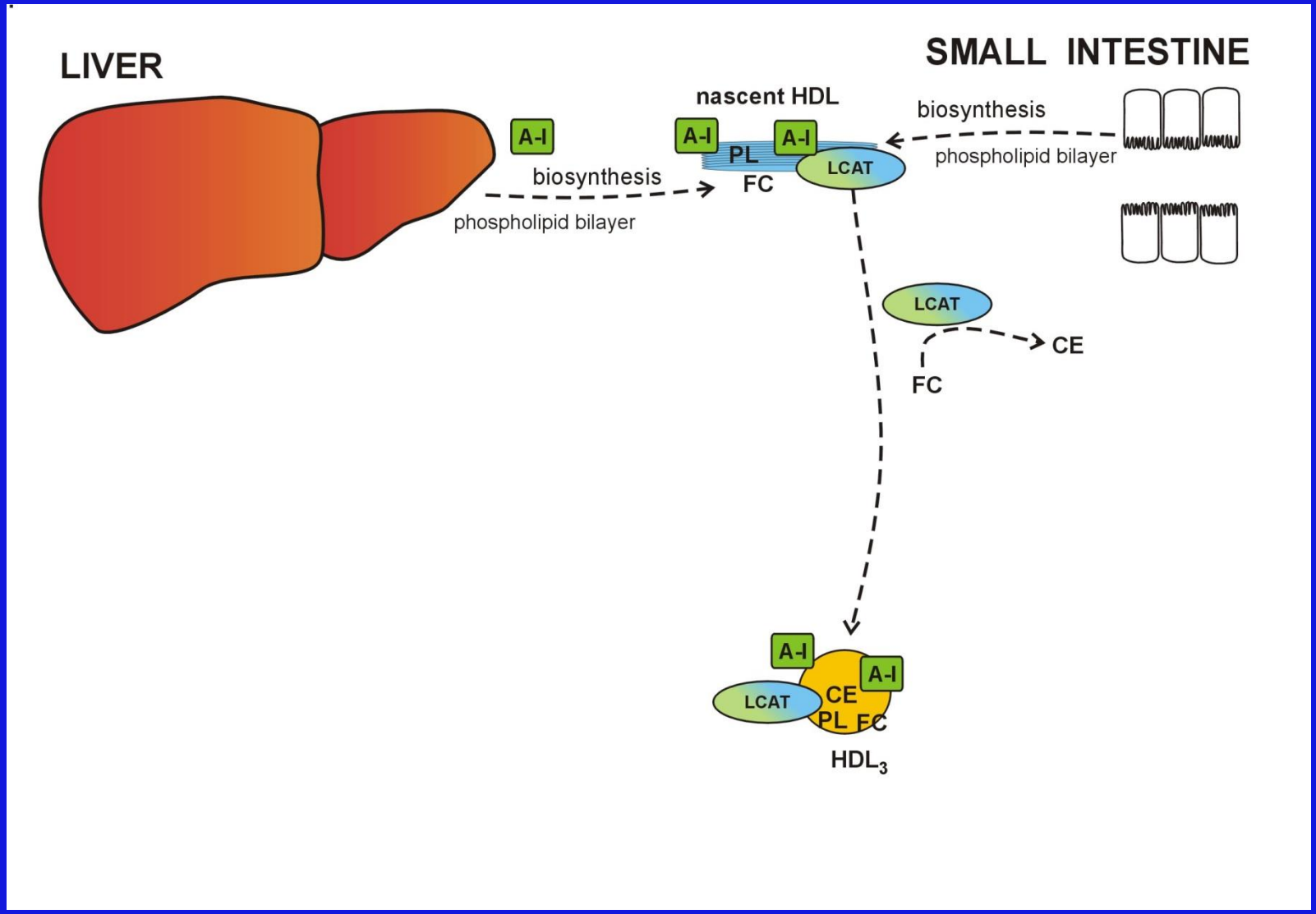

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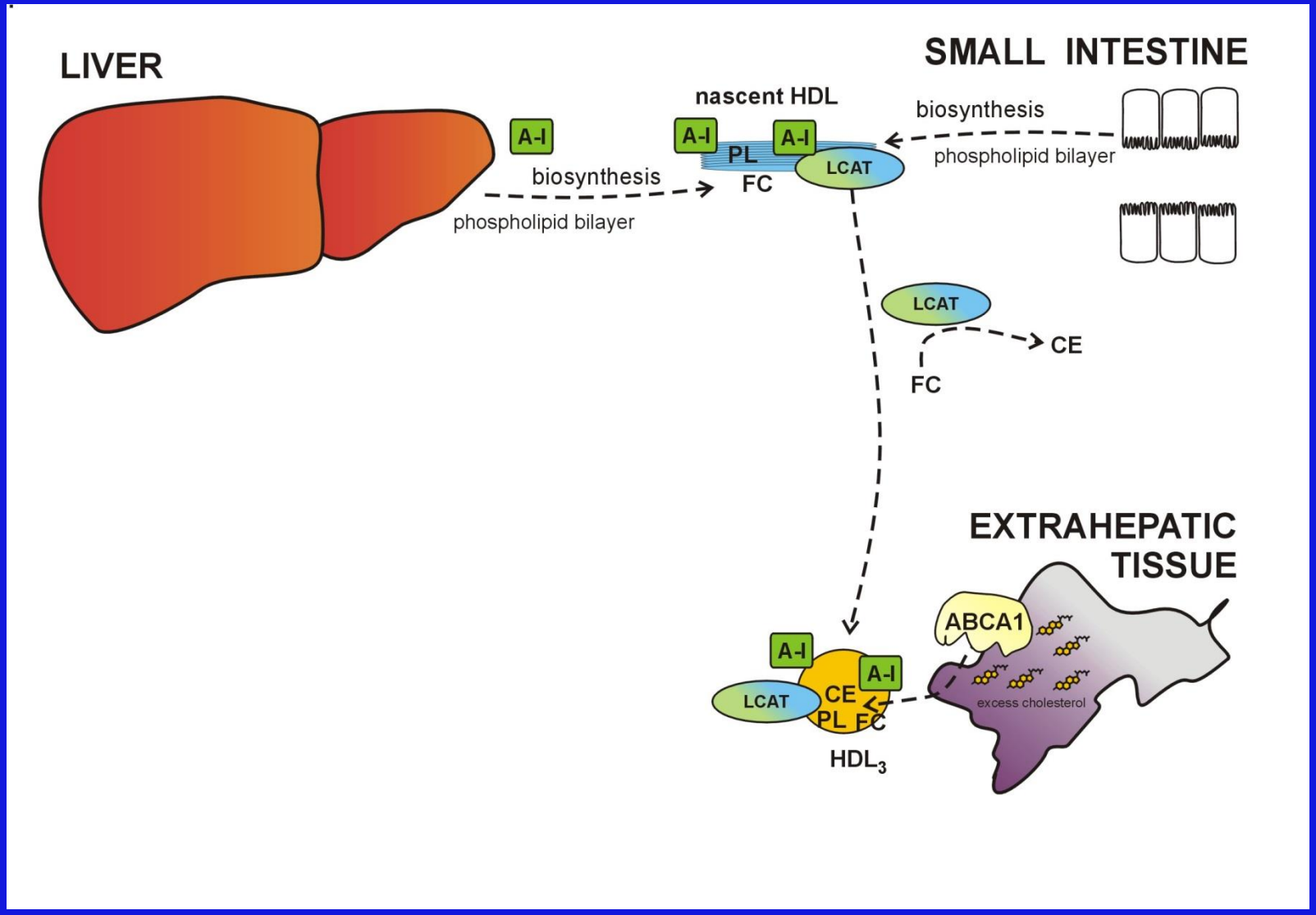

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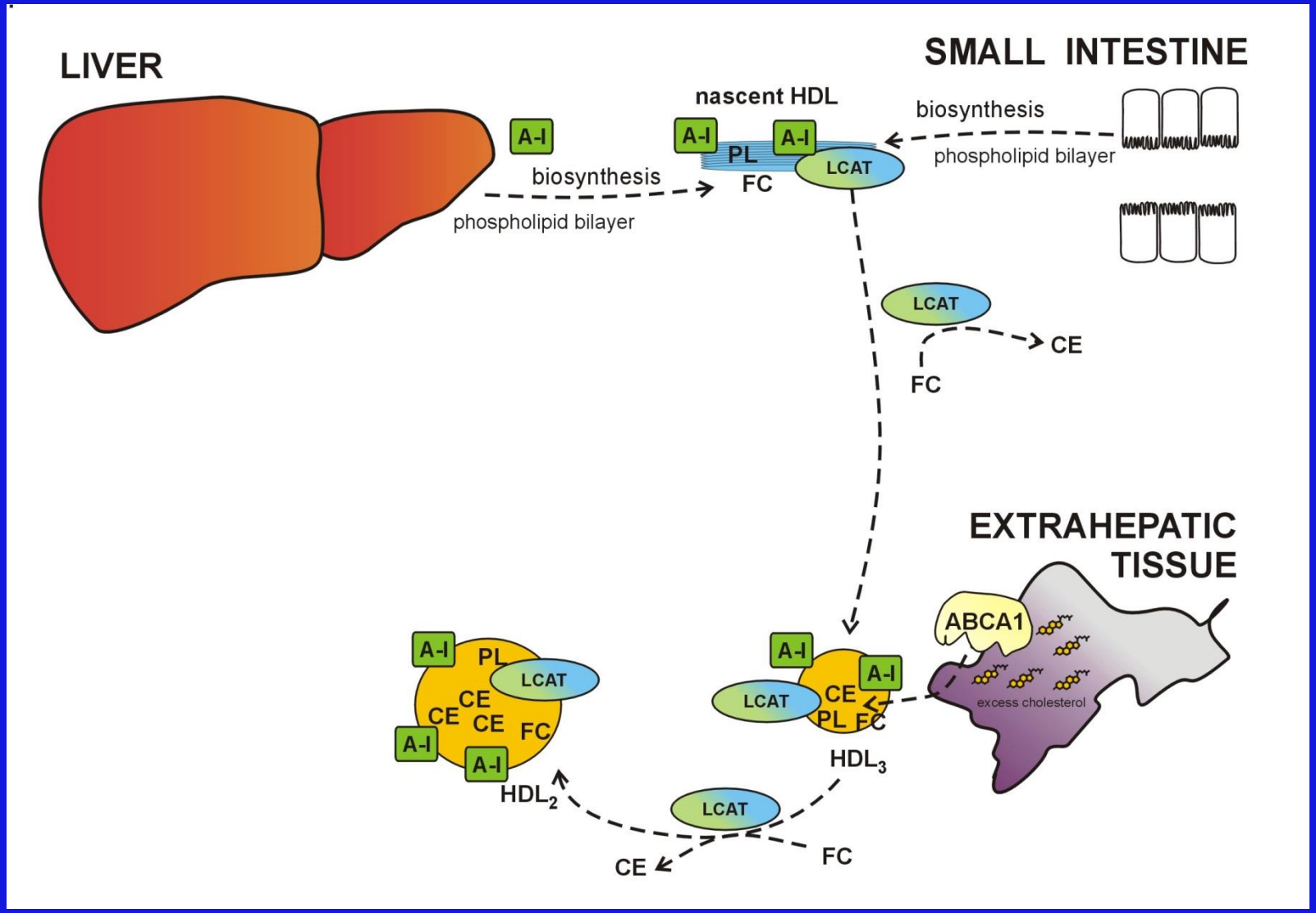

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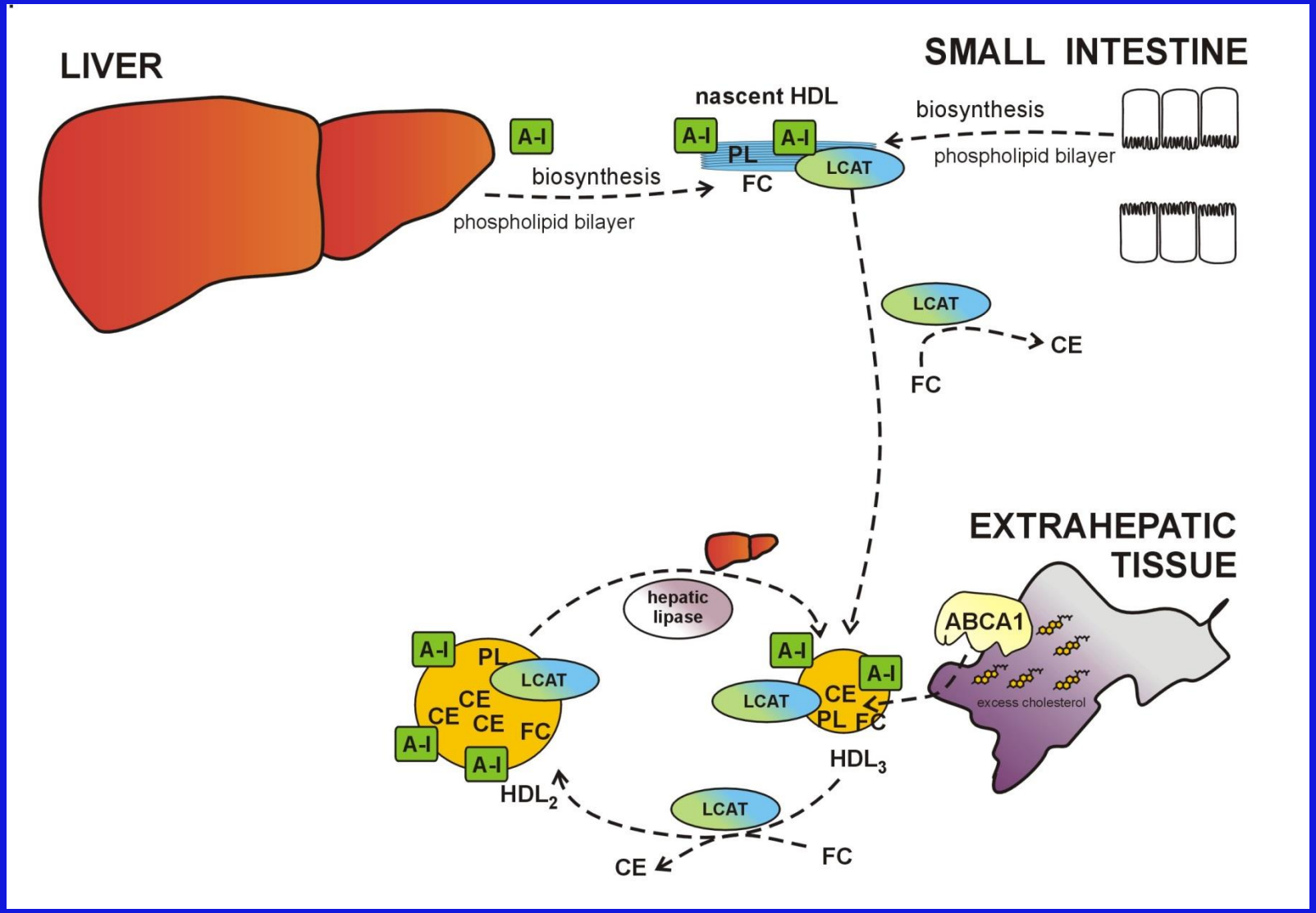

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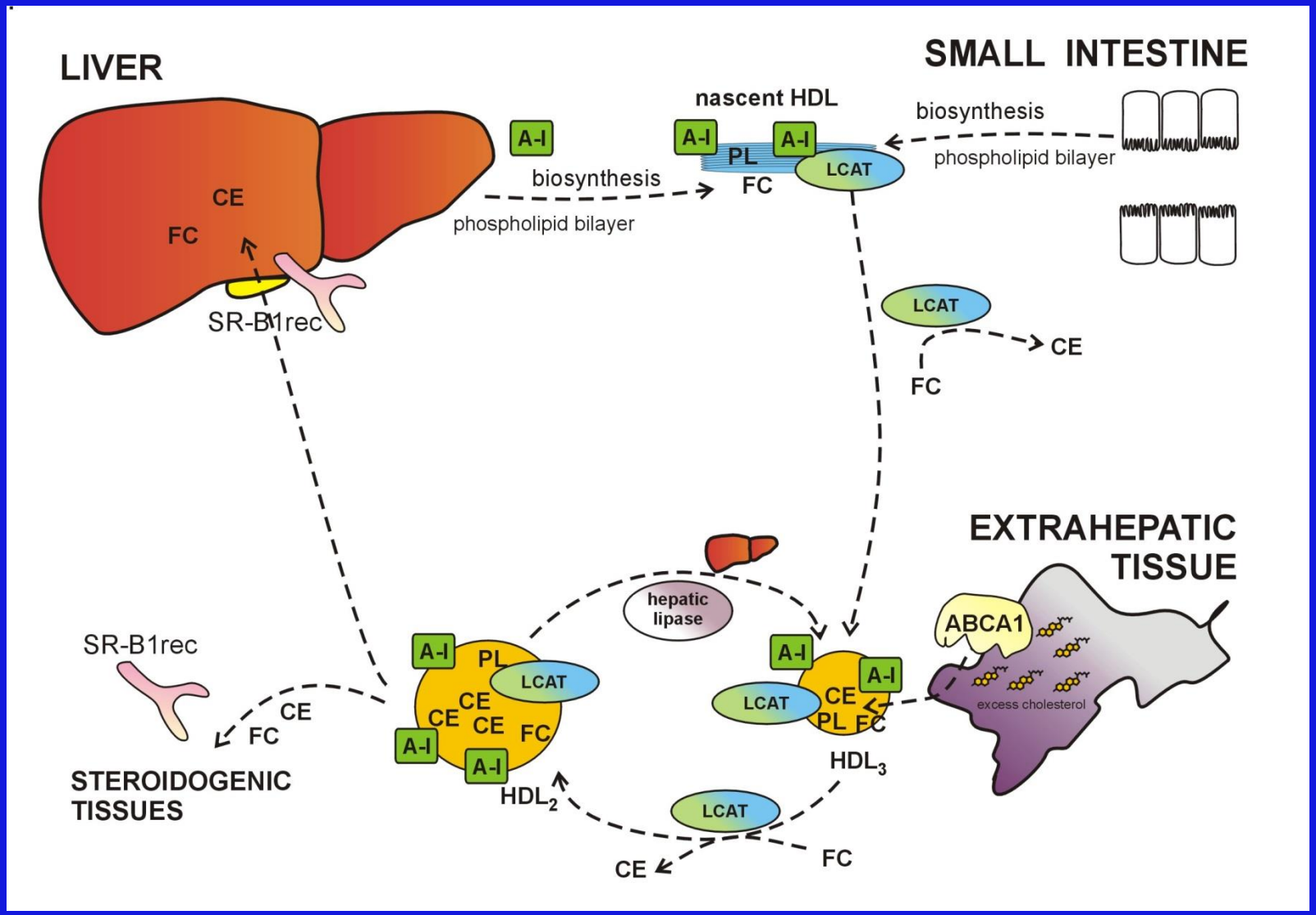
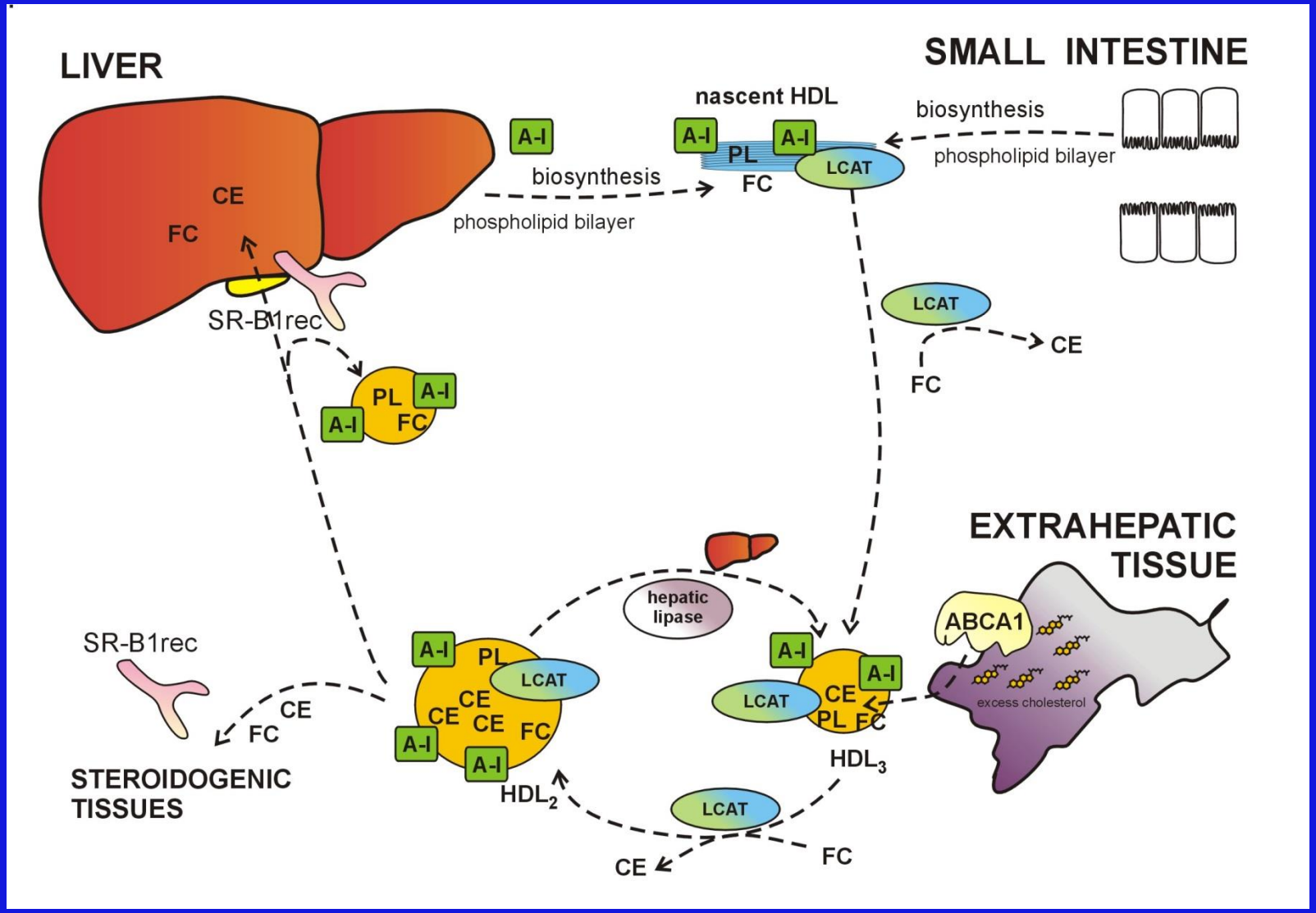


HDL and reverse cholesterol transport



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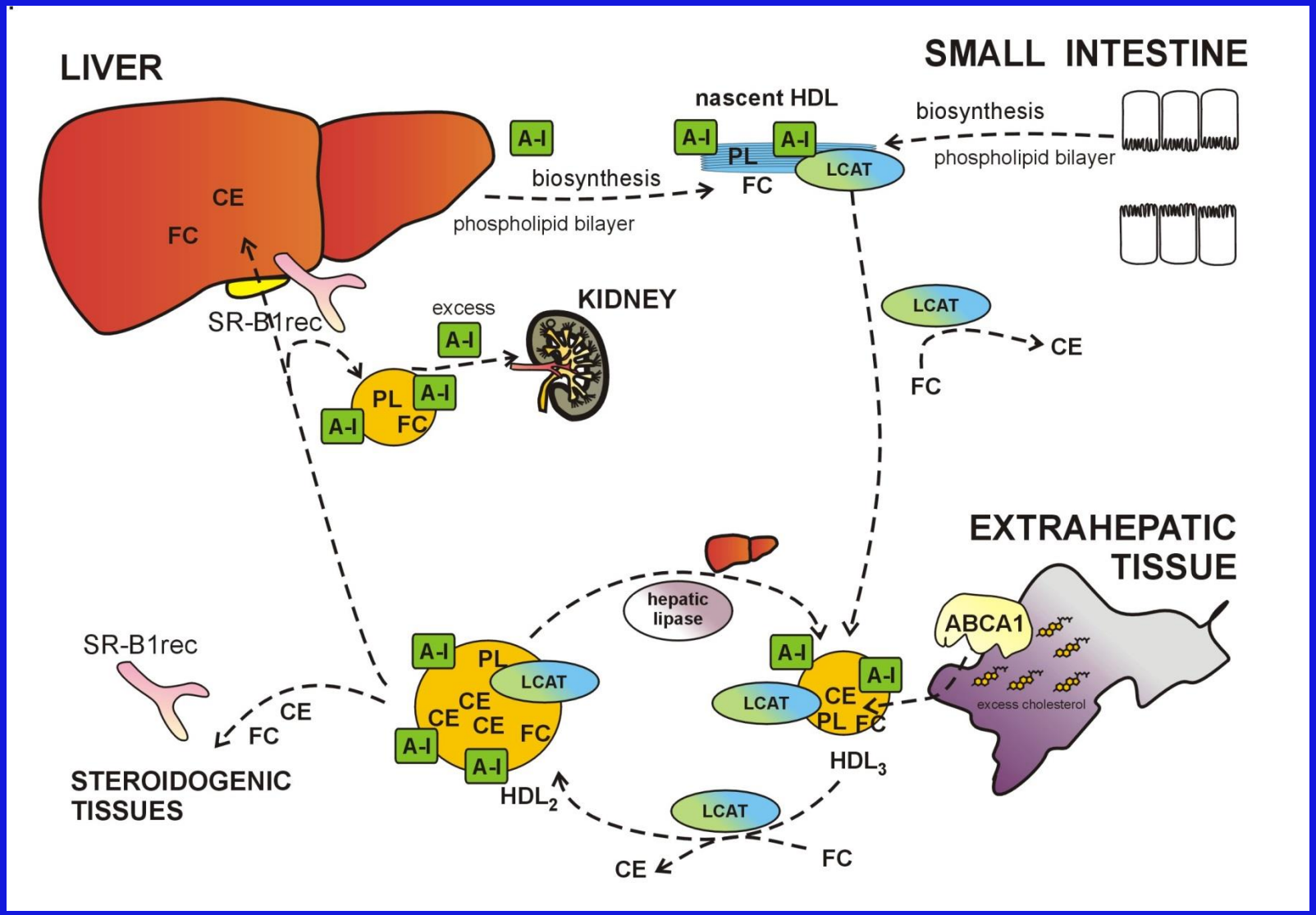

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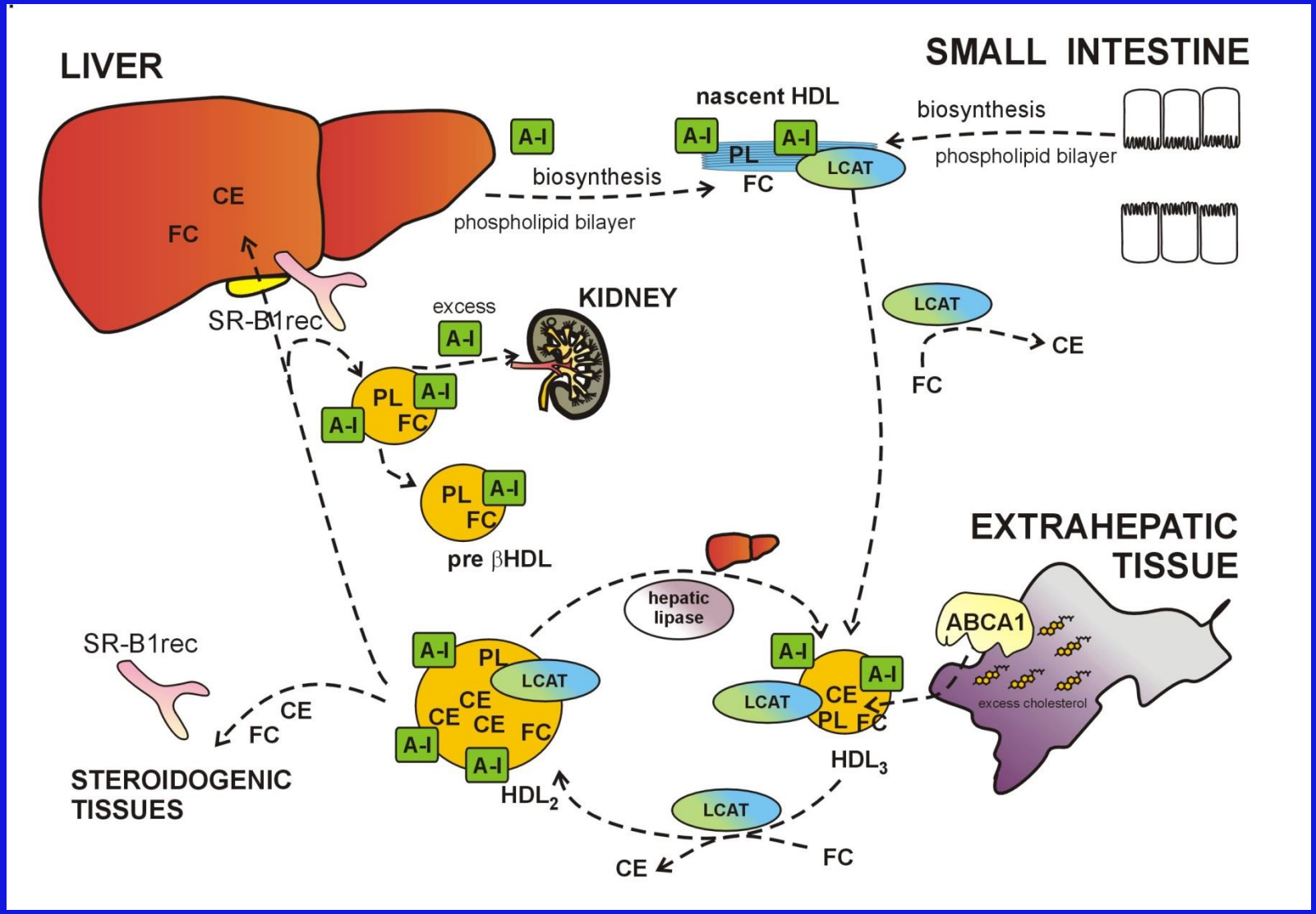

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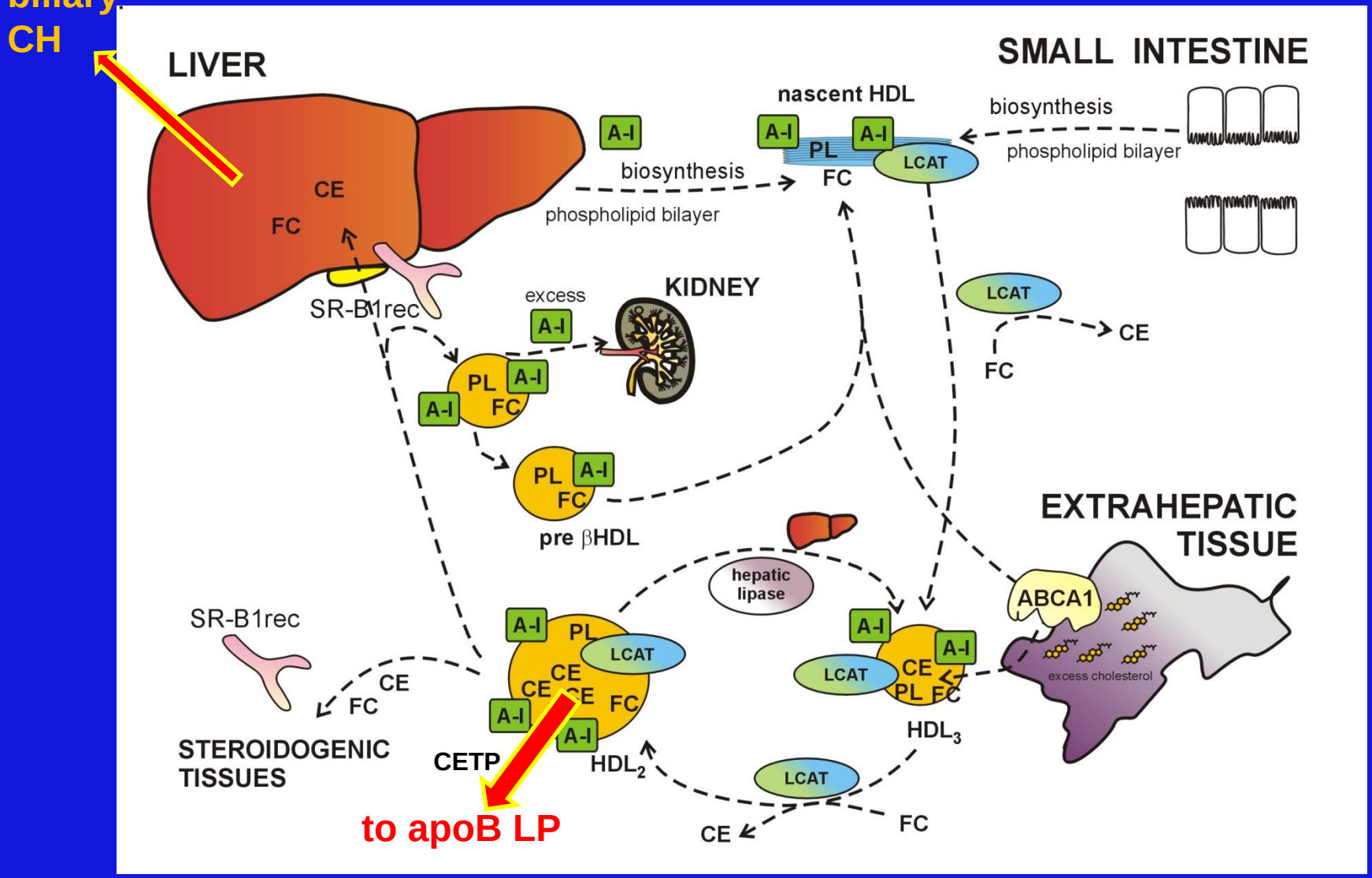
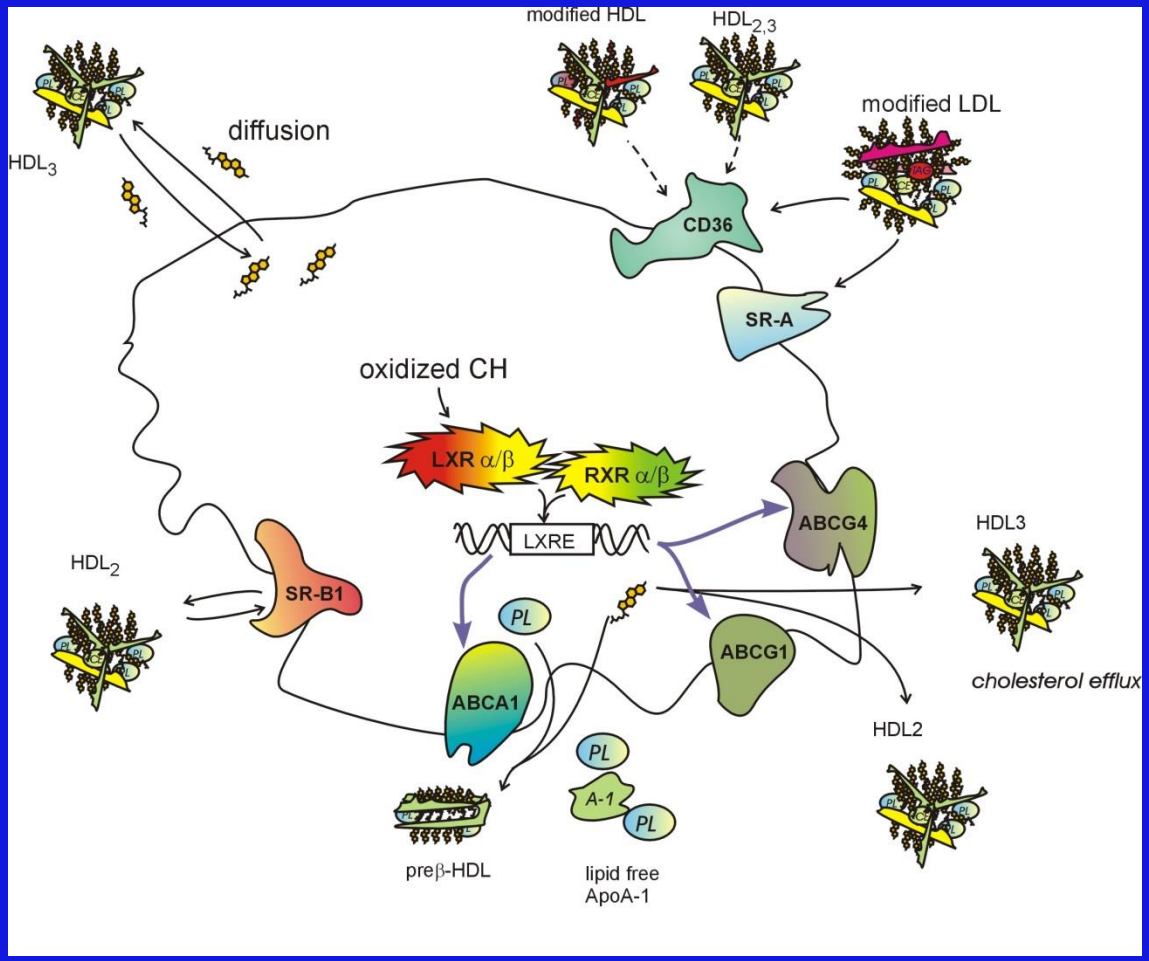


Reverse cholesterol transport

sterol transport from macrophages



Other roles of HDL

Exchanges of lipid classes

- facilitating reverse cholesterol transport (LCAT)
- TAG depletion of VLDL/LDL rich particles (CETP)
- remodelling of HDLs (PLTP)

Antioxidant properties

oxPL (LDL) $\rightarrow$ oxPL (HDL)

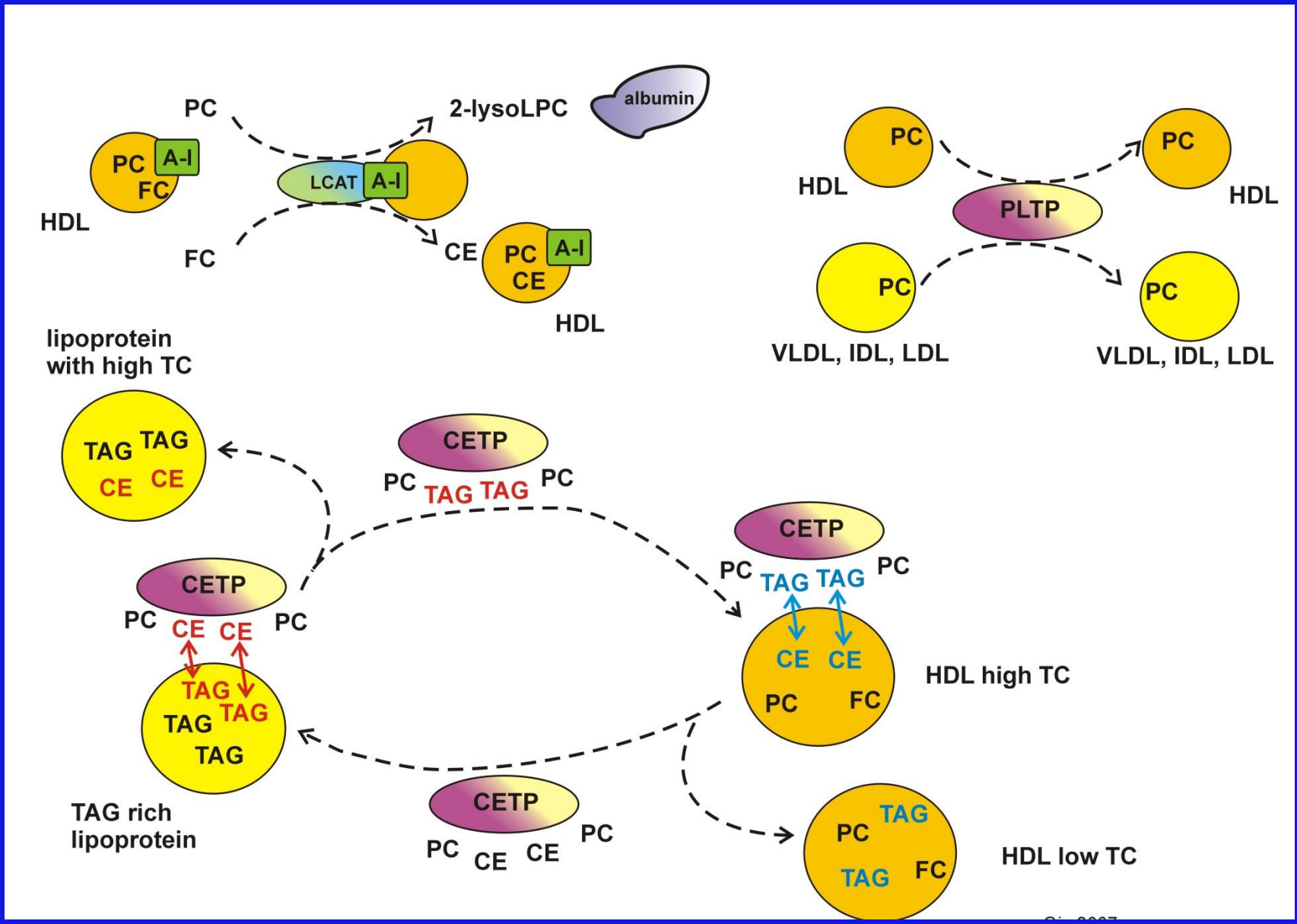
- liberation of oxidized FA from oxPL molecules (PON-1, PAF-AH)

Particle remodelation

- part of acute phase response (SAA for PON-1)

Antiinflammatory/antithrombotic vasodilatory activity

Exchanges of lipid classes



HDL and oxidative stress

1. Removal of oxidised PL from LDL (oxLDL)

oxPL (LDL) → oxPL (HDL)

sdHDL are easy acceptors for oxPL (oxLDL/membranes)

2. Inactivation of oxidised PL

- *via* redox active residues in apo A-I (Met)

  PLOOH → PLOH

- *via* liberation of oxidized FA from oxPL molecules

  paraoxonase (PON-1)

  hydrolysis of oxPUFA from oxPL/oxCE

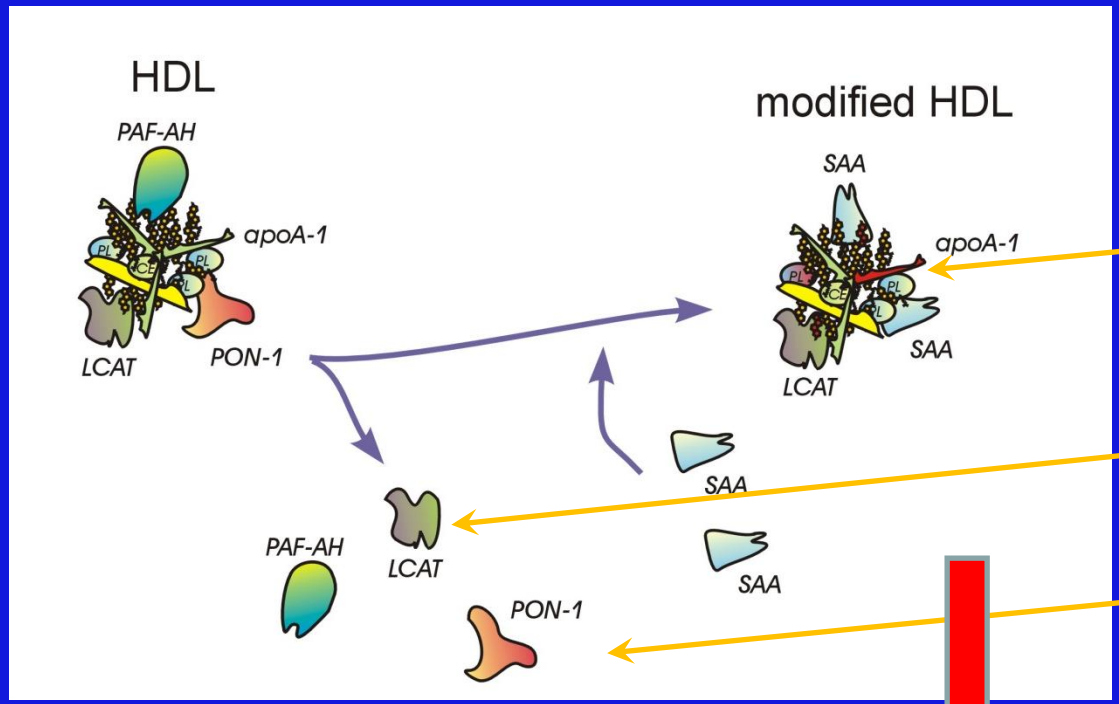
  platelet-activating factor acetylhydrolase (PAF-AH)

  hydrolysis of short chain oxFA from *sn*-2 position in ox PL

HDL remodelation

functionally defective HDL particles

acute phase response/inflammation



modification by glycation oxidation

decreased capacity for RCT

decreased antioxidant capacity of HDL

HDL particles lacking antiatherogenic functions

Lipoprotein receptors I (LDL rec family)

diffusion: CE/TAG
phagocytic mechanisms: modified LP

LDL receptor

- needed for receptor mediated endocytosis of LP (LDL)
- recognizes apoB-100, apoE
- influenced by intracellular cholesterol levels
- *mutations: autosomal dominant FH*
- *defective recycling/endocytosis: autosomal hyperCH*

LRP1 = LDL receptor related protein 1

- needed for receptor mediated endocytosis of LP (CH_R)
- recognizes apoE

also many other molecules

VLDL receptor

- needed for receptor mediated endocytosis of VLDL ($VLDL_R$)
- recognizes apoE, apoJ
- influenced by estrogen, thyroid hormone

apoE

- three alleles ε2/ε3/ε4 (ε2 binds weakly – risk of $VLDL_R$ / CH_R slow catabolism; ε4 - Aβ aggregation - risk factor for Alzheimer disease)

Lipoprotein receptors II (scavenger receptors)

SR-AI receptor

- phagocytic receptor (macrophages → foam cells)
- recognizes modified/oxidized LDL, LPC, PS, FC
- regulates macrophage functions
- *mutations: esophageal cancer?*

SR-B1 = HDL receptor

- needed for transfer of CE into the cell (no degradation of particle)
- steroidogenic tissues, liver, macrophages
- recognizes HDL_2

CD36 receptor (SR-B2)

- expressed in many cell types
- recognizes HDL, mildly oxidized LDL, LP, FA, thrombospondin …

LOX1 receptor

- in highly vascularized tissues, induced by inflammation
- recognizes oxidized LDL

Special lipoproteins

1. Lp(a)

apo (a) attached to apo B-100 with S-S bond
competes with plasminogen for fibrin binding sites
carries oxPL in plasma? ⇒ high Lp(a) = high CVD risk ?
high interindividual concentration variability

2. abnormal lipoproteins

modified/oxidized/negative LDL

LOOH → peroxidation of lipids/apoB-100
easily endocytosed by scavenger receptors

Lp-X, Lp-Y

in liver diseases (albumin + FC (LCAT deficiency))

β-VLDL

in type III HLP (ε2 binds weakly → apoE enriched circulating VLDL/CM)

DISORDERS OF LIPOPROTEIN METABOLISM

DEFINITION AND SIGNIFICANCE OF DISORDERS OF LP METABOLISM

CLASSIFICATION

I. According to changes in lipid/lipoprotein classes:

a) hyperlipoproteinemia (HLP)

b) dyslipoproteinemia (DLP)

II. According to the cause:

a) primary HLP/DLP - independent, genetically determined diseases (60 - 90 %)

b) secondary HLP/DLP - consequence of disease (state) altering metabolism of LP

Definition of hyperlipoproteinemia, hyperlipidemia and dyslipoproteinemia

Hyperlipoproteinemia

= state connected with elevation of one or more LP classes

Hyperlipidemia

= state, when concentrations of TC and/or TAG exceed borderline concentration [defined by 90/95th percentiles]

Dyslipidemia

a) = state, characterised by lowered concentration of HDL-C
HDL-C ≤ 0.9 mmol/l in M (resp. 1.10 mmol/l for F)

b) more generally, any disorder of LP

Pathogenesis of lipoprotein disorders

I. ↑ synthesis of cholesterol and/or triacylglycerols → ↑ secretion of LP

II. disturbed metabolism of lipoproteins

- changes in remodelation of particles → abnormal composition:

LP-X (liver cirrhosis), small dense LDL

- ↓ catabolism of lipoproteins

III. combination of abovementioned mechanisms

+ interaction of genetically susceptible background and non genetic effects (nutritional, metabolic, disease states)

Classification of phenotypes of hyperlipoproteinemias

Primary HLP

Phenotype	Lipoprotein cholesterol					Primary cause
	CM	VLDL	IDL	LDL	HDL	
I	↑			↓	↓	deficiency/inhibitor of LPL deficiency of apo C-II deficient apo A-V, LMF1
IIA				↑		FHC (LDLr def.), PHC, deficient B-100
IIB		↑		↑↑		familial combined hyperlipidemia
III	↑ (CH-R)	β-VLDL	↑			familial HLP III type (apoE ε2) familial deficiency of HL
IV		↑			↓	FHTG (polymorphisms of LPL) polymorphisms of apo A-V
V	↑	↑		↓	↓	FHTG (decompensation) deficiency of apo C-II, A-V

LPL – lipoprotein lipase, LMF1 – lipase maturation factor 1, HL – hepatic lipase, CH-R – chylomicron remnants, FHC – familial (= monogenic, "receptor") hypercholesterolemia, PHC – polygenic hypercholesterolemia, FHTG – familial hypertriacylglycerolemia

Classification of phenotypes of hyperlipoproteinemias

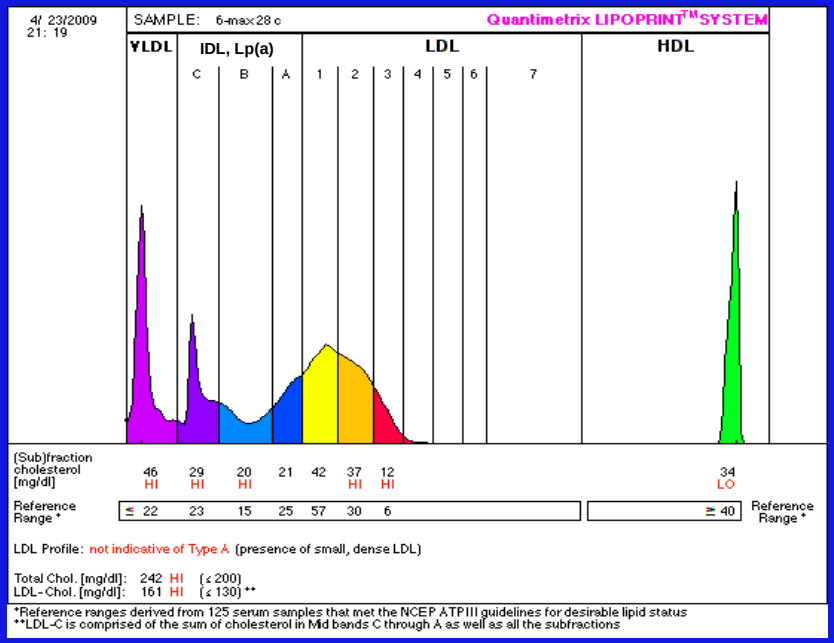
Secondary HLP

Phenotype	Lipoprotein cholesterol					Secondary cause
	CM	VLDL	IDL	LDL	HDL	
I	↑			↓	↓	systemic lupus erythematodes (rarely)
IIA				↑		hypothyreosis, anorexia nervosa
IIB		↑		↑↑		nephrotic syndrome, anorexia nervosa, DM
III	↑ (CH-R)	b-VLDL	↑			hypothyreosis, DM, obesity
IV		↑			↓	DM, chronic renal insufficiency
V	↑	↑		↓	↓	EtOH abuse, diuretic treatment, estrogens (hormonal contraception, hormonal replacement therapy)

DM – diabetes mellitus

Analysis of cholesterol in LP classes

electrophoresis



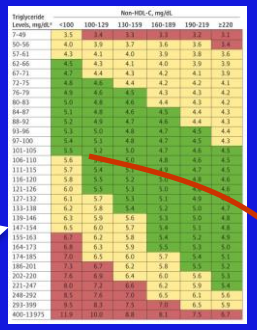
plasma at 4°C overnight

I **IIA** **IIB** **III** **IV** **V**

CM↑ **LDL↑** **VLDL↑** CM↑ **VLDL↑** **CM+VLDL↑**
HDL↓ **LDL↑** **IDL↑** HDL↓ HDL↓

NMR, HPLC, UC
- very expensive, time consuming

Direct LDL-C
- now possible biased at high TAG



linked with

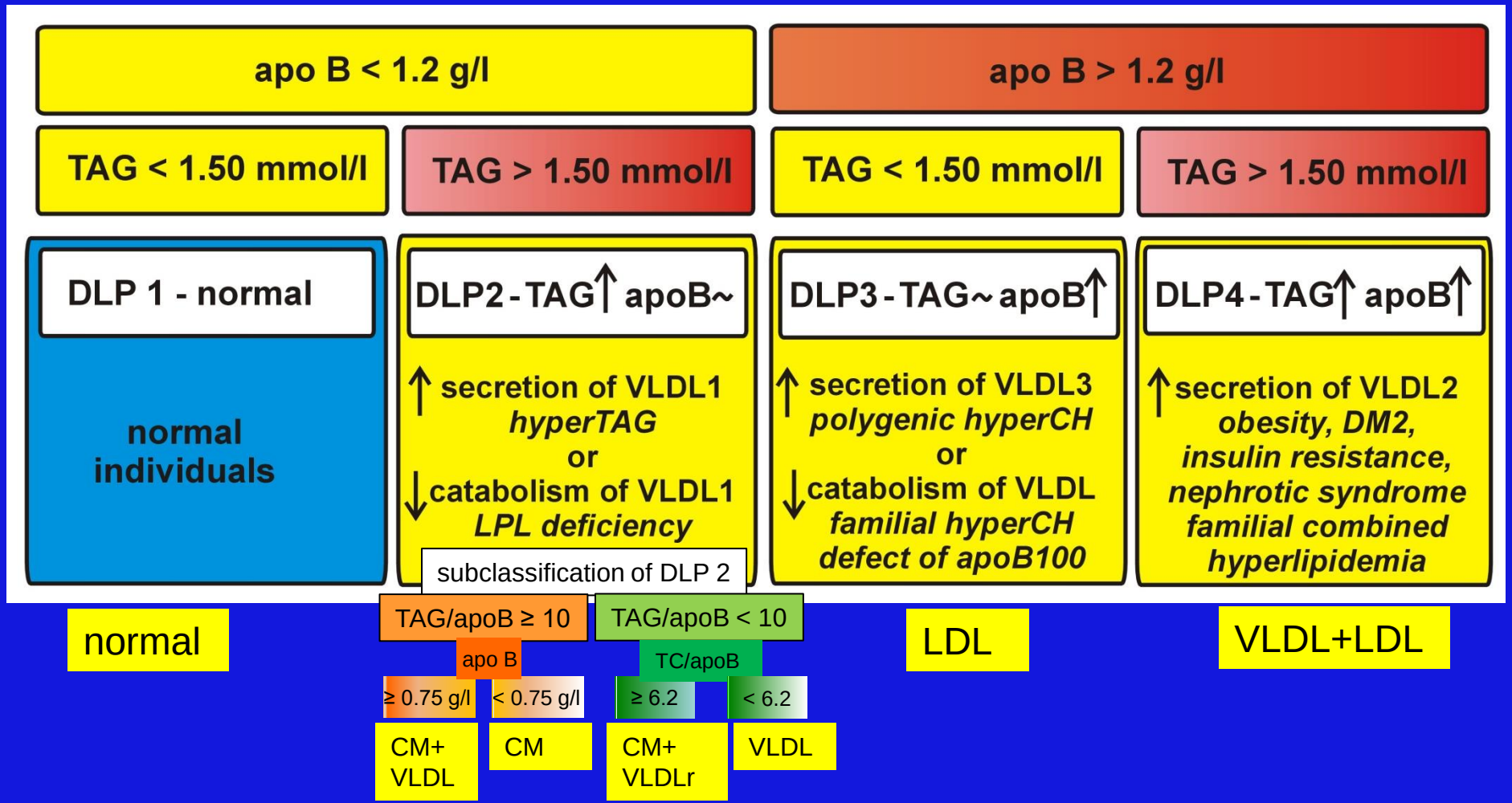
known TAG negligible ?? known

Friedewald: TC = VLDL-C + ~~IDL-C~~ + LDL-C + HDL-C

LDL-C estimation (mg/dL): LDL-C = TC – HDL-C – TAG/5

Chylo-C, VLDL-C, IDL-C – missing information?

CLASSIFICATION OF DISTURBED LIPOPROTEIN METABOLISM by Sniderman



VLDL1, VLDL2, VLDL3 – subpopulations of VLDL particles

Classification of hyperlipidemias

Type of hyperlipidemia	Disorder in lipoprotein class	Primary cause
Hypercholesterolemia	LDL rarely HDL	Familial hypercholesterolemia (LDLr def.) Polygenic hypercholesterolemia Autosomal dominat hypercholesterolemia (PCSK9 mut.) Sitosterolemia (ABCG5/G8 def.) Famiilal defective ApoB
Hypertriacylglycerolemia	VLDL rarely VLDL + CM rarely CM	Familial endogenous hypertriacylglycerolemia Familial mixed hypertriacylglycerolemia Familial hyperchylomicronemia (LPL def.)
Mixed hyperlipidemia	VLDL + LDL rarely IDL	Familial mixed hyperlipidemia Familial dysbetalipoproteinemia (apoE ε2) Familial hepatic lipase deficiency

LDL – low density lipoproteins, VLDL – very low density lipoproteins, CM - chylomicrons, IDL – intermediary density lipoproteins, HLP - hyperlipoproteinemia

Low concentration of TC and TAG

Abetalipoproteinemia

- = Bassen-Kornzweig syndrome (autosomal dominant)
- mutations in MTTP gene (assembly of apoB LP)
  - ⇒ neither apoB-100 nor apoB-48 in plasma
  - ⇒ fat malabsorption (incl. vitamins A, K, E)

Hypobetalipoproteinemia

- missense mutations in apoB gene (VLDL/CH secretion/circulation)
  - ⇒ truncated versions of apoB-100 *(„apoB-2 to apoB-89“)*
  - ⇒ LDL-C↓ or ↓↓
  - ⇒ fat malabsorption (incl. vitamins A, K, E)

Low concentration of HDL-cholesterol

Genetic factors

- deficiency/abnormal structure of apo-A-I (e.g. Apo A-I $_{Milano}$)
- Tangier disease (deficiency of ABCA1)
- deficiency of LCAT familial vs. ”fish eye disease” (mild)
- deficiency and mutations of LPL
- cholesteryl ester storage diseases (lysosomal CEH)
- Niemann-Pick disease (A, B, C variants)

Non genetic causes

- obesity, hypertriacylglycerolemia
- renal insufficiency
- smoking
- decreased physical activity
- enhanced intake of SFA/diminished supply of PUFA n-3, PUFA n-6
- drugs (thiazides, α-methyl DOPA, spirolactone, phenothiazins)

Endocrinopathies

Metabolic syndrome

3 or more present

↑ waist (abd.obesity) + ↑ TAG + ↓ HDL-C + ↑ Glc (IR) + HTN

→ altered metabolism of TAG rich particles

Insulin resistance

Liver:

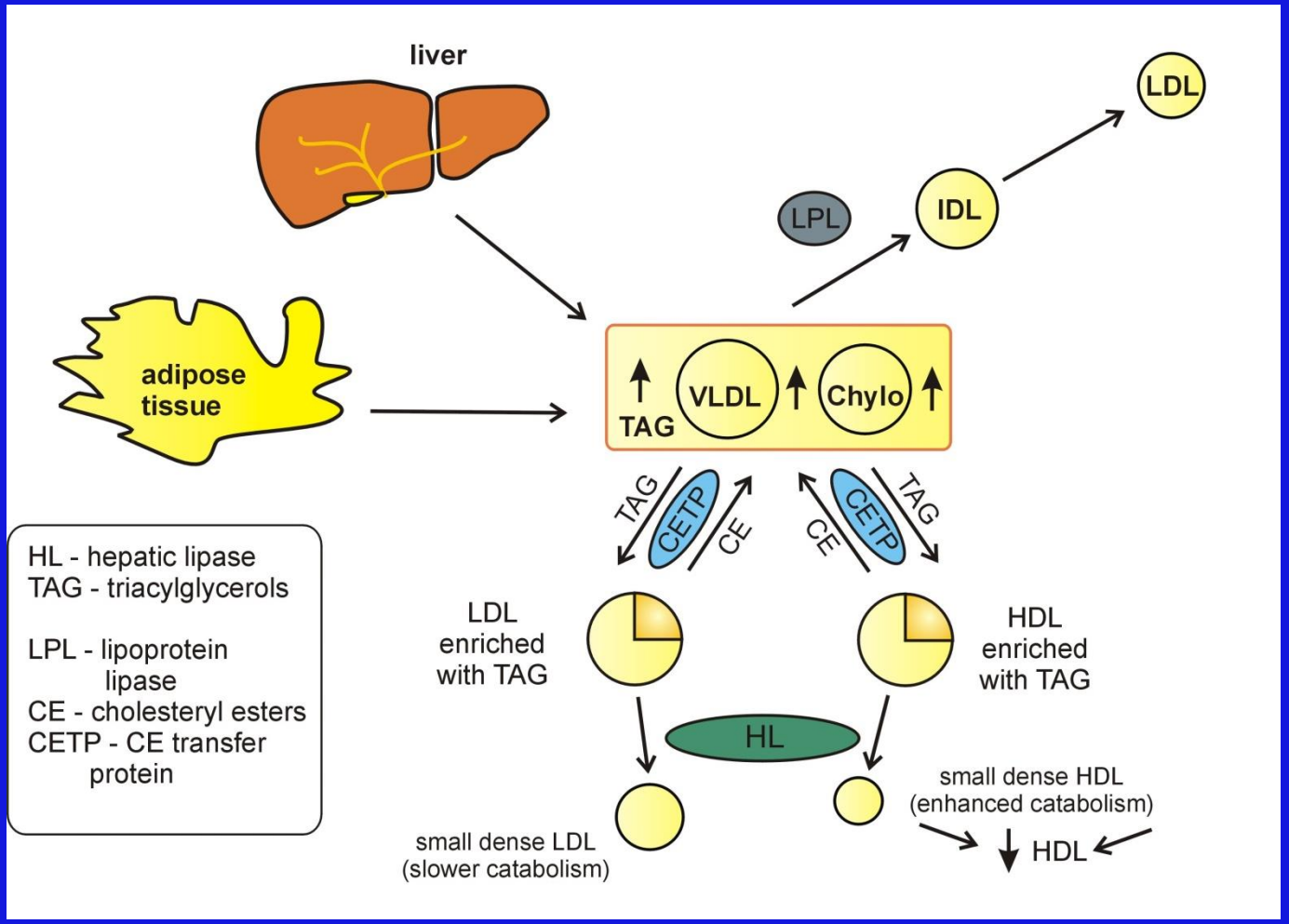
impaired insulin mediated apoB-100 degradation → ↑ VLDL particle biosynthesis/stability

↑ FFA flux from adipose tissue → ↑ hepatic steatosis

Adipose tissue:

impaired insulin antilipolytic effects (HSL inh., FFA uptake) → ↑ FFA flux from adipose tissue

Effect of higher concentrations of particles rich in triacylglycerols on LDL and HDL metabolism



Endocrinopathies

Hypothyreosis

➡ ↓ activity of LDL receptors and LPL (HLP IIA > IIB, III, > IV)

never phenotype HLP I and V, <10% no LP change

with E2/E2 ➡ HLP type III

relatively high frequency

(4, resp. 8 % persons with hypercholesterolemias)

Estrogens (hormonal contraception,gravidity)

➡ ↑ VLDL, ↑ LDL and ↑ HDL (FCH) (phenotype IIB, IV)

gravidity

➡ physiological secondary HLP

(estrogens, progesterone, IR, hyperinsulinemia, human placental lactogen)

Lipid metabolism during fasting

Mobilization of lipid stores

adipose tissue

activation of HSL: TAG → glycerol + 3 NEFA

albumin

liver

gluconeogenesis *(for brain, muscles)*

ketone bodies *(for brain)*

depletion of glycogen

muscle proteins → AA

TCA cycle intermediates (oxaloacetate) are used for gluconeogenesis

acetylCoA excess

Further reading

Textbooks, monographs

Biochemistry of Lipids, Lipoproteins and Membranes (6th Ed); Ridgway ND, McLEod RS (Eds.), Elsevier, Amsterodam (The Netherlands) 2015

Lehninger Principles of Biochemistry (6th Ed); Nelson DL, Cox MM (Eds.), Susan Winslow, New York (U.S.A.) 2013

Harper´s Illustrated Biochemistry (28th Ed); Murray RK, Bender DA, Botham KM, Kennely PJ, Rodwell VW, Weil PA (Eds.), McGraw-Hill, New York (U.S.A.) 2009

High Density Lipoproteins: From Biological Understanding to Clinical Exploitation; Eckardstein A, Kardassis D (Eds.). Springer Open, London (UK) 2015

Lipoproteins in Health and Disease; Betteridge J, Shepherd J, Illingworth R (Eds.). CRC Press, London (UK) 1999

Articles

Mu H, Høy CE: The digestion of dietary triacylglycerols. *Progr Lipid Res* 2004; **43**: 105–133.

Alwaili K, Alrasadi K, Awan Z, Genest J: Approach to the diagnosis and management of lipoprotein disorders. *Curr Opin Endocrinol Diab Obes* 2009, **16**: 132–140.

Hegele RA: Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet* 2009; **10**: 109-121.

Hachem SB, Mooradian AD: Familial Dyslipidaemias: An Overview of Genetics, Pathophysiology and Management. *Drugs* 2006; **66**: 1949-1969.

Sniderman AD: Applying apoB to the diagnosis and therapy of the atherogenic dyslipoproteinemias: a clinical diagnostic algorithm. *Curr Opin Lipidol* 2004; **15**: 433–438.

Web sources

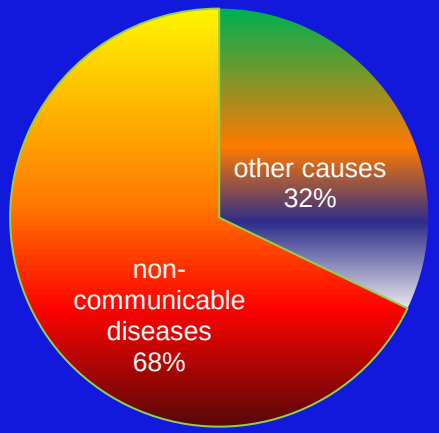
http://themedicalbiochemistrypage.org - the Medical Biochemistry Page

ATHEROSCLEROSIS
pathogenesis
risk factors

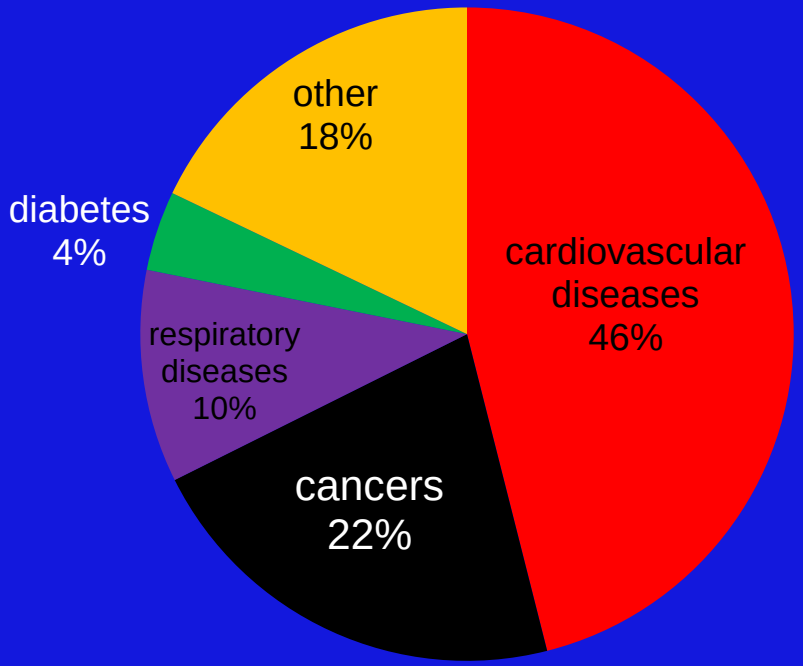
Marek Vecka

Cause of death and burden of disease

worldwide mortality



non-communicable diseases



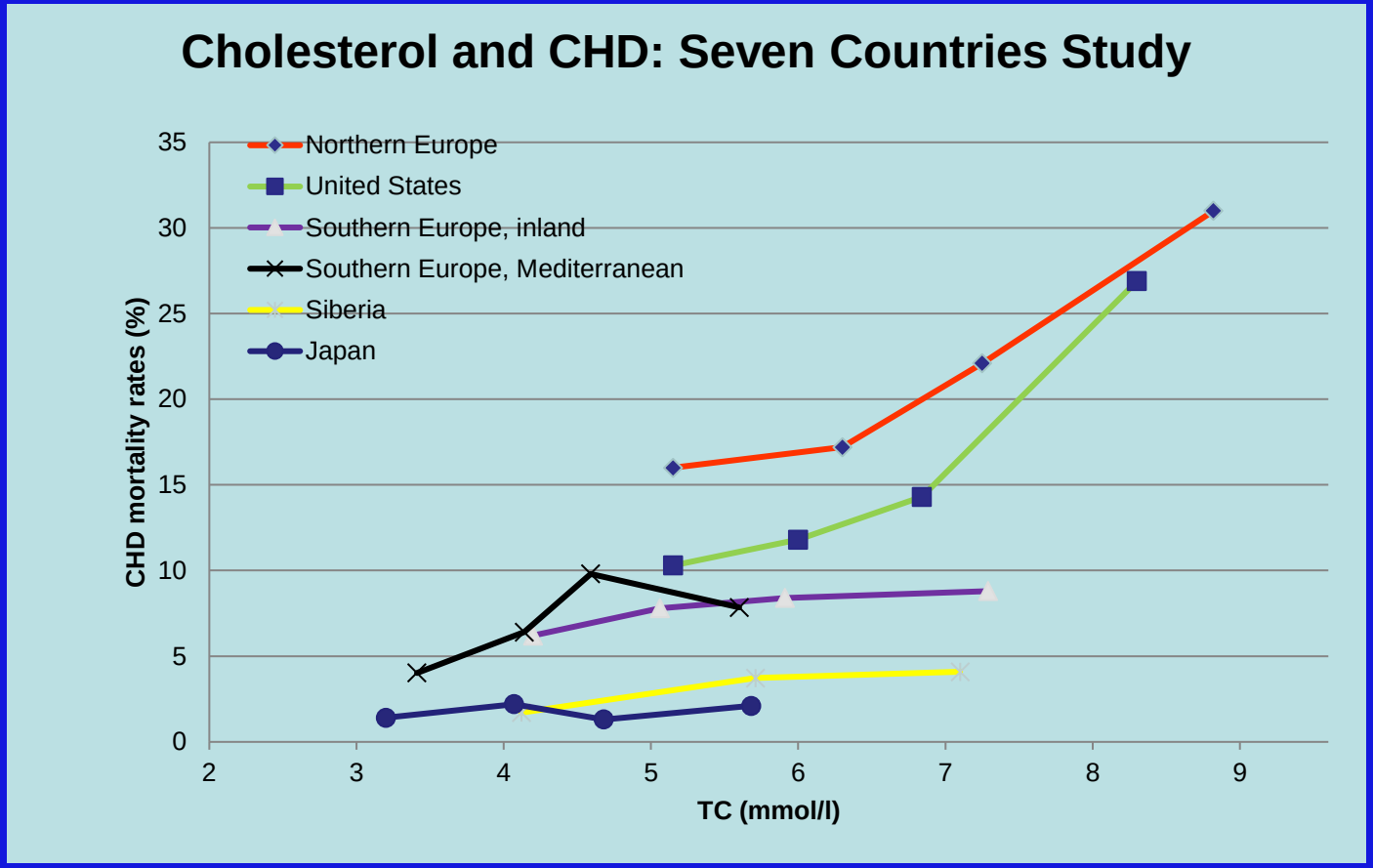
Atherosclerosis

Cardiovascular diseases

atherosclerosis is most important cause

cholesterol plays a crucial role in the pathogenesis of atherosclerosis

Seven countries study: cholesterolemia and mortality



Atherosclerosis

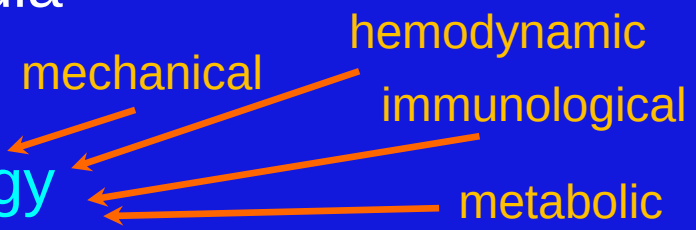
Definition

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

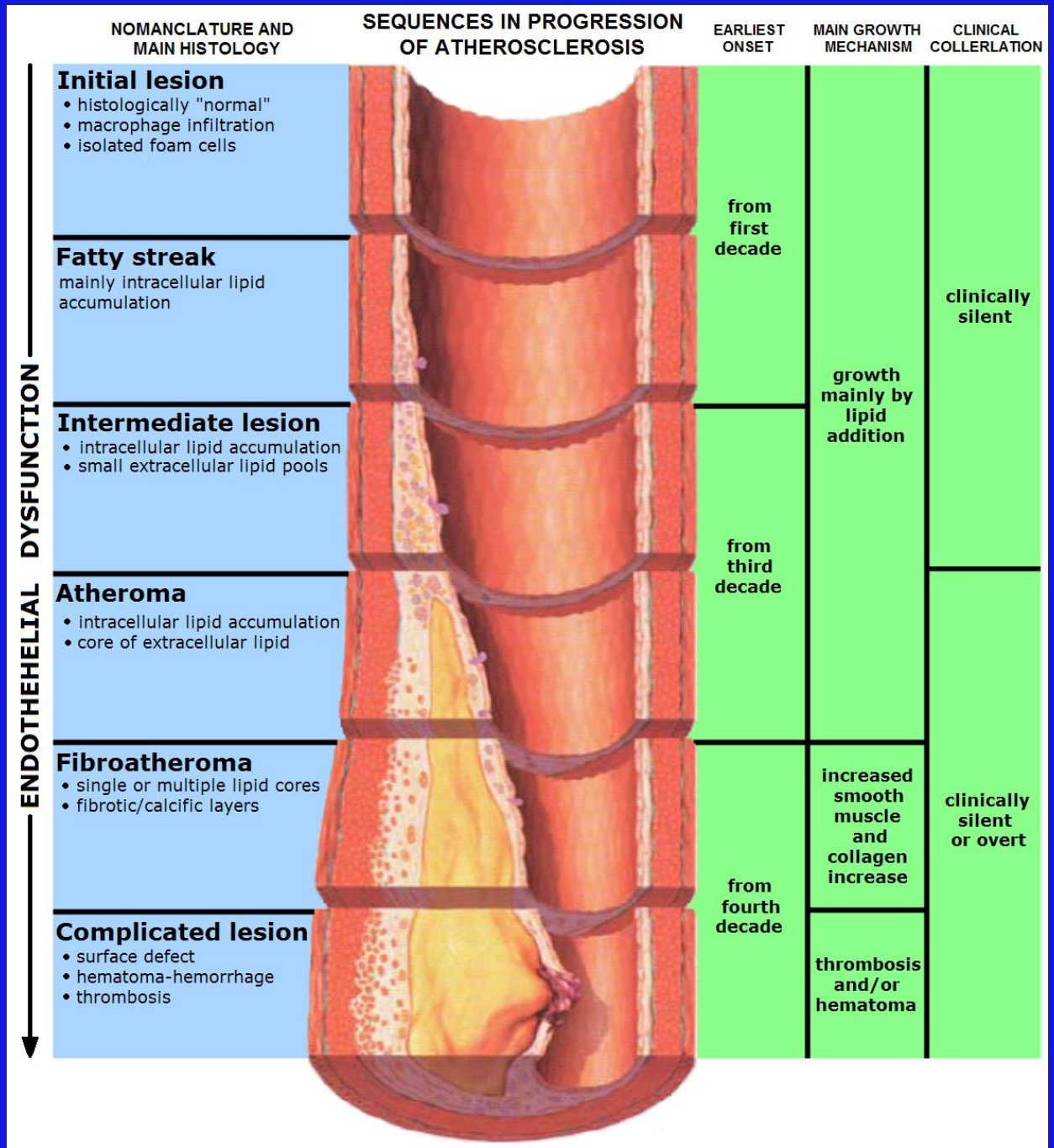
lipid/matrix accumulation

the key role – oxidized lipoproteins

Phases of atherosclerosis

early phase* – *accumulation of lipids

***late phase* – intimal proliferation and adjacent thrombosis**



from Wikipedia/en

Early phase of atherosclerosis

1st type of lesion – isolated foam cells derived from macrophages

2nd type of lesion (*fatty streak*) – accumulation of foam cells

intracellular lipid accumulation

3rd type of lesion (*intermediary lesion)* – small amounts of extracellularly deposited lipids (debris from foam cells)

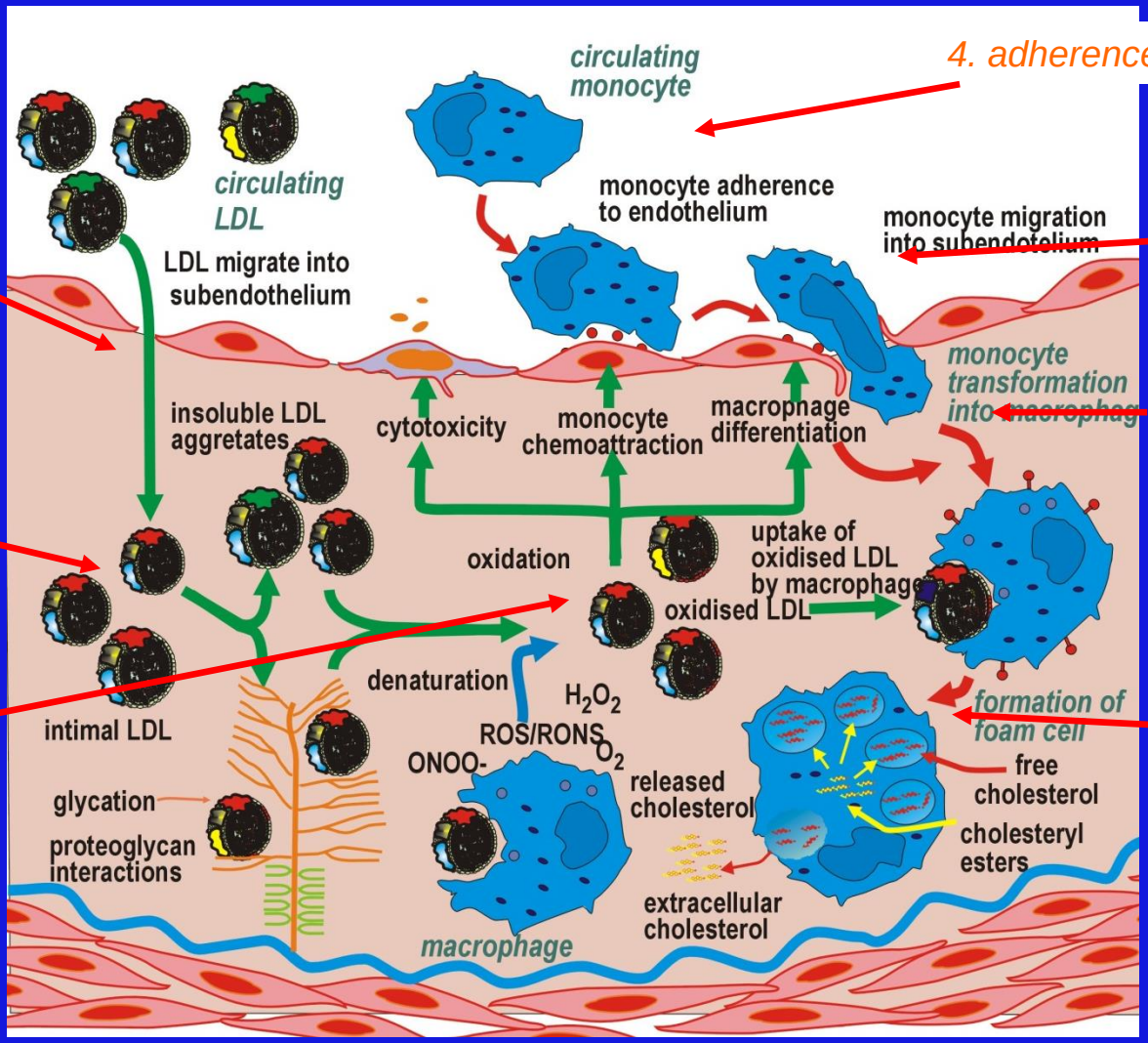
4th type of lesion (*atheroma*) – lipid core localised in the basis of the lesion (almost only extracellularly accumulated lipids)

DEVELOPMENT OF FATTY STREAK

1. transendothelial transport of LP

2. retention of LP

3. oxidative modification of LP



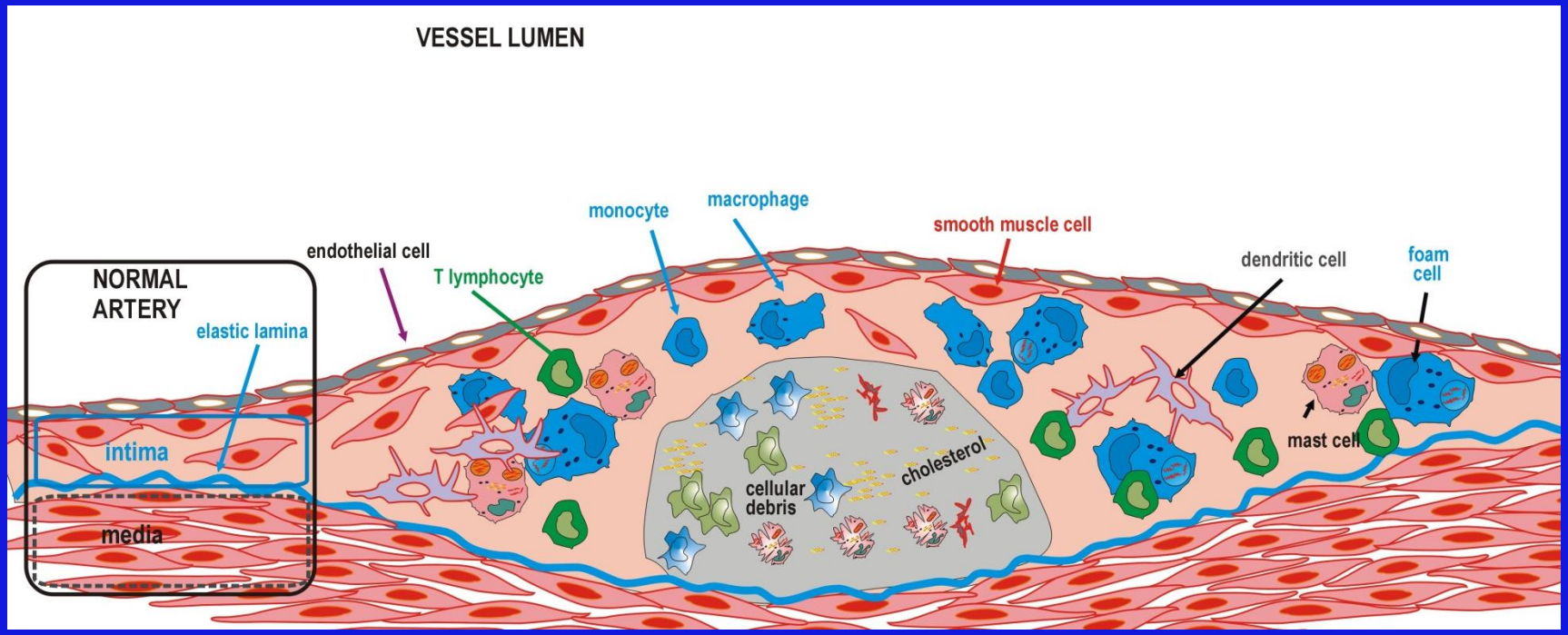
Late phase of atherosclerosis

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

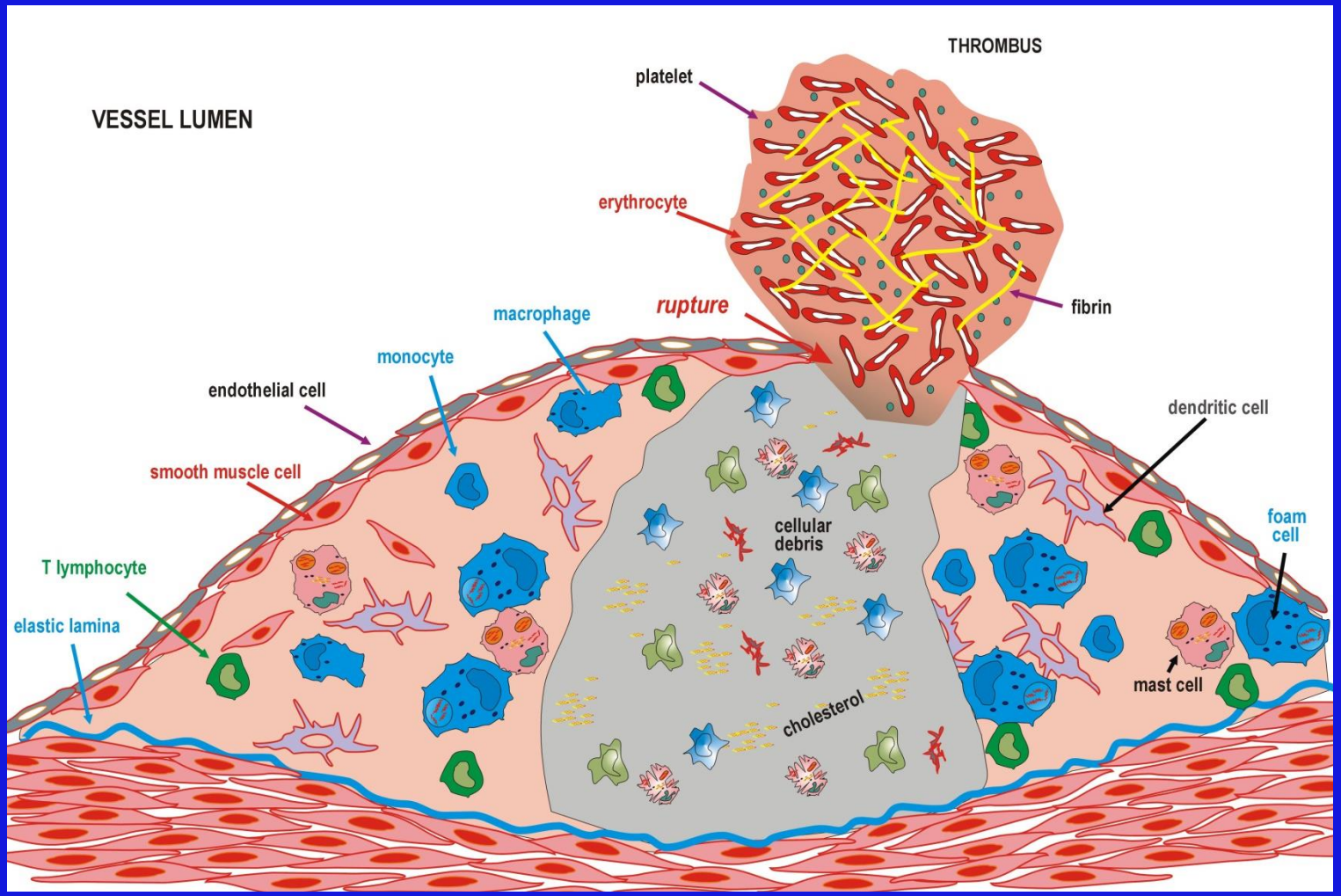
6th type of lesion *(complicated lesion)* - exulceration, hemmorhage into plaque, calcification of necrotic material and artery wall thrombosis

unstable plaque – *see further*

ATHEROSLEROTIC PLAQUE



PLAQUE THROMBOSIS



Atherothrombosis

sudden/impredictable rupture of atherosclerotic plate → platelet activation and thrombus formation

erosion of the plate

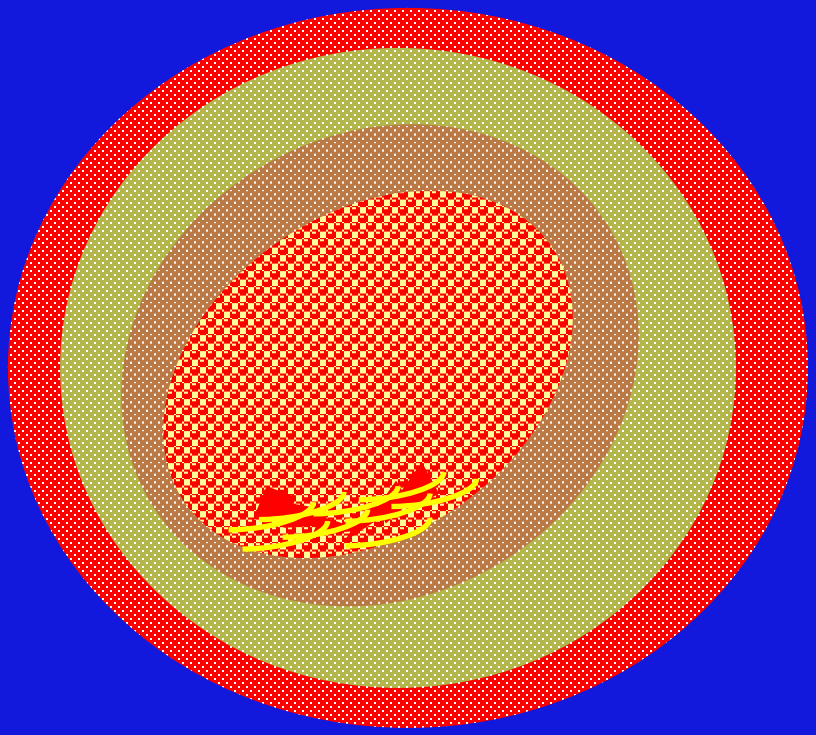
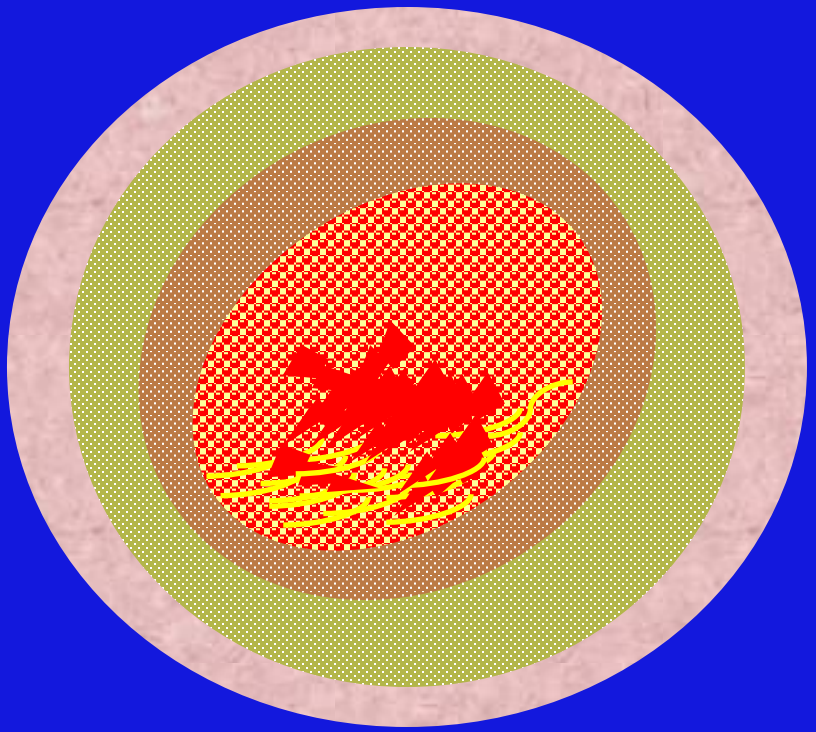


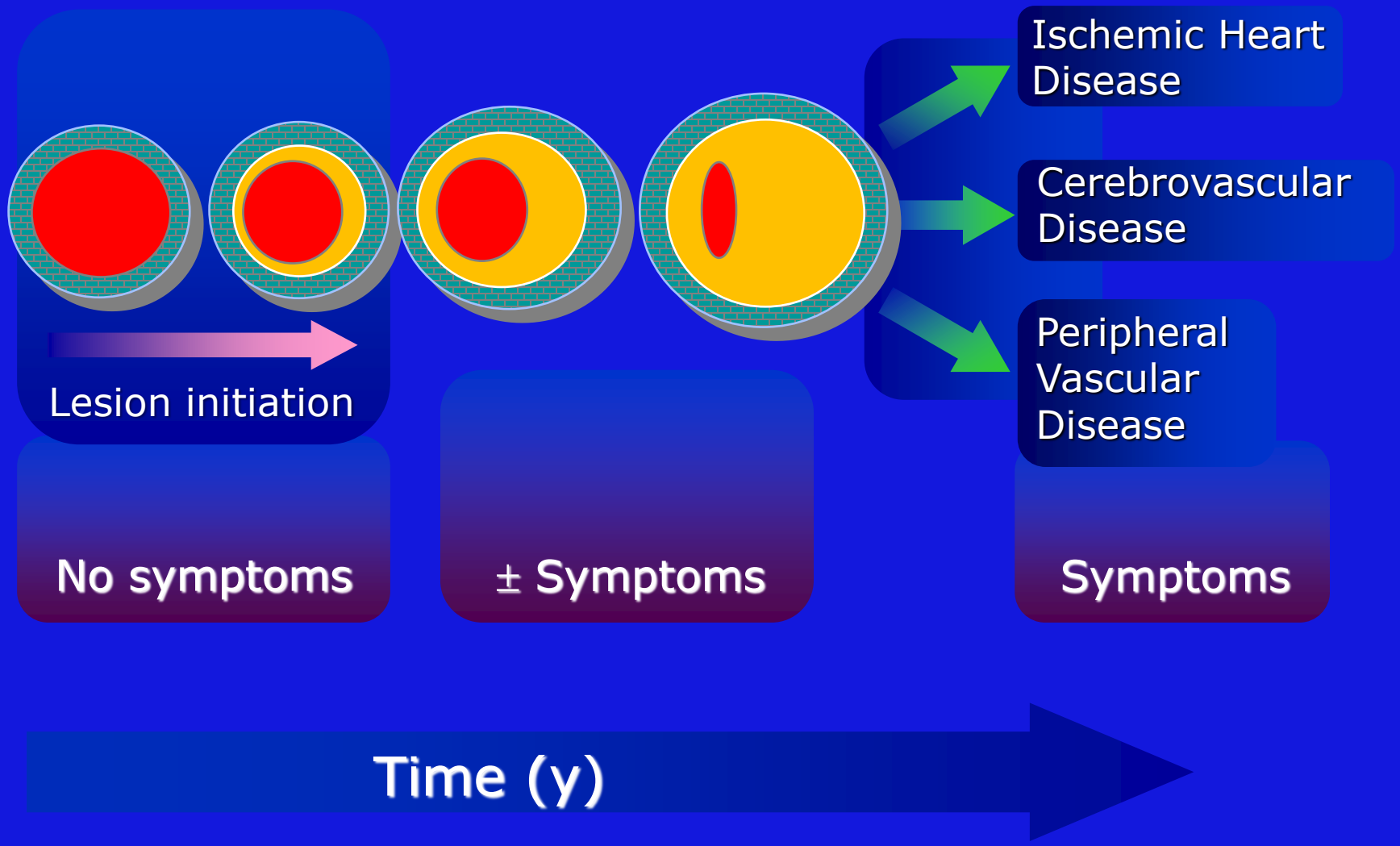
plate rupture



Characteristics of unstable plate in coronary artery

	unstable plate	stable plate
size	30 - 40 % stenosis eccentric	
core lipids	cca 40 % (FC cryst.)	cca 10 %
monocytes/ macrophages/ foam cells	30 % (v/v)	10 % (v/v)
vascular SMC	3 – 5 %	10 – 15 %

Schematic Time Course of Human Atherogenesis



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 atherosclerosis during clinical/laboratory examination

present risk factor ⇒ ↑ relative risk of future atherosclerosis manifestat.

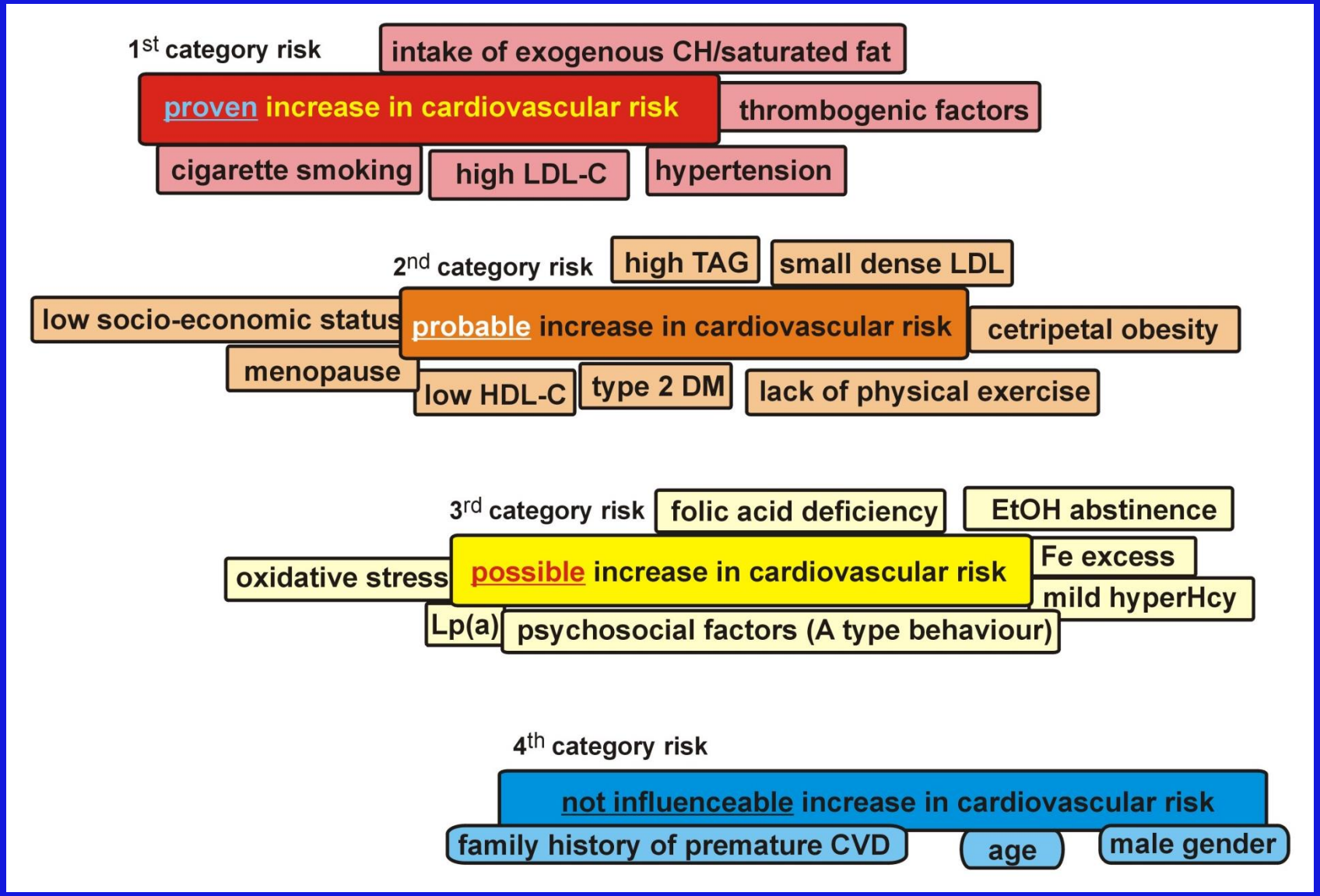
not causally connected
neither denies one another

manifestation of atherosclerosis

this must be supported by intervention studies

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

Categories of cardiovascular risk factors



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

Risk factors of CVD for stratification of risk in primary prevention of CHD

A. Unmodifiable risk factors

I. Age and gender

age: > 45 years in men,

> 55 years in women

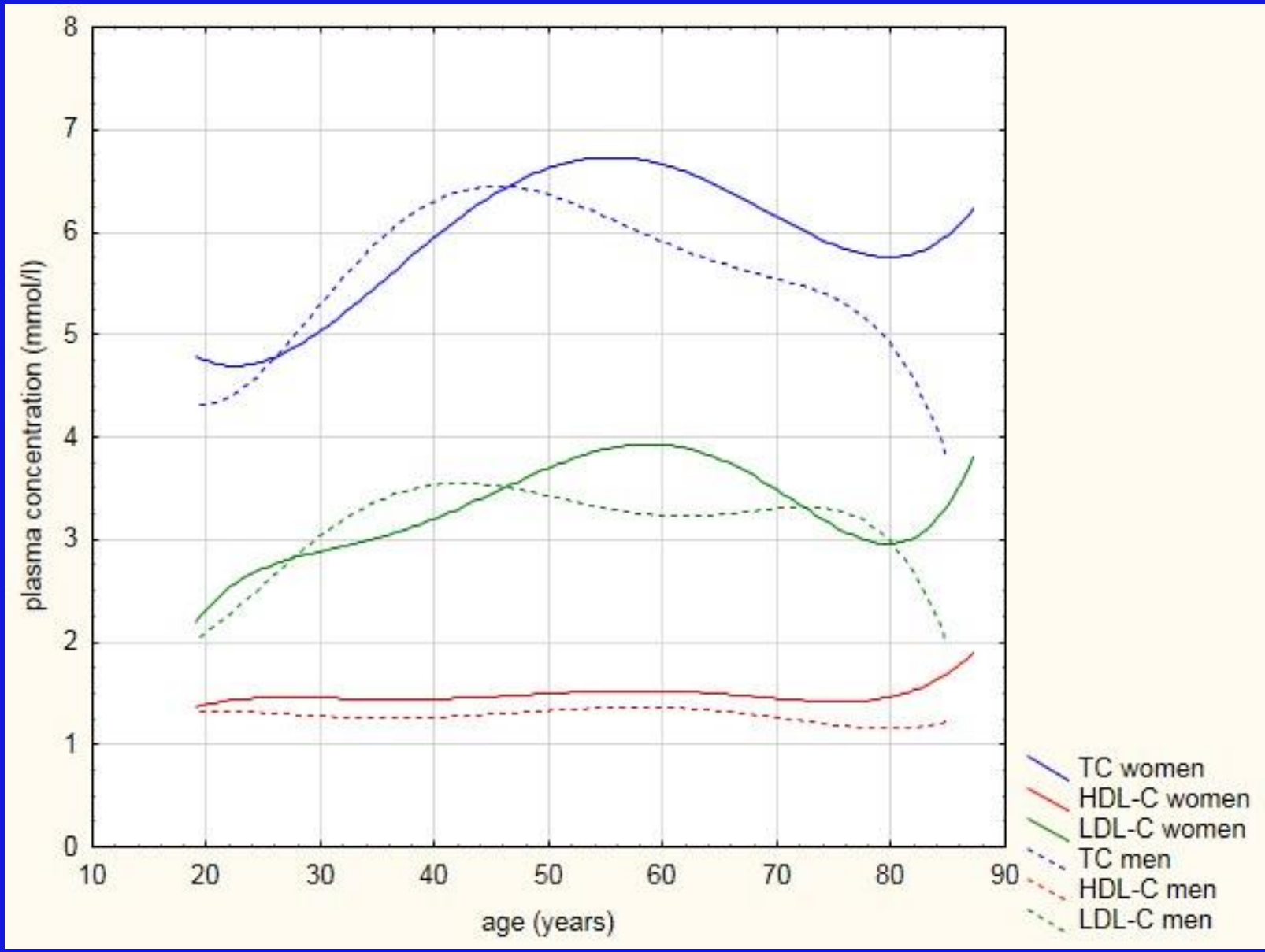
II. Family history of early CHD

< 55 years in male first-stage relatives

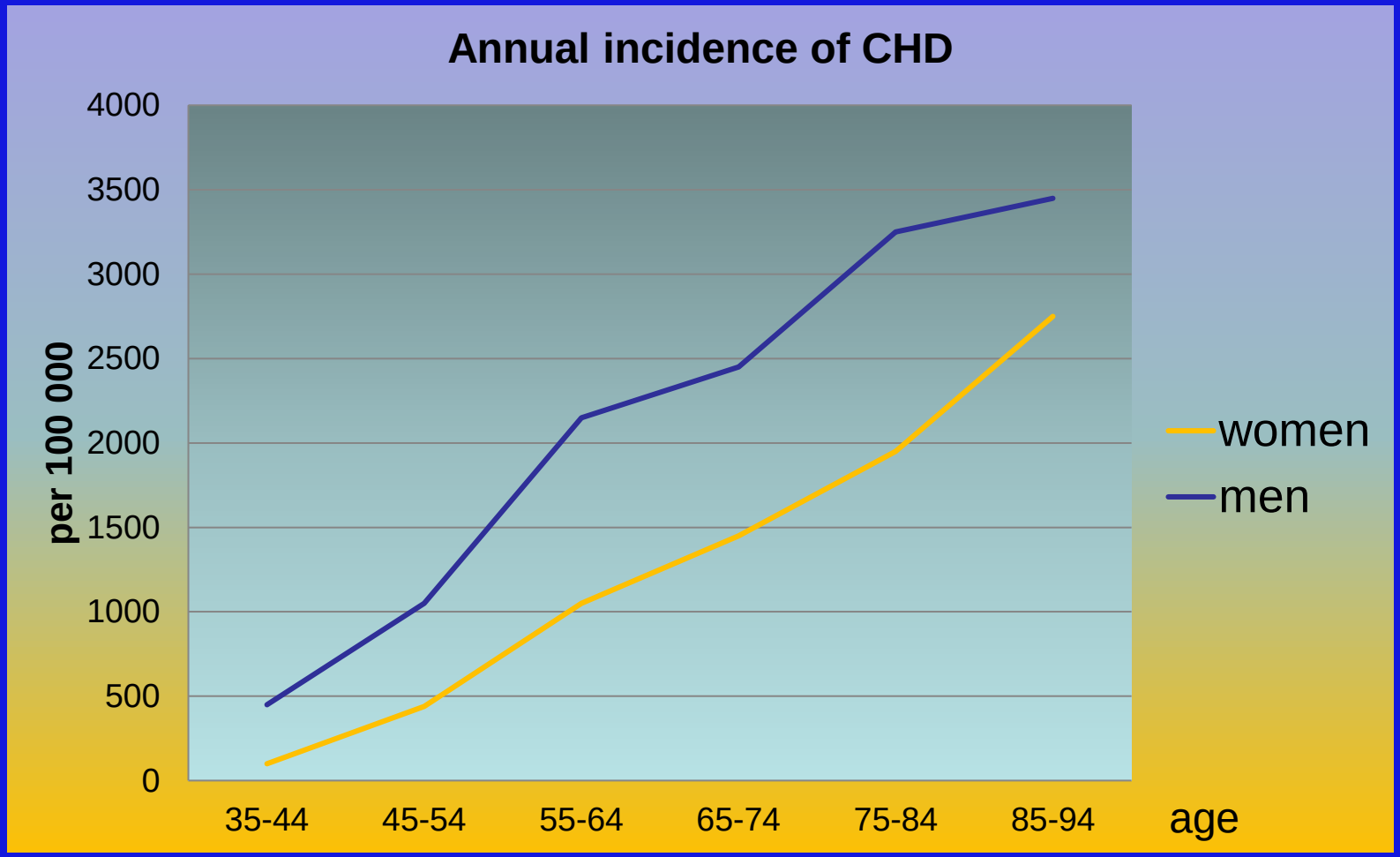
< 65 years in female first-stage relatives

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)

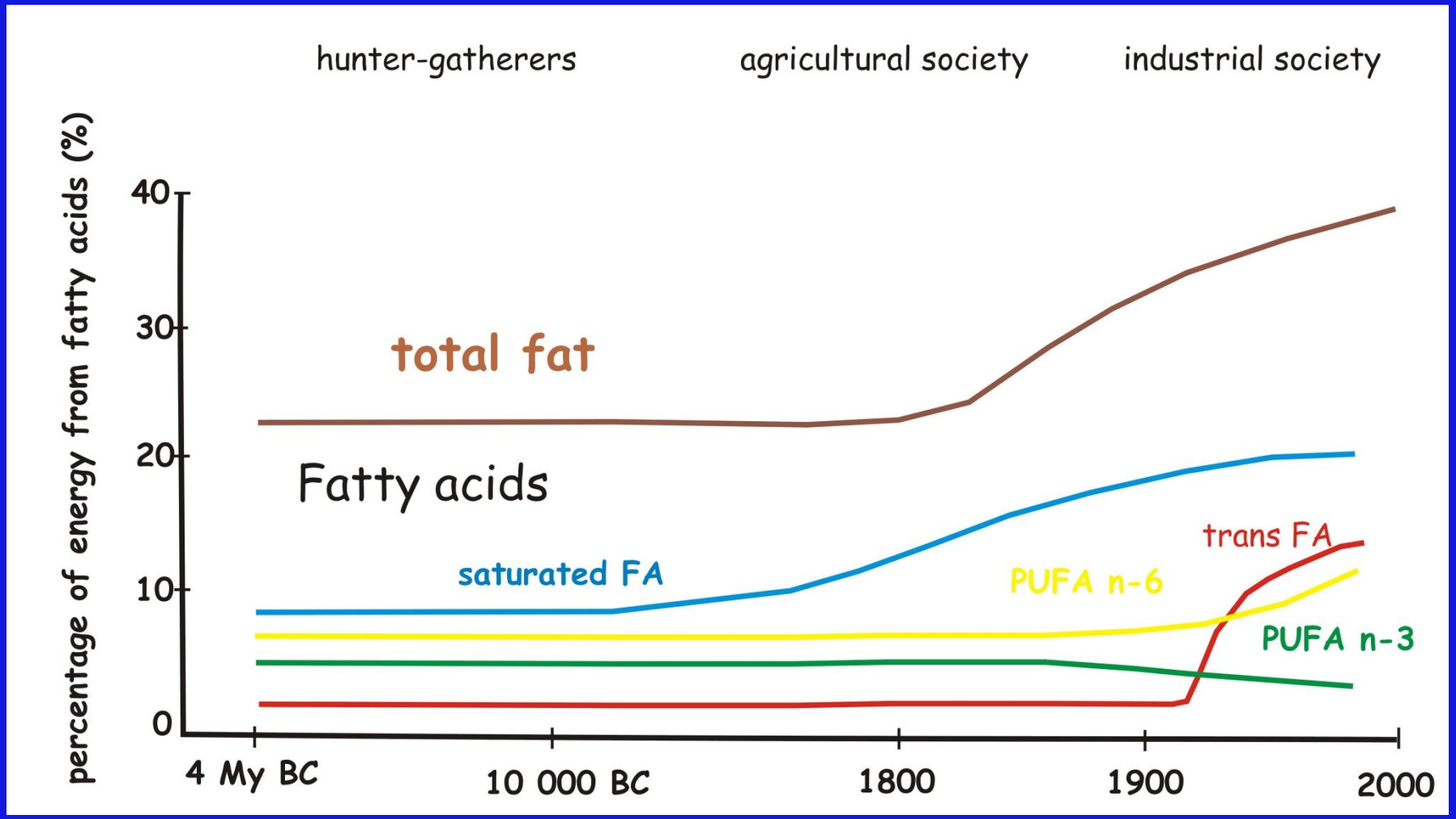
Membrane Transporters (ABCA1)

Gender (ESR1)

Other (CRP, ADIPOQ)

B. MODIFIABLE RISK FACTORS

Intake of fatty acids



Excessive intake of saturated fats

potentiates the rise in plasma TC:

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

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 → ↑ NADH in liver → ↓ FA oxidation → fat=TAG excess)

induction of HTAG → ↑ VLDL (↑ synthesis in hepatocytes)

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

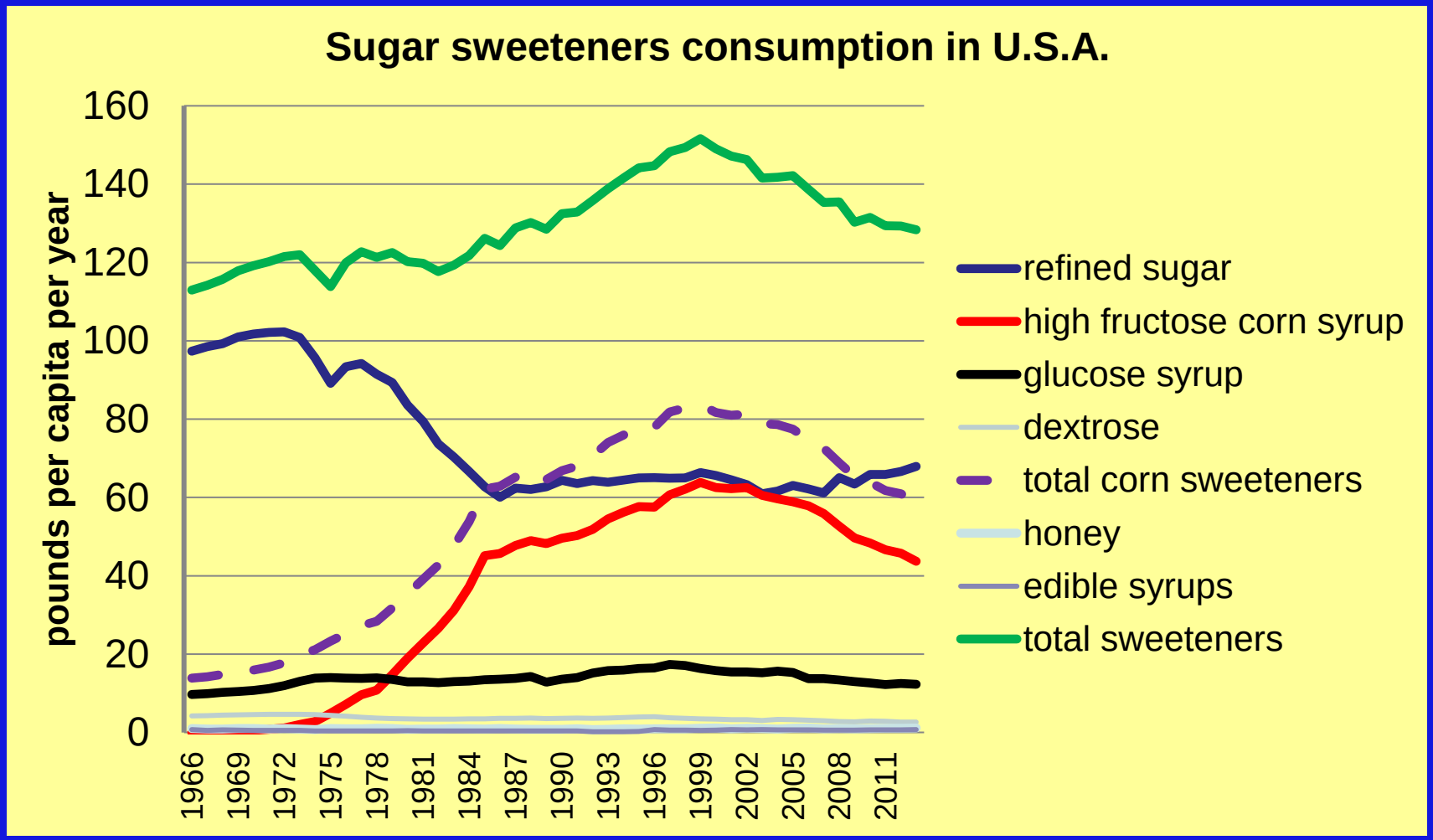
Zieve syndrome

can be a result of chronic EtOH abuse

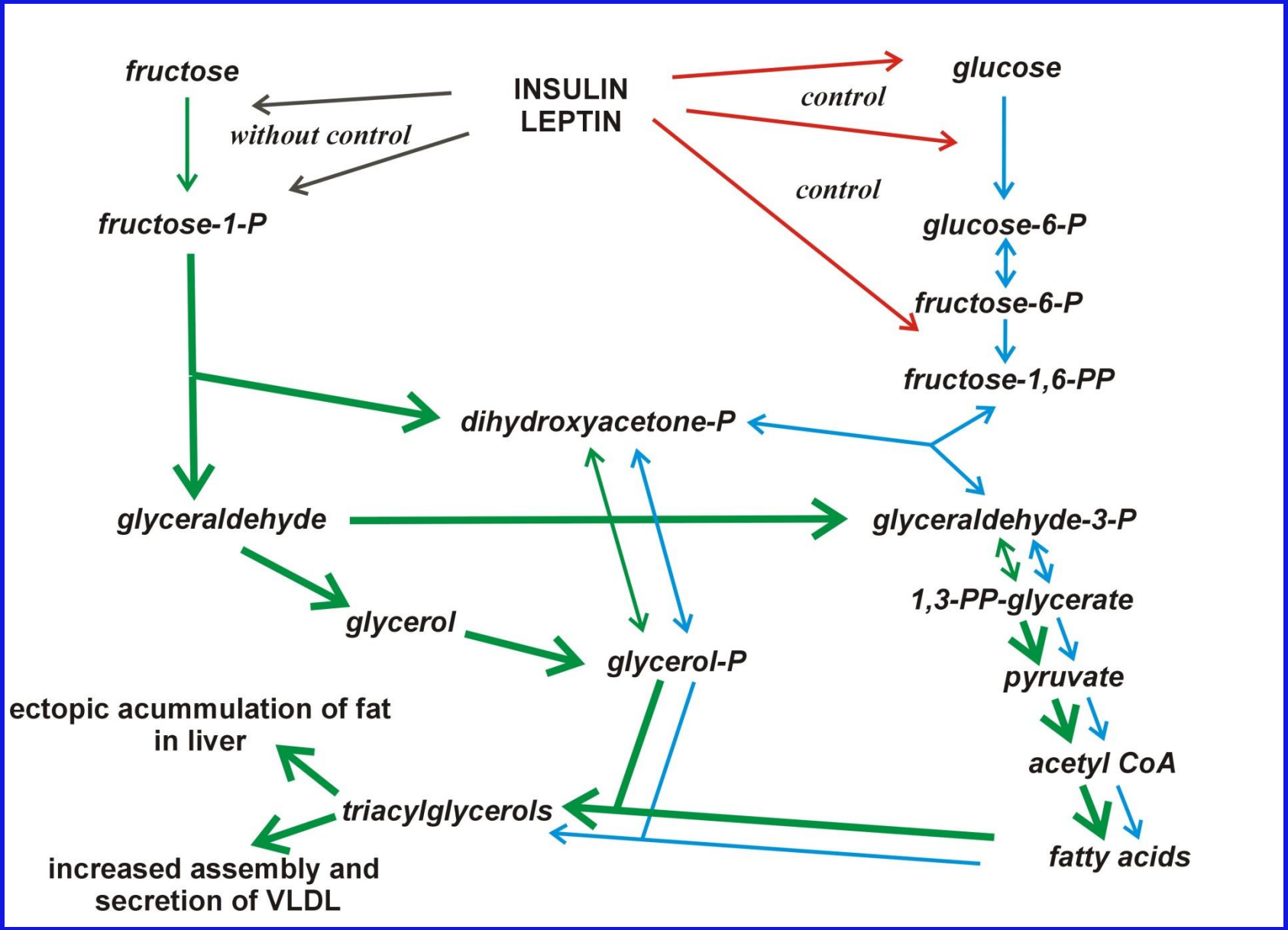
- hyperlipoproteinemia with high CH/VLDL-C and low HDL-C
- secondary deficiency of LCAT
- jaundice and reversible hemolytic anemia

healthy liver → (EtOH) steatosis → (EtOH) cirrhosis → cancer

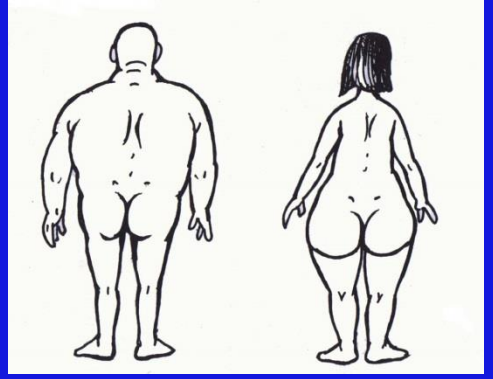
Intake of sugar



Intake of fructose



Overweight and obesity



Gynoid obesity - **only increased TAG and VLDL**

Android obesity - **often with ALP**

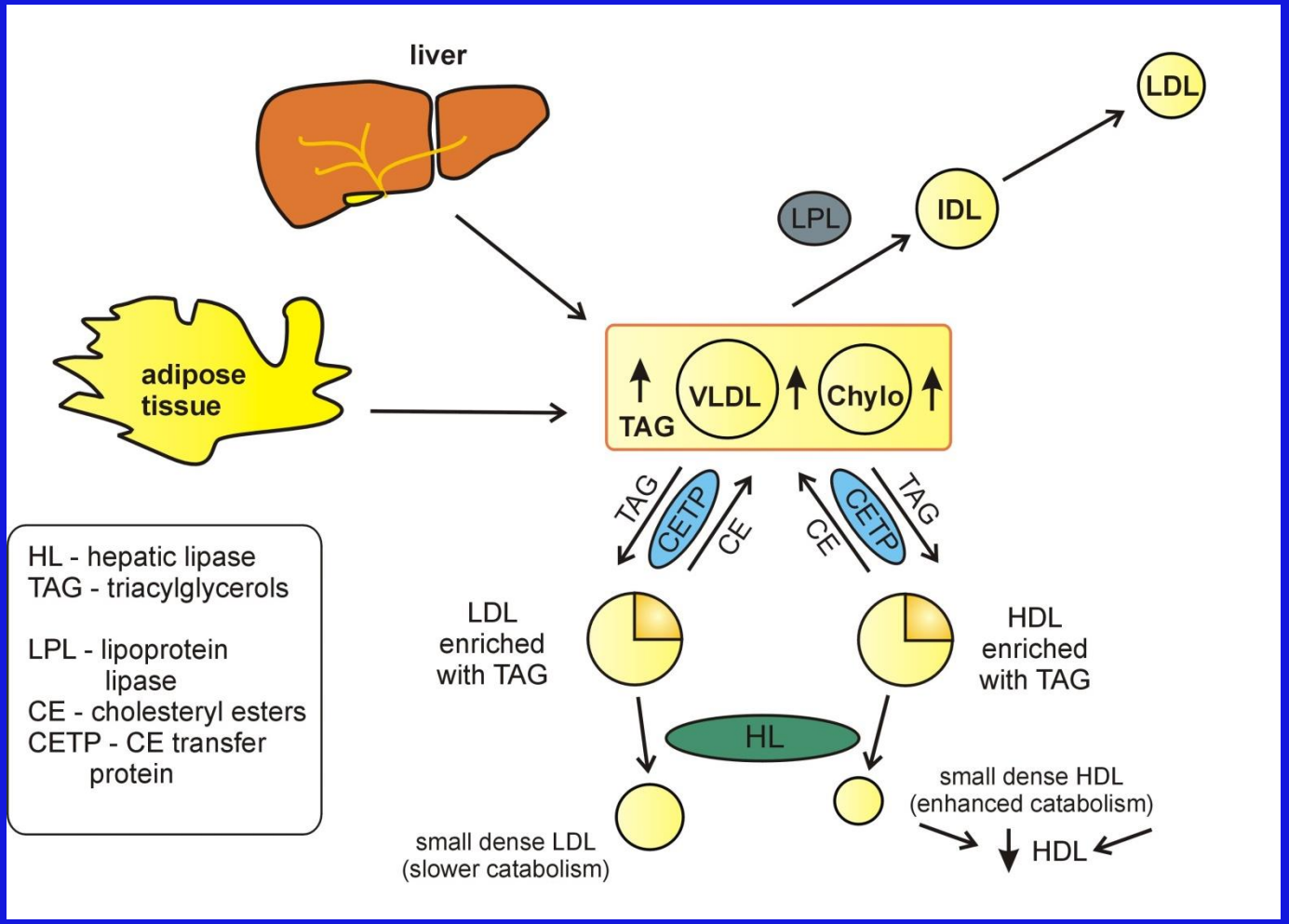
(oxidative stress, ↑ coagulability, chronical inflammation)

atherogenic lipid phenotype (ALP)

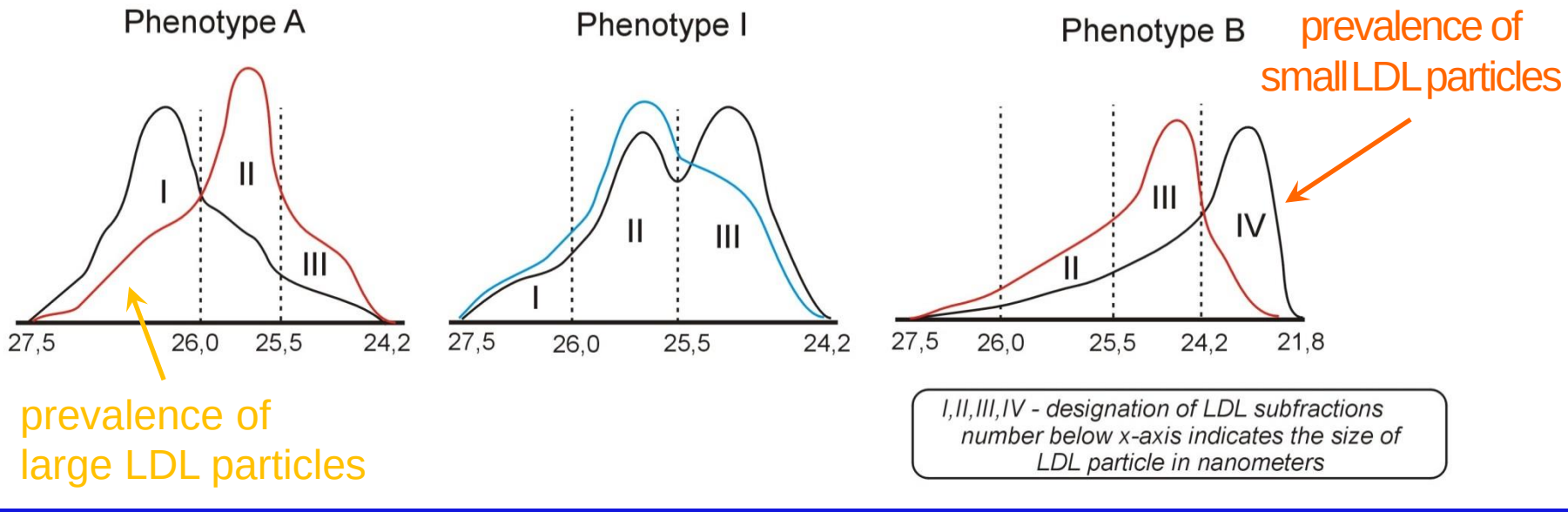
↑ TAG (VLDL) + ↓ HDL-C + ↑ sdLDL

(↑ NEFA, ↑ LDL- apoC-III+)

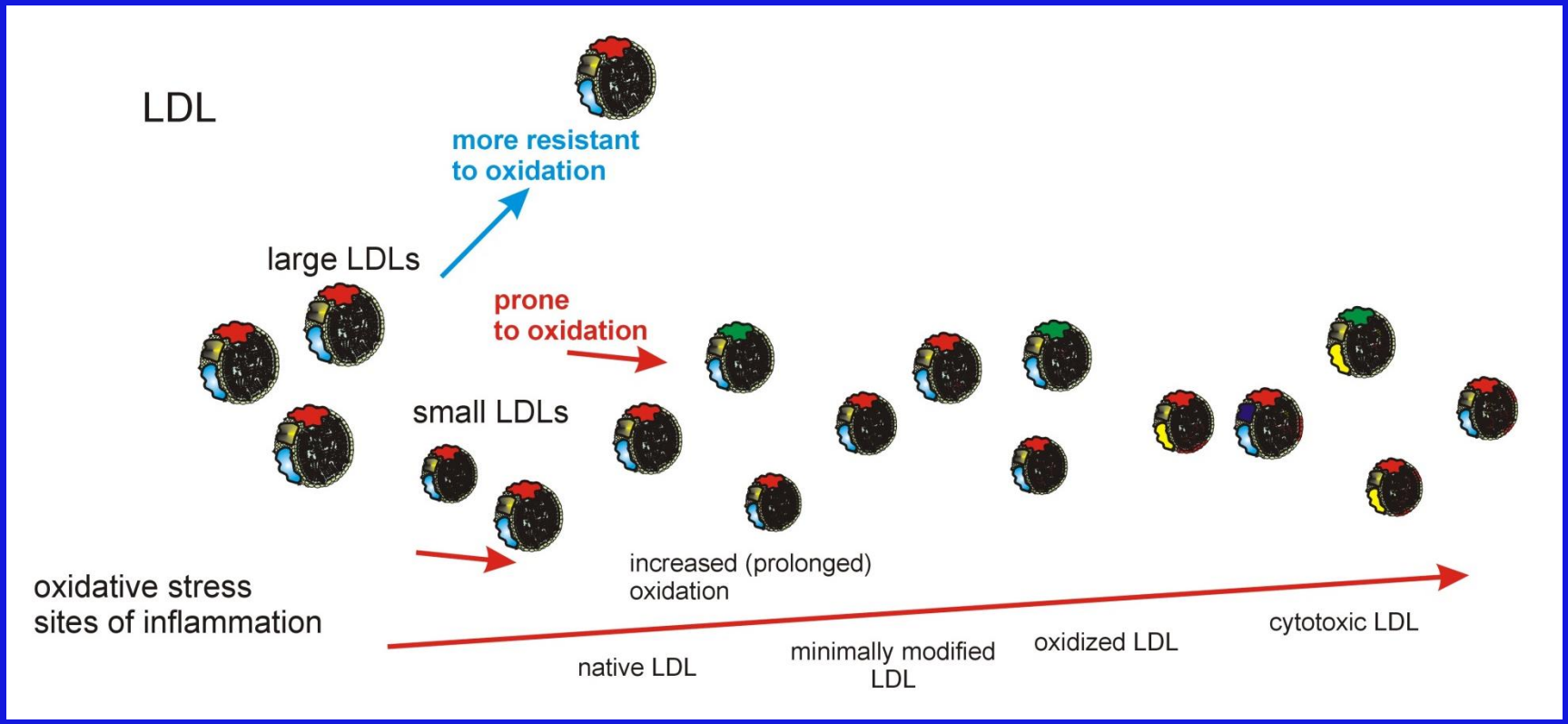
Effect of higher concentrations of particles rich in triacylglycerols on LDL and HDL metabolism



Phenotypes of LDL size



Generation of oxidatively modified LDLs



Properties of oxidatively modified LDL

immunogenicity

endothelial cytotoxicity

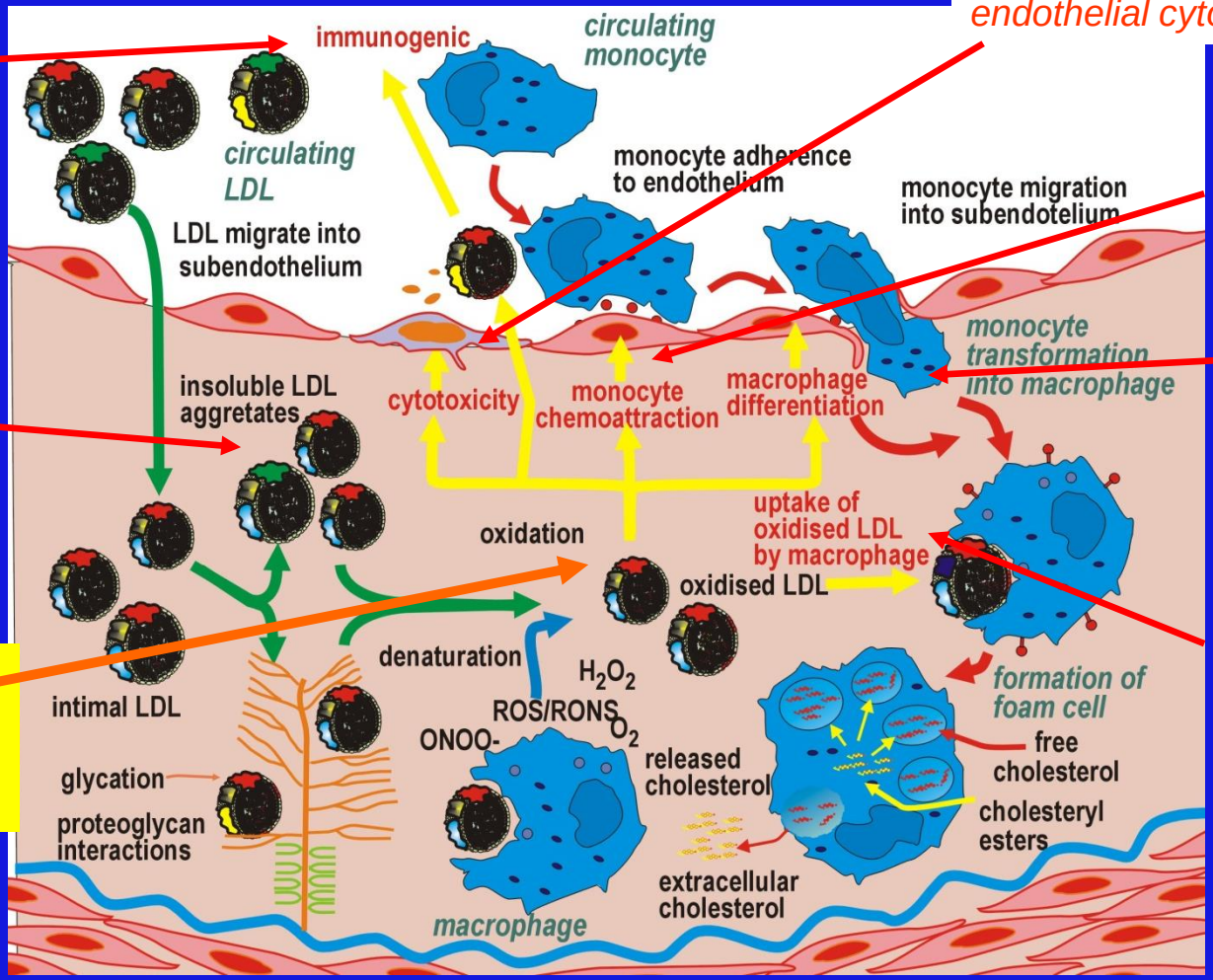
monocyte chemoattraction

macrophage differentiation

enhance retention of LP

catabolism via SR-BI

oxidative modification of LP



Mechanisms of antiatherogenic effect of HDLs

I. Direct effects on lipoprotein metabolism

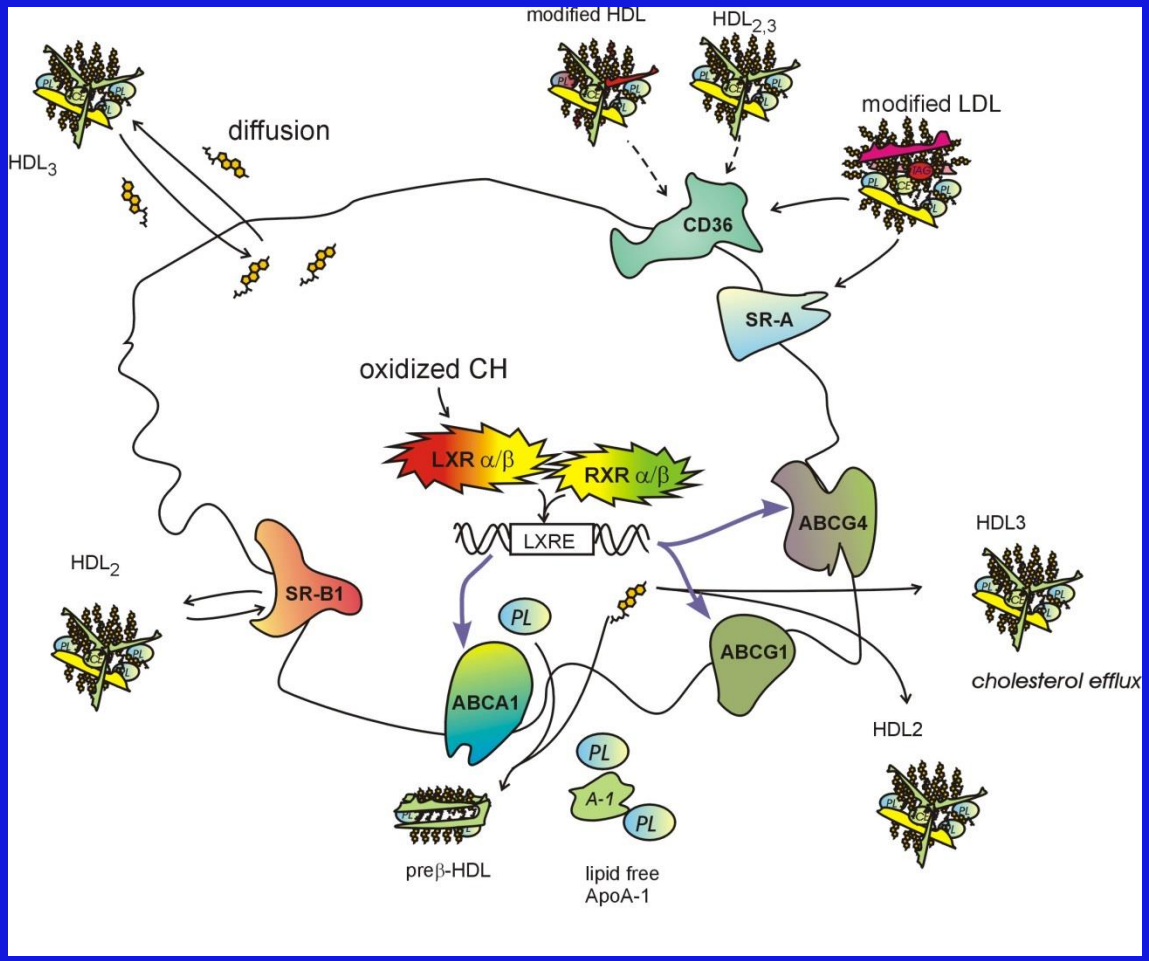
- **reverse transport of CH to liver** *(CH acceptor from cells)*
- **↑ catabolism (VLDL → IDL → LDL)** *(TAG acceptor via CETP)*
- **block transendothelial LDL transport** *(closure of junctions)*
- **VLDL,LDL protection to oxidation** *(PON-1, PAFAH carrier)*
- **oxLDL cytotoxicity inhibitor** *(PON-1, PAFAH carrier)*

II. Other effects

- **anti- and dysaggregative effects on thrombocytes**
- **antiarrhytmic 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

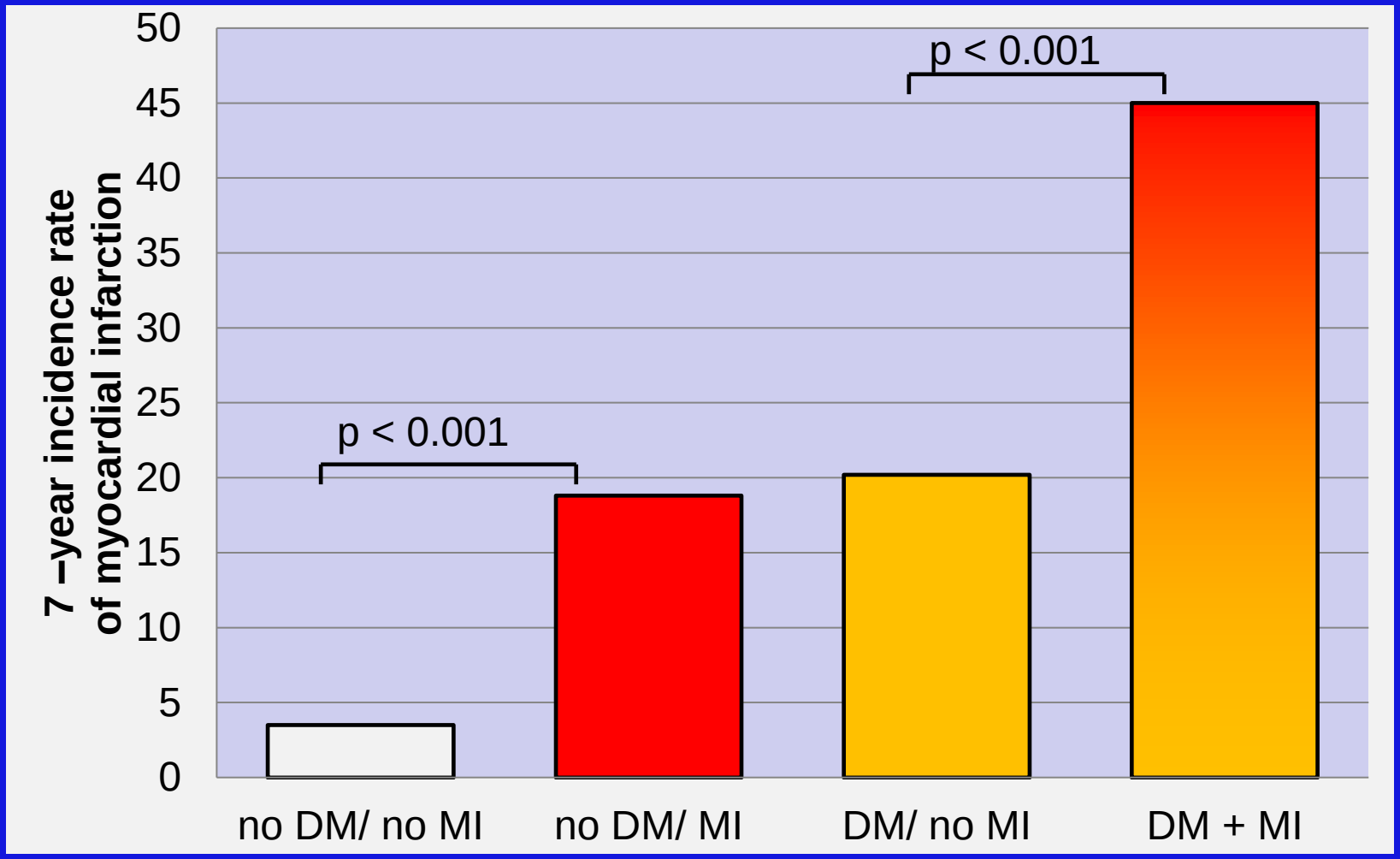
B. Modifiable risk factors

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 atherosclerosis
- an independent risk factor for CVD
- increases CVD risk about two-fold

ESC/EAS Guidelines, 2019

Incidence of myocardial infarction in diabetics



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

< 4.2 mmol/l – 4.2-6.1 mmol/l – > 6.2 mmol/l

ideal f(age,SBP,gender) high

f(smoking.HDL-C)

ESC/EAS Guidelines, 2019

Risk factors of CVD for stratification of risk in primary prevention of CHD

B. Modifiable risk factors

Untreated LDL-levels desirable levels

LDL-cholesterol target values

< 3.0 mM	< 2.6 mM	< 1.8 mM	< 1.4 mM
low risk	moderate risk	high risk	very high risk

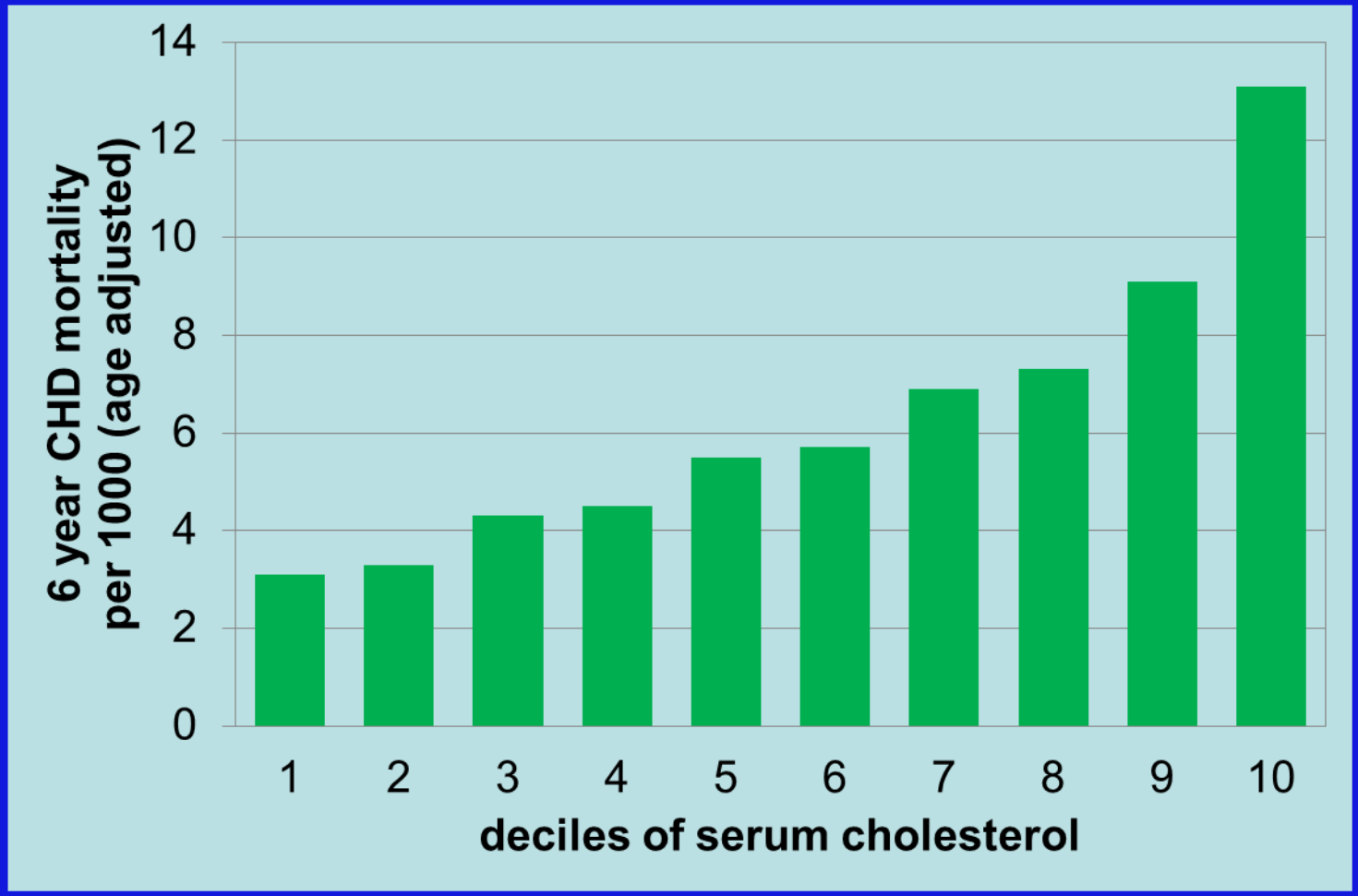
HDL-cholesterol

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

subtracts 1 RF from risk calculation

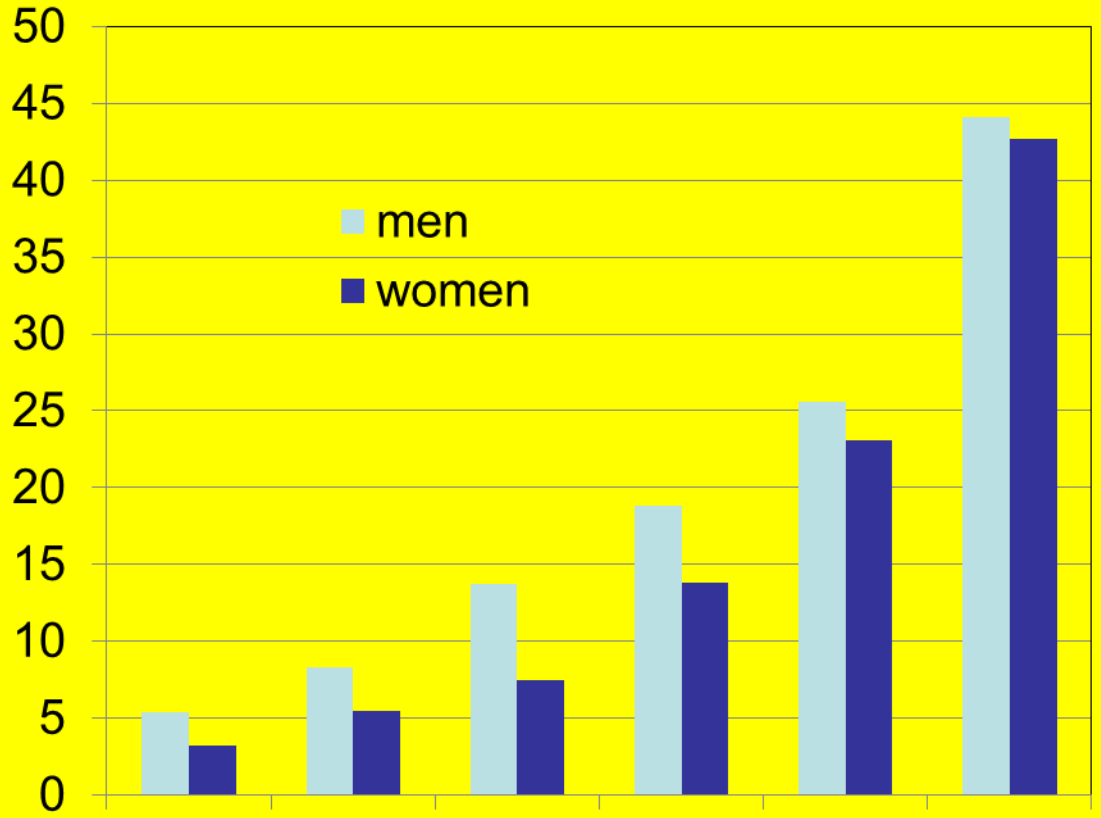
ESC/EAS Guidelines, 2019

Cholesterolemia and mortality



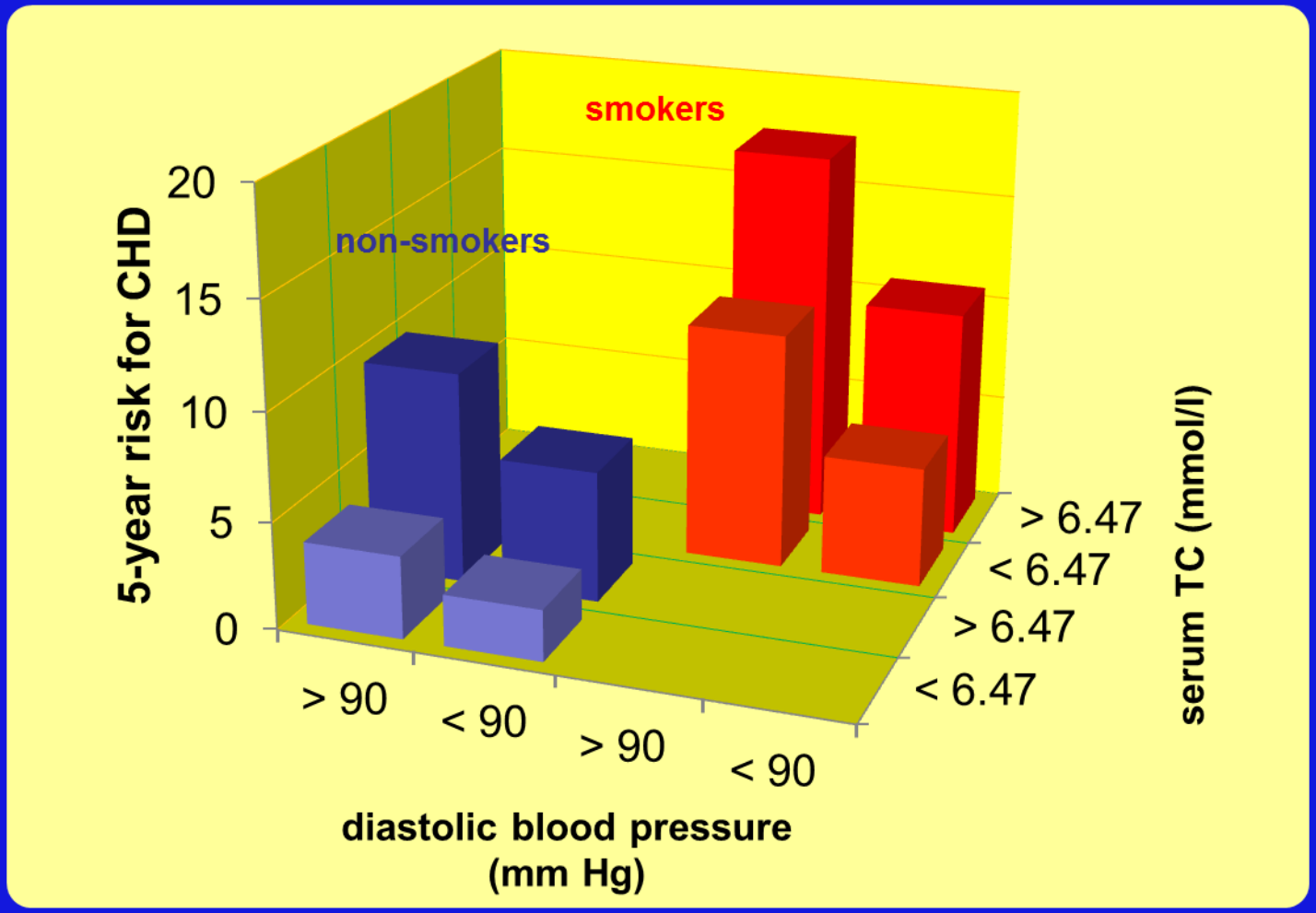
Additive properties of risk factors

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



blood pressure 150-160 mmHg	+	+	+	+	+	+
HDL 0.83-0.90 mmol/l	–	+	+	+	+	+
TC 6.20-6.77 mmol/l	–	–	+	+	+	+
cigarette smoking	–	–	–	+	+	+
diabetes mellitus	–	–	–	–	+	+
left ventricular hypertrophy	–	–	–	–	–	+

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(a)

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*

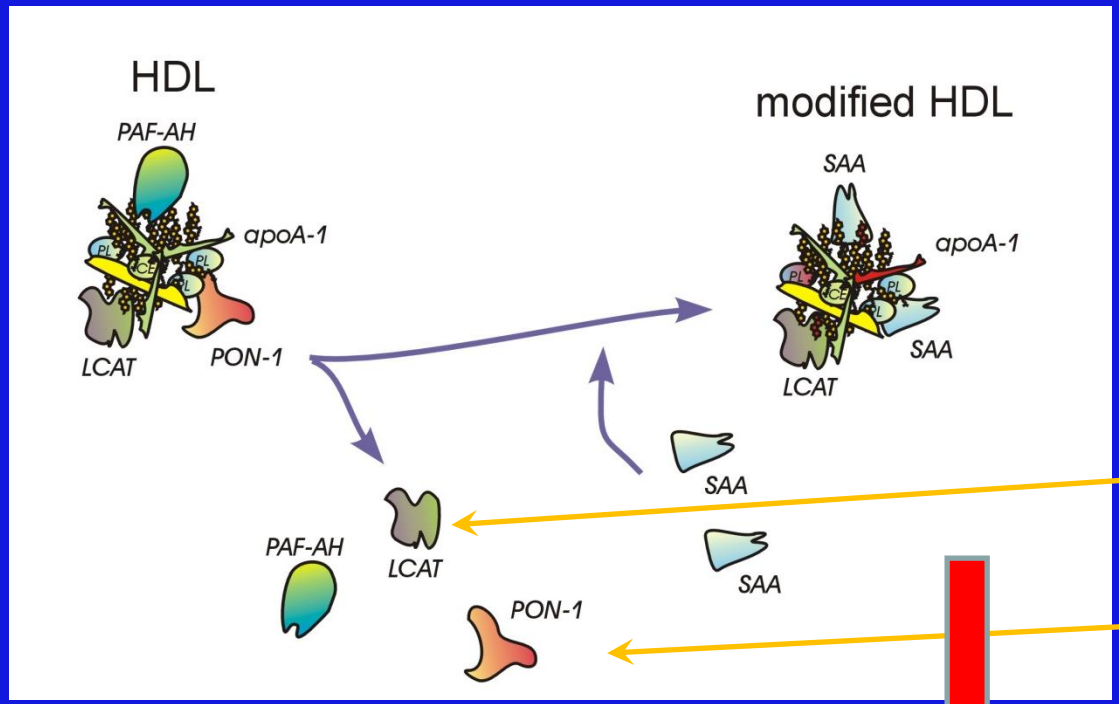
carrier for oxPL in plasma?

high Lp(a) → high risk for cardiovascular disease

SAA and HDL remodelation

functionally defective HDL particles

acute phase response/inflammation



decreased capacity for RCT

decreased antioxidant capacity of HDL

HDL particles lacking antiatherogenic functions

Homocysteine

1. Hcy is noncoding amino acid
 - has SH group → redox balance

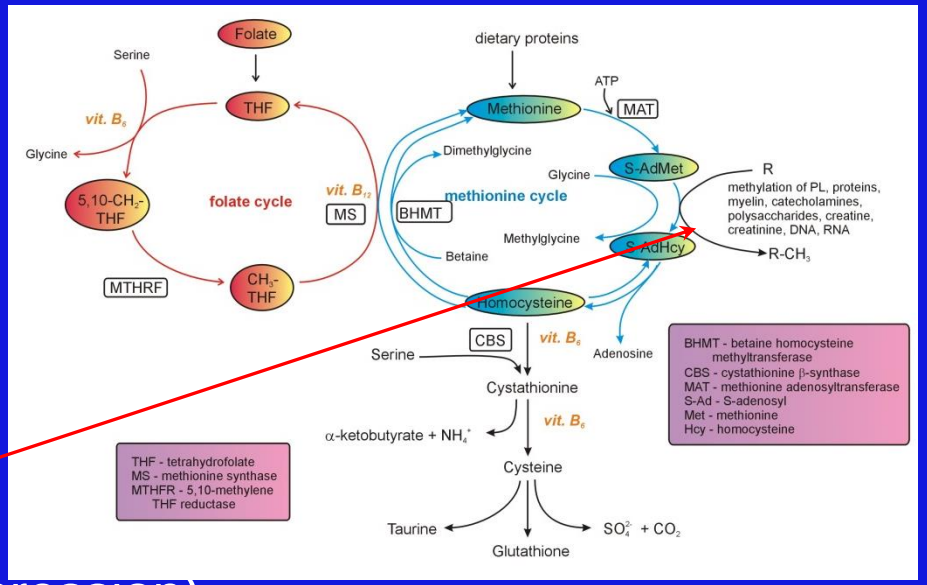
↓

connection with oxidative stress

2. Hcy is linked to methylation events

↓

possible DNA methylation (gene expression)

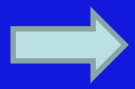


Mild hyperhomocysteinemia suggested as a risk factor for atherosclerosis
- controversial results of interventional studies
- important factor in those with DM + nephropathy, thrombosis

Hypercoagulable states

= states with venous/arterial thrombosis

predisposition for thrombosis
damaged vessel wall



easy thrombus formation

a) mutations in factor V (Leiden), prothrombin
- linked to higher risk of MI/CAD (in younger?)

b) hyperhomocysteinemia (see previous slide)

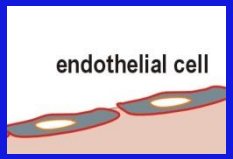
c) antiphospholipid syndrome/SLE
- associated with CAD, stroke

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**

catecholamines — **Endothelium Derived Relaxing Factor**

angiotensin, vasopresin — **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, TNFα, β (T-cells, Mf)**
- **differentiating factors: MCP-1, MCSF-1 (oxLDL)**
- **adhesion molecules: ELAM (ox-LDL)**

Cell types involved in atherogenesis

Thrombocytes:

- ***hypercholesterolemia*** → **megakaryocyte ABCG4↑** → **platelets ↑**

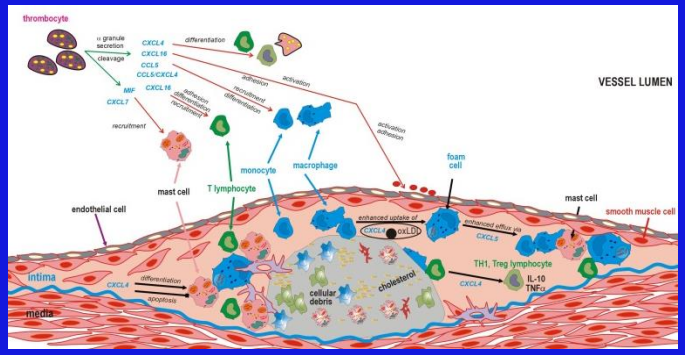
monocyte/macrophage

platelet activation by PAF, 12-HETE → **secretion of TxA_2, 5-HT**

aggregation and degranulation

releasing of growth factors for SMC – PDGF

many chemokines affecting monocytes/macrophages/T cells



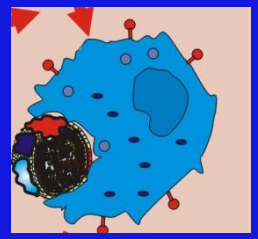
Cell types involved in atherogenesis

Monocytes/macrophages:

I. monocytes

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

II. macrophages

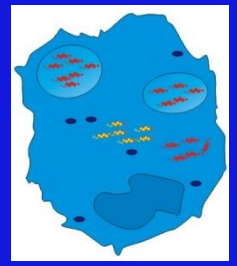


- express receptors for LP: β-VLDL receptor, Ac-LDL-receptor, B/E-receptor Fc-receptor (for complex Ab-LDL)

- synthesize PAF, Il-1, Il-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 → dysruption of lysosomes → apoptosis → cellular debris

Cell types involved in atherogenesis

Smooth muscle cells (SMC):

***phenotype switch*:** ← *atherogenic stimuli*

contractive → synthetic (active) type of SMC

↓

migration from media → intima

proliferation and production of

glykosaminoglycans, colagen

elastin, growth factors, cytokines

proliferation of SMC

stimulation

PDGF 12-HETE

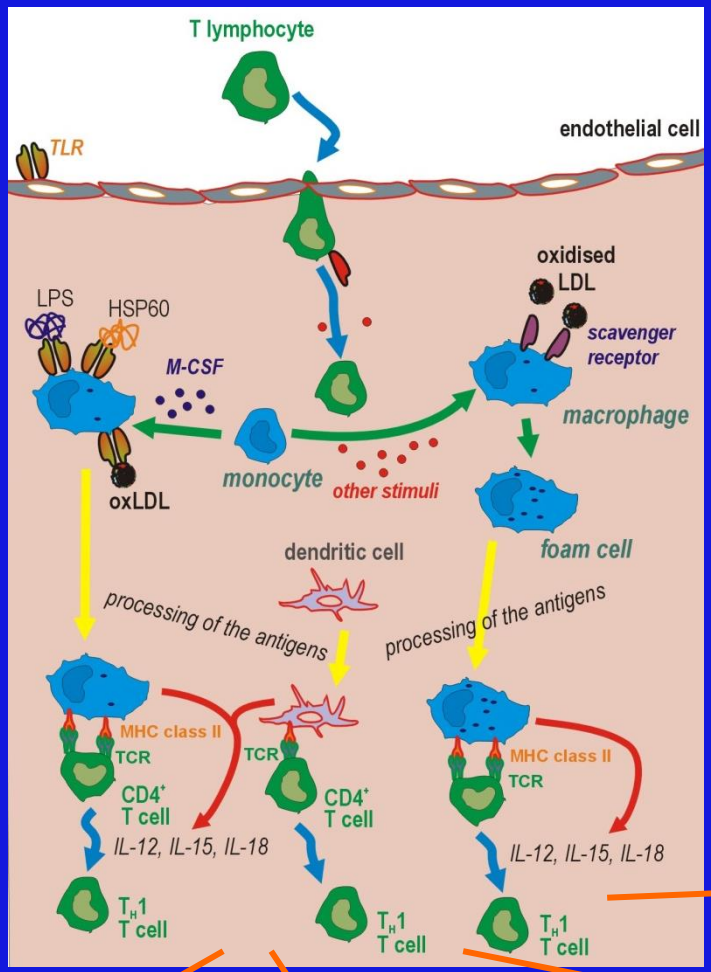
IGF-1

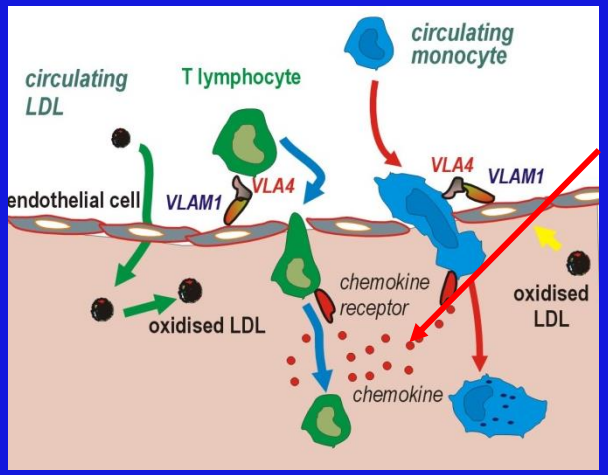
inhibition

heparin NO

PGI_2 INFγ

Mobilisation and activation of immune cells in atherosclerotic plate





migration of monocytes T cells into arterial tissue is supported by locally produced chemokines

proapoptotic factors

proteases

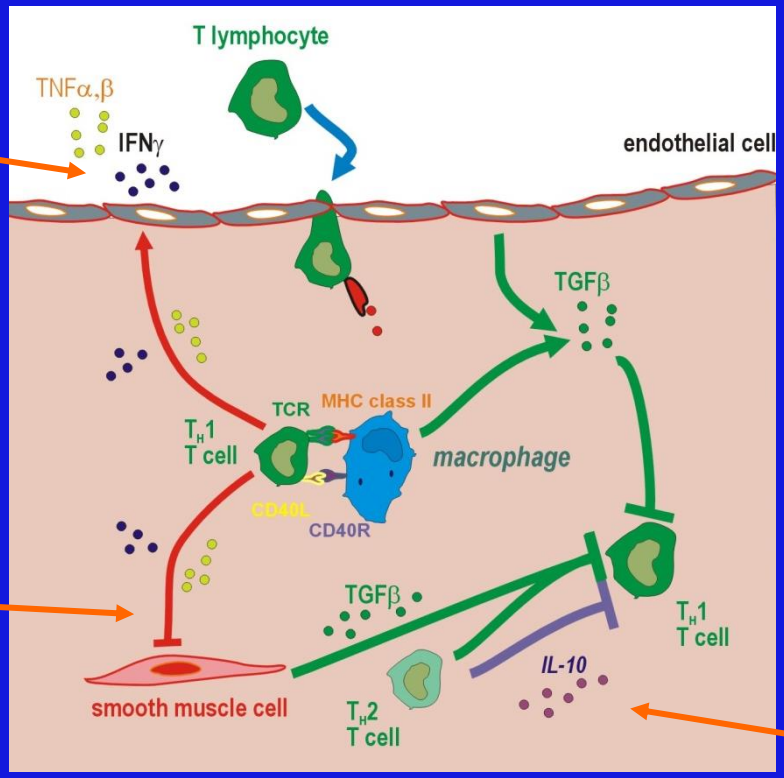
procoagulants

proinflammatory cytokines

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 α/β → vessel vasodilatation

same spectrum of risk factors for CAD, but worse for women in:

smoking: *(↓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) → ~↓CHD risk

Further reading

Textbooks, monographs

Lehninger Principles of Biochemistry (6th Ed); Nelson DL, Cox MM (Eds.), Susan Winslow, New York (U.S.A.) 2013

Harper´s Illustrated Biochemistry (28th Ed); Murray RK, Bender DA, Botham KM, Kennely PJ, Rodwell VW, Weil PA (Eds.), McGraw-Hill, New York (U.S.A.) 2009

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Stamler J, Wentworth D, Neaton JD, for the MRFIT Research Group: Is relationship Between Serum Cholesterol and risk of Premature Death from Coronary Heart Disease Continuous and Graded? *JAMA* 1986; 256: 2823-2828.

Web sources

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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)