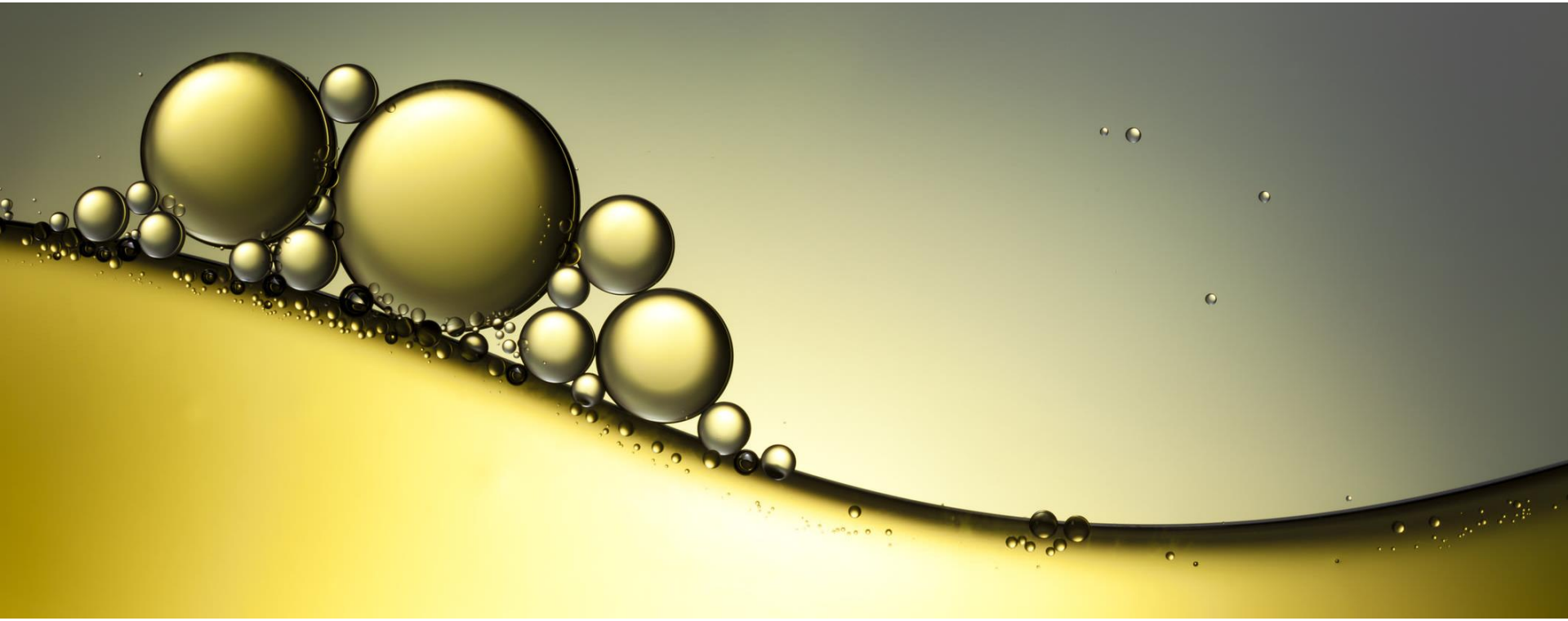
Theoretical basics of clinical medicine - lipidology



Ondrej Kyselak, M.D.
Dept. of Clinical Biochemistry,
St. Anne's University Hospital Brno

Introduction, basic terms

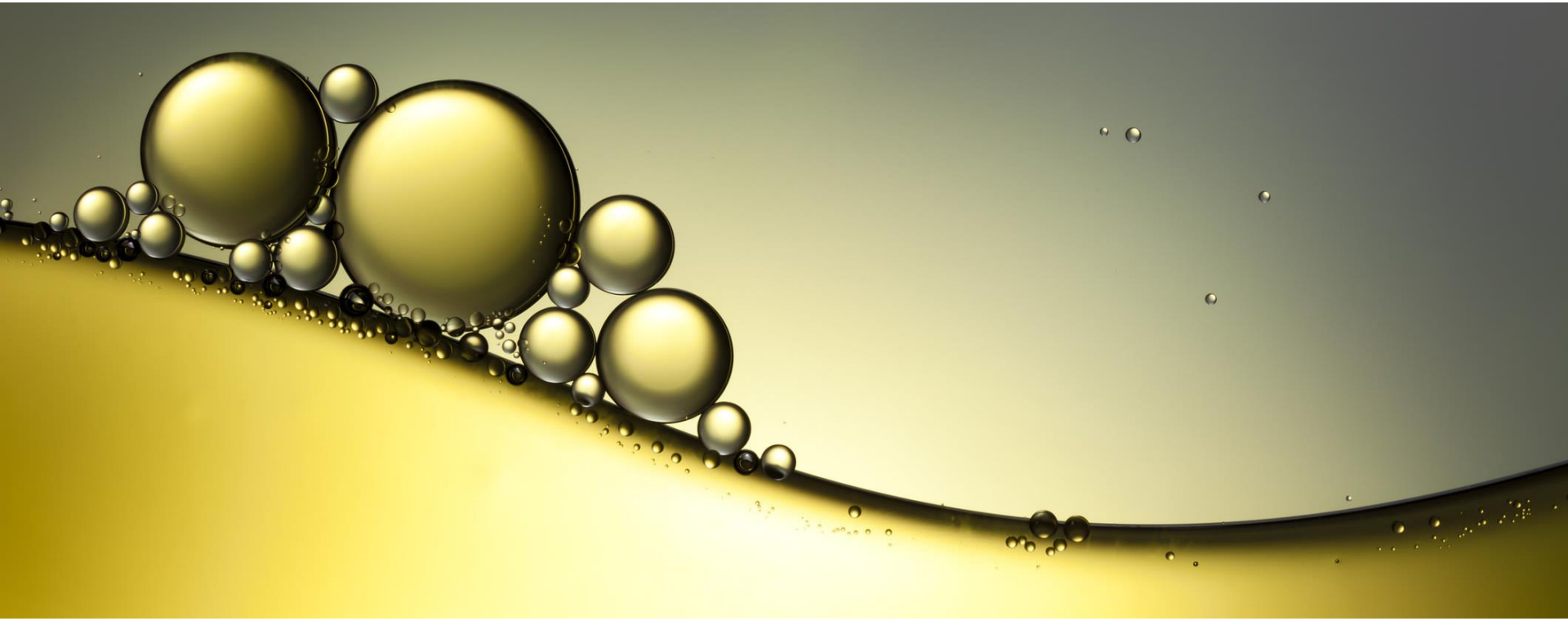
Dyslipidemia = deviation (↓/↑) in blood lipid values. General term, which includes:

- Hypercholesterolemia
- Hypertriglyceridemia

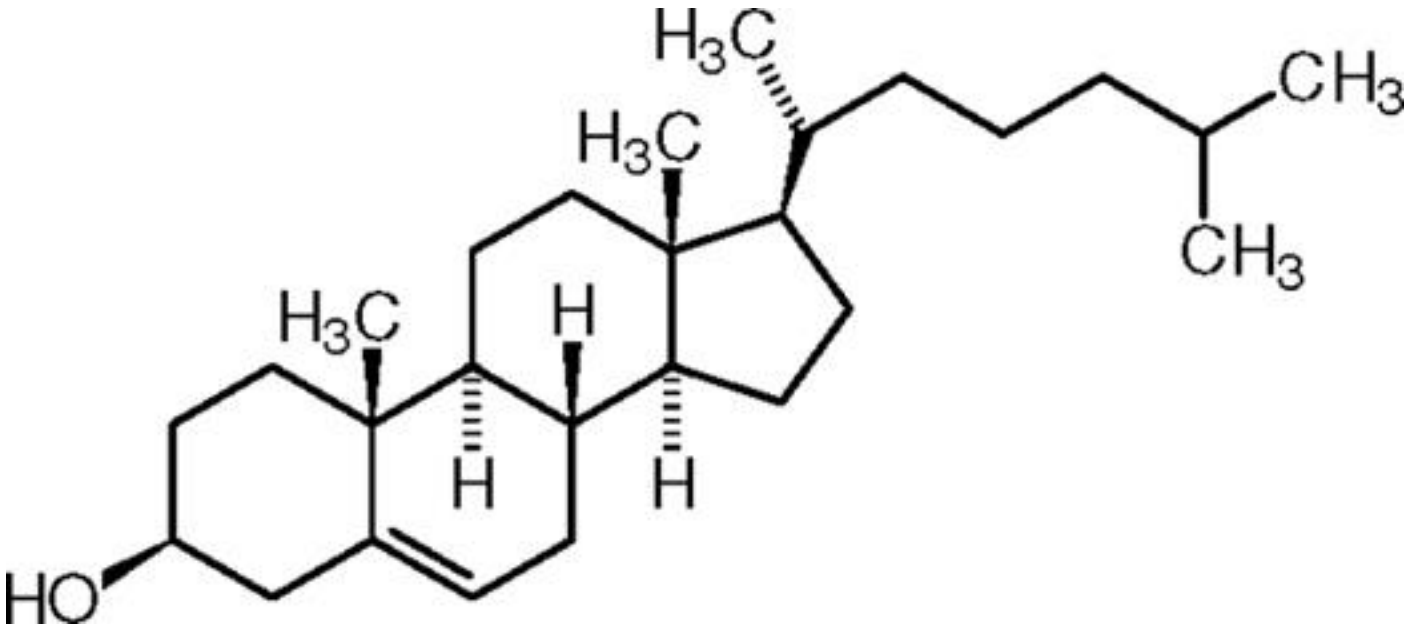
… and less often:

- Rare forms of dyslipidemia
  - familial hypercholesterolemia (FH)
  - dysbetalipoproteinemia (DBL)
  - familial deficit of lipoprotein lipase (LPLD)

Part I.
Metabolism of cholesterol



Cholesterol



Cholesterol

- steroid structure

Sources in human body:

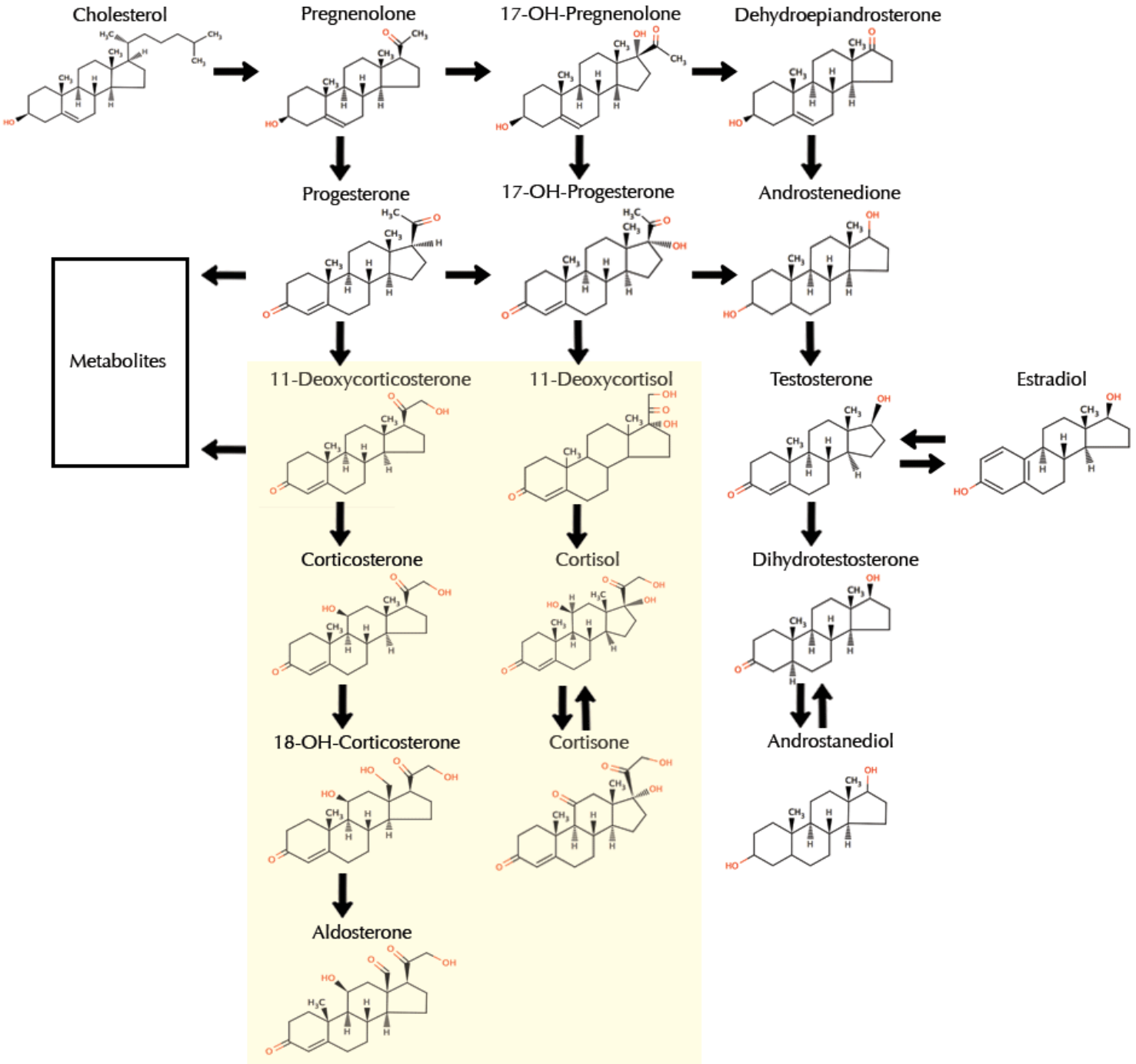
- own synthesis… enzyme HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase)
- food intake

Why do we need cholesterol?

Many substances in our body are derived from cholesterol structure:

- steroid hormones
  - androgens – testosterone
  - estrogens – estradiol
  - gestagens – progesteron
- corticoids
  - mineralocorticoids (aldosterone)
  - glucocorticoids (cortisol, corticosterone)
- vitamins (vit. D)
- bile acids (cholic acid, deoxycholic acid)

Cholesterol is a part of cell membrane as well.


Cholesterol
Pregnenolone
17-OH-Pregnenolone
Dehydroepiandrosterone
Progesterone
17-OH-Progesterone
Androstenedione
Metabolites
11-Deoxycorticosterone
11-Deoxycortisol
Testosterone
Estradiol
Corticosterone
Cortisol
Dihydrotestosterone
18-OH-Corticosterone
Cortisone
Androstanediol
Aldosterone

Why can be cholesterol dangerous?

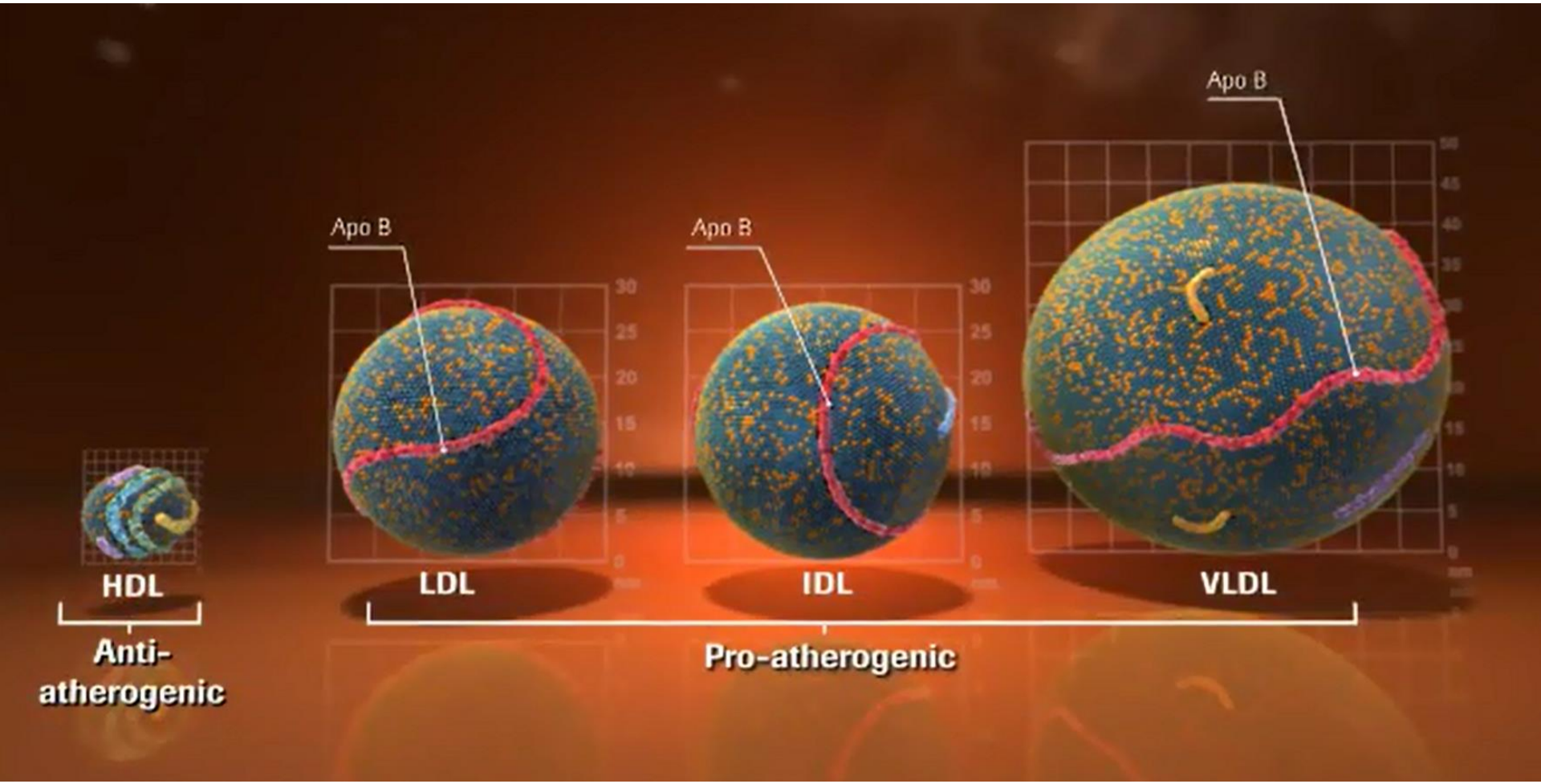
- atherosclerosis (complex process in attendance of immune system – foam cells, pro-inflammatory cytokines)
- vessel injury by atherosclerotic plaques
- CVD, MI, stroke, PAD

How is cholesterol transported in the blood stream?

- … by lipoprotein particles

Depending on density we distinguish these groups of lipoproteins

- Chylomicrons
- VLDL particles (Very Low Density Lipoproteins)
- IDL particles (Intermediate Density Lipoproteins)
- LDL particles (Low Density Lipoproteins)
- HDL particles (High Density Lipoproteins)


Apo B
Apo B
Apo B
HDL
LDL
IDL
VLDL
Anti-
atherogenic
Pro-atherogenic

LDL vs. HDL particles

LDL particles

- cholesterol rich ones
- distribution of cholesterol to peripheral tissues
- strongly proatherogenic – too much of LDL-C causes endothelial injury

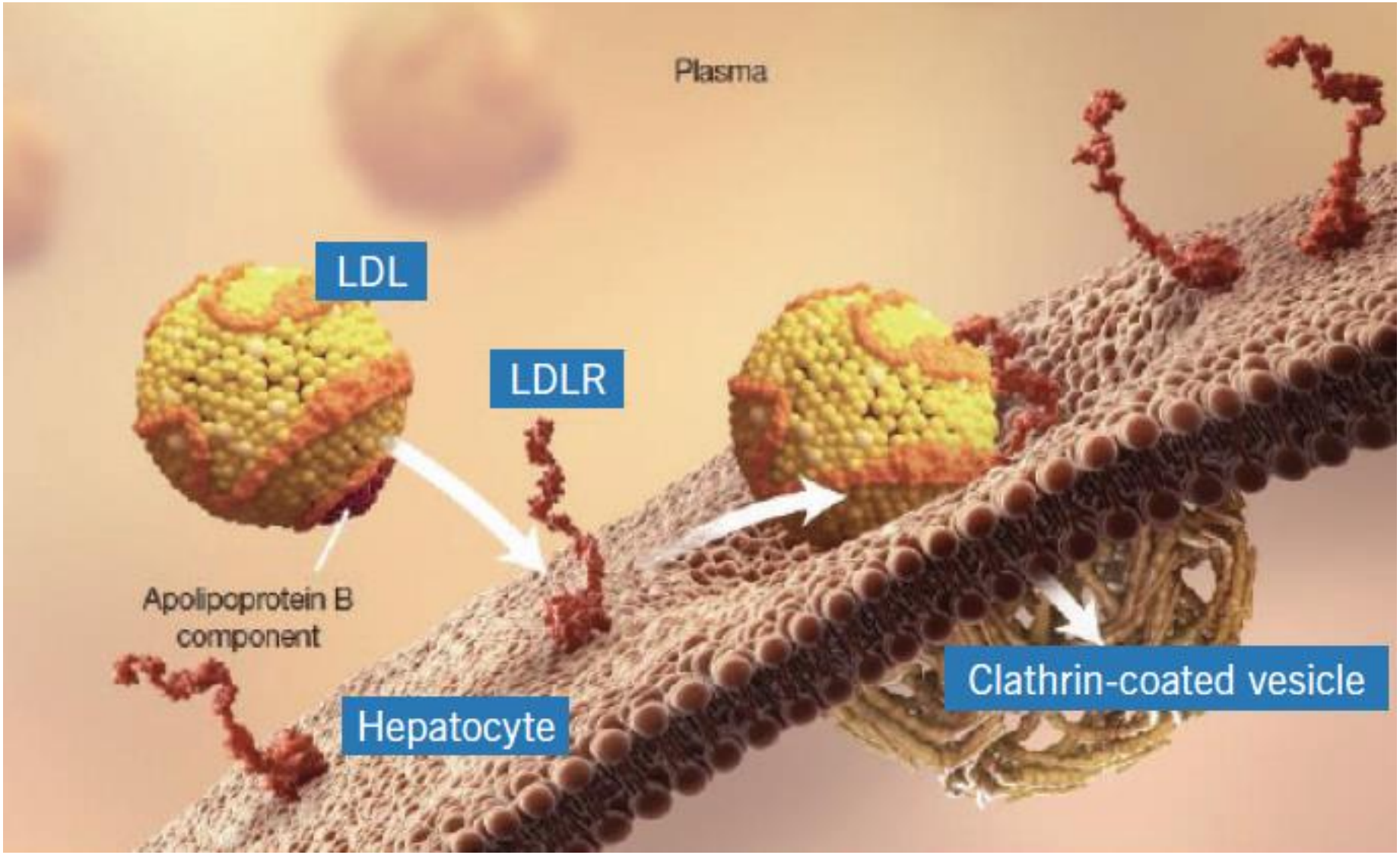
HDL particles

- cholesterol poor ones
- transport of cholesterol from periphery to liver, where cholesterol is metabolised

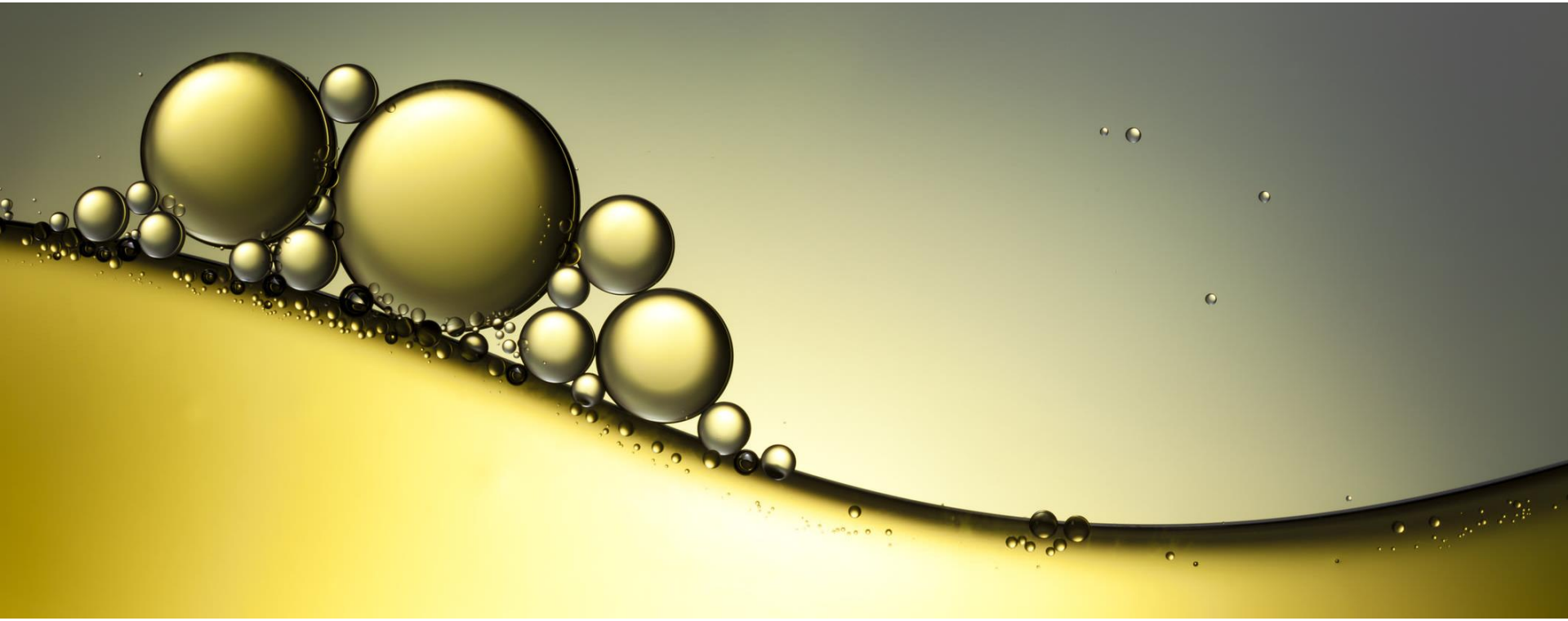
Remember

… molecule of cholesterol is still the same. Biological effect in organism is determined by the transport form (it depends in which type of lipoprotein particles – LDL/HDL the cholesterol is contained).

Degradation of cholesterol



Part II.
Hypercholesterolemia



Hypercholesterolemia

Prevalence of hypercholesterolemia in the Czech Republic (T-CH ≥ 5,0 mmol/l) 53,9 % (without reference to gender) *)

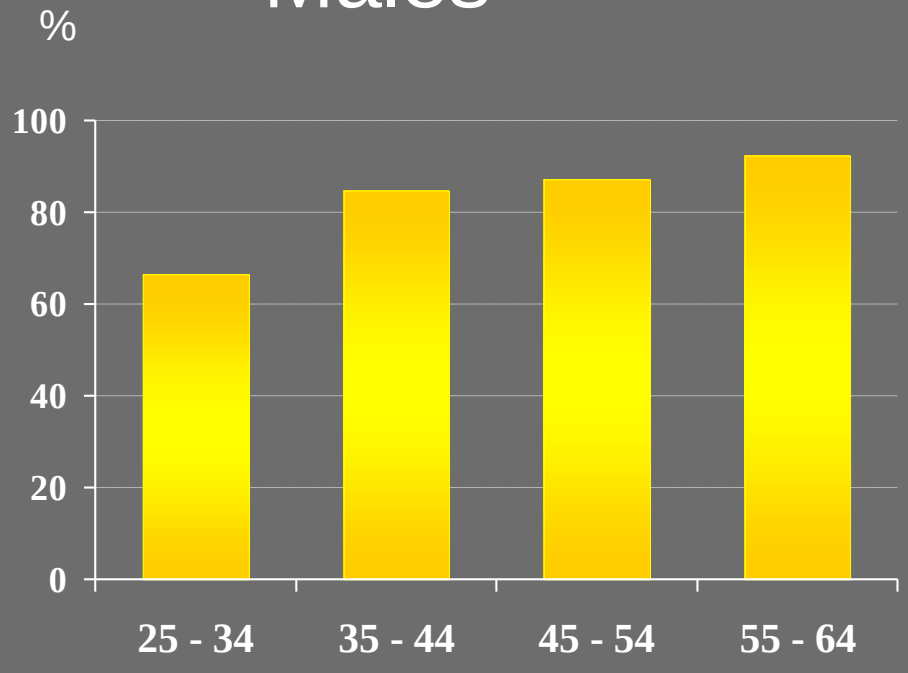
Cholesterol values are dependent on age and gender

---

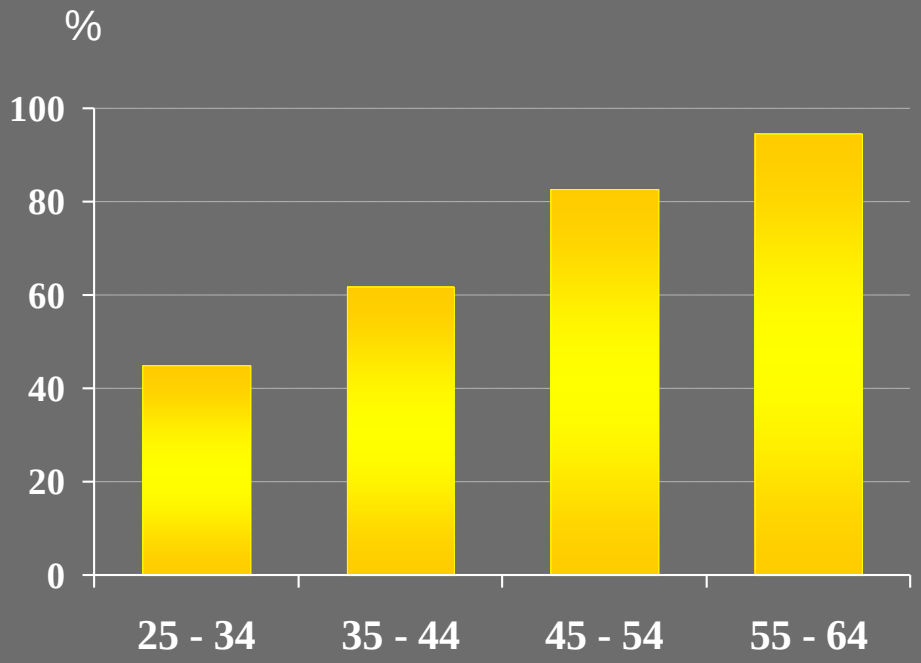
*) WHO: http://www.who.int/en/

Dyslipidemia in Czech population

Males



Females



T-CH. ≥ 5.0 or HDL-ch. < 1.0 or LDL-ch. ≥ 3.0 or TG ≥ 2 mmol/l or lipid - lowering treatment

Hypercholesterolemia

Sub-type of dyslipidemia

- pathologically increased blood level of cholesterol
- risk factor of atherosclerosis and CVD

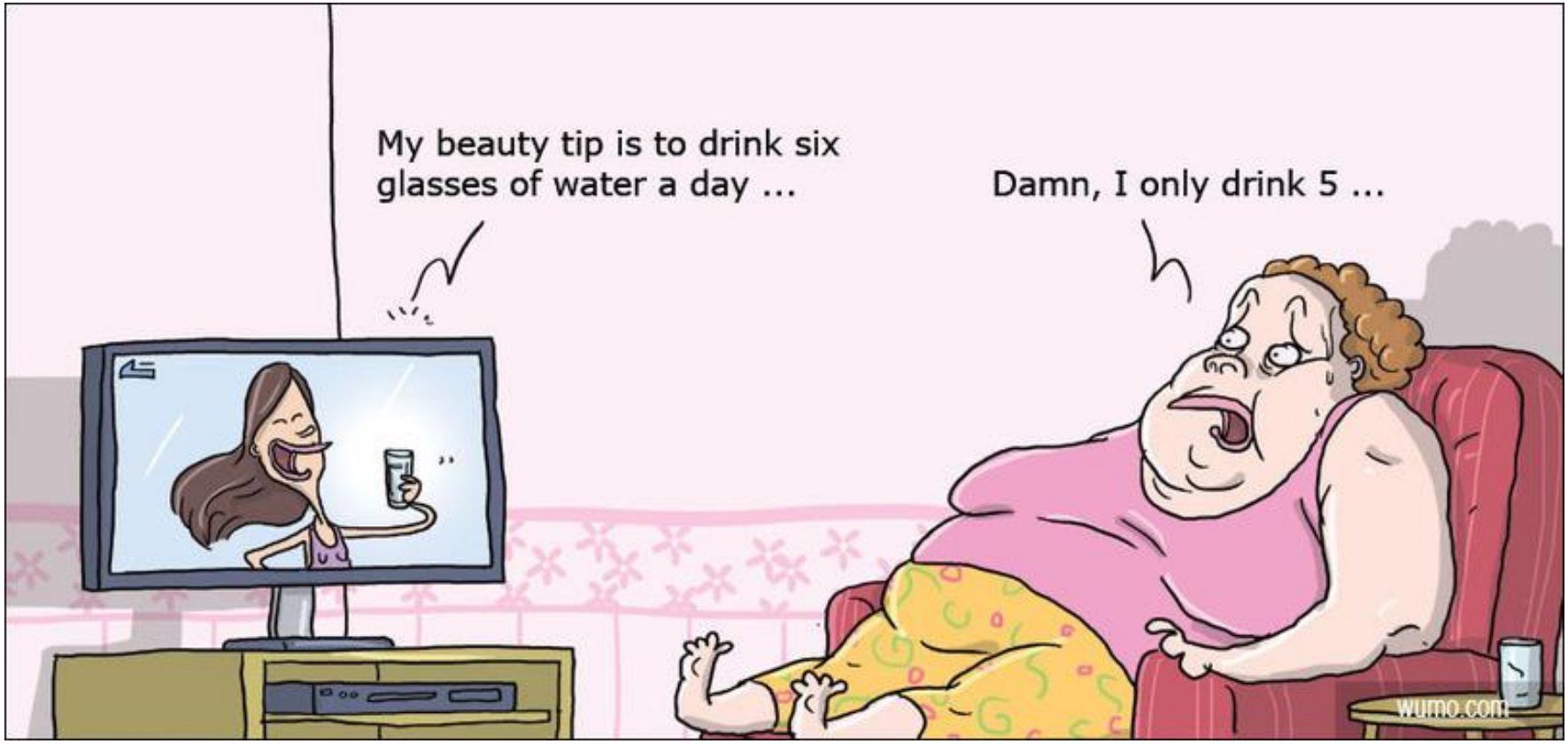
2 groups of dyslipidemia:

- Polygenic
- Monogenic (inherited) – severe DLP

Polygenic hypercholesterolemia

- the most common in population
- influence of many genes with a small effect = genetic predisposition and environment (lifestyle)

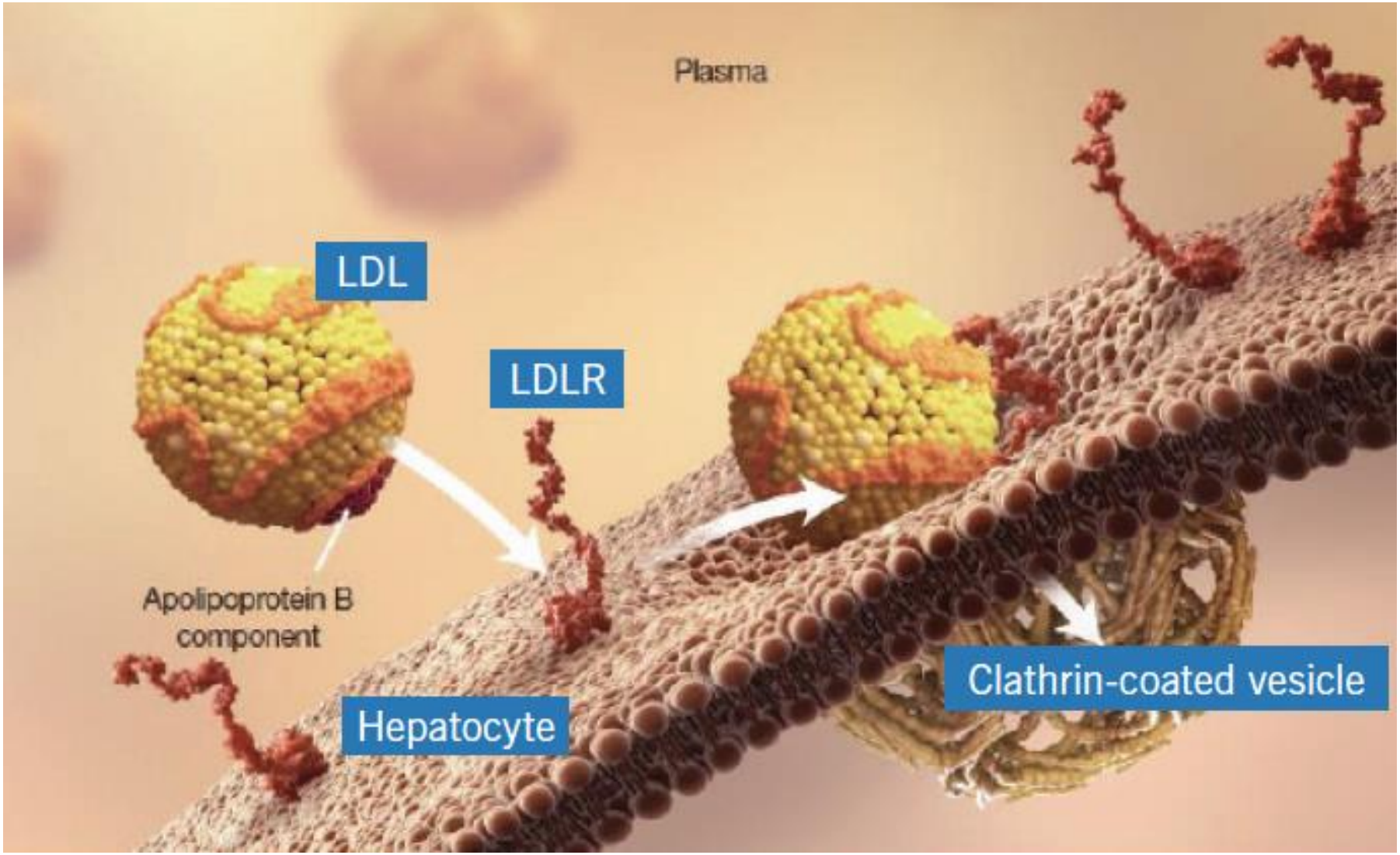
Reduce weight is sooo easy



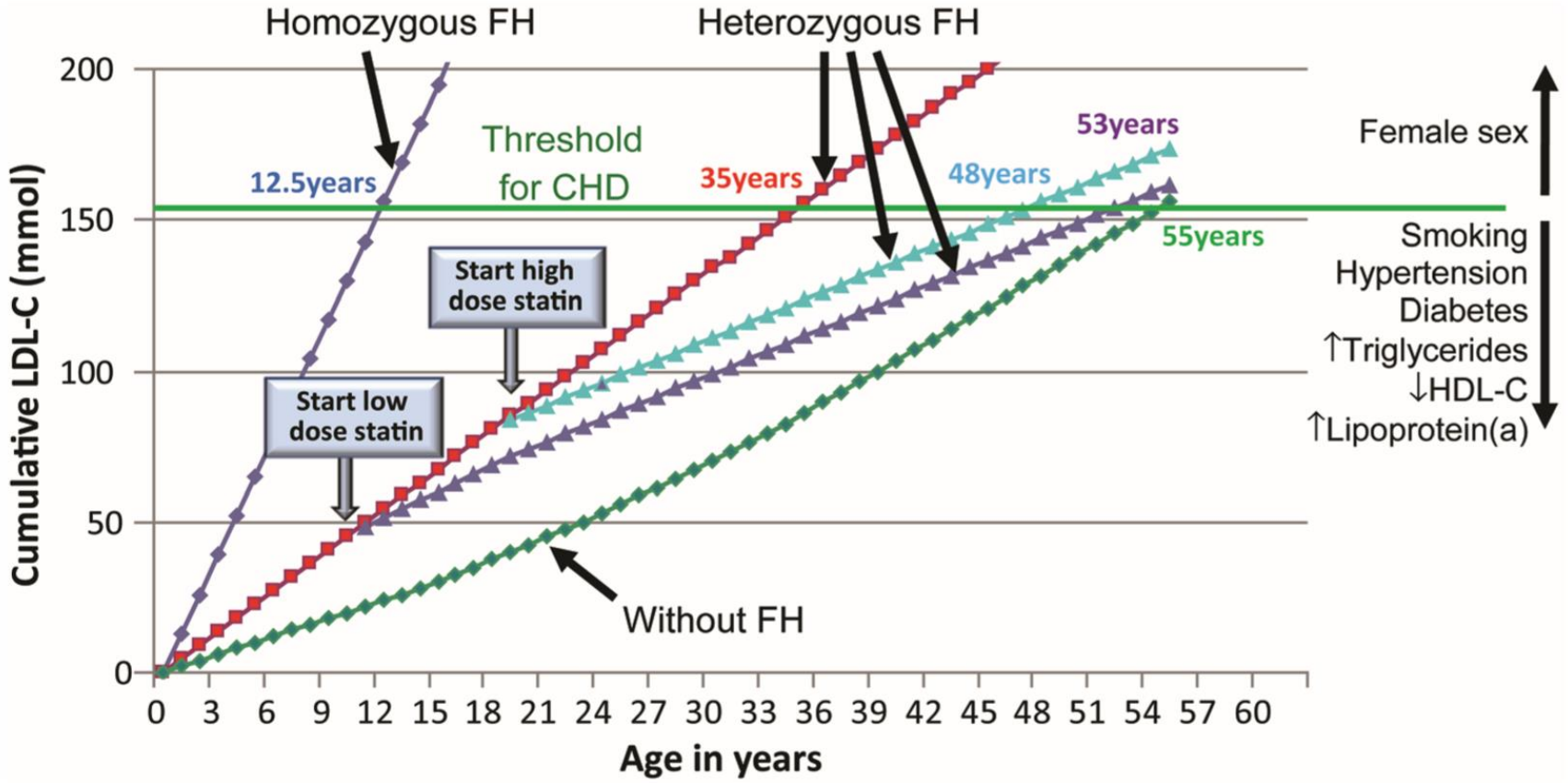
Familial hypercholesterolemia

- monogenic type of DLP
- frequency in population = 1:200 – 1:500
- mutation in DNA leads to defects in structures, which are responsible for cholesterol metabolism
- genes for LDL receptor, apoB, PCSK9
- severe hypercholesterolemia – strongly proatherogenic
- patients with these mutations reach cumulative dose of cholesterol very early – fatal MI, strokes in early age (-teens)
- premature CVD in family history

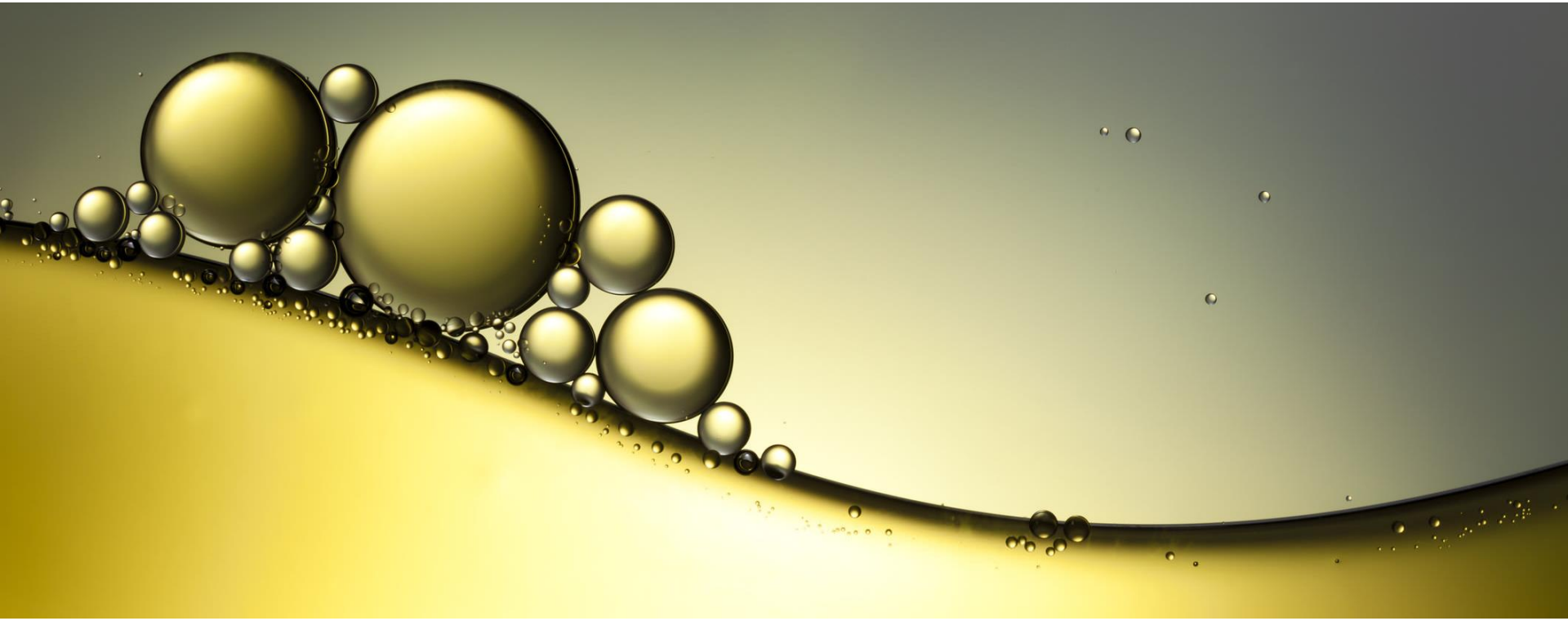
Defective LDLR / apoB in patients with FH



Cumulative dose of LDL – C

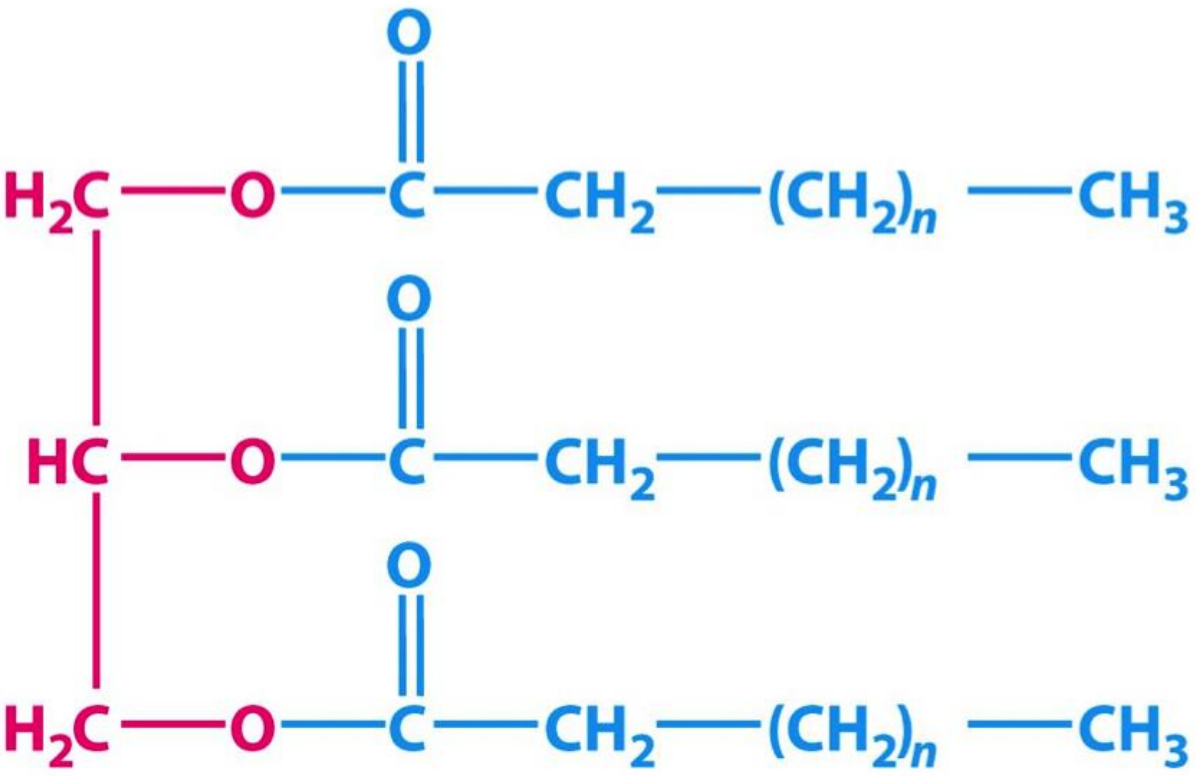


Part III.
Triglycerides



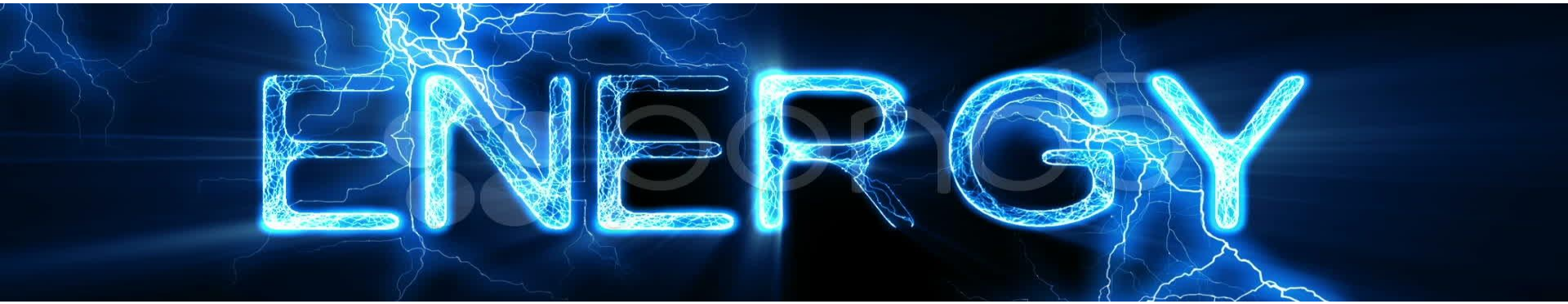
Triglycerides

- Type of blood lipids
- TGs contains glycerol esterified by fatty acids (FA)



What are TGs good for?

- energetic pool (FA are energy - rich substances).
- the only way how to store FA (and energy contained in their structure) are TGs.



Typical patients with hypertriglyceridemia are…

Poorly compensated diabetics and patients with affected glucose metabolism – increased pool of glucose

Patients with increased alcohol consumption

- increased pool of acetyl-CoA = basic substance for lipid synthesis
- high concentration of FA (used for synthesis of TGs)
- alcoholic beverages (beer, wine) – highly caloric, provide substrate for synthesis of TGs.

Patients with polygenic dyslipidemia

Obese patients (abundance of energetic substrates)

Clinical correlations of hypertriglyceridemia

- slightly increased value of TGs (up to 10 - 11 mmol/l) = risk factor for CVD
- very high serum TGs > 11 mmol/l = RF for acute pancreatitis*)
- incidence of AP caused by hyperTG 40 cases / 100 000 adults and year (data from US)**)

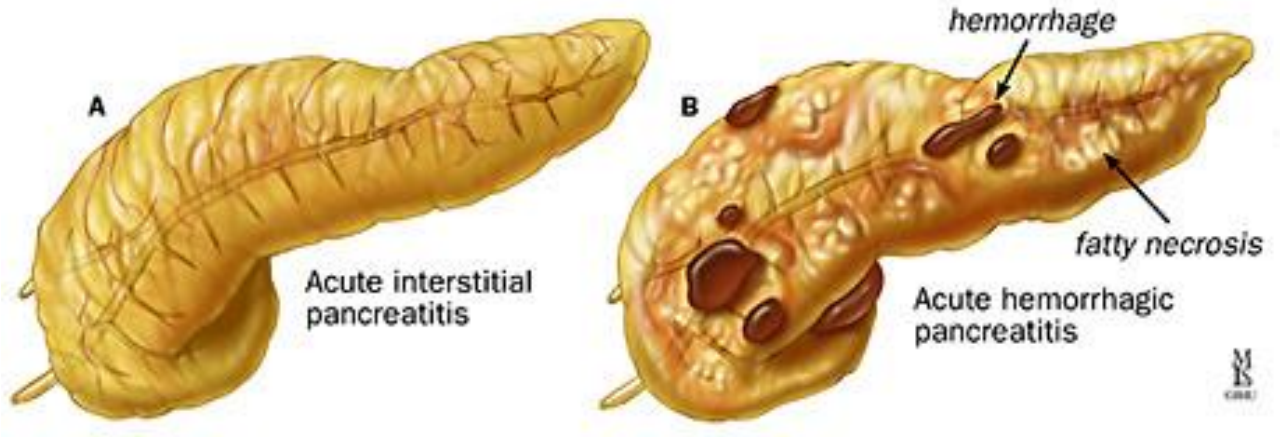
---

*) Kota SK, Jammula S. et al. Hypertriglyceridemia-induced recurrent acute pancreatitis: A case-based review Indian J Endocrinol Metab. 2012 Jan-Feb; 16(1): 141–143.

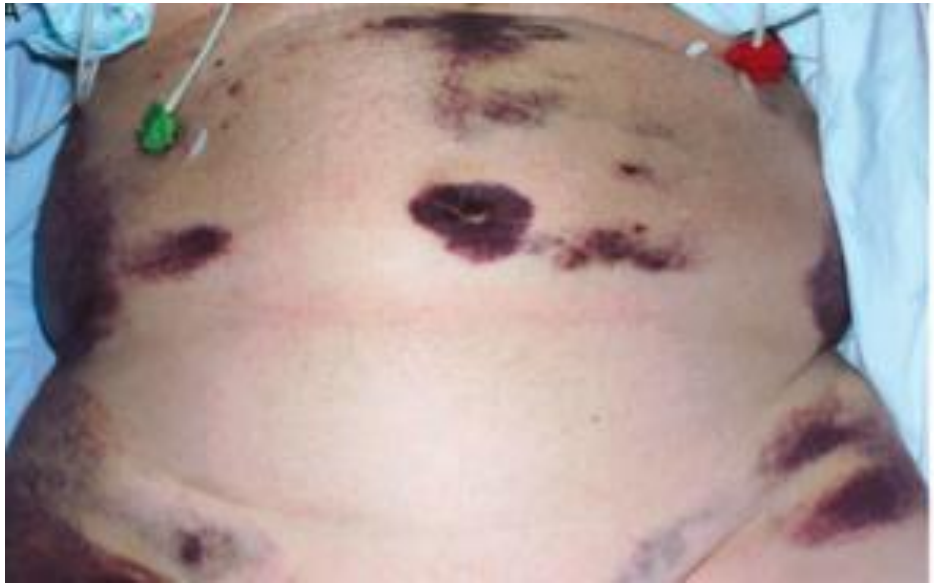
**) Granger J, Remic k D., Acute pancreatitis: models, markers, and mediators. Shock. 2005 Dec;24 Suppl 1:45-51.

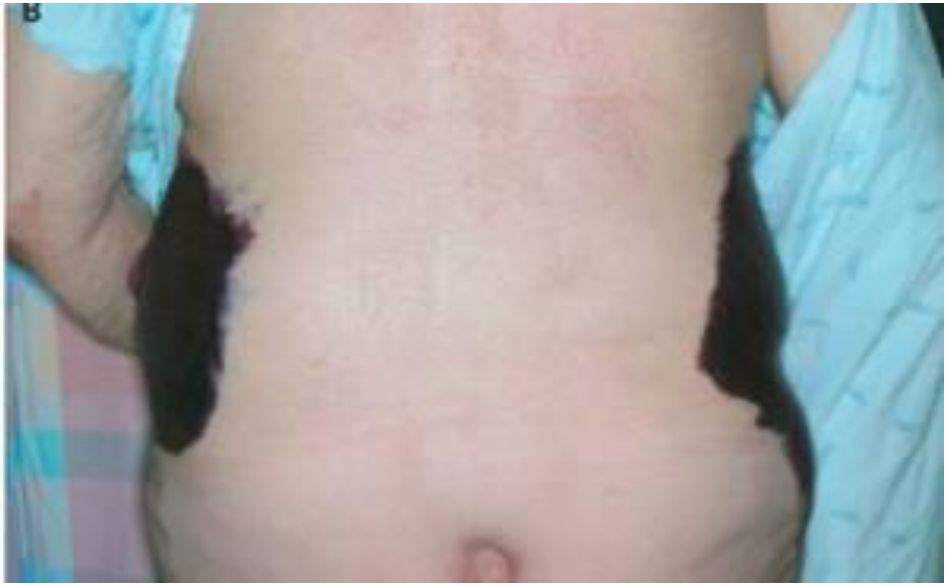
Acute pancreatitis

- very serious and acute situation!
- strict diet without any lipids!
- requires hospitalisation!



Acute pancreatitis
- Cullen´s sign
- Grey-Turner´s sign





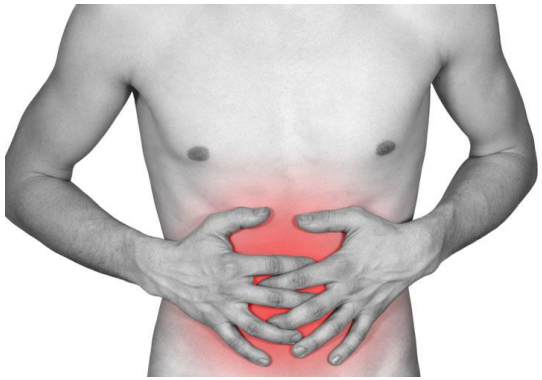
Take home message

Hypercholesterolemia

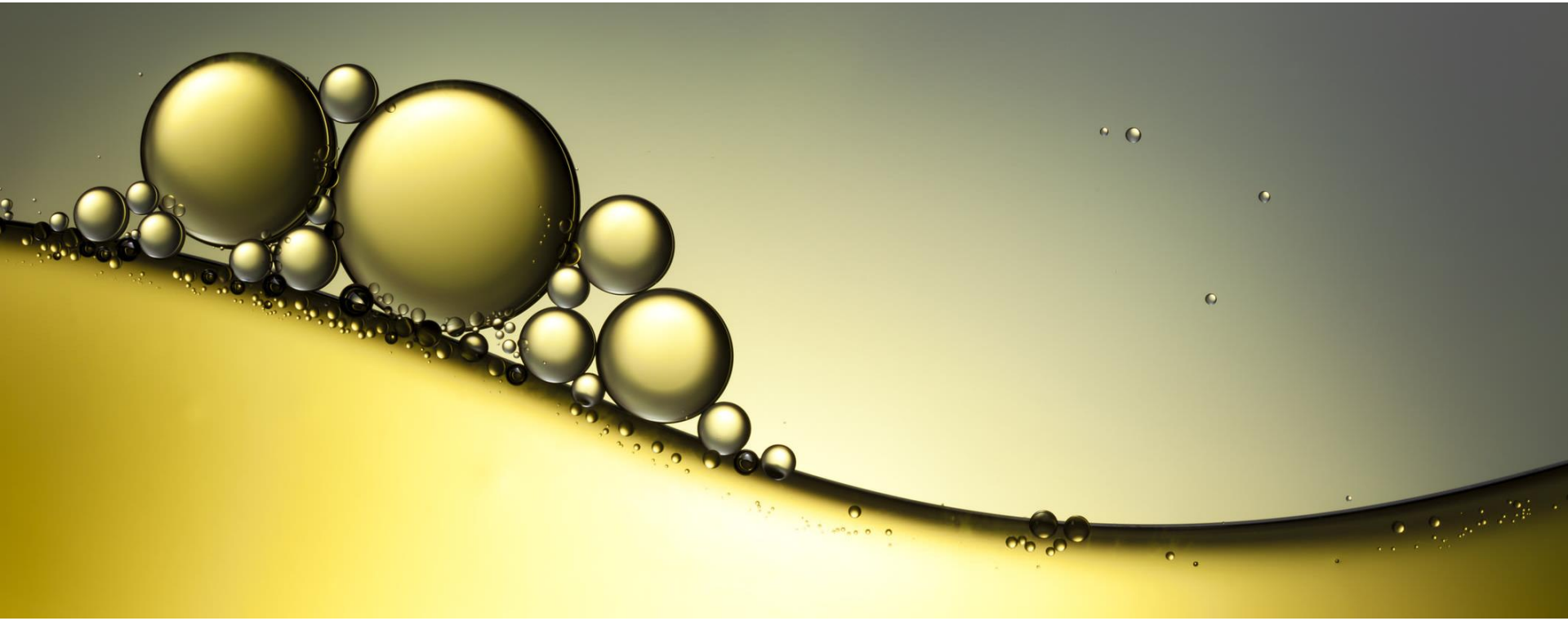
- independent risk factor for CVD (MI, stroke, PAD)

Hypertriglyceridemia

- mild = RF for CVD
- severe = RF for acute pancreatitis



Part IV.
Fatty acids



FA – fatty acids

- mostly part of triglycerides
- source of energy
- substrate for synthesis of many other substances

FA can be divided into many groups

Length of carbonaceous chain:

- short chain FA
- long chain FA – 12 and more carbons in chain

Number of double bonds in chain:

- SAFA (saturated FA)
- MUFA (monounsaturated FA)
- PUFA (polyunsaturated FA)

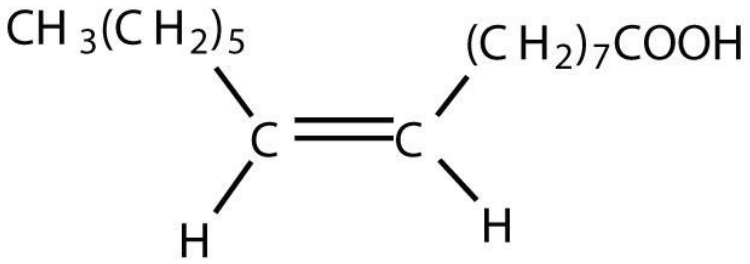
Configuration of FA chain

cis FA – plant lipids, oils

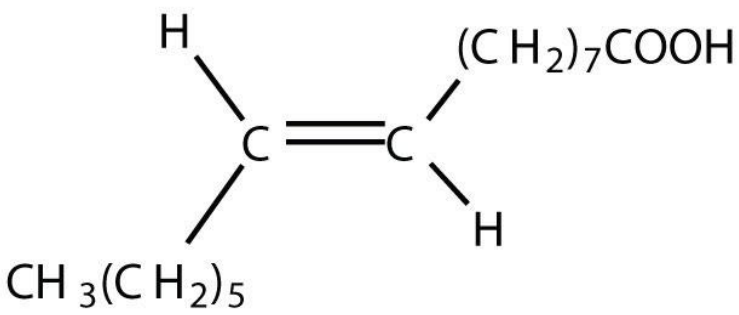
trans FA – less often, milk lipids

- toppings, chocolates , butter, beef.
- strongly proatherogenic

Cis x trans FA



cis fatty acid



trans fatty acid

Synthesis in organism

Essential FA:

- we´re not able to synthetize
- PUFA
- fish, plant oils

Non – essential FA

- we can synthetize
- SAFA, MUFA
- animal lipids

Sources of FA

Vybrané nasycené mastné kyseliny (SAFA)

Zkrácený zápis	Triviální název	Systematický název	Výskyt
4:0	máselná	butanová	mléčný tuk
6:0	kapronová	hexanová	mléčný tuk
12:0	laurová	dodekanová	kokosový tuk
14:0	myristová	tetradekanová	kokosový tuk
16:0	palmitová	hexadekanová	většina tuků
18:0	stearová	oktadekanová	většina tuků
20:0	arachidová	ikosanová	většina tuků
24:0	lignocerová	tetrakosanová	sfingolipidy

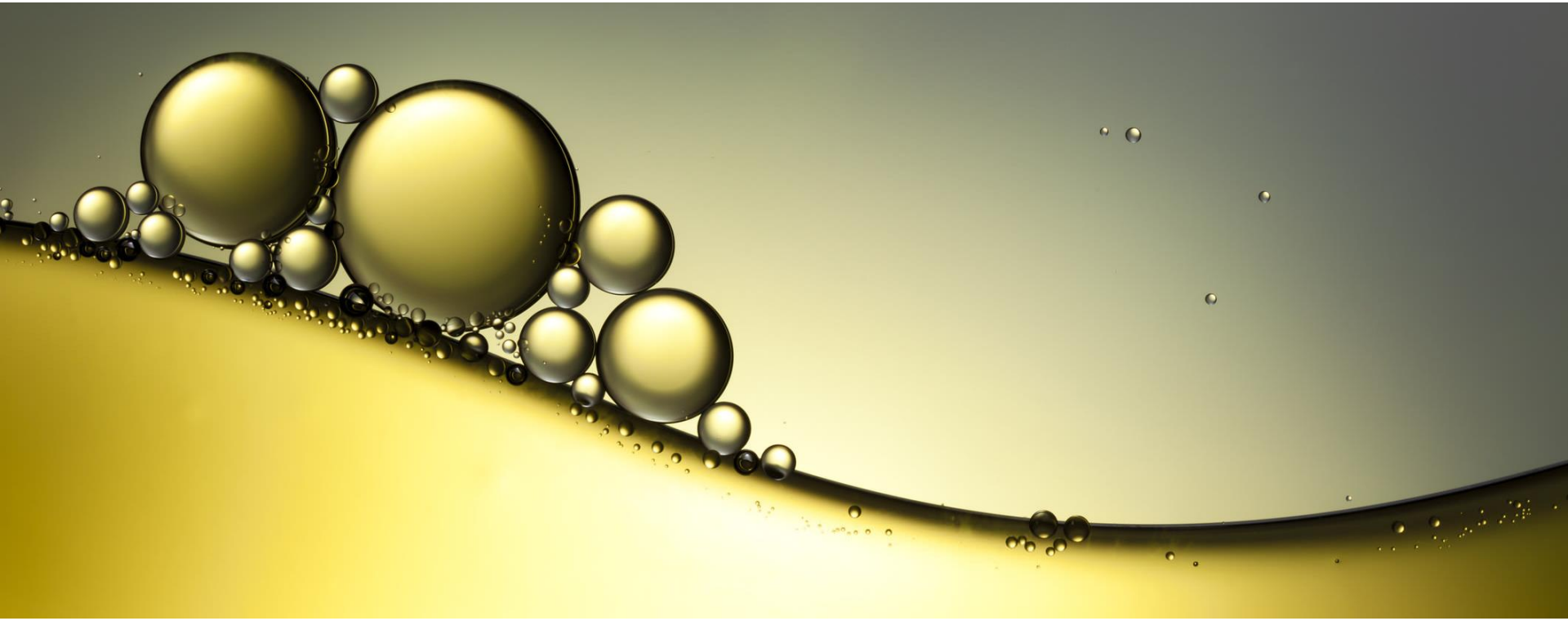
Sources of FA

Vybrané nenasycené mastné kyseliny (MUFA, PUFA)

Zkrácený zápis	Řada	Triviální název	Systematický název[a]	Výskyt
16:1(9)	n-7	palmitolejová	hexadec-9-enová	rostlinné oleje
18:1(9)	n-9	olejová	oktadec-9-enová	rostlinné oleje
18:2(9,12)	n-6	linolová	oktadeka-9,12-dienová	rostlinné oleje
18:3(9,12,15)	n-3	α-linolenová	oktadeka-9,12,15-trienová	rostlinné oleje
18:3(6,9,12)	n-6	γ-linolenová	oktadeka-6,9,12-trienová	rostlinné oleje
20:4(5,8,11,14)	n-6	arachidonová	ikosa-5,8,11,14-tetraenová	fosfolipidy
20:5(5,8,11,14,17)	n-3	EPA[b]	ikosa-5,8,11,14,17-pentaenová	rybí tuk
24:1(15)	n-9	nervonová	tetrakos-15-enová	sfingolipidy

[a]Konfigurace všech dvojných vazeb je *cis*. [b]Z angl. eicosapentaeneoic acid.

Part V.
Atherosclerosis, CV risk



Atherosclerosis

- vessel disease
- complex pathophysiological process (with participation of immune system, pro-inflammatory cytokines)

Storage of cholesterol

↓

Calcification

↓

Increases rigidity of arterial wall

Atherosclerosis

Arterial obliteration by atherosclerotic plaques

↓

Plaque rupture, thrombocyte activation, thrombus

↓

Arterial occlusion

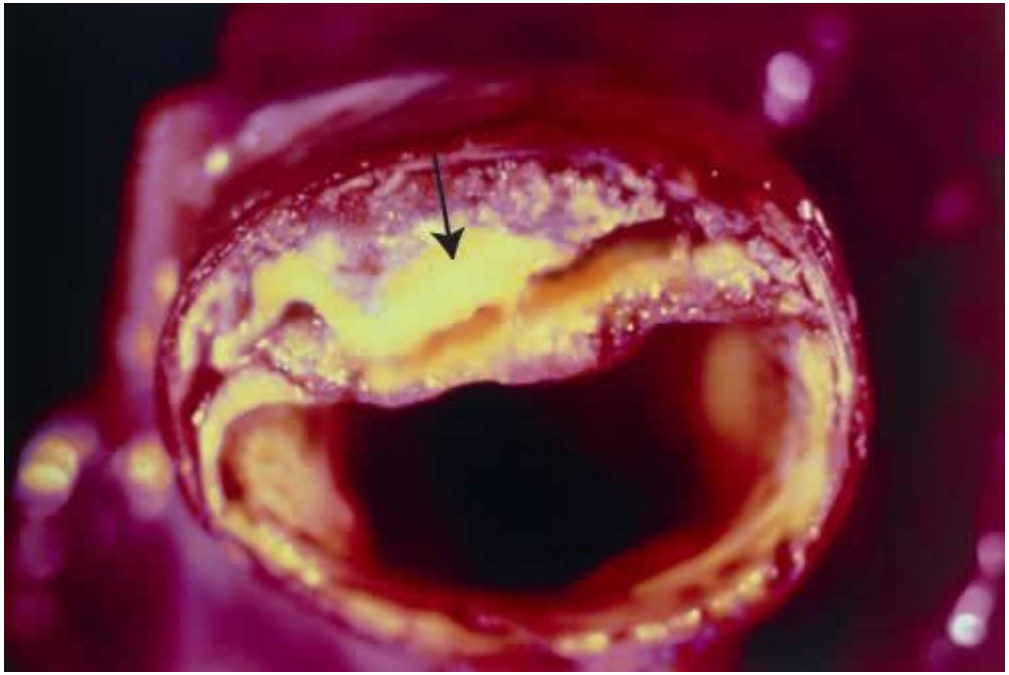
↓

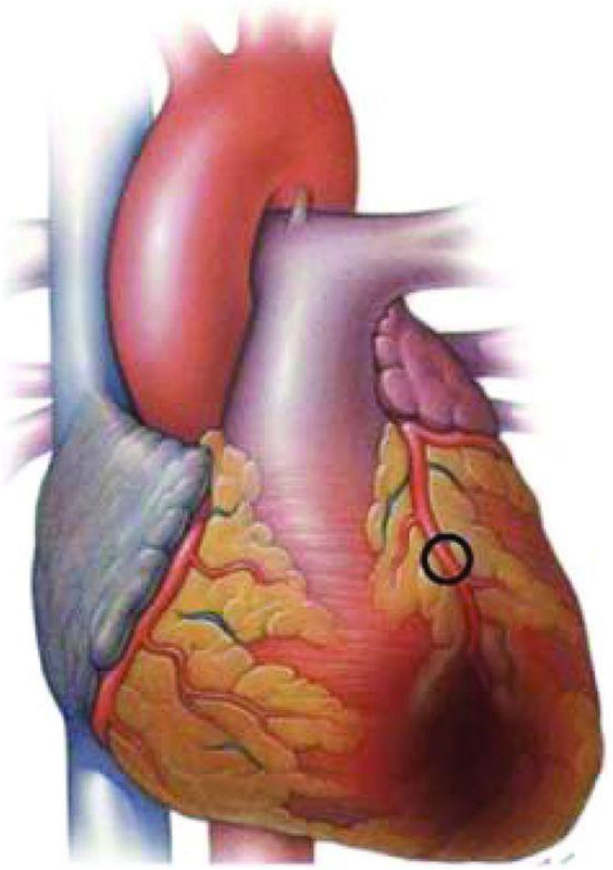
Ischemia of tissues behind the occlusion

↓

Tissue infarction if the blood stream is not restored immediately

Atherosclerosis





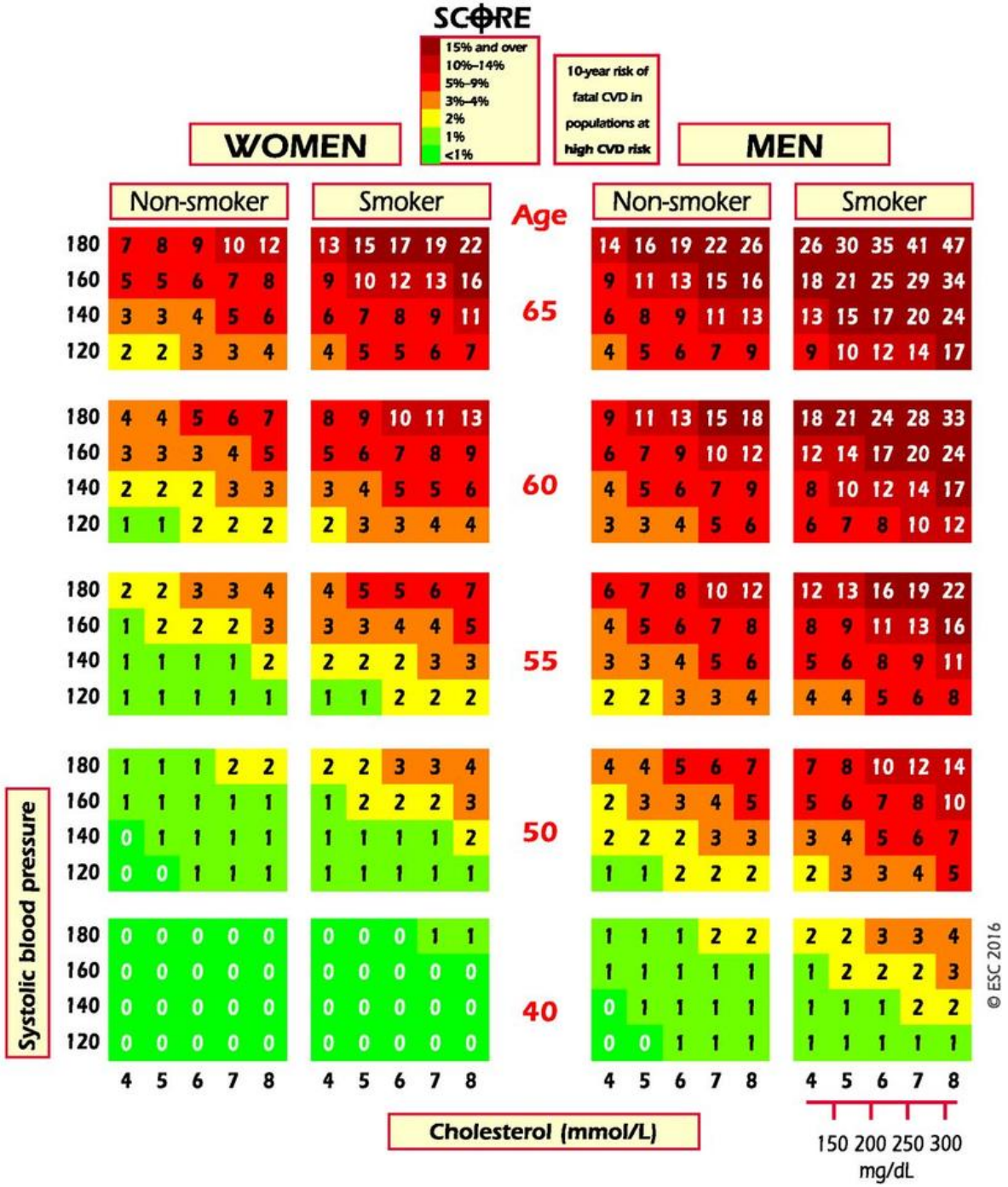
Risk factors for atherosclerosis

Bad lifestyle:

- smoking
- alcohol consumption
- obesity
- kidney disease
- diabetes mellitus
- hypertension
- hypercholesterolemia

Estimation of Cardiovascular risk

- probability of fatal MI or stroke in 10 years
- SCORE charts (Systematic COronary Risk Evaluation)
- need to know: sex, smoking habits, age, systolic BP, T-CH value + other RF (individually adjusting CV risk)


SCORE
15% and over
10%–14%
5%–9%
3%–4%
2%
1%
<1%
10-year risk of fatal CVD in populations at high CVD risk
WOMEN
MEN
Non-smoker
Smoker
Non-smoker
Smoker
Age
65
60
55
50
40
Systolic blood pressure
180 160 140 120
4 5 6 7 8
Cholesterol (mmol/L)
150 200 250 300
mg/dL
© ESC 2016

CV risk categories

- Low CV risk (up to 1 %)
- Intermediate CV risk (1-5 %)
- High CV risk (5 – 10 %)
- Very high CV risk (more than 10 %)

The higher CV risk, the lower value of cholesterol is needed to be reached to protect arteries from the damage by atherosclerotic plaques.

Smoking as a RF of CVD

- Smoking is a psychiatric diagnosis (ICD, International Classification of Diseases: F17)
- Health problem (CVD, lung cancer, infection of respiratory system, COPD, mental and physical addiction
- Economic problem (price of cigarettes, costs for treatment of one smoker)
- Social problem (smell, acceptation by non-smokers)

Smoking and CV risk

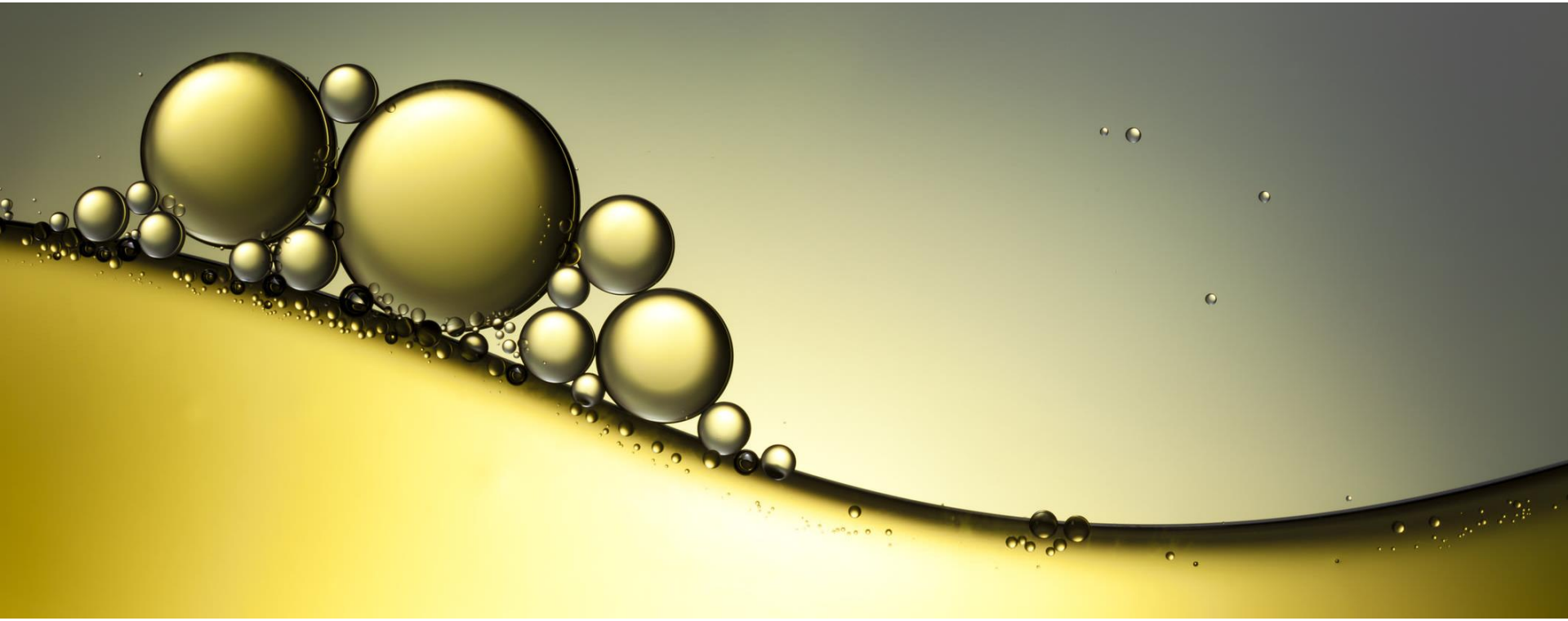
- Smokers have a double CV risk compared to non-smokers!
- Abandon smoking is the basic key to lower the CV risk!

Hypothyreosis and DLP

Hypothyreosis can cause secondary DLP

- Thyroidal hormones are involved into metabolism of nutrients in our body
- Low values of thyroidal hormones can slow down metabolism of lipids.

Part VI.
How to diagnose DLP



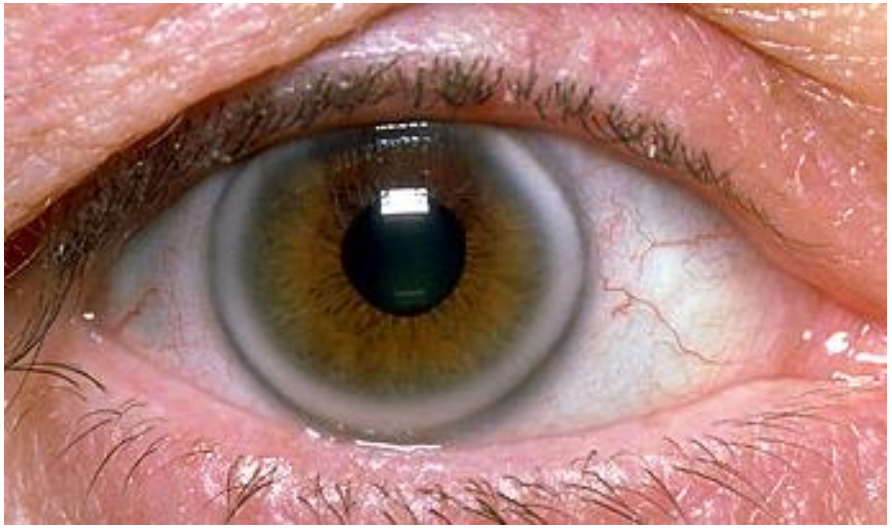
How to make a diagnosis

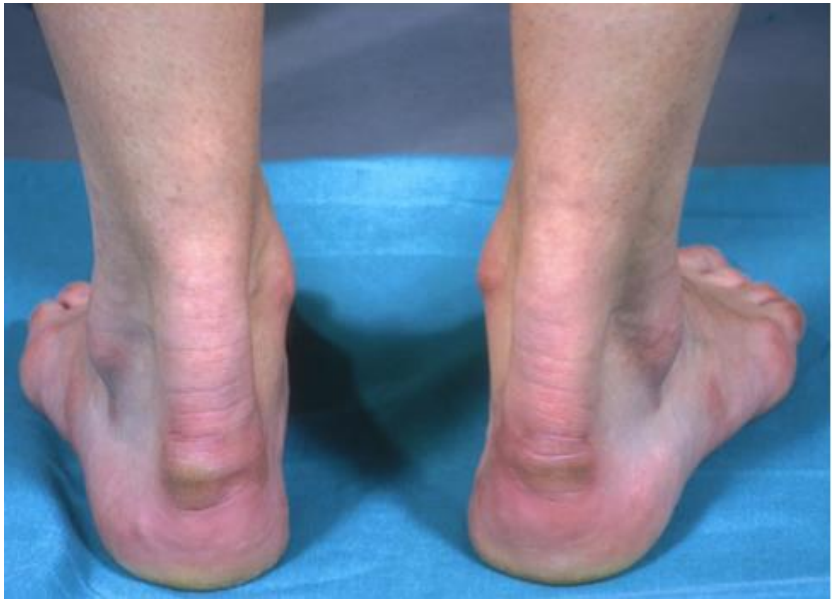
- Anamnesis (premature CVD in family history)
- Clinical examination (presence of clinical sings typical for DLP)
- Laboratory exams (+DNA analysis)
- Other methods (ultrasound of carotid arteries, ergometer, calcium score, coronarography)

Anamnesis

- Focused on premature CVD in family history (CVD in first – degree relatives before 55 years of age in males and before 60 years of age in females)

Clinical signs of HLP





Laboratory exams

- fasting: 12 hrs. prior examination without meal, drink pure water
- 3 days prior examination stop drinking alcohol, stop with any physical exercise
- don´t smoke before taking the blood samples
- use the pills (except of insulin → risk of hypoglycaemia)
- first examination – patient should be without hypolipidemics to know origin values of lipids,
- control examination – patient should take prescribed lipid lowering therapy regularly to see the effect of therapy
- avoid to examine lipid values during acute disease

Chylous serum

Which parameters to examine

- fasting plasma glucose
- kidney parameters (urea, creatinine)
- liver enzymes (AST, ALT, ALP, GGT)
- lipid spectre (TCH, LDL-CH, HDL-CH, TG, apolipoprotein A, apolipoprotein B, non-HDL CH)
- thyroidal parameters (TSH, peripheral hormones)

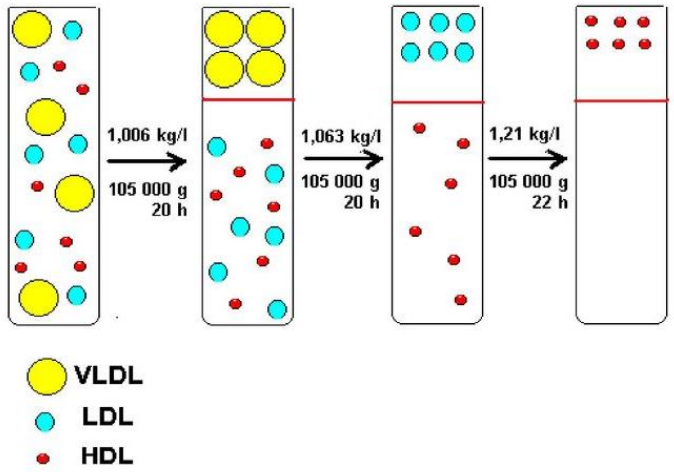
DNA analysis

In case of suspiction of an inherited DLP:

- FH (genes for apoB, LDLR, PCSK9)
- familial dysbetalipoproteinemia (apoE)
- familial defective lipoprotein lipase (LPLD)

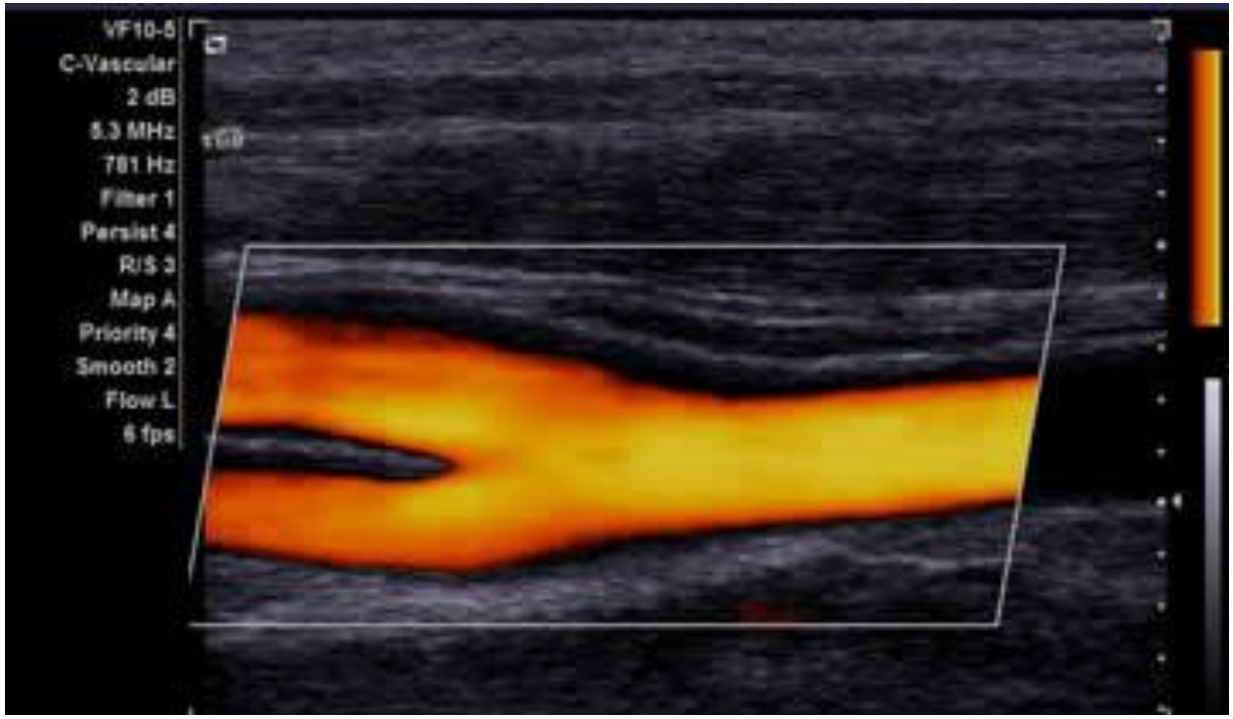
Lipoprotein ultracentrifugation

- enables to separate lipoprotein particles depending on different density given by lipid/protein ratio
- with increasing density the size of lipoprotein particles is getting smaller
- for this type of analysis is used very high gravity acceleration
- used as a diagnostic method for dysbetalipoproteinemia



Carotid ultrasound

- carotid arteries are well accessible, it is not invasive
- intima/media thickness, presence of atherosclerotic plaques (detection of subclinical atherosclerosis)
- velocity and direction of the blood stream

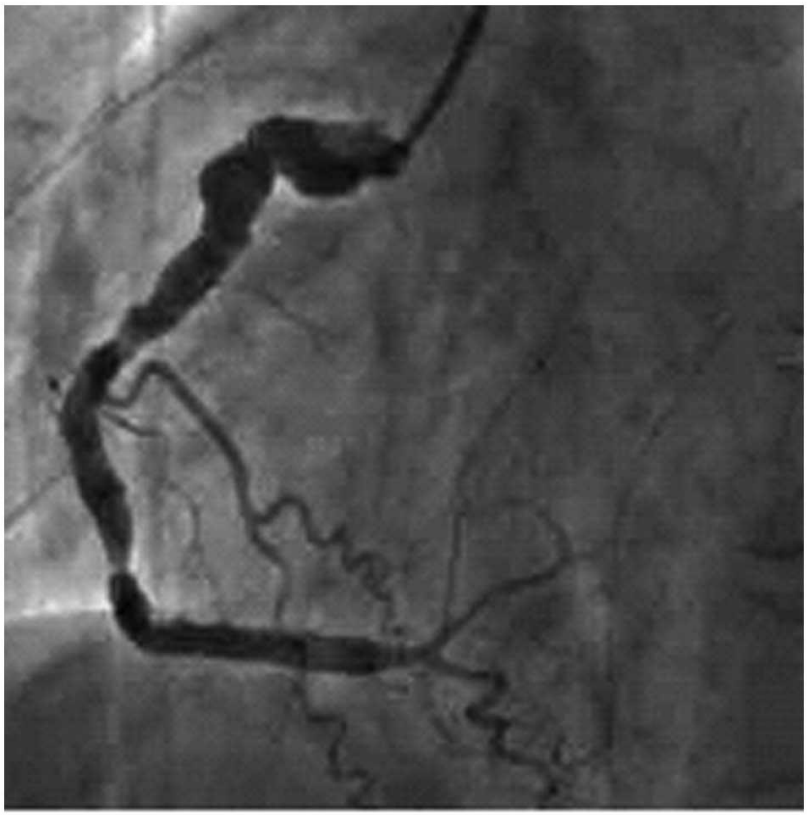


Ergometer

- bicycle exercise
- monitoring of ECG, BP
- if ECG changes are present (arrhythmia, changes in ST segment), than arterial coronarography should be performed

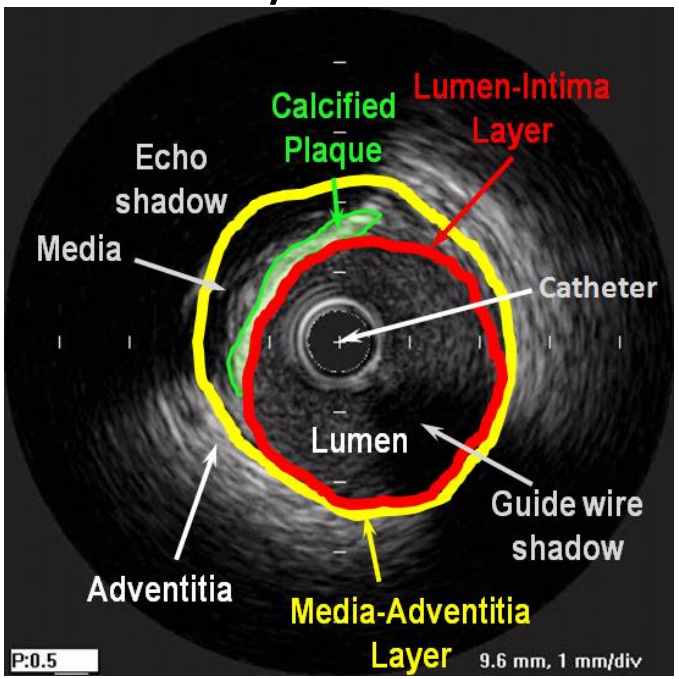
Selective coronarography

- detecting occlusions / obstructions in coronary arteries, which can lead to coronary ischemia or myocardial infarction
- with contrast liquid

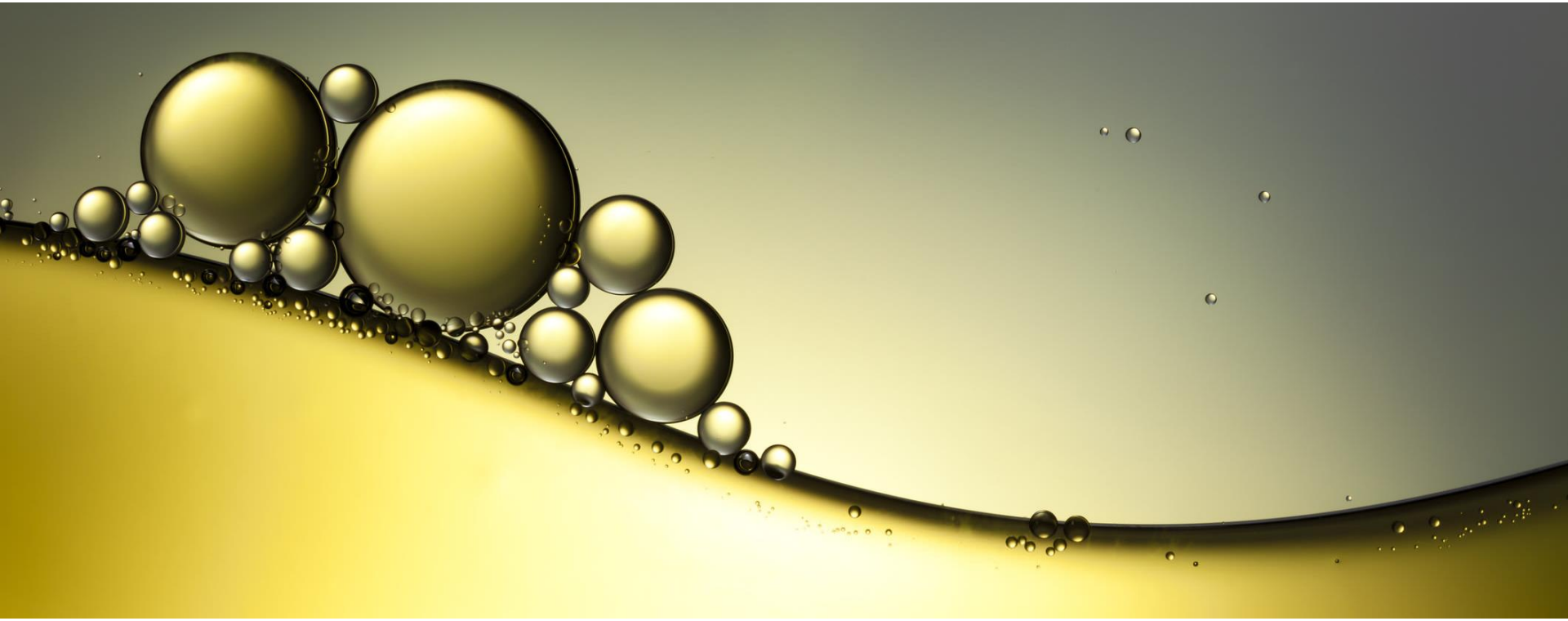


Advanced diagnostic technologies - IVUS

- intravascular ultrasound
- direct exploration of coronary arteries with using ultrasound probe
- evaluating lumen of arteries, presence of atherosclerotic plaques, quality of coronary arteries



Part VII.
Target levels of cholesterol



Guidelines

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

2016 ESC/EAS Guidelines for the Management of Dyslipidaemias

The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS)
Developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR)

Target levels of LDL-C

- recommended by ESC/EAS guidelines 2016
- „safe“ values of LDL-C when progression of atherosclerosis is slowed down or stopped (in optimal case)

LDL-C target levels

The higher CV risk

↓

the higher probability of acute CVD (MI, stroke)

↓

more aggressive hypolipidemic treatment is needed to reach the low levels of LDL-C to protect arterial damage

LDL-C goals (simplified)

Low + intermediate CV risk

- LDL-CH < 3,0 mmol/l

High CV risk

- LDL-CH < 2,6 mmol/l

Very high CV risk

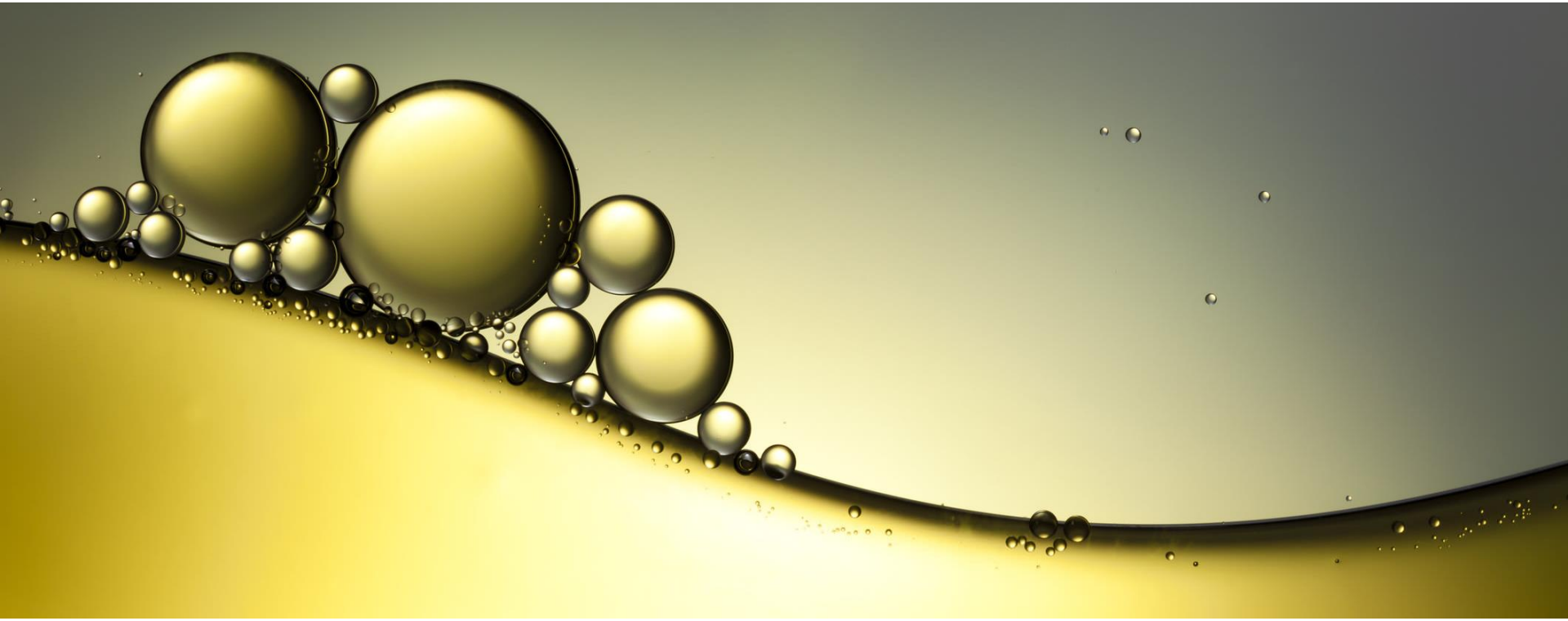
- LDL-CH < 1,8 mmol/l

Concept of treatment

LDL-C:

The lower, the better

Part VIII.
Therapeutic options, modifying CV risk, elimination of RFs



Changes in lifestyle only (without LLT – lipid lowering therapy)

- ESC/EAS guidelines: patients with CV risk

  < 5 % (without any other RFs, which would rise up the CV risk)
- diet + physical activity, weight reduction

Modifying CV risk, elimination of RFs

We can affect:

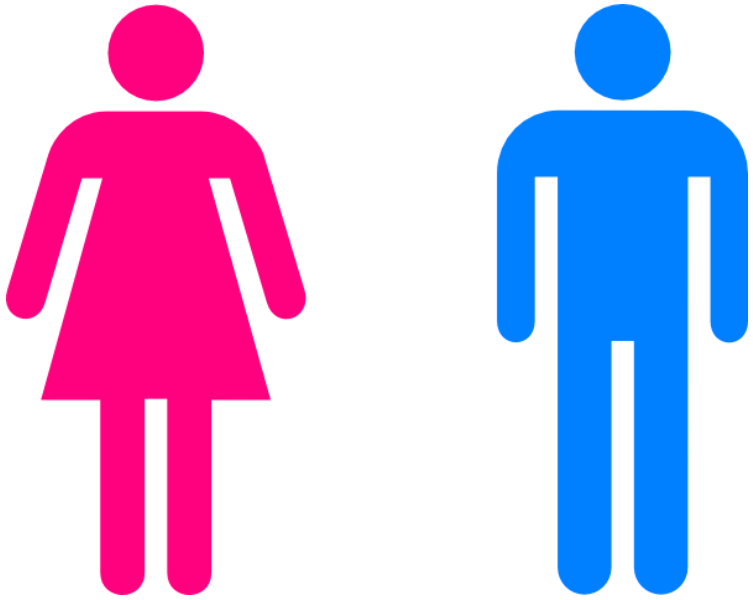
- Smoking – decrease CV risk up to 50 %
- Hypertension (can be treated)
- Cholesterol (can be decreased – with or without LLT)

Other RFs:

- DM (can be treated)
- Hypothyreosis (can be treated)

We can´t affect

- Sex
- Age
- DNA (not yet)



Smoking

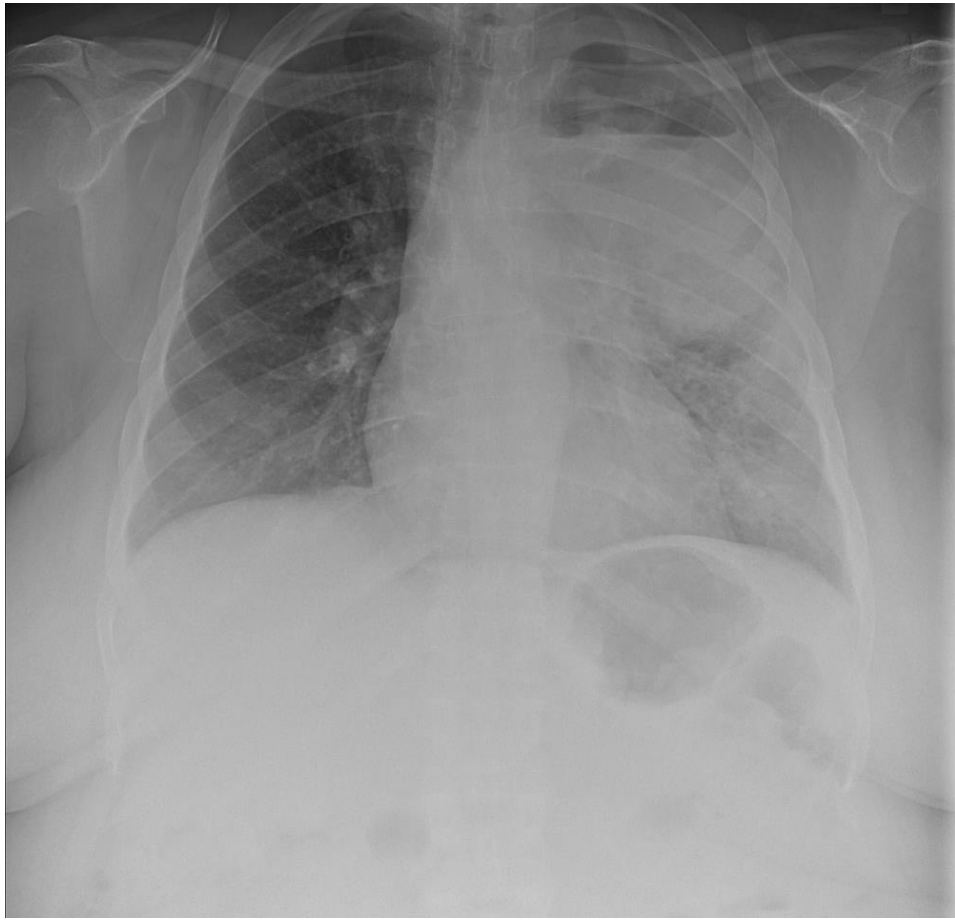
Why do I want to stop smoking?

- because of me (health risk, social handicap, social isolation from non-smokers, financial burden)
- because of the others (smell, health risk – passive smoking)

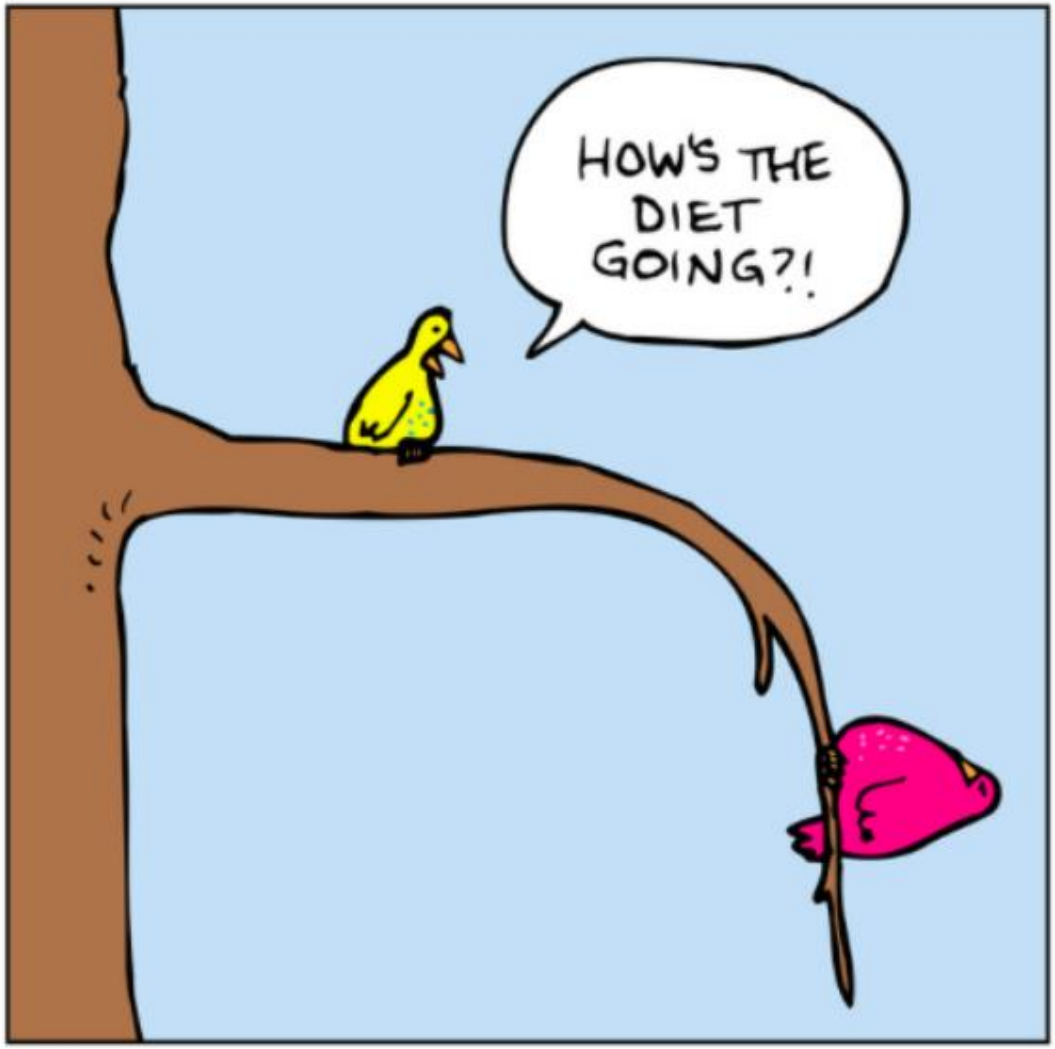
Abandonment of smoking has usually higher impact than making cholesterol levels lower.

Social isolation – „cage“ for smokers?

Just choose your own way



Diet recommendations



Diet recommendations

Reduce intake of:

- saturated lipids (FA) (fried food, butter, chocolate topping, sweet creams, cakes…) …it contains lot of saturated FA, which are strongly proatherogenic
- sugar (sweet bakery products, cookies, sweetened beverages!)

Lipids in nutrition

FA are rich of hydrogen (a lot of energy can be released from their structures)

- need to receive essential FA from plant oils, which we are not able to synthetize by ourselves
- reduce intake of animal lipids in general

Sources of essential FA

Plant oils:

- flaxy
- rapeseed
- olive
- others (sunflower, soya…)

Olive oil is not so rich about essential FA.

Potravinářský tuk	SAFA (%)	MUFA (%)	PUFA (%)
Sádlo	50	45	5
Máslo[a]	60[b]	35[b]	2[b]
Kokosový tuk[c]	90	9	1
Emulgované tuky (margariny)[d]	20-30	20-50	20-40
Řepkový olej	10	60	30
Olivový olej	15	75	10
Slunečnicový olej	10	25	65

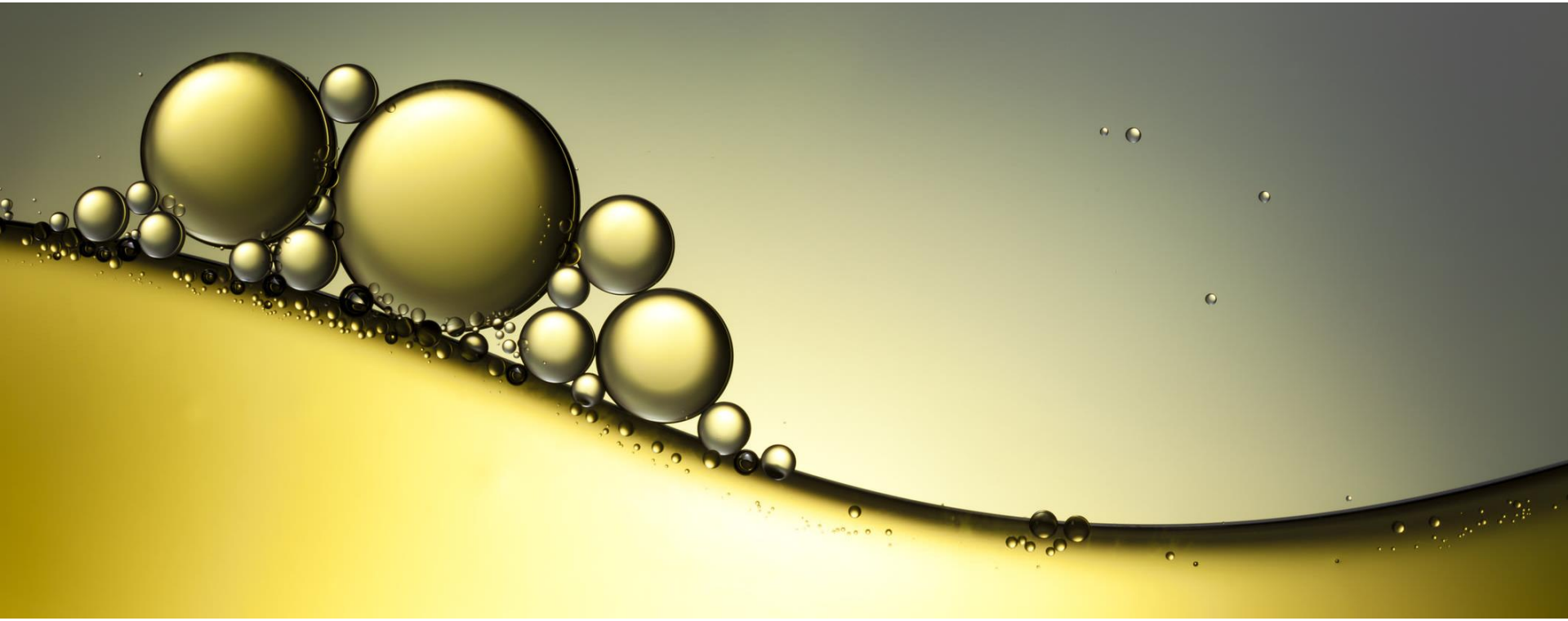
[a] Obsahuje 20 % vody. [b] Zbytek do 100 % mastných kyselin tvoří cca 3 % *trans*-mastných kyselin.
[c] Výborná surovina na výrobu mýdla. Z hlediska výživy nevhodný; součást mražených krémů, nanuků aj.
[d] Obsah vody značně kolísá, klasické margariny 20 %, nízkotučné (light) až 70 %.

Physical activity

Diet is very important part of lifestyle, but only diet is not enough. It is better to combine it with physical activity.

Diet + physical activity = weight reduction, changing muscle/fat ratio in body

Part IX.
Lipid lowering therapy (LLT)



LLT

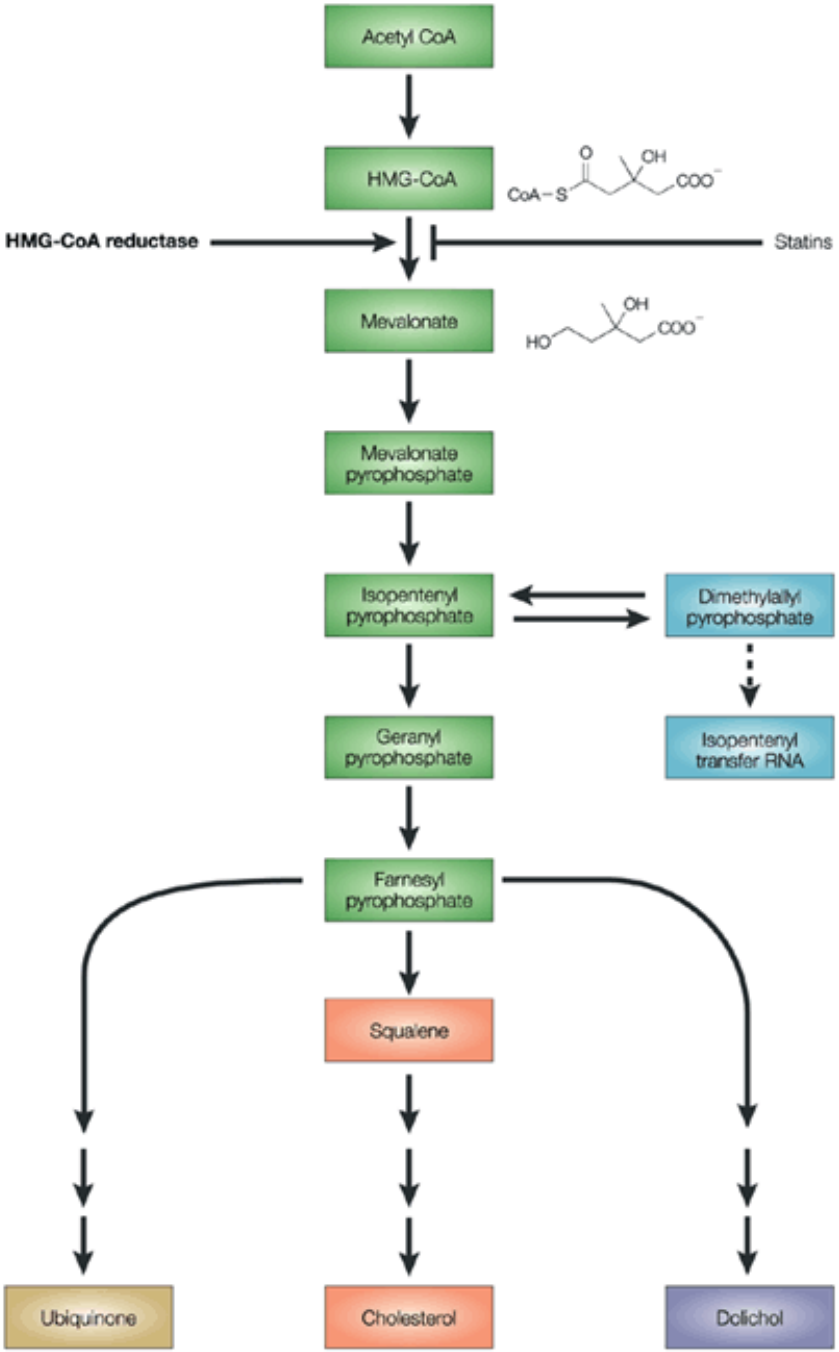
It doesn´t matter which kind of LLT is chosen, healthy diet and lifestyle + physical activity is essential for every patient with HLP.

LLT is recommended for patients with CV risk (SCORE) ≥ 5 %, or in case of severe HLP (FH).

Statins

Fundamental drug for patients with hyperlipidemia

- inhibitors of HMG-CoA reductase
- decreasing own production of cholesterol in liver
- many data about statins from clinical trials
- effective and safe


Acetyl CoA
HMG-CoA
CoA–S
O
OH
COO⁻
HMG-CoA reductase
Statins
Mevalonate
HO
OH
COO⁻
Mevalonate pyrophosphate
Isopentenyl pyrophosphate
Dimethylallyl pyrophosphate
Isopentenyl transfer RNA
Geranyl pyrophosphate
Farnesyl pyrophosphate
Squalene
Ubiquinone
Cholesterol
Dolichol

Statins

Statin therapy, which makes LDL-C levels 1 mmol/l lower, decreases the CV risk in average by 20 - 25 %.*)

---

*) Baigent C., Blackwell L., Emberson J. et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials. Lancet 2010; 376: 1670 - 81.

Statin group of LLT

- Atorva - statin
- Simva - statin
- Fluva - statin
- Rosuva - statin
- Prava - statin
- Pitava - statin

Ezetimibe

Ezetimibe (Ezetrol®)

- inhibitor of absorption of cholesterol from intestinal lumen to blood stream
- can be used in patients who don´t tolerate statins
- weak effect in monotherapy (cholesterol synthesis in liver is compensatory increased)
- synergistic effect with statins (blocking two metabolic ways of cholesterol – synthesis + resorption from food)

Ezetimibe

IMPROVE-IT study

- patients with acute coronary syndrome in personal history
- by adding ezetimibe to statin, the level of LDL-C was decreased in average by 24 %. *)
- combination of simvastatin + ezetimibe significantly decreased the risk of CV events *)

---

*) Cannon CP., Blazing MA., Giugliano RP. et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. N Engl J Med 2015;372:2387 - 97.

Resins

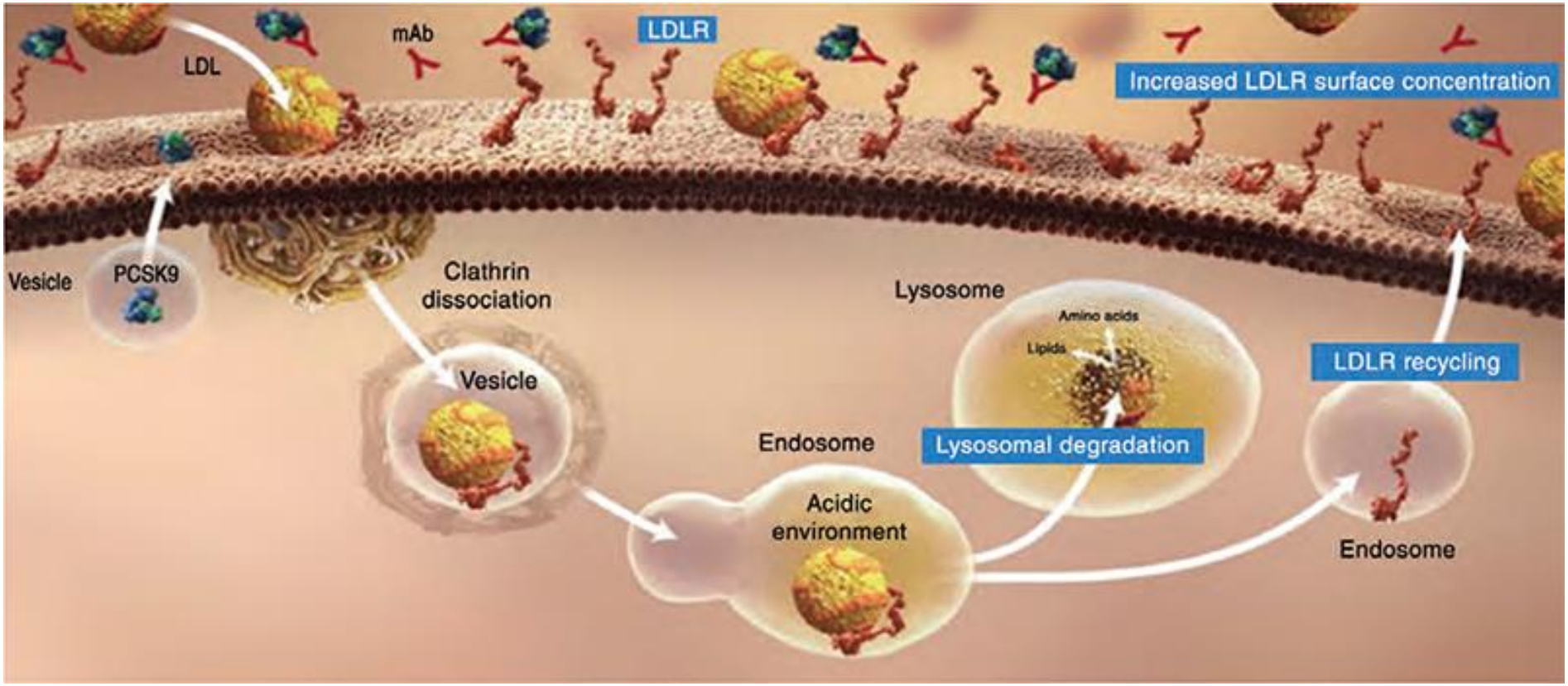
- bad taste
- weak hypolipidemic effect
- only for pregnant women and children with severe HLP (FH)
- Cholestyramin (Vasosan P®)

PCSK9 inhibitors

PCSK9 = proprotein convertase subtilisin kexin type 9. Fully humanised antibodies against PCSK9

- evolocumab (Repatha®)
- alirocumab (Praluent®)

How does it work?



PCSK9 inhibitors

- decreasing LDL- C in average by 50 – 60 % *)
- used in combination with statins
- no adverse events known yet

---

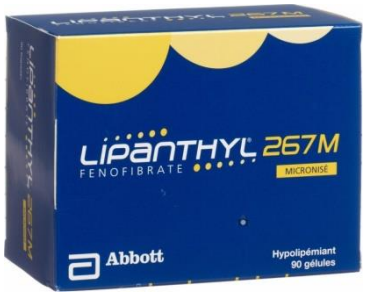
*) Whayne TF. Jr. PCSK9 inhibitors in the current management of atherosclerosis. Arch Cardiol Mex. 2016 Dec 27.

How to use it?

- subcutaneous injection once per 2-4 weeks
- prefilled pen

LLT for hypertriglyceridemia

- fenofibrate
- activator of PPAR – α (peroxisome proliferator-activated receptor-alpha)
- decreases levels of TGs by inhibition of their synthesis and increasing of their clearance
- induces β – oxidation of FA and decreases their availability for synthesis of triglycerides and for VLDL particles
- stimulates activity of LPL, which attacks TGs.
- decreases risk of diabetic microangiopathy – benefit for patients with DM



Polypills

- 2 or more drugs in one pill
- comfortable for patient, increases adherence to therapy

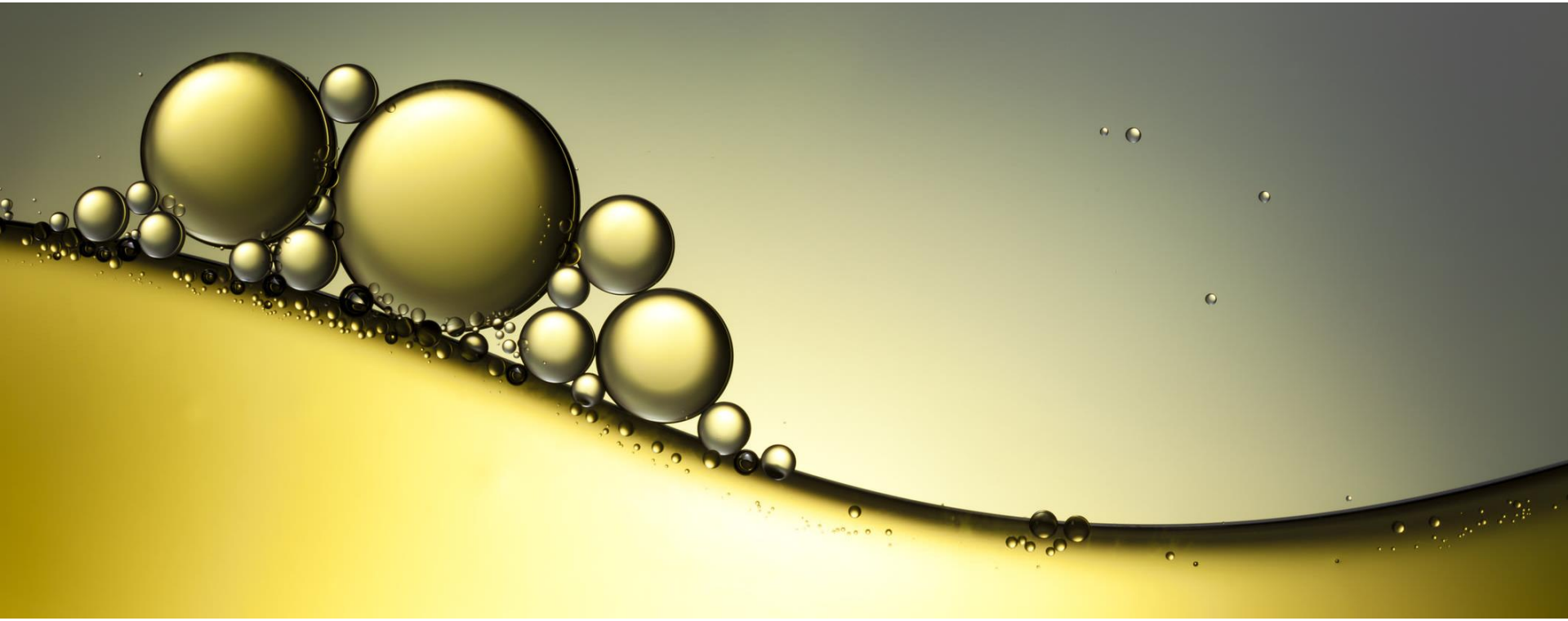
Statins are available in combination with these drugs:

- antihypertensives
- fibrates

Development of new drugs

- Modifying production of LDL particles on the genetic level (silencing RNA – siRNA)

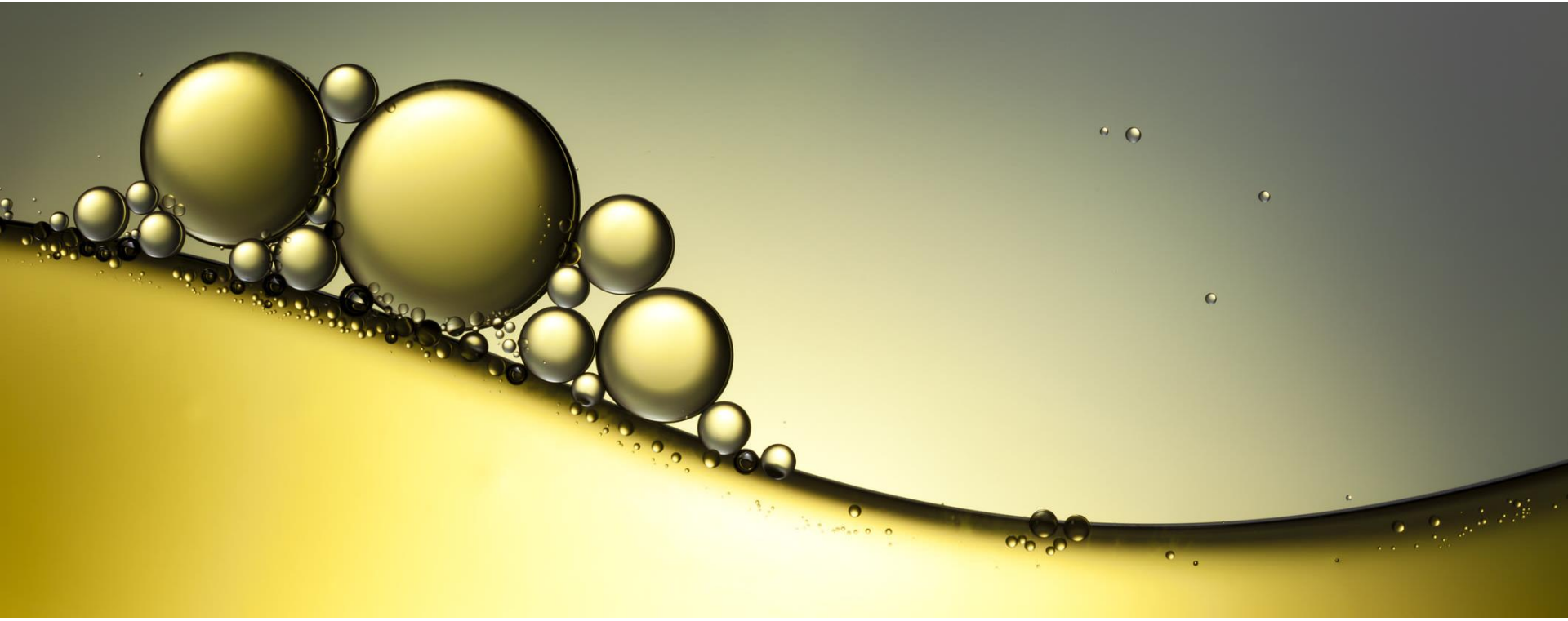
Part X.
Nutrition supplements



Nutrition supplements

- ω - 3 unsaturated FA
- red yeast rice
- plant sterols and stanols

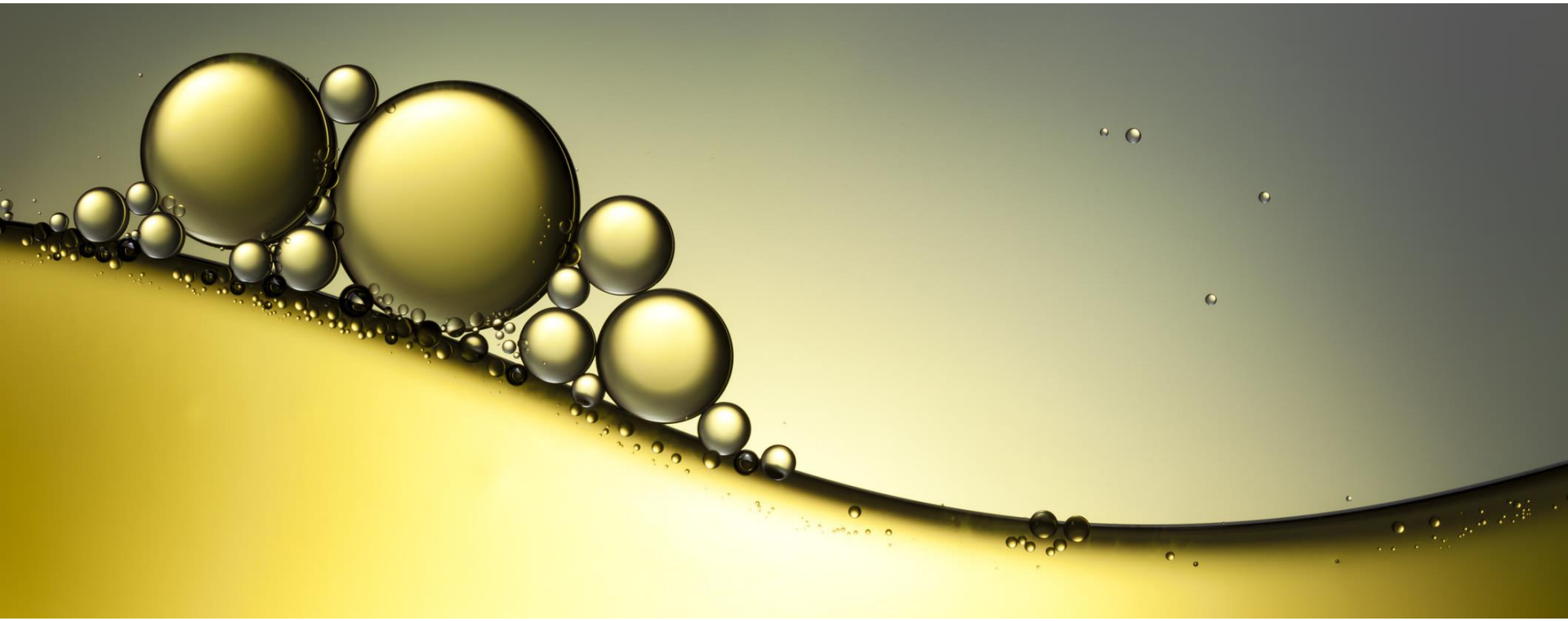
Part XI.
The MedPed project



MedPed project

- Make early diagnosis to Prevent early deaths in MedicalPedigrees
- active searching for patients with inherited HLP. Making diagnose, perform treatment
- prevention of premature deaths due to acute cardiovascular events

Thank you for your attention!



Ondrej Kyselak, M.D.
Dept. of Clinical Biochemistry,
St. Anne's University Hospital Brno
ondrej.kyselak@fnusa.cz