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Cholesterol – a molecule that is known, yet dangerous and essential at the same time – a multidimensional approach

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Summary:

Excessive amount of cholesterol in human organism, leading to hypercholesterolemia, stands as the cause of multiple ailments observed among people living in highly industrialised states, namely circulatory system ailments, coronary atheromatosis, peripheral arterial disease, cerebrovascular disease or biliary lithiasis. Lipid disorders may be associated with single lipoprotein fractions or they may cover all of them. As far as clinical practice is concerned, the main attention is paid to LDL fraction cholesterol, nonetheless, an increasing number of data tend to show that both VLDL and HDL (when their concentration is small) contribute significantly to the development of arteriosclerosis. LDL cholesterol is the main target within treatment focused on decreasing plasma cholesterol concentration. Despite the unfavourable opinion, taking into consideration its biochemical aspect, cholesterol plays important and irreplaceable functions in human organism. It constitutes an important content of cell membranes, a substrate related with biosynthesis of bile acids, steroid hormones and vitamin D3. Disorders related with biosynthesis of cholesterol lead to numerous anomalies in multiple organ structures – it participates in embryonic development. Increased cholesterol level is not a condition sufficient to create a sclerotic plaque, and creation of cholesterol crystals, predisposing to biliary lithiasis, does not depend solely on the degree of bile saturation with this sterol

Key words: cholesterol, statins, biosynthesis of cholesterol, fibrates, ion-exchange resins, cholesterol lithiasis, hypercholesterolemia, nicotinic acid.

Introduction

When undertaking the biomedical and pharmaceutical issue concerning cholesterol, its advantages and disadvantages, it is impossible not to mention the statement by Michael Brown and Joseph Goldstein, Nobel Prize laureates from the year 1985, who stated that: "Cholesterol is the most "awarded" little molecule biology has ever seen. Thirteen Nobel Prizes have been awarded to subsequent researchers, who devoted major part of their scientific careers to cholesterol. Since 1784, when cholesterol was isolated from bile stones, it became a sort of hypnotic fascination among researchers from various fields of science and medicine… Cholesterol is a molecule with a face of Janus [Roman god of war with two faces]. Its feature, which makes it useful in cell membranes, namely its complete insolubility in water, at the same time makes it a killing compound".

What is cholesterol like?

Cholesterol occurs in tissues and serum lipoproteins as free cholesterol or in connection with fatty acids having long carbon chain, as cholesterol esters [1].

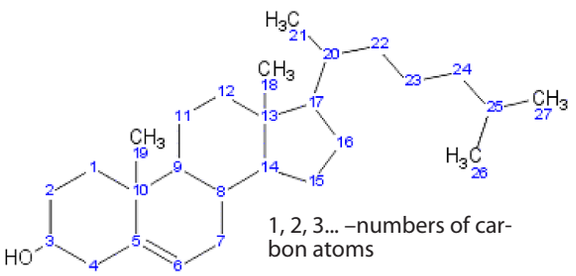

Figure 1: Structural formula of cholesterol molecule.

Proper concentration of the most important representative among sterols in serum ranges between 3.9 and 7.2 mmol/l (150-280mg%). A regular diet covers about 1.5-2g of cholesterol. On average, human organism contains about 60g of this compound, and ⅔ of the above can be found in skeletal muscles, fat tissue and skin. Cholesterol is a noteworthy element of cell membranes. In the above it plays the role of flow and flexibility regulator. Its rigid, hydrophobic, polycyclic molecule with a small hydroxyl group on one end and flexion aliphatic chain on the other end, adjusting into the membrane by means of placing cholesterol rings perpendicularly to the plane of the membrane, and as a result the cholesterol molecule perturbs membranous balance, and isolates a certain number of phospholipid aliphatic chains. Cholesterol hydroxyl group creates hydrogen bond with oxygen atoms of the polar phospholipid part, while the flexible aliphatic tail destroys the crystalline structure within the hydrophobic part of the membrane. Cholesterol not only counteracts membranous crystallization, but also limits membranous movements and migrations of lipid and protein molecules.

Cholesterol is also present in liver, where, among others, it supports biosynthesis of bile acids and steroid hormones, namely sex hormones and corticosteroids. What concerns the nervous tissue, it stands as a part of myelin sheath, constituting 10-15% of the dry matter in the brain [2].

Cholesterol found in human organism may be both of exo – and endogenous origin. Total serum concentration of this steroid is a resultant of lipoprotein molecule metabolism responsible for its transport between the liver (cholesterol synthesized in the system) and intestines, where it is being absorbed during travelling processes (dietary cholesterol) [3]. Under physiological conditions about 60% of cholesterol (in case of regular diet) is absorbed from ingesta (mainly of animal origin), while the rest is excreted with faeces. Absorption process is influenced by multiple factors: bile, essential fatty acids (EFA), content of neutral fats and other lipids in the food, as well as presence of other steroids (sterols of plant – phytosterols and cholestanol) and proteins.

Both the unabsorbed dietary cholesterol, as well as cholesterol excreted to the intestine with bile undergoes certain transformations under the influence of intestinal flora:

1) some part is reduced into coprostanol and in this form it is excreted with faeces,
2) some part is broken into small fragments, which are once again absorbed and participate in synthesizing the endogenous cholesterol.

All these transformations are known as "hepatic and intestinal cholesterol circulation" and are of considerable significance as far as the cholesterol management within the whole organism is concerned [4].

Biosynthesis of cholesterol

Cholesterol originates in a more or less the same proportion from *de novo* synthesis and from diet. Depending on demand, about 800mg/24h of cholesterol is created in the organism, whereas 10% originate in liver, and almost 15% in intestinal mucous membrane. Virtually all cells with nucleus are capable of synthesizing cholesterol. Cytosol fractions and microsomal cells are responsible for this process.

The route of cholesterol synthesis can be divided into five stages:

1) Acetyl-CoA gives HMG-CoA and mevalonate.
2) Melavonate transforms into 3-isopentenyl pyrophosphate.
3) Squalene synthesis from six isoprenoid units.
4) Squalene transforms into lanosterol.
5) Lanosterol transforms into cholesterol.

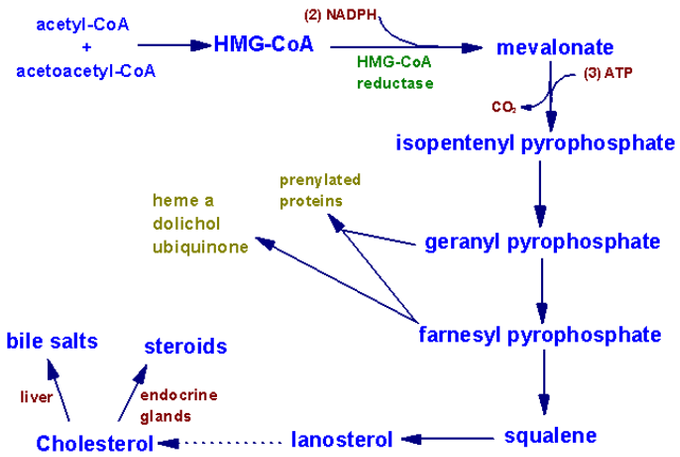

Figure 2: The route of cholesterol biosynthesis [5].
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Stage1.
Synthesis of cholesterol in cytosole begins from condensation of two *acetyl-CoA* particles, and this gives *acetoacetyl-CoA*. This reaction is catalysed by **acetoacetyl-CoA thiolase**. With participation of **HMG-CoA synthase** enzyme, acetoacetyl-CoA connects with acetyl-CoA and creates *3-hydroxy-3-methylglutaryl-CoA (HMG-CoA).*

HMG-CoA occurs in hepatic cells, both in cytosole, as well as mitochondria. Mitochondrial pool plays the role of precursor related with synthesis of ketone bodies, whereas *mevalonate* is created from the pool present in cytosole. This irreversible reaction occurs with the use of NADPH and microsomal **HMG-CoA reductase** enzyme and is not only considered as the stage limiting the pace of cholesterol biosynthesis' route, but it also is the environment where majority of efficient drugs decreasing the cholesterol level, inhibitors of HMG-CoA reductase, operate.

Stage2.
Mevalonate transforms as a result of Tyree subsequent phosphorylations with participation of ATP into *3-isopentenyl-pyrophosphate*.

Stage 3.
Within this stage synthesis of cholesterol begins from isomerisation of isopentenyl-pyrophosphate (active isoprenoid unit) to *dimethylallyl pirophosphate*. These two isomeric units condense into *geranyl pyrophosphate*, and as a result they create *farnesyl pyrophosphate*. The last reaction of *squalene* synthesis is the condensation of two *farnesyl pyrophosphate* molecules with participation of NADPH.

Stage 4.
Squalene transforms into *epoxid* in smooth endoplasmic reticulum. This reaction, catalysed by oxydase, known as **squalene epoxidase,** requires the presence of molecular oxygen. Then the squalene epoxidase transforms with participation of **cyclase** into a cyclic form — *lanosterol.*

Stage 5.
Within the last stage changes take place in steroid ring and in lateral chain and finally lanosterol transforms into *cholesterol.*

Control concerning biosynthesis of cholesterol

The pace of cholesterol synthesis in liver or intestinal mucous membrane mainly depends on cellular level of this compound, which is regulated in many ways:

1) Control concerning the quantity and activity related the reductase of HMG-CoA – enzyme catalysing the creation of mevalonate – namely:
   - feedback: hepatic HMG-CoA reductase is inhibited by mevalonate and cholesterol(?);
   - phosphorylation-dephosphorylation: phosphorylation decreases the activity of reductase; this enzyme is "deactivated" by protein kinase activated by AMP – hence when the ATP concentration is small cholesterol synthesis declines;
   - repression concerning transcription of HMG-CoA reductase gene;
   - degradation of reductase: significant mevalonate and cholesterol concentration causes sudden enzyme degradation.

2) The intracorporeal cholesterol production is influenced by variable amounts of this compound in food: as the cholesterol supply increases, the hepatic biosynthesis decreases.
3) Cholesterol contained in LDL, collected by means of LDL receptors inhibits the endogenous creation of this sterol.
4) Esterification of cholesterol through ACAT (Acyl-CoA cholesterol acyl transpherase).

5) Using cholesterol to synthesize Rother steroids: hormones, bile acids in liver.
6) Collecting free cholesterol from lipoprotein into cell membrane increases the cellular level of this sterol, whereas the influence of cholesterol from membranes into HDL decreases its concentration [1,6,7].

Biomedical significance of cholesterol

Structure of cholesterol does not allow it to move independently in blood into tissues, and hence it has to be transported by special molecules known as lipoproteins. These are aggregates of macromolecules, whose protein constituents – apoproteins condition, among others, solubility of hydrophobic lipids in blood plasma. They also play the structural and metabolic functions, conditioning lipoprotein transformations as enzymatic cofactors or as ligands for particular receptors [8].

There are several types of lipoproteins, which have different contents, different type of function, and most of all different meaning in pathogenesis of diseases.

Chylomicrons
They are created in intestinal mucous membrane during absorption of fat digestion products. These are huge lipoprotein complexes, which enter the circulation through lymphatic vessels. Lipoprotein lipase enzyme hydrolyses these aggregates in plasma, while the rest, saturated with cholesterol, called chylomicron remnants is collected through liver.

Lipoproteins of very low density (Very Low Density Lipoproteins)–VLDL
They are created in liver and they transport the excess of cholesterol and triacylglycerols to plasma. This is where they are hydrolysed by lipases on the level of blood vessel surface. As a result, molecules with depleted triacylglycerol amount and enriched with cholesterol esters called lipoproteins with intermediate density (**I**ntermediate **D**ensity **L**ipoproteins). Liver collects half of these aggregates, while the other half is changed into LDL.

Low Density Lipoprotein – LDL
As the main cholesterol carrier in blond it provides cholesterol to peripheral tissues. Whereas LDL receptors are to control how this sterol is metabolised in cells. Long-lasting clinical researches revealed a direct connection between LDL cholesterol concentration and ischaemic heart disease. This particular cholesterol fraction plays a crucial role in pathogenesis underlying the creation of sclerotic plaque.

Origination of sclerotic plaque is initiated by damage of the endothelial tissue (internal membrane lining the vascular lumen). It is assumed that oxygenated LDL cholesterol molecules have cytotoxic properties and they stand as the main factor causing endothelial damage. Damage of the endothelium leads to origination of inflammatory process, and subsequently it leads to release of inflammatory mediators. This place is being infiltrated by macrophages (so-called scavenger cells). Macrophages capture the LDL cholesterol molecules. Surrounded with oxygenated cholesterol molecules they esterify the above, which results in origination of the so-called foam cells, and these again lead to "fatty infiltrations". The above mentioned and other changes cause accretion of sclerotic plaque. It decreases the lumen in blood vessels. With time, sclerotic plaques are calcified and undergo further lesions resulting in partial or total occlusion of artery [9,10].

High Density Lipoprotein – HDL
Synthesis of this lipoprotein takes place in liver and intestinal epithelial cells. Physiological role of HDL differs from the one played by LDL. HDL carries cholesterol in direction opposite to LDL, namely from tissues and vessels into the liver, where it is properly transformed, and then secreted from the organism. That is why HDL is called the "good cholesterol", as opposed to LDL, which is known as the "bad cholesterol". Some specialists believe that HDL may also collect cholesterol from sclerotic plaque, hence decreasing the plaque and limiting arteriosclerosis. That is why there is a common belief that both in prophylaxis, as well as in treating cardiac and vascular diseases it is essential to obtain a high HDL concentration. HDL cholesterol concentration is lowered in tobacco smokers, while it increases in people performing regular physical activity [3,10,11].

Lipoprotein (a) – Lp(a)
Lp(a) is a variation of LDL. Lipoprotein(a) is considered as a significant genetically conditioned

factor increasing the risk of arteriosclerosis in arterial and cerebral vessels, incidence of heart attack and cerebral crisis. Atherogenic activity of Lp(a) is revealed when its concentration exceeds 30 mg/dl (7, 11-13), and it depends not only on the Lp(a) level, but also on simultaneous presence of other risk factors (f. ex. high LDL cholesterol level, arterial hypertension, smoking) [12].

Table 1: Border values concerning the main lipid parameters related with increased risk of ischaemic heart disease according to the latest recommendations of the International Atherosclerosis Society [13].

Triglycerides	150-400 mg/dl (1.7-4.5 mmol/l)
Cholesterol	> 200 mg/dl (5.2 mmol/l)
LDL-cholesterol	> 135 mg/dl (3.5 mmol/dl
HDL-cholesterol	< 35 mg/dl (0.9 mmol/l) – Males
Cholesterol / HDL-cholesterol	< 40 mg/dl (1 mmol/l) – Females > 5
Lp(a)	> 30 mg/dl

Cholesterol has a bad opinion due to the fact that it supposedly responsible for diseases present in highly industrialized states, namely blood circulation disorders, arterosclerosis, peripheral arterial sclerosis, diseases related with cerebral vessels or cholelithiasis.

However, this sterol plays important and irreplaceable functions in human organism. Disorders relating biosynthesis of cholesterol lead to multiple anomalies in structure of numerous organs. It has been recently discovered that cholesterol plays a crucial role in embryonic development by regulating the activity of signal proteins. Smith-Lemli-Opitz syndrome is a genetic disease. It is characterized by intellectual development delay, changes in facial expressions and many irregularities in structure of multiple organs. The deficit in activity of 7-dehydrocholesterol reductase (DHCR7), an enzyme catalysing the last stage of cholesterol biosynthesis, stands as the reason of congenital defects [14,15].

Hypercholesterolemia

We can talk about lipid disorders when we state quantitative or qualitative changes in lipid profile based on conducted laboratory tests. An excessive amount of lipids signifies hyperlipidemia, whilst an insufficient amount means hypolipidemia. Lipid disorders may relate to single lipoprotein fractions or they or cover all fractions at one time. It is crucial to focus on the type of disorders, nevertheless, due to their frequency and association with arteriosclerosis, the most worrying include hyperlipidemias:

- Hypercholesterolemia – high concentration of total cholesterol
- Hypertriglicerydemia – high concentration of triglycerides
- Mixed hyperlipidemia – high concentration of cholesterol and triglycerides.[10]

As far as clinical practice is concerned, the main focus is attributed to LDL, nonetheless, more and more data reveal that both VLDL, as well as HDL (when their concentration is small) contribute to development of arteriosclerosis. LDL chol is the main aim in case of treatment decreasing cholesterol concentration in plasma [16].

Table 2: Diagnosing hypercholesterolemia.

Form	Cholesterol concentration	
Benign	Tchol: 200-250 mg/dl LDLchol > 135 mg/dl TG < 200 mg/dl	(5.2-6.5) mmol/l) (> 3.5 mmol/l) (< 2.3 mmol/l)
Moderate	Tchol: 250-300 mg/dl LDLchol: 135-215 mg/dl	(6.5-7.8 mmol/l) (3.5-5.5 mmol/l)
Severe	Tchol > 300 mg/dl LDLchol >215 mg/dl	(> 7.8 mmol/l) (> 5.5 mmol/l)

In case of people older than 20-years-old, it is essential to measure total cholesterol (Tchol), LDL cholesterol (LDLchol) and HDL cholesterol (HDLchol) and triglycerides on an empty stomach at least once every 5 years. More frequent measures are crucial in people with numerous risk factors relating ischaemic heart disease [17].

What concerns most cases of severe Hypercholesterolemia, the genetic factor plays an important role.

What concerns disorders enumerated in group covering hypercholesterolemias, it is crucial to differentiate between familial Hypercholesterolemia and apoB-100 defect. The first disorder is associated with LDL receptor defect, whereas the second one is connected with ligand defect of this receptor (B-100 apolipoprotein). The receptor's

Table 3: Genetic diseases associated with extremely high plasma LDLchol concentration (>=190 mg/dl) [18].

Clinical condition	Clinical features and consequences	Treatment
familial heterozygotic hypercholesterolemia	• results from a mutation of gene receptor for LDL (expression of half of proper receptor molecules) • frequency of occurrence: 1/500 people in USA • LDLchol concentration: exceeds twice the standard range (f. ex. 190-350 mg/dl) • Tchol concentration finally increases to 300-500 mg/dl • frequent occurrence of xanthomas (*) in sinews • often premature coronary disease: – 30-40 year of life among men – 40-50 year of life among women	• initial use of medications decreasing the LDLchol concentration in Young adults • lifestyle change recommended in all patients • statins become the first-choice drugs (with simultaneous therapeutic diet) • ion-exchange resins (in association with statins) • if necessary, it is recommended to consider triple drug therapy (statin + Ion-exchange resin + nicotinic acid)
familial homozygotic hypercholesterolemia	• it results from a mutation of LDL receptor gene on both chromosomes • frequency of occurrence: 1/1 million people in USA • LDLchol concentration: 4 times greater (f. ex. 400-1000 mg/dl) • Tchol concentration: 700-1200 mg/dl • xanthomas: in sinews, nodular, skin • severe, disseminated sclerosis (covers numerous arterial beds) • severe clinical course of arteriosclerosis • aortic valve defect	• diet based treatment is inefficient • ion-exchange resins are inefficient • nicotinic acid reveals small effectiveness • statins may be moderately efficient in certain patients • hepatic transplantation is effective but impractical • plasmapheresis is currently used, as it selectively removes VLDL and LDL from plasma
familial defective apolipoprotein B-100	• results from mutation of gene for apo B-100 (LDL molecules have smaller affinity to LDL receptor) • frequency of occurrence: 1/700-1/1000 people • LDLchol concentration: 1,5-2 times increased (160-300 mg/dl) • xanthomas in sinews • premature arteriosclerosis: – 40-65 year of life among men – not confirmed among women	• therapeutic lifestyle change is recommended • all drugs decreasing the LDLchol concentration are efficient • pharmacological combined treatment proves to be essential less frequently than in case of familial heterozygotic Hypercholesterolemia
multi-gene hypercholesterolemia	• results from multiple genetic polymorphisms (often with simultaneous improper diet) • frequency of occurrence: 1/10-1/20 people (depending on the age) • LDLchol concentration: >=190 mg/dl • frequency of occurrence in case of coronary disease: 3-4 more than the average range among population	• therapeutic lifestyle change is recommended • it is recommended to consider administration of LDLchol decreasing drugs, if LDLchol concentration equals >=190 mg/dl despite therapeutic diet • combined treatment is rarely necessary

(*) Xanthomas are cholesterol bumps, which are deposited under the skin, around eyes and along sinews [19].

defect may be diagnosed through evaluation of its activity or by stating the presence of genetic mutation responsible for breaking the synthesis or perturbing receptor's activity. Determining the activity may stand as the only or the first-choice examination conditioning future tests on the mutation level. In clinical practice these tests are performed extremely rarely due to their cost and hence limited availability [20].

It is very important to detect congenital lipid and lipoprotein transformation disorders in young age (even directly after birth) and early implementation of prophylaxis. This may significantly decrease the risk of revealing the clinical form of ischaemic heart disease in the future [21].

Among patients with hypercholesterolemia we can observe associated lesions in cholesterol and phospholipid values in erythrocyte membrane with increase of cholesterol relation to phospholipids. Cholesterol of erythrocyte membrane is in dynamical balance with plasma cholesterol.

Adult erythrocyte without nucleus is not capable of synthesizing cholesterol. Hyperlipidemia causes changes in red blood cell membrane properties – erythrocyte becomes less flexible and loses its capability to adapt its shape to vascular diameter, which leads to worsened microcirculation and may hasten the development of arteriosclerosis. Researches reveal an influence of statins (atorvastatin and simvastatin) on the content of discussed

sterol in erythrocyte membranes. By decreasing the total cholesterol in plasma, medications from this group decrease the membranous cholesterol value and prevent damage of cell membranes [22]. Researches performed on rabbits with hypercholesterolemia, caused by diet rich in cholesterol, showed damage of oral mucosa, which may lead to gum inflammations [23].

Undoubtedly, lipid and lipoprotein disorders constitute an important atherogenic factor. It is assumed that Hypercholesterolemia increases frequency of calcifications in sclerotic plaques, which are more advanced developmental lesions. It has been proven that calcifications are related with death of cells, which have the capability of phagocytosis and are destructed due to cholesterol overload. Necrotic cellular residues may then undergo calcification. It has been shown that Hypercholesterolemia is connected with calcified aortic exits, which unfortunately are not reduced after implementing intensive treatment with medications decreasing the blood cholesterol level [24,25].

Overlying genetic factors and improper diet (high-fat diet), smoking, lack of physical activity, not only pose a significant influence on failure of pharmacological treatment based on hypolipidemic drugs, but also increase the risk of ischaemic heart disease. The fight with Hypercholesterolemia begins with implementing multifactor therapeutic lifestyle changes.

Factors influencing cholesterol concentration in the organism:

Diet

For decades numerous researches have been devoted to the enormous influence it poses on cholesterol concentration in human organism and the meaning of particular grocery products in fighting hypercholesterolemia. High-calorie diet, rich in saturated fats contributes both to the occurrence of lipid disorders and obesity, which results in low plasma HDLchol concentration, as well as to the increase of prothrombotic blood activity. What is most significant is the fact that visceral (abdominal) obesity is associated with the so-called polymetabolic syndrome, characterized by conditions such as hypercholesterolemia [26,27]. Currently we can indicate groups of products not only increasing LDLchol concentration, but also the ones enabling to decrease the above (Table 4.).

Table 4: Diet decreasing lipid level.

Principle	Quantity	Nutritive sources
↓ total fat ↓ saturated fat ↓ *trans* fatty AIDS	<30 % energy 7 – 10 % energy	Avoiding butter, hard margarines, full milk, cream, whole cheese, fat meat, poultry, products containing hydrated oils, palm and coco nut oil; food produced from partially hydrated oils, as well as products fried on hydrated fats (source of *trans* fatty acids)
↓ consuming protein foods of small saturated fats content		Fish, chicken, turkey, veal, venison
↑ complex carbohydrates ↑ fruit and vegetable fibre (soluble fibre) ↑ leguminous	About 3.5g of fibre a day	All fruits, with dried, fresh and frozen vegetables, lens, seeds from leguminous plants, unrefined cereal products, including the one from oat
↓ dietary cholesterol	<300 mg/day	It is allowed to consume 2 egg yolks a week and a liver 2 times a month.
Moderate increase in consumption of oils rich in monounsaturated (they are the only fats that can be both fried and baked) or polyunsaturated fatty acids and margarine derivatives	Monounsaturated 10–15% energy-Polyunsaturated 7-10% energy	Olive oil, oil, canola oil, margarine derivatives, peanuts (monounsaturated)Sea fish, cod liver oil, soyean oil, sunflower oil and safflower oil (polyunsaturated)

Recently conducted research revealed that vegetable stanols, obtained from soyean and pine oil decrease the LDLchol serum concentration. Esterised vegetable stanols and sterols are present in specially enriched margarine available on the market. These compounds stand as inhibitors of dietary cholesterol absorption and are perceived as therapeutic option — 2g a day.

Currently various researches tend to focus on n-3 polyunsaturated fatty acids (omega-3), present

in soy, rapeseed oil, walnuts, fish oil, and their ability to decrease the risk of arteriosclerosis [17,28,29].
Despite numerous diversities concerning the influence dietary cholesterol poses on lipid level in blood serum, it is nowadays commonly believed that using low saturated fats diet and low *trans* fatty acids diet, as well as implementing rich oleic acid diet results in considerable decrease of total cholesterol and LDL cholesterol fraction concentration, influences Triglycerides and shows slight positive influence or no influence on HDL cholesterol fraction [30].

Dietary and pharmacological treatment of hyperlipidemia is enumerated among one of the most important elements related with primary and secondary prophylaxis of circulatory system diseases. Effectiveness of both the dietary and pharmacological hypolipidemic therapy differs in individual patients. It is possible that genetic factors may prove crucial in case of diverse lipid and lipoprotein responses, and as far as the above is concerned researchers recently focus on polymorphism of apolipoprotein E, which plays a crucial role in lipid metabolism. Depending on the occurring gene alleles, people reveal greater inclination to increased levels of certain cholesterol fractions [31].

Physical activity and body mass control
Regular physical activity helps to maintain good physical and psychical condition. It has a positive influence on blood lipid profile. It causes increase in HDLchol fraction concentration.

Smoking tobacco
Apart from exacerbating the sclerotic plaque development by direct influence on walls of blood vessels, smoking also decreases the concentration of HDLchol. As a result of chronic exposure to tobacco smoke and its contents, concentrations of atherogenic lipoprotein fractions (VLDL, LDL) tend to rise, and this leads to increased total cholesterol values.
Whereas inhibition of enzymes regulating the cholesterol esters management in plasma, the tobacco smoke increases the concentration of free cholesterol [32].

Alcohol
Certain researches showed that moderate alcohol consumption lead to increased HDL cholesterol concentration and probably decreases plaque aggregation. Results of these researches suggest that drinking one or two alcoholic drinks (14-26g of ethanol) may decrease the risk of ischaemic heart disease by 30-50%. However, due to proven fact of harmful influence alcohol has on health, this method evokes many reservations [28].

It is essential to diagnose each person with increased LDLchol concentration or other form of hyperlipidemia in order to exclude secondary dyslipidemia. Main reasons for secondary dyslipidemia include: diabetes, hypothyroidism, nephritic syndrome, cholestatic jaundice, chronic renal failure. Medications (that can increase LDL cholesterol concentration or can cause other dyslipidemias): progestagens, anabolic steroids, corticosteroids, protease inhibitors used in treating HIV infection [17].

Pharmacological treatment of hypercholesterolemia

If it is possible to obtain the target LDLchol concentration only by means of therapeutic changes in ones lifestyle, it is recommended to consider addition of a medication. Usually, statin will be this drug, yet alternatively it is possible to implement ion-exchange resins, binding bile acids and nicotinic acid (Figure 3).

The history of introducing statins into medicinal therapy begins with a Japanese, Akiro Endo, who, inspired by Fleming – penicillin discoverer, and his works, searched for substances decreasing cholesterol level among goods produced by saprophytic fungi. In 1973, after long researches he finally managed to isolate a compound from *Penicilium citrinum*, which was later known as mevastatin. It was the first drug from statin group that was enumerated among 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors. This substance directed the research conducted by the Merck Company, which used the structure of this new agent to produce and market the first acknowledged hypolipidemic preparation – lovastatin [33].

Statins — HMG-CoA reductase inhibitors

- **The main mechanism underlying its activity:** by inhibiting biosynthesis of cholesterol they

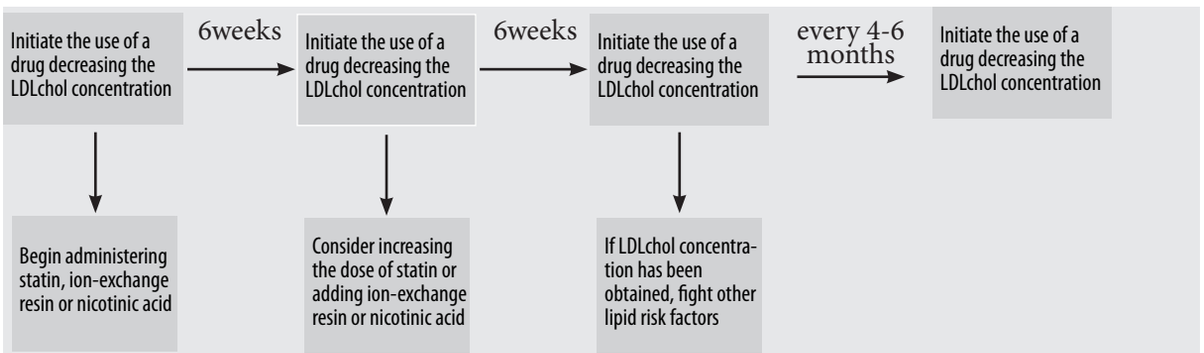

Figure 3: Algorithm presenting pharmacological treatment of hypercholesterolemia

decrease its pool in hepatic cells, and as a result of stimulating the LDL receptor creating process – they intensify removal of LDL cholesterol fraction molecules from circulating blood.

- **Influence on plasma lipid profile**: LDL cholesterol decreased by 18-55%, HDL cholesterol increased by 5-15%, Triglycerides decreased by 7-30%.
- **Main usage:** to decrease the LDL cholesterol concentration.
- **Contraindications:**
  - absolute: active or chronic hepatic disease;
  - relative: simultaneous use of ciclosporin, macrolide antibiotics, various antifungal medications and P-450 cytochrome inhibitors (be cautious when using simultaneously with fibrates and nicotinic acid).
- **Efficiency:** decreases the risk of coronary heart disease and cerebral stroke.
- **Safety:** minimal side-effects during clinical tests.
- **Main adverse events:** myopathy, increased activity of aminotranspherases in serum.
- **Maximum daily dosage approved by the FDA:** 80 mg (for all statins) [33-36].

Ion-exchange resins

- **The main mechanism underlying its activity:** In gastric pH they produce quaternary ammonium salts, which exchange anions with bile acids, binding them irreversibly in the intestine and inhibiting their reversed absorption. Decreasing the bile acid level in liver supports subsequent conversion of further cholesterol portions into bile acids and hence its elimination from the system. Reduced content of this sterol in liver stimulates intensified LDL lipoprotein binding and decreases their serum concentration.
- **Influence on plasma lipid profile:** LDL cholesterol decreased by 15-30%, HDL cholesterol increased by 3-5%, triglycerides not affected or increased.
- **Main usage:** to decrease LDL cholesterol concentration.
- **Contraindications:**
  - absolute: familial dysbetalipoproteinemia, triglyceride concentration >400 mg/dl,;
  - relative: triglyceride concentration >200 mg/dl.
- **Efficiency:** decreased risk of coronary disease confirmed in clinical researches.
- **Safety:** without overall toxic activity in clinical researches: frequent abdominal-intestinal syndromes.
- **Main side effects and adverse events:** frequent symptoms from upper and lower segment of the alimentary tract, decreased absorption of other drugs.
- **Maximum daily dosage:** Colestyramin 24 g, Colestypol 30 g, Colesevelam 4.4 g [33-36].

Nicotinic acid and its derivatives

- **The main mechanism underlying its activity:** Inhibits VLDL synthesis in liver. This results from decreased inflow of free fatty acids and increased VLDL catabolism, which is caused by activation of lipoprotein lipase. And as a consequence this leads to limited VLDL molecule conversion to LDL and decrease of their blood level.
- **Influence on plasma lipid profile:** LDLchol decreased by 5-25%, HDLchol increased by 15-35%, triglycerides decreased by 20-50%
- **Usage:** in majority of lipid disorders.

- **Contraindications:**
  - absolute: chronic liver disease, severe form of gout;
  - relative: hyperurycemia, type 2 diabetes (large doses).
- **It is enumerated** among the most efficient hypolipidemic drugs.
- **Safety:**
  - crystalline form: rarely any long-term side effects;
  - controlled release preparations: more frequent severe hepatotoxicity may appear.
- **Main side effects and adverse events:** skin blushing, hyperglycemia, hyperurycemia or gout, disorders within the upper alimentary tract, hepatotoxicity (especially when using controlled release preparations).
- Maximum daily dosage:
  - nicotinic acid in crystalline form 4.5g;
  - nicotinic acid with controlled release 2 g; c) nicotinic acid with prolonged release (Niaspan) 2 g [33-36].

Fibric acid derivatives (fibrates)

- **The main mechanism underlying its activity:** they inhibit VLDL lipoprotein synthesis in liver and hasten their catabolism by influencing the lipoprotein lipase activity. Apart from the above they also reduce synthesis of cholesterol in hepatic cells.
- **Influence on plasma lipid profile:** LDLchol decreased by 5-20% (in people without hypertriglyceridemia); in people with hypertriglyceridemia this may increase; HDLchol increased by 10-35% (greater effect in severe hypertriglyceridemia); triglycerides decreased by 20-50%.
- **Main usage:** hypertriglyceridemia, atherogenic dyslipidemia. They currently do not constitute a standard in treating hypercholesterolemia.
- **Contraindications:** severe renal or hepatic failure.
- **Efficiency:** moderate decrease the risk of coronary disease confirmed in clinical researches.
- **Safety:** it seems that there are no long-term side effects, although results of the first. researches suggested an increased risk of death related with non-coronary causes.
- **Main side effects and adverse events:** indigestion, various ailments related with the upper alimentary tract, creation of cholesterol gallstones in bile ducts, myopathy.
- Maximum daily dosage:
  - Gemfibrozil 1200 mg;
  - Fenofibrate 200 mg;c) Clofibrate 2000 mg [33-36].

Cholesterol lithiasis and biliary lithogenic index

Cholesterol lithiasis *(cholelithiasis)* can be called a social disease. It is a considerable health issue in Western Europe states, in USA and in Poland. The frequency of its occurrence increases with age (weakened sensitivity of muscle fibres within the gall bladder walls to the activity of cholecystokinin) and is 2-3 times higher among women than among men. And this is related with the influence posed by sex hormones on bile over saturation with cholesterol and on weakened gall bladder motility. What is also of considerable significance is the hormonal contraception, numerous pregnancies and hormone replacement therapy during post-menopausal period.

Pathomechanisms underlying the occurrence of cholesterol concrements are well known. They are created as a result of disorders within biosynthesis of bile acids and cholesterol crystallization [37,38].

The most common place where gallstones are deposited is the gall bladder (here usually cholesterol gallstones are created), although they can also originate within intrahepatic and extrahepatic ducts (mainly pigment stones). In case of the latter ones, bile is not over saturated with cholesterol and the bile acid content is also normal. The cause of pigment lithiasis is an increased bilirubin hydrolysis.

Creation of cholesterol gallstones

1. Chemical stadium

Physiologically proper bile contains three basic constituents:

- Bile acids;
- Phospholipids (mainly lecithin);
- Cholesterol.

Cholesterol — obtained from food, synthesized in liver or removed by this organ from the circulatory system – it is almost entirely excreted in bile as cholesterol or bile acids. Free cholesterol is entirely insoluble in water environment, such as bile, and hence it has to be integrated in micelles

of lecithin-bile salt. Lecithin is not soluble in water systems, but may be soluble by bile salts in micelles.

Large quantities of cholesterol present in human bile are being dissolved in these mixed micelles, and this enables proper transport of this sterol without its precipitation in human bile and without transfer through bile duct to the intestine. Nonetheless, this system has a limited dissolving capability, which depends on relative proportion of bile salts, lecithin and cholesterol, as well as water content in bile. [39]
Relation between cholesterol content and bile salts ranges between 1:20 and 1:30. In case of changes within the scope of these proportions and due to other various reasons this relation is decreased (f. ex. when bile is over saturated with cholesterol or decreased amount of bile acids), lithogenic bile is created, from which micro crystals of cholesterol precipitate. Tendency to crystallize within bile changed this way is quite considerable, but this is not the only condition essential for concrements to occur. Crystallisation activators include glycoproteins of cholecystic mucus, cholecystic IgA, aminopeptidase, C phospholipase and fibrinopectins. This process leads to occurrence of cholesterol monohydrates (biliary microlithiasis) [40].

2. Physical stadium
When bile has significant cholesterol excess, we can observe a spontaneous and quick creation of crystals, which are created "homogenous" gallstone nucleus. Cholesterol may also deposit around the "heterogenous" nucleus, which is created by desquamated epithelial cells, calcium salts, bacteria or foreign matter.

3. Gallstone development stadium
Accumulation of cholesterol crystals in gallbladder, as well as their aggregation leads to occurrence of macroscopic concrements. Mucopolysaccharydes secreted into the lumen of the gallbladder are considerably significant within this stage, as they create a gel system that binds crystals. Protein and calcium bilirubinate complexes may constitute material binding the gallstone layers created from cholesterol crystals [41,42].

Cholesterol gallstones are of yellowish and white colour and in about 90% they are made of cholesterol, in 3-4% of pigments, and in 1-2% of calcium and trace amounts of magnesium, sodium, potassium and fatty acids.

Bile lithiasis in child age occurs as classical complication related with haemolytic anaemia. Factors predisposing to cholesterol lithiasis in children include bile duct pathology: improper structure of bile ducts, conducing biliary stagnation and predisposing the creation of concrements; altered bile content and recurring infections [43,44].

Biliary lithogenic properties

Creation of cholesterol crystals does not depend solely on the degree of bile's saturation with this sterol. The following factors may also be decisive as far as biliary lithogenic properties are concerned:
- creation of instable micelles, which are more prone to rupture with cholesterol precipitation;
- increased sterol synthesis in liver, which is evaluated basing on activity of HMG-CoA reductase;
- decreased hepatic pool of bile acids;
- lecithin deficiency leading to imbalanced proportion in lipidous bile constituents [45].

Due to the above-mentioned factors cholesterol cannot dissolve in lecithin-biliary micelles. By using the triangular coordinate system, Redinger and Small were able to determine the maximal solubility of cholesterol in bile. Only the small, coloured fragment approximately reflects the phase concentrations of the main bile constituents reflecting the standard range. Within the single micellar liquid phase, bile content equals: 5% cholesterol, 15% lecithin and 80% bile salts. Within any other point of this triangle we may observe disadvantageous phase transformations, where we can either report cholesterol excess

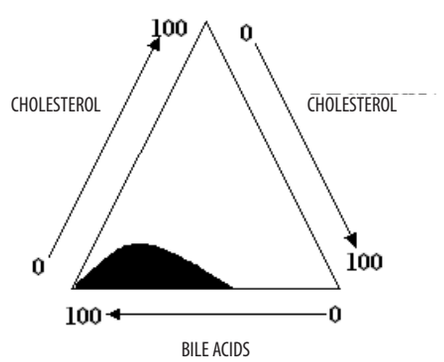

Figure 4: Solubility of cholesterol in bile (according to Redinger and Small) [39,46].

either in over saturated solution or in precipitated form — crystal or liquid crystals (Figure 4).

According to Linbladt, physiological balance between bile components in healthy individuals is expressed with the following molar quotient:

$$\frac{\sum cholesterd}{\sum cholic\,acids + \sum lecithin} = 0.7(mol:mol)$$

Maintaining this balance (the denominator holds a system of a certain HLBand ΔG_m^0) is the condition underlying proper colloidal biliary condition (single phase micellar colloid) and constitutes the border of permanent solubilisation of cholesterol – **biliary lithogenic index**.
This balance is changed in certain conditions [47].

In **obese** people synthesis of cholesterol is strictly correlated with fat tissue resources: about 20mg of cholesterol is synthesized on every additional kilogram of the fat tissue. Increased metabolic turnover of cholesterol leads to its increased concentration and secondary to biliary lithiasis (Figure 5).

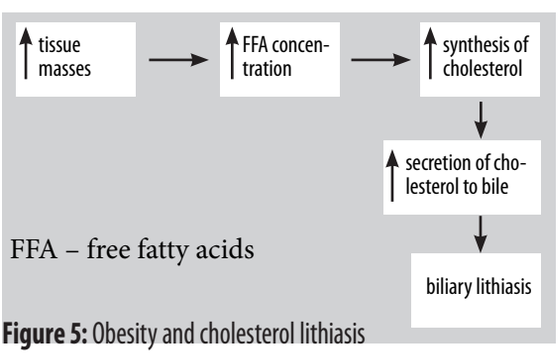

Figure 5: Obesity and cholesterol lithiasis

Consuming excessive amounts of certain foods (monosaccharides, chocolate, honey, as well as Animals FAT) or insufficient amount of others (unsaturated fatty acids, vegetable oils, dietary fibre) results in a decreased level of lecithin in bile (balance disorders according to Linblandt) [37,48,49]. Apart from the fact that dietary fibre (wheat, oat bran) improves constriction of the gallbladder (which prevents bile concrements), it also influences metabolism of bile acids by shortening the time essential for the food to pass the intestines. As a consequence, this decreases the synthesis of secondary bile acids in intestines (mainly the deoxycholic acid) and their return into liver within hepatic and intestinal circulation. Hence it increases the synthesis of primary acids, mainly the chenodeoxycholic acid, which reveals litothic activity [38,50].

As far as obese patients are concerned, decreased amount of bile acids (impairment of their synthesis in liver or disorders in hepatic and intestinal circulation of bile acids) is perceived as the fundamental reason underlying disorders related with biliary lithogenic properties. Surgical procedures performed on bile ducts and long-lasting diseases of the alimentary tract, associated with the loss of bile acids, lead to exhaustion of systemic compensating mechanisms and secondary increase of biliary lithogenicity.

Type IV hyperlipidemia (according to Friedrickson) is enumerated as yet another factor. Patients reveal proper pool of bile acids, next to intensified hepatic cholesterol synthesis (almost twice growth concerning the activity of HMG-CoA reductase).

Risk of cholesterol lithiasis increases under conditions of administering certain hypolipemic medications, f. ex. from the group of fibric acid derivatives, which increase concentration of cholesterol in bile and simultaneously decrease the synthesis of bile acids.

The influence of **starvation** on breaking the balance between bile contents depends on the time of starvation – long lasting starvation results in the lack of stimulus to empty the gall bladder, and so bile tends to condensate in bladder and the so-called biliary mud, namely cholesterol microlithiasis. Additionally, the quantity of bile acids supplied to the liver is decreased. This causes decreased secretion of lecithin into bile and increased cholesterol secretion.

Frequent cases of lithiasis among women are justified by the influence of **hormones** on pathomechanism underlying this disease. Increased lithogenic bile is observed in the second and third trimester of pregnancy, as well as during the use of oral contraception. This is related with increased cholesterol secretion, decreased daily hepatic and intestinal cycle of bile acids, as well as delayed gallbladder emptying.

Symptoms of cholesterol lithiasis:
If the gallstone blocks the bile duct leads to disorders in bile transport, which results in a sudden

pain in the right hypochondrium of the abdominal cavity, radiating to the right shoulder blade; recurring symptoms of indigestion, intensified nauseas and vomiting, sometimes it leads to jaundice.

Treatment of biliary lithiasis covers:

1) prophylaxis in people from high risk group,
2) symptomatic treatment in attack of biliary
3) colic and during the period between the attacks,
4) treatment of direct consequences of lithiasis,
5) conservative attempts of gallstone lysis,
6) endoscopic removal of concrements,
7) mechanical Cushing of concrements,
8) operational treatment.

Search for new, well-tolerated by patient and effective preparations dissolving or solubilising cholesterol concrements, requires appropriate physio-chemical methods, enabling preclinical evaluation of these preparations. Numerous *in vitro* studies and clinical observation were performed basing on the information concerning cholesterol solubility, which allowed using – despite certain contraindications – urso – and chenodeoxycholic acid to produce preparations of the following kind: Chenofalk, Ursofalk, Delursan, since prolonged use of these preparations enables dissolving cholesterol concrements (complete efficiency is observed in 20% of treated patients). In some patients lithiasis recurred after finished treatment.

Chenodeoxycholic acid discovered in geese (from Greek *chen* — goose), is a natural component of bile in humans, which participates in maintaining cholesterol in micellar solution. It has been shown that it has the capacity to dissolve cholesterol gallstones leading to:

1) decreased cholesterol synthesis in liver by inhibiting HMG-CoA reductase,
2) enlarged pool of circulating bile acids and improved biliary lithogenic properties,
3) increased concentration of ursodeoxycholic acid, which is its metabolite.

Chenodeoxycholic acid administered in large doses may cause hepatic damage.

Ursodeoxycholic acid reflects a Progress in conservative treatment of cholesterol lithiasis. It occurs in large concentration in bear's bile (from Latin ursus – bear), which was used in Japanese traditional medicine for ages as a drug in alimentary tract diseases. Mechanism underlying its activity lies in decreasing cholesterol resorption from intestines by creating crystals in the intestine, but it also acts by inhibiting cholesterol in liver. The drug proved to be entirely safe, it does not reveal hepatotoxic activity. Therapy based on the above-mentioned drugs can be successful only in case of cholesterol gallstones [38,41].

Another method of non-operational treatment is to crush gallstones by means of shock wave generated from the outside and using ultrasound waves. The sand resulting from stone crushing is then dissolved with drugs. One of the methods is based on introducing a catheter directly into the gallbladder and administering a solution of stone dissolving substance. This method makes stones disappear within several hours. Surgical treatment lies in resection of the whole gallbladder with gallstones and in inspection of the whole bile duct and removing all possible stones (cholecystectomy with bile duct revision). Currently, a special gallbladder accessing technique known as laparoscopic cholecystectomy is utilized, which allows minimizing surgical interference, and the patient returns home after 1-2 days after the procedure [51].

Summary

Cholesterol stands as the precursor of steroid in the organism: corticosteroids, mineral corticoids, sex hormones, bile acids and vitamin D_3. It undergoes biosynthesis from acetyl-CoA. The pace of complex transformations is limited with reaction catalysed by HMG-CoA reductase. This stage is also the place where drugs reducing systemic cholesterol concentration are captured. Dietary cholesterol is incorporated in the overall pool of this sterol in human organism. Tissues maintain cholesterol balance between factors causing cholesterol increase and factors leading to its loss.

Excessive amount is secreted from liver in bile as cholesterol or as biliary acid salts, which then enter the hepatic-intestinal circulation. Increased concentrations of cholesterol fractions participating in its transport to tissues is considered as the one of the most important risk factors related with ischaemic heart disease. Forms of

acute hypercholesterolemia are usually associated with genetically conditioned defects in lipoprotein metabolism (mainly LDLchol). Overlying genetic factors and improper nutrition (high fat diet), smoking, lack of physical activity, pose a considerable influence on insufficiency resulting from pharmacological treatment based on hypolipemic drugs, as well as increase the risk of ischaemic heart disease. That is why it is essential to begin treatment with introduction of multifactor changes in patient's life style.

Pathomechanisms underlying the occurrence of cholesterol lithiasis are well known and do not depend solely on the degree of bile saturation with cholesterol, namely on disorders in balance between basic biliary components (bile acids, phospholipids and cholesterol). This influences the change in biliary lithogenic properties and the limited solubility of cholesterol, lipids and lipolithic substances. That is why it is extremely important to improve biliary lithogenic index in drug form technology, by adding proper solubilisers.

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