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N.A. Vorobyeva, A.S. Vorontsova, A.A. Murashkina

HEALTHY LIFESTYLE AS A PREVENTIVE MEASURE FOLATE METABOLISM DISORDERS

Study guide

Arkhangelsk
2025

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Authors:
N.A. Vorobyeva*,** Dr Sci Med, Professor, Head of the Department of Clinical Pharmacology and Pharmacotherapy, NSMU; ***A.S. Vorontsova, Assistant, Department of Clinical Pharmacology and Pharmacotherapy, NSMU; ***A.A. Murashkina,*** Clinical Laboratory Diagnostics Doctor

Reviewers:
A.A. Popov*,** PhD, Associate Professor of the Department of Clinical Biochemistry, Microbiology and Laboratory Diagnostics, NSMU; ***O.N. Popova, Dr Sci Med, Professor of the Department of Hygiene and Human Ecology, NSMU

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The study guide presents key aspects of lifestyle that influence the functioning of the folate cycle, the pathogenetic pathways of these effects, as well as the probable outcomes of folate metabolism disorders.

The study guide is for international students on the educational programme of professional education on the basis of the Federal State Educational Standard of Higher Education – specialty on the field of training 31.05.01 General Medicine.

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Н.А. Воробьева, А.С. Воронцова, А.А. Мурашкина

ЗДОРОВЫЙ ОБРАЗ ЖИЗНИ КАК СПОСОБ ПРОФИЛАКТИКИ НАРУШЕНИЙ ФОЛАТНОГО ОБМЕНА

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Авторы:
Н.А. Воробьева, д-р мед. наук, профессор, заведующая кафедрой клинической фармакологии и фармакотерапии СГМУ; ***А.С. Воронцова,*** ассистент кафедры клинической фармакологии и фармакотерапии СГМУ; ***А.А. Мурашкина,*** врач клинической лабораторной диагностики

Рецензенты:
С.Л. Совершаева, доктор медицинских наук, профессор кафедры нормальной физиологии Северного государственного медицинского университета; ***О.Н. Попова,*** доктор медицинских наук, профессор кафедры гигиены и медицинской экологии Северного государственного медицинского университета

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В учебном пособии представлены ключевые аспекты образа жизни, оказывающие влияние на функционирование фолатного цикла, патогенетические пути этих воздействий, а также вероятные исходы нарушения фолатного обмена.

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Contents

Abbreviations list

BHMT – betaine homocysteine S-methyltransferase;
BMI – body mass index;
BP – blood pressure;
CβS – cystathionine-β-synthase;
CI – confidence interval;
CSE – cystathionine-γ-lyase;
DBP – diastolic blood pressure;
HC – homocysteine;
HDL – high density lipoproteins;
HHC – hyperhomocysteinemia;
HS – hemorrhagic stroke;
IS – ischemic stroke;
LDL – low density lipoproteins;
MTHFR – methylenetetrahydrofolate reductase;
MTR – methionine synthase;
MTRR – methionine synthase reductase;
SAM – S-adenosyl methionine;
OR – odds ratio;
SBP – systolic blood pressure;
TC – total cholesterol;
TG – triglycerides;
THF – tetrahydrofolate;
WHO – World Health Organization.

Physiological basis of folate metabolism

The folate cycle is a multi-stage complex cascade process catalyzed by enzymes that have cobalamin and folic acid derivatives as coenzymes. There are two important functions of folate metabolism (Fig. 1). The first function is associated with the synthesis of nucleotides, the second is associated with the process of remethylation of homocysteine to methionine [1,2].

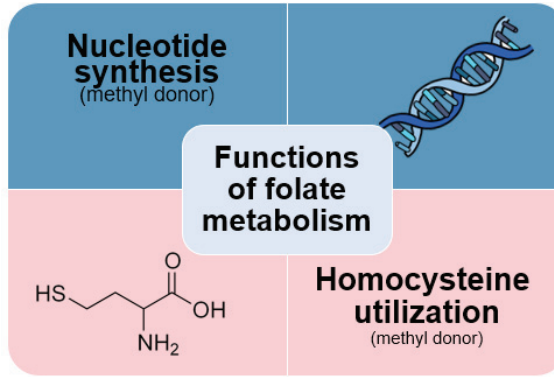

Fig. 1. Functions of folate metabolism [1]

The following enzymes and vitamins are involved in the process of phosphate exchange: methylpentyrohydrofluorodecate, methionine synthase, methionine synthase reductase, transport agents of the pholates, active metabolites of folic acid and cobalamine [3].

The main biochemical marker reflecting the work of folate metabolism is homocysteine. Homocysteine was first synthesized by the American biochemist Vincent du Vigneaud. The new amino acid was obtained by treating methionine with sulfuric acid. The resulting compound differed in one carbon atom from cysteine, which is why it was called homocysteine. In 1955, the scientist was awarded the Nobel Prize in Chemistry for his work in the field of synthesis and study of the homocysteine molecule [4,5]. **Homocysteine** is a sulfhydryl-containing non-proteinogenic amino acid that is not used for protein synthesis. Homocysteine is an intermediate product in the metabolism of the amino acids methionine and cysteine [6]. Homocysteine is not found in food products, so it does not enter the human body

with food. The physiological process of biosynthesis of the amino acid homocysteine occurs from the essential amino acid methionine [7]. Methionine enters the human body only with food. The source of methionine are products of animal origin (meat, cheese, eggs). After animal feeding, the concentration of methionine in the blood is increased for a short time, and between meals methionine is metabolized and its concentration decreases [8,9]. Methionine is first alkylated with adenosyl triphosphate, using the enzyme methionine denozyl transfecrate (Fig.2).

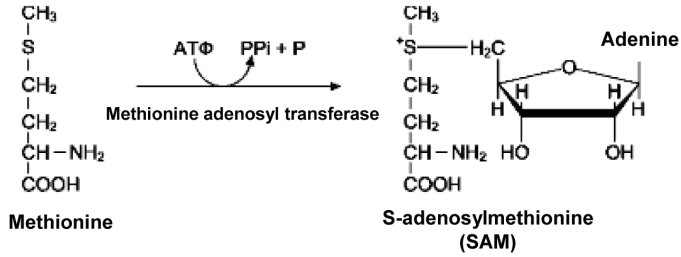

Fig. 2. Scheme of the reaction of S-adenosylmethionine formation [10]

This process produces S-adenosylmethionine (SAM), which is a donor of methyl groups (CH_3) necessary for the synthesis of nucleic acids, phosphatidylcholine, sphingomyelin, creatinine, detoxification of xenobiotics and other important reactions. More than two hundred biochemical processes in the human body take place with the participation of SAM. To participate in these processes, S-adenosylmethionine, under the action of the enzyme cytinosyl-5-methyltransferase, gives up its methyl group and becomes S-adenosylhomocysteine, which is then broken down by the enzyme adenosylhomocysteinase to homocysteine and adenosyl [11,12].

In the cell, homocysteine is present mainly in the reduced form. Excess homocysteine is secreted from the cell into the blood through special transport systems. Strict regulatory folate-dependent remethylation pathways operate inside the cell, which do not allow the concentration of homocysteine to increase. As soon as the intracellular concentration of homocysteine begins to increase, the level of adenosineglycine immediately increases, which in turn blocks transmethylation with the help of adenosinemethionine. After homocysteine leaves the cell and enters the blood, it is oxidized under the influence of high concentrations of oxygen. Thus, 99% of ho-

mocysteine in plasma is in the oxidized form as disulfides, which quickly combines with other plasma proteins, such as albumin, and only about 1% of the total amount of homocysteine in plasma is in the reduced form. Oxidized homocysteine must be neutralized and removed from the body. The main organs where homocysteine is metabolized are the liver, which absorbs most of the homocysteine bound to plasma proteins, and the kidneys, which in turn neutralize homocysteine disulfides. Folate-independent mechanisms for homocysteine utilization operate in these organs [13].

It should be noted that homocysteine is necessary for the body, as it is a methyl acceptor, but its excess concentration can initiate a number of pathological processes: oxidative stress, cell apoptosis, genetic mutations (Fig. 3) [14].

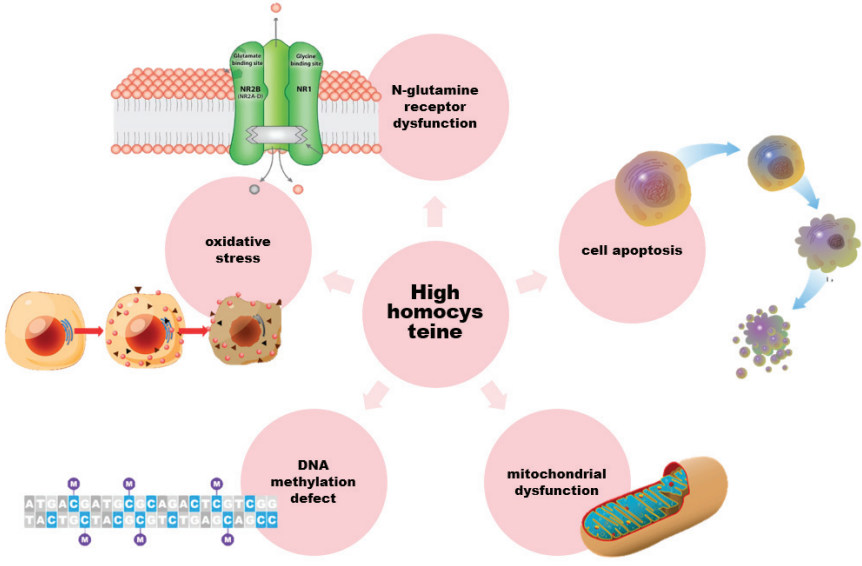

Fig. 3. Pathological processes caused by hyperhomocysteinemia [14]

Due to the fact that homocysteine is a toxic compound, dynamic processes of its neutralization function in the body. Currently, the processes of **remethylation** and **transsulfation** of homocysteine are known, which keep the level of homocysteine in blood plasma stable and low [15].

The homocysteine methylation process is important and involves neutralizing a highly cytotoxic compound. Three enzymatic ways of remethylation of homocysteine are currently described. The main pathway involves the **folate cycle** enzymes and alternative ways of utilization of homocysteine occur through the enzymes **betaine-homocysteine-S-methyltransferase** and **betainehomocysteine-S-methyl transferase 2**. Moreover, remethylation by means of folate exchange occurs in all tissues of the human body, and methylation by means of betaine-homocysteine-S-methyl transferase takes place only in the liver and kidneys [3]. Another pathway of metabolism is transsulfatization, which is catalyzed by the cystation-beta-synthase enzyme [15]. Figure 4 shows the pathways of homocysteine neutralization in the body [9].

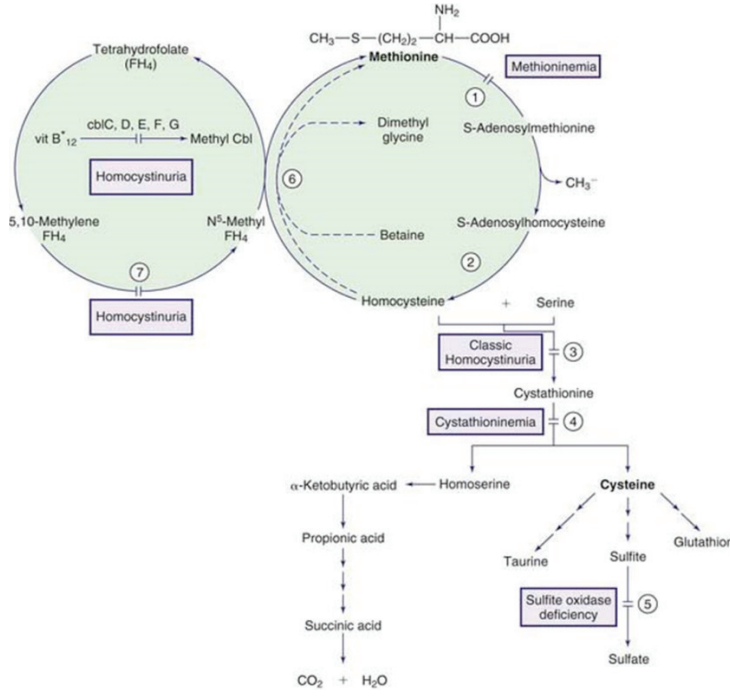

Fig. 4. Ways to neutralize homocysteine in the body. Enzymes: (1) methionine denosyltransferase (MAT), (2) S-adenosylhomocysteine hydrolase (SAHH), (3) cystathionine synthase (CβS), (4) cystathionine-γ-lyase (CSE), (5) sulfite oxidase, (6) betaine homocysteine methyltransferase (BHMT), (7) methylenetetrahydrofolate reductase (MTHFR) [9]

Folic acid is a necessary source for folate metabolism. Folates enter into the human body with food. The origin of the flocks is more vegetal and less animal. This chemical compound is a complex molecule that consists of a pteroid derivative (pteroid acid) and one (monoglutamate) or several (polyglutamate) residues of glutaminic acid. In the mouth cavity of the thin and iliac rectum, glutamic acid residues are chipped off and converted into folate monoglutamate, which is absorbed into the blood by means of a PCFT and FRC1 folate transporter. The folate monoglutamate is reduced to tetrahydrofolate (THF). THF is subsequently converted to a single-carbon serine fragment and 5,10-methylententetrahydrofolate is synthesized [16].

The next participant in the process is the enzyme methylenetetrahydrofolate reductase (MTHFR). With the help of this enzyme, an active metabolite of folic acid 5-methyltetrahydrofolate is formed from 5,10-methylpenteterydrahydrofolate. After methylation, the active metabolite of folic acid enters the cell, where it serves as the main source of methyl groups and tetrahydrofolate. Tetrahydrofolate is an acceptor of monocarbon fragments, converted into different types of folates, which participate in reactions of synthesis of purine bases and the pyrimidine base of nucleic acids thymine [17].

The key role in the reaction of remethylation of homocysteine to methionine is played by the active metabolite of folic acid 5-methyltetrahydrofolate. The remethylation reaction is catalyzed by the enzyme methionine synthase (MTR). The active form of vitamin B_{12} – methylcobalamin is necessary for the functioning of the enzyme [2]. It is an intermediate carrier of the methyl group. During the transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine, homocysteine is converted to methionine and cobalamin is oxidized, as a result of which methionine synthase becomes inactive. The enzyme methionine synthase-reductase (MTRR) is next included in the work of the folate cycle. This enzyme is used to restore the function of the methionine synthase enzyme. The recovery of methylcobalamin occurs with the participation of the activated form of methionine – S-adenosylmethionine [1].

Influence of the nutritional status on the folate cycle

Nutrition is an essential part of a person's lifestyle, which affects their health and life expectancy. Inadequate and irrational nutrition in the form of excess, undernutrition and nutrition with nutrient deficiencies is a global problem for the world's population. According to the World Health Organization, in 2017, 11 million deaths were associated with malnutrition, while improving diet could prevent every fifth death worldwide. The leading causes that increase the risk of death are high salt intake and low intake of plant foods such as whole grains, vegetables, seeds, nuts and fruits [18].

Different countries in the world have very different eating patterns. In most countries, the prevalence of vegetarian diets does not exceed 10%, the largest number of vegetarians is traditionally observed in countries with a warmer climate, and the lead in this belongs to India, where the prevalence of vegetarianism is about 32.8%, but in different regions varies from 10.1 to 47.5%. Among vegetarians, the majority of men (56.5%), which is different from the sex structure of other countries. In countries with a more contrasting climate, the prevalence of vegetarianism is much lower. In the US, 7% of the adult population follow a vegetarian diet: 8.4% women and 5.5% men. In recent years, however, in countries like the US, the UK and Mexico, where meat and animal products make up a large part of the diet, vegetarianism is not uncommon and has become more widespread. In countries with a climate similar to that of Russia, the prevalence of vegetarianism is significantly lower. An example is Finland, where 3.3% of the adult population is vegetarian. In Russia, the proportion of adherents of vegetarian diets is still low. According to "Random observation of the diet of Russians" in 2013 only 3% of respondents said themselves to vegetarians (2% men and 4% women) [19].

Culture, customs, traditions and religious worldviews also have an undeniable influence on eating behavior in various populations of the world. For example, the denial of animal food is an important part in some Asian cultures, such as where people profess Hinduism, Jainism and Buddhism. While most Muslims and Christians adhere to the meat diet with certain restrictions. However, the global trends are not only in the increase of adherents of vegetable diets, but also in the increase of people reducing consumption of red meat, while not respecting vegetarian diet as a whole [19,20].

India is known to be the world's number one vegetarian consumer, but various studies have shown that consumption of many food groups has varied across regions. Sweets and snacks were more likely to characterize diets in East and South India, while fruits, vegetables, rice and legumes were more likely to characterize diets in the North and West. The diet of the East and South was also determined more by consumption of meat or fish than that of the North and West. However, in India, most diets are vegetarian with a high content of fruits, vegetables and legumes, with added added sweets and snacks. Snacks in India are usually fried foods with a high content of fat and salt, which can also contain a large amount of trans fats, and this may explain their association with a number of different health effects. On the other hand, a varied diet with a high content of fruits, vegetables, legumes and nuts has been linked to lower total cholesterol (TC), indicating that traditional diets may have a healthier profile. A review by Green R et al in 2016 included studies of men, women and children from all regions of India, pooling data from a number of different modelling studies that used similar food groups to study nutrition models and the relationship between nutrition and health. The study showed that higher body mass index (BMI) was associated with dietary patterns including high intake of high-fat and high-sugar foods, which was associated with increased obesity as well as hypertension and diabetes, while a more traditional diet based on fruits, vegetables and legumes was associated with positive health outcomes and prevention of non-communicable diseases [21].

A 2014 study by Vecchio MG focused on vegetarianism in India, showing the typical food and dietary profile of the average Indian vegetarian, commonly referred to as a Hindu vegetarian. India is a country with more than 28 diverse regional cuisines, each of which has its own local dietary practices. The majority of the population eat a variety of cereal-based diets. Basic products such as wheat, barley, millet and maize are commonly consumed in the northern regions, while in the southern regions rice predominates. These cereals are the main source of carbohydrates. Dried pulses/lentils, known as the gave, are prepared in almost all households and contain a significant portion of protein and carbohydrates. Most recipes also include green leafy vegetables, other cooked or raw vegetables and oils, rich in micronutrients, minerals, fiber and beneficial fats. Cheese and milk are also part of the normal diet. When all these components are consumed

together, the body's needs for vitamins, micro- and macro-elements are usually met.

It is important to pay attention to the shortcomings of vegetarianism. Not all vegetarian practices lead to nutrient deficiencies, but some diets that completely exclude animal products, such as eggs and dairy products, are at higher risk for nutrient deficiencies. The study showed that 78.7% of vegetarians were deficient in nutrients and needed dietary supplements such as iron (22.86%), vitamin B_{12} (25.71%), calcium (8.57%), biotin (5.71%) and polyvitamins (37.14%). The study highlights the potential dietary risks associated with protein and micronutrient deficiencies in a vegetarian diet. Vitamin B_{12} deficiency is a common problem among both Indian and Western vegetarians. The study also reported that vegetarians tend to have less skeletal muscle mass, which increases the risk of osteoporosis and fractures, especially in postmenopausal women. This may be due to lower levels of vitamin D and overall protein intake among vegetarians. The study showed that vegetarianism has become popular and associated with various health benefits, including normalization of BMI, lowering cholesterol levels, reducing the risk of hypothyroidism. A shift to vegetarianism can have positive health outcomes, with people benefiting from weight loss, improved cholesterol and reduced risk of cardiovascular disease. Vegetarian diets are associated with a lower risk of developing diabetes compared to non-veggie diets.

Awareness of the health benefits of vegetarian diets varied among the studied population: some participants perceived vegan diets as healthier, while others did not share this belief. This perception was influenced by religious practices and demographics. The typical dietary profile of Indian vegetarians, characterized by a variety of cereals, legumes, vegetables and dairy products, provides a balanced combination of macro- and microelements. Although vegetarianism has many benefits, it is important to address potential nutrient deficiencies, especially among communities that completely avoid animal products. Supplements with vitamins and minerals such as iron, vitamin B12 and calcium may be needed to overcome this deficiency. It is essential to recognize the potential risks of protein and micronutrient deficiency in a vegetarian diet and take appropriate measures to ensure full and balanced nutrient intakes. Overall, the results highlight the positive impact of vegetarianism on health outcomes while underlining

the importance of meeting nutritional needs to maintain optimal well-being [20].

Karamnova N.S. et al summarized in their work data on the benefit of vegetarian diet. The publication shows that vegetarians have the lowest rates of nutritional risk factors for diseases such as cardiovascular disease, diabetes mellitus, cancer and others. Among vegetarians, there are fewer people with obesity, arterial hypertension, elevated triglycerides (TG), and low-density lipoprotein cholesterol (LDL). Meta-analysis of 40 studies showed that veganes have lower BMI (by -1.72 kg/m2 [-2.30; -1.16]), waist circumference (by -2.35 cm [-3.93; -0.76]), high density lipoprotein cholesterol (HDL) (by -0.49 mmol/l [-0.62; -0.36]) (at -0.14 mmol/l [-0.24; -0.05]), blood glucose level (at -0.23 mmol/l [-0.35; -0.10]), systolic BP (at -2.56 mm Hg [-4.66; -0.45]) and diastolic BP (at -1.33 mm Hg [-2.67; -0.02]) with $p<0.0001$ for all values. The meta-analysis of 11 randomized clinical trials demonstrated the possibility of vegetarian diet in lipid profile correction. The combined reduction of TC was 0.36 mmol/l (-0.55; -0.17; $p<0.001$); LDL – -0.34 mmol/l (-0.57; -0.11; $p<0.001$); HDL – -0.10 mmol/l (-0.14; -0.06; $p<0.001$), non-HDL cholesterol – -0.30 mmol/l (-0.50; -0.10; $p=0.04$); whereas the decrease in TG was significant. It has been shown that vegetarians have a lower risk of cardiovascolary pathology, which is associated with an optimal lipid profile. Pescatarians – adherents of a vegetarian diet with added fish also had lower risk of ischemic heart disease, myocardial infarction, stroke and heart failure compared to persons on a mixed diet [18].

The most efficient way of preventing cardiovascular diseases is the Mediterranean diet, which is based on the food habits of countries bordering the Mediterranean Sea, i.e. Spain, Italy and southern Greece. The Mediterranean diet is characterized by high consumption of whole grains, olive oil, high consumption of vegetable products (fruits, vegetables, herbs, legumes, cereals, nuts and seeds), moderate consumption of fish, seafood, dairy products, poultry and eggs and low content of red meat and sweets, as well as almost complete absence of saturated fat, processed refined grains and sugar. The Mediterranean diet has been found to be beneficial as a dietary model, rather than as a one-nutrient supplement, because it provides by its nature antioxidants, reduces inflammation of the vascular wall, modulates the proterogenic genes, changes the gut microbiota and improves lipid panels by reducing LDL and increasing HDL.

Nutritional genomic studies show that stricter adherence to the Mediterranean diet prevents the development of an adverse cardiometabolic phenotype in genetically susceptible people with polymorphism of cyclooxygenase-2, interleukin-6, apolipoprotein A_2, cholesterol ether transfer protein in plasma.

Between 2002 and 2005, a large-scale interpopulation study was conducted to assess the pattern of nutrition in Poland, Russia and the Czech Republic. A total of 28,945 middle-aged men and women participated in the study. Among the Russian participants showed the lowest consumption of fruits, vegetables. Very rarely Russians consumed olive oil and nuts. In addition, the Russian cohort had significantly higher overall mortality and mortality from circulatory system diseases than the Polish and Czech cohorts. The study revealed a correlation between mortality rates and consumption of sufficient amounts of plant-based food, especially among smokers. The strongest association was found between consumption of plant foods and mortality from stroke. However, the strict Mediterranean diet, which implies consumption of a small amount of dairy products is not acceptable for northern countries, with limited sunlight due to the increased prevalence of osteoporosis, especially among women in the postmenopausal period. Olive oil is extremely expensive in many countries, so its use is limited. In India, mustard oil, which reduces the risk of developing ischemic heart disease by 50%, and rice bran oil, which has hypolipidemic properties, are used more often.

The consumption of sufficient quantities of vegetables, herbs and fruits in the daily diet is a problem for many countries of the world. There is a particular shortage of plant food in the northern countries due to the high cost of imported vegetables and fruits. It is known that countries in the Mediterranean region, such as Greece and Italy, have had lower mortality from circulatory diseases compared to the Nordic countries, such as Finland and the USA, probably due to different dietary patterns [22,23].

Nutrition in Russia among young people is the subject of numerous studies. As shown by the study conducted by Pokida A.N. et al., only 24.2% of men and 27.2% of women (aged 15 and older) consume a sufficient amount of fruits and vegetables per day. Fast food restaurants are very popular among young people, with up to 87% of students regularly eating fast food. This food is known to contain a lot of harmful sub-

stances, such as carcinogens, trans fats, excess salt and sugar. Fast food is rich in fats and simple carbohydrates, which negatively affects the physiological processes in the body. The priority for young people is not the use, but the taste and cost of food, so there is a great demand for fast food [24].

Shmandina K. V. and co-authors in their publication indicate that statistics of recent years demonstrate a sharp increase in the consumption of high-calorie foods, cereals, bakery products, pasta, confectionery, sugar among young people and a decrease in the consumption of animal products. This imbalance of eating behavior has led to deficiencies in vitamins, micronutrients and proteins. The study provides data on dietary insufficiency of essential foods. Recommended amount of sour milk products, eggs, milk consumed only 29% of respondents. 31% of students reported insufficient consumption of meat, meat products, fish. An analysis of the actual dietary intake revealed a vitamin deficiency, especially in group B and vitamin A [25].

Folic acid and cobalamine are known to be essential vitamins for maintaining the physiological process of the metabolism of homocysteine in the folate cycle.

Folate is the name given to folic acid found in natural products. Folate takes its name from the Latin word folia – leaf. This is due to the fact that folates were first isolated from spinach leaves. Folic acid enters the human body with food. Leafy vegetables, lentils, turnips, asparagus, broccoli, as well as beef liver, egg yolk, etc. are rich in folic acid. The modern diet, which includes a large amount of potatoes, meat and a small amount of plant foods – vegetables and fruits, leads to hypovitaminosis of group B. Major studies in America and Europe have shown that people of all ages consume less folate than is necessary for normal body function. However, natural folates from food are highly unstable. They lose their biological activity during storage and thermal processing of products. Up to 90% of folic acid is destroyed in cooking [26]. It should be noted that synthetic folic acid is chemically more stable, not subject to oxidation, resistant to heat treatment, does not lose its activity for many months. It is monoglutamate, metabolized in the cells of the intestine into an active form. Synthetic folic acid has been successfully used for flour fortification in the USA and Canada for many years [27].

It has been proven that the folates are synthesized by E. coli, Bacteroides spp., Lactobacillus ssp. Streptococcus thermophiles, Bifidobacterium spp., Fusobacterium spp. in thick and to a lesser extent thin intestine, but their number is minimal and insufficient for closing the organism's need. Intestinal flora absorbs and uses most of the vitamin B_9 [28].

The daily requirement for vitamin B9 varies significantly depending on age. According to WHO recommendations, the daily requirement for folic acid for adults and children over 12 years of age should be 400 μg, for children under 12 years of age – 200 μg, for children in their first year of life – 40-60 μg [29]. According to the clinical recommendations of the Ministry of Health of the Russian Federation «Folic acid deficiency anemia», the prophylactic dose of folic acid for children under 3 years of age is 25-50 μg/day, for children 4-6 years of age 75 μg/day and 100 μg/day for children 7 years of age and older, it is prescribed to children suffering from chronic inflammatory diseases and intestinal diseases with malabsorption syndrome. For adults, the prophylactic dose of folic acid is 150-200 μg/day [30]. Methodological recommendations of Rospotrebnadzor MR 2.3.1.0253-21 «Norms of physiological needs for energy and nutrients for various groups of the population of the Russian Federation» define the physiological need for adults as 400 μg/day. The physiological need for children is from 50 to 400 μg/day [29]. As we can see, the stable ideal daily requirement for folic acid varies. It is necessary to remember that no more than 200 μg of folates per day can be metabolized in the intestine, the rest of the synthetic folic acid will be absorbed into the bloodstream in a free unreduced form, which can lead to inhibition of the transport of endogenous active folates, resulting in the formation of functional deficiency of vitamin B_9 [31].

The main part of folates is absorbed in the small intestine. Two mechanisms are responsible for the absorption of folates – specific (saturable) and non-specific (unsaturable). Folic acid from food is known to enter the human body as polyglutamate, which in the gut undergo hydrolysis to monoglutamate. Monoglutamate in turn can be recognized by the receptors of intestinal epithelial cells. When the folates are overdosed, the number of folate receptors is reduced and the carrier proteins become inactive. All this leads to a significant decrease in the uptake of folic acid in the intestine. The potentiality of the saturating mechanism is limited, with its help up to 200 μg of vitamin B_9 per day [27].

The non-specific (unsaturated) mechanism is activated in the iliac. This mechanism has unlimited capabilities. Absorbed and transferred almost all the fused in the body, and metabolized, and non-metabolized forms. The non-specific mechanism leads to an excess of unmetabolized folic acid in the body, which accumulates in the blood serum [32].

Excess folic acid in the body has a number of negative consequences. Currently, there is evidence that excess free folic acid increases the risk of developing cancer and affects the decrease in immunity in postmenopause. Inactive metabolites of folic acid have a negative effect on the fetus. An increased risk of developing asthma and respiratory infections in early childhood has been identified [33].

Long-term use of large doses of folic acid masks vitamin B_{12} deficiency, resulting in megaloblastic anemia. The oxidized form of synthetic folic acid increases the risk of cognitive impairment and depression. Many clinical and epidemiological studies show a relationship between the intake of large doses of folic acid and malignant neoplasms. A dose-dependent effect is observed, on the one hand, taking folic acid at least 100 μg/day significantly reduced the risk of developing such oncological pathology as colorectal cancer, esophagus, larynx, trachea, stomach, pancreas, breast, endometrium, ovaries, kidneys and prostate. On the other hand, receiving folic acid in doses exceeding 400 μg per day is associated with the risk of developing the above-mentioned oncological diseases. European scientists from the Universities of England and Italy conducted more than 200 studies on the properties of folates and came to the conclusion that the use of synthetic folic acid in inappropriately high doses during pregnancy can increase the likelihood of mental disorders, obesity and insulin resistance in children [31].

A phenomenon called the “methyl trap” or “folic acid trap” has been described in the literature. This phenomenon occurs with a deficiency of vitamin B_{12}. It is known that there is a close metabolic link between vitamin B_{12} and folic acid. Both substances are members of the folate cycle, in which homocysteine is remethylated to methionine. Under the action of the methylenetetrahydrofluorooctase enzyme, an active metabolite of folic acid (5-methyltetrahydrofolate) is synthesized and the methyl group is transferred to vitamin B_{12}, which then, through the enzyme methionine synthase, transfers its homocysteine to turn it into methionine. In the case

of a kobalamine deficiency, there is no transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine. There is a situation in which a large number of active folic acid metabolites are accumulated in the cell, but they cannot be used for nucleotide biosynthesis and methionine. DNA synthesis and cell division are slowed down. Vitamin B_9 pseudodeficiency occurs. When the dose of folic acid increases, the situation is not corrected, but further aggravated and pathological processes progress. High doses of folic acid (more than 1,000 μg/day) can cause its non-metabolized form to enter cells and make it difficult to diagnose B_{12} deficiency [33].

The role of folates is difficult to overestimate. Folic acid, in particular its active metabolite, participates in carrying out vital functions. These functions include methylation, replication, DNA repair. Vitamin B_9 is necessary in the process of hemopoiesis, folate exchange, participates in the synthesis of certain amino acids (serine, tryptophan, methionine, glycine, etc.), purines and pyrimidines, neuromediators (dopamine, adrenaline, serotonin). Without folates, it is impossible to fully form rapidly dividing cells (epithelium of the skin, mucous gastrointestinal tract, bone marrow). Folates are of great value during pregnancy, they play an important role in the formation of placenta, pregnancy and fetal development. Folic acid deficiency is no less dangerous than excess folic acid [34].

Deficiency of folates leads to hyperhomocysteinemia (HHS) and, as a consequence, the risk of cardiovascular disease. The lack of folates inevitably leads to a failure of the folate exchange, which results in a failure of the remethylation of homocysteine. When the concentration of homocysteine in plasma increases the risk of developing adverse vascular events such as myocardial infarction, stroke, venous thromboembolism, arterial hypertension [35].

Folate deficiency leads to the disruption of DNA replication, repair and methylation, which negatively affects the proliferation of fast-dividing cells. Damage to their genome increases the risk of malignant neoplasms. There is accumulated data on the link between deficiency of folates and risk of developing breast cancer, colorectal cancer, lung cancer [36]. The results of a study conducted in the USA show that the risk of developing cervical cancer in patients with high-risk carcinogenic HPV, low levels of folic acid and vitamin B_{12} in blood plasma is 70% higher, than women with normal concentrations of folates and cobalamine. Numerous population

studies show a significant reduction in the risk of developing oncological diseases of different localization when consuming at least 100 μg of folates per day [37].

Folic acid deficiency is associated with the development of neurological and mental disorders such as neuropathy, depressive disorder, Alzheimer's disease. The reason for this is a disturbance of the conductance of nerve impulses, associated with myelin methylation disruption, which occurs when folates are lacking. Folic acid is also associated with the synthesis of monoamines such as serotonin, adrenaline and noradrenaline. Low folate status is associated with the risk of developing schizophrenia and autism [38].

Vitamin B_9 deficiency leads to hemopoietic disorder. Erythrocyte hematopoietic growth inhibition leads to megalobular anemia, and leukocyte growth inhibition leads to immune system dysfunction. The negative effect of deficiency of folates in embryogenesis, which leads to the formation of defects in fetal development has been proven. First of all, the association between folate deficiency and defects of neural tube growth (spina bifida, anencephalia, cerebral hernia) was identified. Other developmental defects are also noted: various abnormalities of the limbs, the genitourinary system, the hearing and vision organs, the maxillofacial system, the nervous and cardiovascular systems. The consequences of insufficient folates can be spontaneous abortion, premature labor, preeclampsia, placental failure [26].

Folic acid is an indispensable nutrient from the B group of vitamins. Folate, as a cofactor, participates in numerous intracellular reactions and this is reflected in various derivatives that have been isolated from biological sources. Folic acid is involved in single carbon transport reactions and serves as a source of single carbon units in various oxidative degrees. The processes involved in the absorption, transport and intracellular metabolism of this cofactor are complex. Most of the folic acid is closely related to enzymes, indicating that this cofactor is not in excess and that its availability in cells is protected and strictly regulated. In animals, the liver controls the uptake of folic acid by first passage metabolism, biliary secretion, and enterohepatic recirculation, as well as by the recirculation of old erythrocytes. Folic acid deficiency can occur for many reasons, including reduced consumption, accelerated metabolism and/or increased needs, as well as

due to genetic defects. The consequences of folic acid deficiency include hyperhomocysteinemia, megaloblastic anemia and mood disorders. Folic acid deficiency is also associated with disorders related to neural tube defects. Cereal supplements, such as cereals, have been undertaken in several countries as a cost-effective means of reducing the prevalence of neural tube defects. Common polymorphisms have recently been found in several genes associated with folate pathways that may play a role in folate deficiency diseases, especially in mild folate deficiencies.

Recent epidemiological and clinical data indicate that people with low folic acid levels and high levels of homocysteine are at increased risk for Alzheimer's disease, but the underlying mechanism is unknown. The hypothesis was tested that disruption of single-carbon metabolism due to folic acid deficiency and high level of homocysteine promotes accumulation of DNA damage and sensitizes neurons to amyloid beta peptide (Aβ) toxicity. Incubation of hippocampal cultures in folic acid deficient medium or in the presence of methotrexate (folic acid metabolic inhibitor) or homocysteine caused cell death and made neurons vulnerable to death caused by Aβ. The methyl donor deficiency caused incorrect uracil incorporation and DNA damage, and also significantly potentiated the toxicity of Aβ as a result of reduced repair Aβ-induced oxidative modification of DNA bases. When a diet with folic acid deficiency was used in mutant transgenic amyl-precursor protein (APP) mice, but not in wild type mice, increased cellular DNA damage and neurodegeneration of the hippocampus were observed. Aβ levels have not changed in the brain of mice with folic acid deficiency APP. The data obtained showed that folic acid deficiency and homocysteine disrupted DNA repair in neurons, increasing their sensitivity to oxidative damage caused by Aβ.

A folic acid deficiency may contribute to the development of several different age-related diseases, including ischaemic heart disease, stroke and cancer. By contributing to the incorrect inclusion of uracil and hypomethylation of DNA, as well as disrupting DNA repair, folic acid deficiency can cause damage to DNA in mitotic cells. Hyperhomocysteinemia is a consequence of folic acid deficiency, which contributes to the pathogenesis of cardiovascular diseases and stroke and possibly Alzheimer's disease and Parkinson's disease. Homocysteine is the metabolite of methionine, an amino acid that plays a key role in forming the metal groups required for

numerous biochemical reactions. Homocysteine can either be resealed to methionine by enzymes requiring folic acid, or catabolized by cysticerone-β-synthase, a vitamin B_6-dependent enzyme, with the formation of cysteine. Patients with severe hyperhomocysteinemia have a wide range of clinical manifestations, including deep neurological disorders such as mental retardation, cerebral atrophy and convulsions. Recent studies have shown that homocysteine can be directly toxic to cultivated neurons; the mechanism may include activation of NMDA-receptors or apoptosis caused by DNA damage.

In Alzheimer's disease, neuronal death in brain regions critical for learning and memory is thought to result from increased production and accumulation of insoluble forms of amyloid β-peptide (Aβ), which can compromise and kill neurons by causing oxidative stress and disrupting cellular ion homeostasis. Analysis of brain tissue of patients with Alzheimer's disease, as well as experimental cell cultures and animal models of Alzheimer's disease provided evidence for the involvement of Aβ and apoptotic biochemical cascades in the neurodegenerative process. DNA damage, which is a powerful trigger for neuron apoptosis, has been documented in studies of patients with Alzheimer's disease as well as in cell cultures and animal models of Alzheimer's disease. In addition, nonrenal cells of patients with Alzheimer's disease demonstrate a defect in their ability to repair DNA damage, suggesting a widespread anomaly in the mechanisms of DNA repair. The cells of patients with Down syndrome are hypersensitive to DNA damage caused by ionizing radiation, and environment-induced DNA damage may contribute to neurofibrillar degeneration in the ABS-Parkinson's complex on Guam. In light of evidence that DNA damage in the neurons that degenerate in Alzheimer's disease is increasing, and evidence that folic acid deficiency and homocysteine can disrupt DNA repair in nonneuronic cells, cell cultures and mouse models of Alzheimer's disease were used to test the hypothesis that folic acid deficiency and homocysteine sensitize neurons to Aβ-induced death. Folic acid deficiency increases homocysteine levels and sensitizes hippocampal neurons to death in APP-mutated transgenic mice. Transgenic mice, hyperexpressing the Swedish mutation in APP under the control of a promoter, demonstrate elevated levels of soluble Aβ1-42 and progressive age-related Aβ in the hippocampus and cortex. In the transgenic line used in this study,

amyloid deposits are first detected in the brain at 10 months of age. In the experiment, 7-month-old APP mutant mice and mice of the same age of wild type were placed on a normal diet or diet with folic acid deficiency and excessive homocysteine. After 3 months of diet, serum homocysteine levels were 10 times higher in both mutated APP mice and wild-type mice on the experimental diet compared to mice on the control diet. The study of cutaway-purple-colored slices showed that APP mutant mice who were on a diet deficient in folic acid had damage to the CA3 hippocampal neurons compared with mutated mice who were on a diet deficient in folic acid, and that there was damage to the mice of the wild type who were on a diet with folic acid deficiency. Stereotyping was performed to determine the numerical density of neurons in CA3 and CA1 regions in mice with mutant APP and mice of wild type, which were maintained on a control diet and folic acid deficient diet. The CA3 hippocampus analysis showed a very significant 20% loss of neurons in mutant APP mice on a folic acid deficient diet compared to mutant mice on a control diet and mice of wild type that were on a folic acid deficient diet. The total number of neurons within the reference volume CA3 that was measured was 888 ± 61 for non-transgenic mice on a regular diet, 880 ± 52 mm^3 for non-transgenic mice on an experimental diet – 896 ± 66 mm^3 for mutant mice APP on a routine diet, and 712 ± 32 mm^3 for APP mutant mice on experimental diet ($p < 0.01$ compared to each of the other values; ANOVA with Shefa post mortem tests). There was no difference in density of neurons in the CA1 region among four different groups of mice. The reference amounts analyzed for specific areas of hippocampus among the four groups of animals were not reliably differentiated ($p > 0.1$); for the CA3 region, the values were 0.0028 ± 0.0007 mm^3 for non-transgenic mice on a normal diet $0{,}0028 \pm 0.0008$ mm^3 for non-transgenic mice on the experimental diet 0.0026 ± 0.0006 mm^3 for APP-mutants on normal diets and 0.0032 ± 0.0009 mm^3 for APP-mutants on the experimental diet. Thus, the decrease in CA3 density is associated with cell loss and was not a result of the change in volume of this structure.

The ability of folic acid deficiency and elevated homocysteine levels to increase the vulnerability of cultivated hippocampal neurons to Aβ-induced death and promote degeneration of neurons in transgenic mice with mutant APP-strain suggests a mechanism, where individuals with low folic acid intake and, as a consequence, increased homocysteine levels may be at an

increased risk of Alzheimer's disease. Aβ may cause oxidative stress and DNA damage in cultured neurons, as well as oxidative stress and DNA damage have been documented in neurons associated with Aβ-containing blobs in the brain of patients with Alzheimer's disease. Analysis of DNA damage and repair shows that folic acid deficiency and homocysteine contribute to the accumulation of DNA damage in neurons, disrupting DNA repair. Thus, the death of neurons caused by a methyl donor deficiency has been mitigated by the addition of purine and thymidin, suggesting that limiting the availability of purines and thymidine for DNA repair plays a key role in threatening the actions of a methyl donor deficiency. The difference in the dynamics of DNA damage caused by a combination of folic acid and Aβ deficiency, compared with the combination of homocysteine and Aβ, may be due to the faster direct action of homocysteine and the more delayed effect of folic acid deficiency, requiring production of endogenous homocysteine. Because cellular RNA can also contain oxidizing bases, it may be a target for Aβ-induced oxidative modification. However, the recombinant GPC enzyme that was used is specific for 8-oxo-carboxylosanosin and the conditions of the alkali comet analysis will not lead to RNA degradation. The immunocoloration protocol we use is also specific for base modifications of DNA, and extranuclear localization of immunoreactivity can reflect oxidative modifications of mitochondrial DNA.

Apoptosis of cultivated neurons has been observed in response to various DNA damaging agents, including ultraviolet radiation, cytokine arabinosid and a topoisomeric inhibitor of camptothecine. It has been shown that postmitotic cells are more vulnerable to DNA damage than mitotic cells, probably due to insufficient DNA repair efficiency. It has been shown that neurons and neuroblastoma cells become extremely sensitive to ultraviolet radiation after terminal differentiation. Patients with hereditary diseases such as Cockaine syndrome and pigment xeroderma, DNA repair deficiency along with increased susceptibility to cancer, have severe neurological abnormalities. There is currently little known about the regenerative capacity of neurons. However, while the ability to repair DNA may decrease during brain maturation, neurons in an adult brain can effectively repair damaged DNA. This means that impaired ability to repair DNA in neurons may be an important factor in the accumulation of DNA damage and neuron death in neurodegenerative disorders. The ability of folic

acid deficiency to potentiate the toxicity of Aβ has been associated with oxidative modification of DNA caused by reduced DNA repair, which is consistent with previous studies on non-renian cells, showing that folic acid deficiency increases genetic damage caused by alkylating agents and γ irradiation. It was found that postmitotic neurons are more vulnerable to death from homocysteine and folic acid deficiency than mitotic astrocytes. In dividing cells, DNA damage can suppress DNA proliferation and repair and lead to mutagenesis and malignant transformation. Thus, folic acid deficiency and elevated homocysteine levels may also have an adverse effect on the mitotic cells of the nervous system, including glial and neural precursor cells.

While the APP-mutated transgenic mice used in this study did not show any signs of degeneration of neurons when they were kept on a regular rodent diet, there was significant loss of neurons in these mice, when they were on a folic acid-deficient diet for 3 months, which resulted in >10-fold increase in homocysteine levels. In contrast, wild-type mice fed a diet deficient in folic acid did not experience significant loss of neurons despite similar increases in circulating homocysteine. The main consequence of mutant APP hyperexpression in mice is an increase in the production of Aβ1-42, which then forms a globule deposit in the hippocampus and cortex of the brain. Soluble levels of Aβ1-42/Aβ1-40 were found to be elevated in the APP mutant mice brain, as were intracellular immunoreactivity levels of Aβ in the neurons of hippocampal mutants. However, Aβ levels did not change in mice with mutant APP that were fed a diet deficient in folic acid. In combination with cell culture data showing that methyl donor deficiency and homocysteine sensitize hippocampal neurons to Aβ toxicity, our results on APP mutant mice suggest that increased production Aβ1-42 in the brain of these mice makes their neurons vulnerable to homocysteine. In this scenario, the death of neurons will be caused when the degree of DNA damage reaches a critical threshold level that is lowered due to folic acid deficient diets and an age-related increase in Aβ accumulation. Selective loss of CA3, as opposed to CA1, neurons in the brain of mutant mice with APP after diets deficient in folic acid may be a result of the pyramidal vulnerability of CA3 hippocampal neurons to DNA damage and/or exocytotoxicity. Previous studies have shown that CA3 neurons are vulnerable to exsytotoxicity and that Aβ increases the vulnerability of neurons to exsy-

totoxicity. This possibility is consistent with data indicating that oxidative stress, DNA damage and p53 activation will mediate cytotoxicity.

Accumulating data shows that cell cycle-related proteins such as cyclins or cykinases are reexpressed in neurons doomed to death in response to various injuries, including Aβ. It has been reported that DNA replication can be initiated in post-mitotic neurons. In proliferating cells, specific proteins detect DNA damage (for example, PARP and p53) and can cause cell cycle disruption and promote DNA repair. If the damage is too extensive to be repaired, the same factors cause apoptosis. This mechanism appears to occur in differentiated postmitotic cells, such as neurons. Aβ-induced cell death can be mediated by p53, which responds to DNA damage and activates the transcription gene program inducing the expression of pro-apoptotic genes. Homocysteine has been shown to cause DNA damage, p53 activation and neuronal cell death. Folic acid deficiency also causes DNA damage with subsequent cell death. The ability of postmitotic neurons to replicate their DNA in conditions of precursor DNA deficiency caused by homocysteine or folic acid deficiency explains our observation of the relatively rapid impact of these treatments on misinclusion and cell death.

There is growing evidence that DNA damage and apoptosis play a role in the pathogenesis of several neurodegenerative disorders, including Alzheimer's disease. Neurons in vulnerable areas of the brain of patients with Alzheimer's disease exhibit several changes indicating apoptosis, including caspase activation, elevated Par-4 proapoptotic protein levels and increased Bax expression. Exposure of Aβ to cultured neurons induces caspase activation and increased production of Par-4 and Bax, each of which appears to play an important role in the process of cell death. Apoptosis caused by DNA damage usually involves activation of PARP and induction, and activation of the tumor suppressor protein p53. Increased PARP activity and p53 levels have been reported in connection with degeneration of neurons in patients with Alzheimer's disease and in cultured neurons exposed to Aβ. In addition, the chemical inhibitor p53 may protect neurons from Aβ toxicity, indicating the key role of this DNA-damaging cell death pathway in the pathogenesis of Alzheimer's disease. The study's findings suggest that by disrupting the ability of neurons to repair DNA, folic acid deficiency and elevated homocysteine levels may lower the threshold level of DNA damage required to trigger neuronal death. From this point of

view, folic acid deficiency and increased homocysteine levels accelerate the accumulation of DNA damage, which is facilitated by aging oxidative stress and accumulation of Aβ. Neurons are more vulnerable to DNA damage than nonneuronal cells, suggesting that the brain may be particularly sensitive to diets deficient in folic acid and other nutritional and genetic factors associated with the metabolism of one carbon.

The normal range of plasma homocysteine concentrations is 5-15 μmol/L in humans, and levels of homocysteine in spinal fluid and brain tissue vary from 0.5 to 10 μmol/L. Homocysteine levels in the blood of wild-type mice and mutant APP mice were not different in this study, ranging from 1 to 3 μmol/l under normal diet. However, homocysteine levels increased to 20-30 μmol/L in mice of the wild type and APP-mutants contained on a methyl deficient diet. Homocysteine can be rapidly absorbed by neurons through a specific membrane transporter and cause DNA strand ruptures, causing a deficiency of thymidine and contributing to the misinclusion of uracil. With age, the level of folic acid in blood plasma decreases and the level of homocysteine increases even more in patients with Alzheimer's disease, this is consistent with the possible contribution of de novo disrupted purin and thymidin synthesis and increased accumulation of damaged DNA to the pathogenesis of several different age-related neurodegenerative diseases. The results of studies of cultivated neurons exposed to Aβ and APP mutant mice, reflect a pathogenic process in humans, then it can be expected that food supplements with folic acid reduce the risk of sporadic forms of Alzheimer's disease, and can also suppress the neurodegenerative process in family cases of Alzheimer's disease [39,40].

The second important vitamin that plays a cofactor role in the folate cycle is vitamin B_{12} or kobalamine. The term vitamin B_{12} is used to describe a group of chemically similar biologically active compounds. All of these compounds are called cobalamins, due to the fact that the structure contains a cobalt atom and a corrinoid ring. Another synonym for vitamin B_{12} is Castle's external factor. A person receives vitamin B_{12} exclusively from animal products. The daily requirement for vitamin B_{12} is 5 μg [28]. Compounds of similar composition (pseudocobalamins) contained in plants cannot be absorbed by the human body. It is a known fact that cobalamin is produced by the microflora of the large intestine. The largest amount of vitamin B_{12} is synthesized by Propionibacterium spp., Klebsiella spp.,

Pseudomonas spp., Citrobacter freundii. However, it is also known that absorption of vitamin B_{12} in humans, unlike in animals, occurs in the terminal ileum. In the large intestine, absorption of cobalamin is minimal, so it is not possible to consider microflora as an endogenous source of vitamin B_{12} [41]. However, in 1980, it was shown by American scientists that the population of healthy Indians, adherents of a vegetarian diet, had less Kobalamin deficiency than the UK vegetarians. This was due to the fact that the small intestine of Indians was more populated with Klebsiella spp. and Pseudomonas spp. [42].

Cobalamin ingested with food is released in the stomach under the action of pepsin and binds to haptocorrin. Haptocorrin is a protein of gastric juice that protects Castle's extrinsic factor from hydrolysis in the acidic environment of the stomach, forming a "cobalamin-haptocorrin" complex. Then, in the distal part of the duodenum, under the action of pancreatic enzymes, the complex formed in the stomach is broken down. Free cobalamin reacts with Castle's intrinsic factor. Castle's intrinsic factor is secreted by the parietal cells of the stomach. A "Castle's extrinsic factor – Castle's intrinsic factor" complex is formed. The complex moves to the terminal part of the ileum and is absorbed into enterocytes there. In the lysosomes of intestinal epithelial cells, cobalamin is separated from Castle's intrinsic factor and secreted into the blood. In the blood, cobalamin binds to the protein transcobalamin and is transported to the cells of tissues and organs in a complex with it. Once in the cells, vitamin B_{12} is converted into the active forms methylcobalamin and 5-adenosylcobalamin. Methylcobalamin serves as a cofactor for the enzyme methionine synthase, which is involved in folate metabolism. 5-adenosylcobalamin enters the mitochondria and participates in the conversion of methylmalonyl-CoA to succinyl-CoA [43].

An important feature of vitamin B_{12} is its ability to be deposited in the liver. The content of cobalamin in the liver varies from 1.0 to 1.5 mg, which makes it possible to maintain an adequate level of the vitamin for 3-4 years. In the general population, vitamin B_{12} deficiency occurs with a frequency of 5.0 to 30.0%. There are several causes of cobalamin deficiency. In economically developed countries, vegetarian and vegan diets are gaining popularity, which inevitably leads to a lack of cobalamin in the body. A significant cause of hypovitaminosis B_{12} are some diseases of

the gastrointestinal tract, such as atrophic gastritis, Zollinger-Ellison syndrome, a condition after gastric resection or gastrectomy, Crohn's disease. When invaded by tapeworms, in particular the broad tapeworm, there is competition with the "host" organism for the absorption of cobalamin, which causes a state of hypovitaminosis B_{12}. Also, the use of certain medications may underlie cobalamin deficiency. These drugs include metformin, proton pump inhibitors, and histamine receptor blockers type 2. Vitamin B_{12} deficiency may be caused by mutations in the genes of cobalamin receptors and transporters [44].

From the point of view of diagnosis of B_{12} deficiency, the classic feature is megaloblastic anemia. The general blood test will record an increase in the average body cell erythrocyte volume (MCV). However, not always with B_{12} – the deficit will be hematological disorders. Therefore, the MCV indicator should not be relied upon when diagnosing B_{12} hypomicinosis. The most common and cost-effective method is considered to measure the total concentration of vitamin B_{12} in blood serum. The main difficulty in interpreting the results of a laboratory study of vitamin B_{12} concentration is due to the fact that no generally accepted reference intervals have been developed. The threshold values vary from 90 to 300 pmol/l in numerous sources. In some cases, it is proposed to raise the threshold to 340 pmol/l. In order to perform a comprehensive assessment of the cobalamine deficiency along with the determination of vitamin B_{12} concentration, it is recommended to determine the concentration of homocysteine and folic acid. The test that determines the concentration of the active form of vitamin B_{12} – golotranscobalamin is now considered to be more accurate for diagnosing a true kobalamine deficiency. Levels below 35 pmol/l are a reliable indicator of hypovitaminosis [41].

Vitamin B_{12} deficiency can lead to various pathological conditions of the body, including hypomethylation, which leads to imbalance in the SAM and SAN cell, which ultimately leads to the development of hyperhomocysteinemia. Kobalamin deficiency is also associated with neurological pathology. Both the central and peripheral nervous systems are affected. A study conducted in India shows that low levels of vitamin B_{12} in mothers are associated with adverse effects on mother and child health. In the mothers there is an increase in homocysteine level, habitual pregnancy failure, gestational diabetes, preeclampsia, in the children – insufficient

body mass at birth and subsequently cognitive disorders, obesity and insulin resistance. The level of actual data confirms the inclusion of vitamin B_{12} in existing nutrition programs in India to improve pregnancy outcomes and progeny health, beyond anemia control. In addition, B_{12} deficiency is associated with cognitive disorders. It has been shown that the absorption of vitamin B_{12} decreases with age, therefore for persons over 65 years of age to control hypomicanosis annual determination of the concentration of cobalamin in blood serum is recommended [45].

Thus, the folic acid and cobalamine deficiency involved in the process of folate exchange can lead to slow homocysteine remethylation, accumulation in blood plasma and development of a hyperhomocysteinemia condition, which, in turn, is a predictor of cardiovascular disease. Overconsumption of synthetic folic acid is also unsafe and associated with a number of pathological conditions.

Effect of tobacco smoking on folate status

Smoking is one of the most significant modified risk factors for many diseases, including cardiovascular, oncological and respiratory. It has a negative effect on the vascular system, contributing to the development of atherosclerosis, inflammation and oxidative stress. Smoking can also affect blood homocysteine levels.

Tobacco smoking is the process of inhaling smoke produced by the combustion of various substances. The main methods of delivering nicotine to the body are: smoking tobacco using cigarettes, cigars, pipes, hookahs, and electronic cigarettes. The most common type of smoking is cigarette smoking. Cigarette smoking remains one of the main causes of preventable diseases and deaths worldwide. According to official statistics, about 1.3 billion people in the world smoke tobacco, which is approximately 17% of the world's population. 34% of men and 6% of women smoke in the world. More than 8 million people die annually from smoking-related diseases, of which 7 million deaths are related to direct smoking and 1.2 million deaths are caused by passive smoking. China and India lead in the number of smokers. In China, about 300 million people smoke, while in Southeast Asia, the prevalence of smoking among men reaches 45%. The tobacco epidemic is one of the most serious public health threats the world has ever faced. By the end of 2030, the number of deaths caused by smoking is estimated to exceed 10 million, 70% of which will occur in developing countries. According to a recent report on the Global Survey of Adult Tobacco Use, in India, about 19% of men and 2% of women currently smoke tobacco. Active and passive smoking is the leading cause of preventable disease and death worldwide and is a major health problem for both adults and children. Smoking cessation benefits the health of smokers who quit before age 35, as their mortality rate is comparable to that of those who have never smoked. The World Health Organization has taken important steps at the micro and macro levels to combat the tobacco epidemic. Attempts to persuade smokers to quit should be part of a broader national tobacco control strategy that focuses on prevention. Clearly, the greatest success in reducing tobacco-related morbidity and mortality is achieved in this way. There are many effective methods for smoking cessation, including pharmacotherapy, such as nicotine replacement therapy, and behav-

ioural approaches such as group or individual counselling and self-help materials [46,47].

Health education and promotion is an effective way to combat the tobacco epidemic at both the national and international levels. It mainly focuses on the behavioral, cultural, social and economic factors that are crucial in causing diseases. An effective approach to behavioral interventions should be theoretically grounded, targeted at specific behaviors and focused on risk groups and vulnerable groups. Research shows that healthy lifestyle models value more informed approaches to personal skills and health literacy among people through health education [48].

India is known to be home to 10% of the world's smokers, and it is the second largest producer and consumer of tobacco in the world, with the greatest variety of forms of tobacco (for smoking and without). Bidis and cigarettes are the two most common forms of tobacco for smoking in India, with bidis accounting for 85% of the total volume of tobacco for smoking. Globally, tobacco causes 71% of lung cancers, 42% of respiratory diseases and about 10% of cardiovascular diseases, accounting for almost 12% and 6% of all disease deaths among men and women. In India, the mortality rate from non-communicable tobacco-related diseases is 190 per 100,000 men and 12 per 100,000 women. Tobacco use accounted for 5.9 per cent (2,081/100,000 population) of the disability-adjusted life years (DALY), with males having a higher prevalence than females. The availability of tobacco products in India is one of the main factors behind its overuse. It has been shown that raising the price of tobacco through higher taxes is the most effective way to encourage tobacco users to stop smoking and prevent young people from becoming involved. It was also stressed that taxes should be raised regularly to compensate for inflation and reduced consumer purchasing power. Worldwide, it has been proven that raising taxes on cigarettes is very effective in reducing cigarette consumption. Thus, the consumption of cigarettes and bidis in India can be reduced by raising the tax and eventually the prices of these products [49].

Electronic cigarettes (vapes, electronic vaporizers) are devices that spray "e-liquid" for inhalation and are becoming increasingly popular among smokers worldwide. Devices range from older, low-power "cigar-like" devices to refillable pens and larger tank devices, as well as newer, smaller, high-strength nicotine salt pods and disposable products. Elec-

tronic cigarettes are used by millions of people around the world, especially young people. Current evidence on electronic cigarettes, including their direct health effects and indirect effects (such as effects on smoking behavior), should be integrated to inform evidence-based policy and practice. Several major reviews on the health impact of e-cigarettes have been published. A total of 400 publications were included in the meta-analysis. Data on the health effects of electronic cigarettes is very limited. Users of electronic cigarettes are at higher risk for a number of adverse health effects. There is strong evidence that nicotine e-cigarettes can cause poisoning and immediate inhalation intoxication (including cramps) especially in children and adolescents, and defective devices can cause injuries and burns; There is strong evidence that nicotine e-cigarettes can cause addiction or addiction in non-smokers. There is strong evidence that electronic cigarettes cause lung damage associated with the use of electronic cigarettes or vapes (EVALI), mainly due to electronic cigarette fluids containing THC (and the vitamin E acetate supplement, which is found in many but not all products containing THC). However, 14% of EVALI cases in the largest relevant study were related to nicotine-containing liquids for electronic cigarettes without these components. There is moderate evidence that nicotine e-cigarettes may cause less serious side effects such as headache, cough, sore throat, dizziness and nausea. Identified environmental effects include waste generation, fires and the release of solid particles into indoor air (from substantial to conclusive evidence). There is insufficient data on changes in respiratory symptoms, exacerbations of respiratory diseases, lung function and other respiratory indicators among smokers who switch exclusively to nicotine e-cigarettes. There is limited or insufficient evidence that the use of nicotine e-cigarettes by non-smokers (mostly people who have never smoked) results in a dramatic deterioration of lung or other respiratory function. There is moderate evidence that nicotine e-cigarettes immediately after use increase heart rate, systolic and diastolic arterial pressure, and artery stiffness in smokers. Published data show that the use of nicotine e-cigarettes increases the risk of adverse health effects, including addiction, poisoning, inhalation toxicity (including cramps), and lung injury (mainly, but not exclusively, due to products containing THC/vitamin E acetate). Devices can cause injuries and burns, mainly due to exploding lithium batteries. There is evidence of adverse effects on the

cardiovascular system (including arterial pressure and heart rate) and lung function. Non-smokers who use electronic cigarettes are about three times more likely to start smoking and become avid smokers than those who do not use electronic cigarettes. Taking into account the caveats regarding observations-based conclusions and the possibility of residual mixing of factors, this relationship was recognized as probable. Non-smokers and young people are most vulnerable to the side effects of electronic cigarettes because they are disproportionately exposed to risks such as addiction, poisoning, inhalation toxicity and increased smoking. There is little potential benefit from quitting smoking. The environmental impact of electronic cigarettes includes indoor air pollution, waste generation and fires.

The evidence for the effectiveness of nicotine e-cigarettes as a means to quit smoking has been obtained mainly in clinically supported studies and has been limited. This finding is consistent with the findings of other major independent reviews (2018-2021) that evidence for the effectiveness of e-cigarettes as a means to quit smoking is limited, not available, similar to evidence for the effectiveness of other smoking cessation methods, are insufficient, amplified, weak, or insufficient. Cochrane Review of Electronic Cigarettes as a Means to Quit Smoking, published in 2022, where high-level evidence was found that nicotine e-cigarettes are more effective as a means to quit smoking, than standard nicotine replacement therapy (six studies), the evidence of a medium degree of confidence that they are more effective than nicotine-free e-cigarettes, and the evidence of a very low degree of confidence that they are more effective, than conventional treatment or behavioral support. However, overall, there is insufficient evidence of the impact of nicotine and nicotine-free electronic cigarettes on many major clinical indicators including cancer, cardiovascular disease, metabolic disorders, mental health, development, reproductive function and neurological disorders. Evidence mainly relates to nicotine e-cigarettes and the effects that may occur within months or years of starting use (including influence on smoking behavior), as well as acute effects, The association with the use of electronic cigarettes may be evident at the individual or group level, such as addiction, poisoning, burns, toxic effects of nicotine and withdrawal syndrome. The authors focused on comparing the effects of electronic cigarettes with the effects of tobacco cigarettes on biomarkers and found that, in general, e-cigarettes were more favourable than tobacco.

Users of electronic cigarettes inhale a complex mixture of chemicals, including nicotine, solvent carriers, flavourings, tobacco-specific nitro, volatile organic compounds, phenolic compounds, tobacco alkaloids, aldehydes, Free radicals, active oxygen forms, furans and metals; many of these are associated with adverse health effects. Studies in humans and animals show that nicotine has an adverse effect on the cardiovascular system, lung function and development and brain function in adolescents. Some of the harm listed here, including device-related, is likely to also apply to nicotine-free electronic cigarettes, as well as uncertainty about other effects [50].

A number of harmful health consequences from the use of electronic cigarettes have been identified. Nicotine addiction and aggressive marketing are at the root of the wide spread and growing popularity of e-cigarettes among young people. Direct health risks, the relationship between e-cigarette use and smoking, and uncertainty about their impact on key health outcomes mean that e-cigarette use by non-smokers, especially children and adolescents, is an important public health issue. The impact of electronic cigarettes on former smokers' health is reduced if they use other smoking cessation methods or if the use of electronic cigarettes is short-term; there is limited evidence that that e-cigarette users are more likely to return to smoking. Smokers who have been able to quit smoking have done so without the use of any aids, and there are many approved smoking cessation aids that are proven to be safe, good in quality and effective. In many countries, the most common model for using electronic cigarettes is smoking both tobacco and electronic cigarettes. The direct impact of co-smoking on health is unknown, and e-cigarettes may promote continued smoking, increasing risks. Smokers are exposed to the negative health effects of electronic cigarettes, while other consequences (poisoning, environmental impact, use by non-smokers) may affect family members and society. Electronic cigarettes may be useful for smokers who use them to completely and quickly quit smoking, but due to limited data in this area, the risks associated with them uncertainties regarding their impact on key clinical outcomes and continued smoking by most users their overall safety and effectiveness remain unclear.

The risks associated with electronic cigarettes are likely to be increased due to certain characteristics of the product, as well as factors that lead

to their wider use by people who do not use them for smoking cessation, including higher concentration of nicotine in liquid for electronic cigarettes; greater volume of liquid for electronic cigarettes; dilution of liquid "at home" and other ways of preparing liquid for electronic cigarettes; falsification of liquids for electronic cigarettes; Poor labelling and packaging, not intended for children; products with a high concentration of nicotine salt; flavourings and other characteristics attractive to children, adolescents and non-smokers; accessibility, advertising and low cost; inadequate enforcement of legal restrictions on the use of electronic cigarettes; the impact of the tobacco and nicotine industries; and disinformation about their health effects [51].

Hookah smoking has been called a global tobacco epidemic by health officials. A study on the characteristics, behaviour and perception of hookah among young smokers was published, involving 280 regular hookah users. Data were collected using a questionnaire of 29 points, composed by three main areas: characteristics (socio-demographic and personal), behavior and perception (about the harm compared to smoking cigarettes). The results of the study showed that the average age at which people started smoking a hookah was 17.3 years; 75% of participants did not receive parental approval; and the most common nicotine-related effects were dizziness, headache and nausea. Thus, 24.6% of participants reported that they smoked a hookah daily. The average time to smoke a hookah was 1 hour 19 minutes. 68.2% of the participants reported that they smoked a hookah in a hookah room, and 35.7% of the participants reported that they smoked a hookah with other people. About 66.7% of participants did not intend to quit smoking. Most of them (71-80%) mistakenly believed that smoking a cigar is safer than smoking cigarettes, and 54-82% of participants did not know about the harm to health. The study concludes that educational activities are needed to raise awareness among young people about the dangers of smoking a hookah [52].

It is a common misconception that smoking hookah is less harmful and addictive than other types of tobacco. However, studies show that people consume the same amount of nicotine, resin and carbon monoxide as they do when smoking 100 cigarettes in a single session. Other studies also show that hookah smoking increases the risk of developing oral cancer, cardiovascular disease, esophageal cancer, chronic obstructive pulmonary

disease, and thrombosis. A study conducted in 2017 found that 32% of the young people and adolescents in their sample believe that a bong is more or less harmful than cigarettes. Hookah tobacco contains nicotine and is addictive, so convincing hookah users that they can quit seems wrong.

A cross-sectional study conducted from 2013 to 2015 in Washington, DC, found that youth who perceived hookah as somewhat (AOR: 5.70; 95% CI: 1.37–23.77) or very much (AOR: 12.36; 95% CI: 2.61–58.50) socially acceptable were more likely to use hookah than youth who perceived hookah as socially unacceptable. Research conducted between 2013 and 2014 found that youth who perceived hookah to cause "little or no harm" were 2.7 times more likely to initiate hookah use one year later compared to youth who perceived hookah to cause "a lot of harm," and that youth in the "low" perceived hookah harm category and those in the "low" and "moderate" perceived hookah dependence categories were more likely to initiate hookah use one year later compared to youth in the "high" categories. While the association between hookah use and perceived harm and dependence is clear, it is unknown how perceived harm and dependence influence the age at which youth initiate hookah use. Earlier hookah use may harm the developing brain, affecting learning, memory, and attention. Many studies show evidence that young people with misconceptions about the harm and addiction of hookah are at higher risk of starting hookah smoking at an earlier age compared to young people with more accurate beliefs about the harm and addiction of hookah. These findings indicate the need for prevention and education campaigns that help young people and inform them about the harm and addiction of hookah in order to delay the onset of its use [53].

A generally recognized risk factor for the development of cardiovascular pathology is smoking. Both active and passive smoking have a negative effect on a living organism. Tobacco smoke contains free radicals and aldehydes, which are stimulators of oxidative stress. A study by Podzolkov V.I. and co-authors showed a positive correlation between the level of total homocysteine and smoking, smoking history, and the smoking index. The study showed that higher concentrations of homocysteine were found in the group of smokers suffering from hypertension and in the group of "healthy smokers" compared to the group of non-smokers [54].

It is known that cyanides contained in tobacco smoke affect pyridoxine and cobalamin, reducing the concentration of B vitamins in the blood plasma, thereby slowing down transsulfation and folate-dependent remethylation of homocysteine in the body. Excess homocysteine causes oxidative stress, disrupts cholesterol biosynthesis, initiates apoptosis of endothelial cells and leads to endothelial dysfunction. The study by Fefelova E. V. and co-authors also demonstrated that the concentration of homocysteine in the blood plasma of smokers is higher (12.22 μmol / l) than in non-smokers (7.83 μmol / l), the concentration of homocysteine in saliva in smokers is 4.4 times higher than in non-smokers (2.13 μmol / l versus 0.48 μmol / l). There is evidence that the level of homocysteine depends on the number of cigarettes smoked per day. Each cigarette smoked increases homocysteine levels by 0.5% in men and 1% in women [55,56].

Abduvakhopova N.R., when conducting a correlation analysis, showed a connection between the level of homocysteine and smoking and coffee consumption. Moreover, the level of homocysteine did not differ significantly in the group of non-smokers and participants who quit smoking, which is important from the point of view of preventing hyperhomocysteinemia and a significant motivational factor for quitting smoking [57].

A study by Indian scientists showed that smokers had significant increases in homocysteine levels and decreased levels of folic acid and vitamin B_{12} compared to non-smokers. Homocysteine strongly correlated with the duration of use and the number of cigarettes consumed. Folic acid and vitamin B_{12} were significantly reduced in patients who smoked for more than 20 years, compared to those who smoked for less than 5 years. Among smokers, there is a positive correlation between homocysteine and thiocytes in the blood plasma and tinuria, as well as a negative correlation between tinuria and folic acid in the plasma. It was concluded that cigarette smoking increases the level of homocysteine, which is strongly correlated with tinuria and plasma thiocyanates. In addition, smokers had a tendency to develop hypofoliemia and B_{12} hypopolymycin, especially when the duration of consumption exceeded 20 years [58].

Passive smoking also has an adverse effect on folate metabolism. Studies show higher levels of homocysteine, as well as reduced levels of folic acid in people who are systematically exposed to tobacco smoke [59]. Other studies have shown that inhalation of tobacco smoke from passive smok-

ing resulted in increased levels of fibrinogen and homocysteine, which are important biomarkers of cardiovascular disease risk. Passive smoking has the same toxic effects as active smoking, which, in particular, is manifested by an increase in the concentration of atherogenic homocysteine in the blood plasma of both passive and active smokers [60]. The effect of passive maternal smoking on the development of congenital malformations in the fetus was studied. The following results were demonstrated. Four population-based studies (Norwegian Facial Cleft Study, 1996–2001; Utah Child and Family Health Study, 1995–2004; Norwegian Mother and Child Cohort Study, 1999–2009; National Birth Defects Prevention Study (USA, 1999–2007)) were pooled to yield 4,508 cleft cases and 9,626 controls. Participants were stratified by first-trimester tobacco smoke exposure, either passive or active. Multivariate logistic models were used, adjusting for potential confounders (maternal alcohol consumption, use of folic acid supplements, age, body size, education, and employment, as well as study fixed effects). Children whose mothers were active smokers had an increased risk of developing oral clefts (odds ratio (OR) = 1.5). 1.27, 95% confidence interval (CI): 1.11, 1.46). Children of passive smoking mothers also had an increased risk (OR = 1.14, 95% CI: 1.02, 1.27). The risk of developing cleft was further increased among children of smoking mothers who were exposed to passive smoking (OR = 1.51, 95% CI: 1.35, 1.70). Using a large pooled dataset, a moderate association was found between first trimester passive smoking and oral cleft, which was consistent across different populations, different study designs, and cleft subtypes. Passive smoking exposure during pregnancy has been shown to be teratogenic [61].

Effect of alcohol drinking on folate status

Alcohol or alcoholic drinks beverages contain **ethanol**, a psychoactive and toxic substance that can cause addiction. Although alcohol has been widely used in many cultures for centuries, its consumption is associated with serious risks and harm to health. 2.6 million deaths were attributed to alcohol use worldwide, including 2 million among men and 0.6 million among women in 2019. The highest alcohol-related death rates per 100 000 population are found in the WHO European and African regions, at 52.9 and 52.2 deaths per 100 000 population, respectively.

Young people (20–39 years) suffer disproportionately from alcohol use: this age group had the highest proportion of alcohol-related deaths in 2019 (13%).

Global alcohol consumption data for 2019 show that 400 million people aged 15 years and older have alcohol use disorders and approximately 209 million people are alcohol dependent [62].

Alcohol is one of the most widely consumed psychoactive substances worldwide. Alcohol abuse, defined as quantity and frequency of consumption, is associated with acute and chronic diseases. Alcohol use disorders are mental syndromes characterized by impaired control over alcohol use and other symptoms. Current etiological views are based on the biopsychosocial model, which emphasizes the interrelationships between genetics, neurobiology, psychology, and the social and community context. There is strong evidence that alcohol use disorders are influenced by genetic factors, but with a complex polygenic structure. There is also strong evidence for environmental influences, such as childhood adversity and maladaptive developmental trajectories. Well-known biological and psychological determinants of alcohol use disorders include neuroadaptive changes that occur with chronic use, differences in brain structure and function, and motivational determinants including overestimation of alcohol reinforcement, acute effects of environmental factors and stress, increased levels of impulsivity, and lack of alternative reinforcers. Social factors include the two-way role of social networks and sociocultural influences such as public health strategies and social determinants of health [63].

Alcohol can penetrate almost all tissues of the body, which leads to changes that entail serious pathophysiological consequences for many

systems. Approximately 3.4% of the global burden of noncommunicable disease-related mortality, 5% of years of life lost, and 2.4% of disability-adjusted life years lost can be attributed to alcohol abuse, with higher burdens associated with liver cancer and cirrhosis [64]. The burden of alcohol-related disease is closely associated with average alcohol consumption, and there is a dose-dependent relationship between the amount and duration of alcohol consumption and the incidence of diabetes, hypertension, cardiovascular disease, stroke and pneumonia [65].

The average rate of elimination of alcohol from the body is ~7 g/h, which corresponds to ~1 drink per hour. Alcohol is metabolized on its first passage through the stomach by the enzyme alcohol dehydrogenase. Most tissues express alcohol dehydrogenase and are capable of metabolizing alcohol. However, most alcohol oxidation occurs in the liver. Alcohol is metabolized to acetaldehyde primarily by alcohol dehydrogenase and cytochrome P450 2E1 (CYP2E1). This latter pathway is particularly relevant after chronic alcohol abuse. Acetaldehyde is converted to acetate in the mitochondria by the enzyme acetaldehyde dehydrogenase type 2 (Fig. 5) [65].

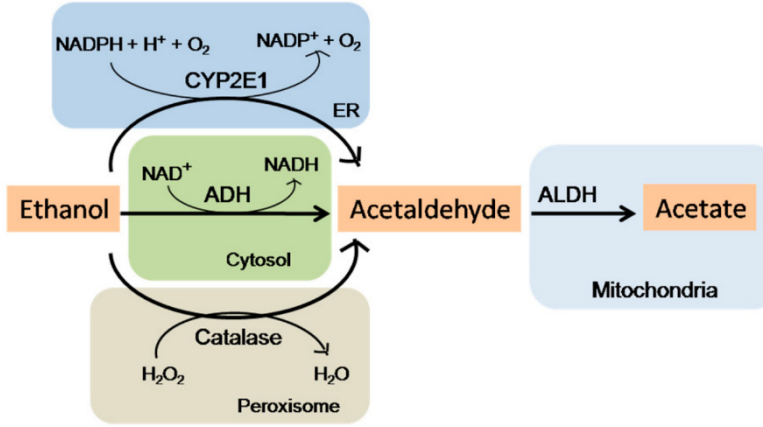

Fig. 5. Pathways of ethanol metabolism in the human body [66]

Most of the acetate formed enters the systemic circulation and is activated to acetyl coenzyme A, a key intermediate metabolite in peripheral

tissues. Acetaldehyde can form adducts that can cause damage by activating immune responses [65].

Excessive chronic alcohol consumption is usually accompanied by malnutrition, impaired intestinal absorption, damage to the liver (the body's main synthetic factory), and the toxic effects of alcohol and its metabolic products [67]. The listed factors influence folate metabolism and, accordingly, the metabolism of the amino acid homocysteine at its various stages: at the stage of assimilation of cofactors of enzymes involved in the utilization of homocysteine, as well as the work of these enzymes themselves; which can lead to hyperhomocysteinemia.

The Effect of Alcohol on the Availability of Vitamins of group B

1. Pyridoxine (vitamin B_6)

Transsulfuration is the main pathway for homocysteine utilization, by which a sulfur atom is integrated into the cysteine molecule. Transsulfuration occurs primarily in the liver and kidneys. The enzymes cystathionine β-synthase and cystathionine γ-lyase require the cofactor pyridoxal **phosphate** for their function [68]. A decrease in the concentration of this vitamin or impaired bioavailability can lead to a state of hyperhomocysteinemia.

Lumeng L et al., when comparing plasma pyridoxal phosphate levels in alcoholics with those in non-drinkers, found a difference in the frequency of vitamin B_6 metabolism disorders. Sixty-six alcoholics were selected based on their normal liver function and hematological parameters. The authors found that the products of ethanol metabolism, namely acetaldehyde, impaired the formation of pyridoxal phosphate (the active form of vitamin B_6) from pyridoxal, pyridoxine in red blood cells. The study showed that the effect of acetaldehyde was mediated by phosphatase, which resulted in accelerated breakdown of phosphorylated vitamin B_6 compounds in red blood cells [68].

The Medici V team's study assessed levels of biochemical markers of liver function, vitamin B_6, folate, vitamin B_{12}, homocysteine, methionine, S-adenosylmethionine, S-adenosylhomocysteine, cystathionine, cysteine in 40 patients with alcoholic liver cirrhosis, of whom 24 had liver biopsy, 26 active alcohol consumers without liver disease and 28 healthy controls. The results showed that vitamin B6 levels were within normal limits in all

groups, however, in the group of patients with alcoholic liver disease, the concentration of the vitamin was significantly lower than in the remaining groups ($p < 0.02$). Serum homocysteine levels were elevated in all alcoholics, while patients with alcoholic liver disease had low vitamin B_6 levels with elevated cystathionine levels and a reduced alpha-aminobutyrate/cystathionine ratio, which corresponds to a decrease in the activity of vitamin B_6-dependent cystathionase [69].

2. Folic acid (vitamin B_9)

One of the ways of homocysteine utilization is remethylation, which requires methyl group donors. **5-methyltetrahydrofolate** (5-MTHF) can be such a donor [70].

Studies by many authors have shown that long-term consumption of alcoholic beverages leads to a decrease in the level of folic acid in the blood [71,72]. A study by Wani NA et al. in humans, monkeys and pigs shows that chronic exposure to excessive amounts of alcohol reduces intestinal absorption of both polyglutamyl folates and monoglutamyl derivatives of folate. Feeding rats with ethanol for 3 months at a dose of 1 g/kg body weight per day reduced total folate levels as well as polyglutamyl folate levels [73]. Ethanol reduces the expression of glutamate carboxypeptidase II (GCPII), which is necessary for the hydrolysis of polyglutamyl folates, and also directly affects the absorption of vitamin B_9 in the intestine, affecting the activity of intestinal folate transporters (SLC46A1; SLC19A1) and receptors [73,74]. A study by Butts M et al. showed that the effect of alcohol on the absorption of vitamin B_9 also occurs at the genomic level through epigenetic mechanisms. After chronic ethanol intake, hypermethylation was present both in the gene associated with folylpolyglutamate hydrolase and in the genes of vitamin B_9 transporters [74].

An analysis by Halsted CH et al. showed that minipigs fed alcohol for 1 year showed signs of alcohol dependence, coupled with decreased translation and activity of the intestinal transporter of reduced folate. Data from another study, also conducted on minipigs fed ethanol, demonstrated an imbalance of methionine and DNA nucleotides in the liver, as well as increased hepatocyte apoptosis. When ethanol feeding was combined with folate deficiency, minipigs developed typical histological features of alcoholic liver disease after 14 weeks, as well as increased plasma homocysteine, decreased hepatic S-adenosylmethionine and glutathione, and

increased markers of DNA and lipid oxidation. Thus, chronic alcohol consumption impairs folate absorption by suppressing the expression of the reduced folate transporter and reducing hepatic folate uptake and renal retention of circulating folate. At the same time, folate deficiency accelerates alcohol-induced changes in hepatic methionine metabolism, contributing to increased oxidative liver injury and histopathological changes in alcoholic liver disease [72].

3. Cyanocobalamin (vitamin B_{12})

An important remethylation enzyme is methionine synthase (MTR), which requires the presence of methylcobalamin for its function [75].

Rajdl D et al. showed that two weeks of moderate alcohol consumption, regardless of the strength of the drink, reduced vitamin B_{12} levels. Male volunteers in this study (n = 117) were randomly divided into five groups: a group that consumed only wine (control, 375 ml of white wine daily for a month), and four groups that combined wine consumption with the intake of one of the supplements (folic acid, betaine, and vitamins B_{12} or B_6). A significant reduction in homocysteine concentrations after a period of wine consumption was found in participants taking both folic acid and betaine. Taking vitamin B_{12} and vitamin B_6 supplements did not result in statistically significant changes in homocysteine levels [76]. In the work of Butts M et al., a tendency towards decreased absorption of vitamin B_{12} with long-term consumption of alcoholic beverages was also noted, however, the mechanisms of the influence of alcohol on the absorption and availability of cyanocobalamin are currently unknown [74].

Zibold J et al. in a study of patients with Leber's hereditary optic neuropathy found that the subgroup with vitamin B_{12} deficiency neuropathy had significantly more smokers and alcohol abusers than those without ($p < 0.05$, respectively). Significantly more patients with Leber's hereditary optic neuropathy had alcohol abuse than asymptomatic mutation carriers ($p = 0.007$). In the subgroup with vitamin B_{12} deficiency neuropathy, 38% had hematological abnormalities such as macrocytosis, and 16% had polyneuropathy [77].

The effect of alcohol on the functioning of folate cycle enzymes

Long-term exposure to various doses of alcohol, according to many researchers, leads to a state of hyperhomocysteinemia [71,76,78], as well

as an increase in the concentration of homocysteine in various types of cells [79,80].

Ji C et al., after intragastric administration of ethanol to mice for a month, found that the plasma homocysteine level in the experimental group mice was seven times higher than in the control group mice [75].

A study by Vatsalya V et al. showed that 27% of patients with alcohol dependence had clinically significant hyperhomocysteinemia. The authors also noted that chronic alcohol exposure in rats resulted in excess accumulation of homocysteine in adipocytes, which caused changes in adiponectin production [78].

The study by Korean scientists included patients who had suffered a stroke and were admitted to a medical center. Ten months after the stroke, a questionnaire was administered and blood samples were taken to determine biochemical parameters. Nutritional status was determined using the Mini Nutritional Assessment (MNA), and nutrient intake was determined using the 24-hour method. Of the 203 patients, 84% were malnourished or at risk of malnutrition, and 26% had elevated homocysteine levels 10 months after stroke. Logistic regression identified factors associated with high homocysteine levels 10 months after stroke: alcohol abuse ($p = 0.02$), low MNA scores ($p = 0.026$), low serum vitamin B_{12} ($p = 0.021$), and low serum folate ($p = 0.003$). Of 156 patients with normal homocysteine levels at admission, 36 patients developed hyperhomocysteinemia 10 months after stroke, which was associated with excessive alcohol consumption ($p = 0.013$). Persistent hyperhomocysteinemia, observed in 22 patients (11%), was associated with male gender ($p = 0.031$), older age ($p = 0.042$), low vitamin B_6 intake ($p = 0.029$), and excessive alcohol consumption ($p = 0.013$) [80].

The effect of alcohol on the functioning of enzymes involved in homocysteine utilization is mediated by epigenetic influences.

Ethanol inhibits methionine synthase (MS), activates betaine-homocysteine methyltransferase (BHMT), and possibly inhibits methionine adenosyltransferase (MAT) (Fig. 6). All of these have the effect of increasing homocysteine levels and depleting liver S-adenosylmethionine (SAM), causing fatty liver disease. Because of the inhibition of methionine synthase, the BHMT pathway becomes more important as a source of SAM and a determinant of homocysteine levels in alcohol users [76].

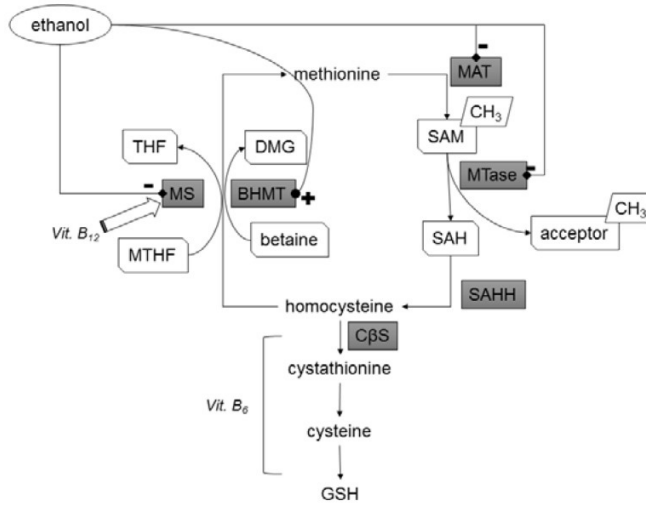

Fig. 6. Effect of ethanol on the work of enzymes involved in homocysteine metabolism [76]

However, another study comparing the effects of alcohol on liver and cerebellar cells yielded different results. In cerebellar cells, alcohol exposure resulted in a 45% decrease in MS expression, a 70% increase in S-adenosylhomocysteine hydrolase (SAHH) expression, and a nearly two-fold increase in MAT expression, while methylenetetrahydrofolate reductase (MTHFR) and BHMT expression levels did not change significantly. Withdrawal from alcohol resulted in a return to normal MS and SAHH expression, but MAT expression remained significantly elevated [79]. In liver cells, the effects of alcohol were somewhat different. Chronic ethanol exposure also resulted in a significant decrease in the expression of MS, MTHFR by 30% and BHMT by 20%, but did not affect the expression of SAHH. Upon alcohol withdrawal, all these changes returned to normal with the exception of BHMT expression in the liver [79].

Thus, alcohol, in addition to the already well-known negative effects, affects the functioning of the folate cycle by reducing the availability of cofactors for the enzymes of this cycle and changing their activity.

The influence of genetic factors on folate metabolism processes

Single nucleotide substitutions (SNP) may occur (substitutions of one nucleotide (A, G, T, C) for another) in genes encoding folate cycle enzymes. These substitutions lead to changes in the primary structure of the protein (amino acid sequence), which causes spatial modifications of the molecule and, most often, a disruption of enzymatic activity. These mutations can cause changes in the methylation profile of other genes [81], which entails disruption of epigenetic mechanisms and an increased risk of disease development. Also, based on the biochemical reactions of homocysteine metabolism, it follows that polymorphism of the genes of folate cycle enzymes affects the level of this amino acid in the blood serum and can lead to hyperhomocysteinemia [82,83,84]. In this section we will consider the following polymorphisms:

- methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphism C677T (Ala222Val, rs1801133) and A1298C (Glu429Ala, rs1801131);
- methionine synthase (*MTR*) gene polymorphism A2756G (Asp919Gly, rs1805087);
- methionine synthase reductase (*MTRR*) gene polymorphism A66G (Ile22Met, rs1801394);
- folate transporter gene polymorphism (*SLC19A1*) A80G (His27Arg, rs1051266).

Methylenetetrahydrofolate reductase (MTHFR) gene C677T (Ala222Val, rs1801133) polymorphism

More than 40 polymorphisms have been found in the methylenetetrahydrofolate reductase gene *MTHFR*, located on the short arm of chromosome 1 [85]. The substitution of cytosine (C) for thymine (T) at position 677 of the *MTHFR* gene is a quite widespread and well-studied mutation and results in altered enzyme activity by modifying the flavin adenine dinucleotide (FAD) binding region. This results in a decrease in the concentration of the reaction product: 5-methyltetrahydrofolate, a methyl donor for homocysteine; and hyperhomocysteinemia [86]. This missense mutation causes a decrease in *MTHFR* enzymatic activity by approximately 70% and 35%, respectively, in carriers of the TT and CT genotypes, which

disrupts folate metabolism [85,87,88]. The prevalence of the pathological T allele in the world is 33.68%, in the European population – 34.84%, and in the Asian – 33.86% [89].

The *MTHFR* C677T gene polymorphism is associated with a number of pathological conditions: hypertension, stroke, coronary heart disease, atherosclerosis, congenital heart defects, periodontitis, diabetes mellitus, cancer and pregnancy disorders.

Hypertension. In a study by Chiu MH et al. on the Taiwanese population (n = 1238), the effect of the *MTHFR* rs1801133 gene polymorphism on the predisposition to hypertension was studied. It was shown that the distribution of the *MTHFR* rs1801133 genotypes (CC, CT and TT) differed significantly depending on the presence of hypertension ($p < 0.0001$): in the hypertensive group, 19.75% have the TT genotype, and in people without hypertension, only 8.42%. The unfavorable TT genotype was associated with an increased risk of developing hypertension (OR: 2.718; 95% CI: 1.503–4.914), while the heterozygous CT genotype was not [90]. Similar results were obtained by Hu XJ et al. when studying a Chinese population (n = 985) from the central Guangxi region. Patients with hypertension were divided into groups with H-type hypertension (homocysteine > 10 μmol/L, n = 528) and without H-type hypertension (homocysteine ≤ 10 μmol/L, n = 78). The frequencies of the TT genotype (22.73%) and T allele (46.21%) in patients with H-type arterial hypertension were significantly higher than in patients in the control group (11.35% and 30.47%, respectively; $p < 0.001$) and the non-H-type hypertension group (10.26% and 28.85%, respectively; $p < 0.001$) [88].

Atherosclerosis. Folate metabolism disorder caused by gene polymorphism is associated with homocysteine metabolism, lipid metabolism and can become a factor provoking the progression of atherosclerosis. This was confirmed in the study by Cai N et al. in China. The analysis revealed that the frequency of the T allele of *MTHFR* rs1801133 in patients with atherosclerosis of several arteries was significantly higher (32.6% vs. 27.1%, $p = 0.012$) than in atherosclerosis of one artery. The results of logistic regression analysis showed that the *MTHFR* rs1801133 TT genotype (TT vs. CC: OR: 1.943, 95% CI: 1.179–3.203, $p = 0.009$) may be an independent risk factor for multiartery atherosclerosis (adjusted for age, gender, smoking, alcohol consumption, hypertension, and diabetes) [86].

Ischemic heart disease. The disruption of metabolic processes due to carriage of unfavorable alleles of the *MTHFR* rs1801133 gene polymorphism was also noted by Luo Z et al. in a 2024 study. The object of their study was people with coronary heart disease. The patient group (n = 430) had older age ($p < 0.001$) and higher levels of systolic BP ($p < 0.001$), diastolic BP ($p < 0.001$), TG ($p = 0.05$), LDL-C ($p = 0.01$), APOB ($p = 0.01$), lipoprotein(a) (LP(a)) ($p < 0.001$), homocysteine ($p < 0.001$) and hs-CRP ($p < 0.001$) than the control group. Among patients with coronary heart disease, the unfavorable T allele was significantly ($p = 0.01$) more common (40%) compared to the control group (32%). In carriers of the TT genotype, the homocysteine level was higher than in carriers of the CC genotype, both in patients with coronary heart disease (TT vs. CC = 16.40 ± 7.63 vs. 13.06 ± 4.46, $p = 0.02$) and in patients without it (TT vs. CC = 16.78 ± 9.61 vs. 13.52 ± 4.51, $p = 0.03$). In addition, CT genotype carriers had higher homocysteine (CT vs. CC = 14.95 ± 4.98 vs. 13.52 ± 5.12, $p = 0.02$), systolic BP (CT vs. CC = 148.29 ± 28.22 vs. 139.34 ± 24.65, $p = 0.02$), LDL-C (CT vs. CC = 2.59 ± 0.99 vs. 2.32 ± 0.77, $p = 0.04$) and hs-CRP (CT vs. CC = 15.14 ± 27.54 vs. 9.34 ± 17.84, $p = 0.05$) and lower APOA1 levels (CT vs. CC = 0.98 ± 0.20 versus 1.04 ± 0.15, $p = 0.05$) than in patients with the CC genotype with coronary heart disease. ROC curve and PR curve showed that hyperhomocysteinemia is a sensitive indicator for predicting the severity of coronary heart disease or multiple vascular lesions. Multivariate logistic regression showed that homocysteine, rs1801133, age, smoking, weight, body mass index, Lp(a), and high-sensitivity C-reactive protein are independent risk factors for coronary heart disease [91].

Stroke. The association of *MTHFR* rs1801133 gene polymorphism with stroke development has been studied for a long time worldwide. In a study of young Indian patients with cryptogenic ischemic stroke, it was found that *MTHFR* rs1801133 polymorphism was statistically significantly associated with cryptogenic juvenile ischemic stroke ($p = 0.0261$) in the Indian population. It was also found that the T allele of the *MTHFR* rs1801133 gene was statistically associated with cryptogenic juvenile ischemic stroke (OR: 1.8725; 95% CI: 1.1858–2.9570; $p = 0.0071$) [92]. Cernera G et al., studying the Italian population: patients with ischemic stroke (n = 282), transient ischemic attack (n = 87) and healthy individuals (n = 430); came

to the conclusion that the *MTHFR* rs1801133 polymorphism is associated with stroke, since the mutant variant of the T gene was significantly ($p = 0.003$) more common in patients with stroke (52.1%) than in the control group (43.8%). In addition, the distribution of genotypes in these groups differed significantly: among patients with ischemic stroke, CT heterozygotes were 2% more common in the healthy study sample, and TT homozygotes were 7.3% more common ($p = 0.01$) [93].

Type 2 diabetes. In addition to cardiovascular diseases, the association of the *MTHFR* C677T gene polymorphism with endocrine diseases was also found: with type 2 diabetes mellitus. Pathak D et al. observed a significant difference in the distribution of genotypes between the main and control groups ($p = 0.0002$): in the group of patients with type 2 diabetes mellitus, the T allele had a higher frequency (0.33) compared to the control group (0.16) [94]. The effect of folate metabolism on the risk of diabetes is also mediated by the effect on homocysteine levels. Hyperhomocysteinemia is often observed in patients with type 2 diabetes. Lapik IA et al. found that plasma homocysteine levels were significantly correlated with age, sex, diabetes duration and blood pressure in patients. Elevated serum homocysteine levels have a damaging effect on the vascular wall caused by oxidative stress. In this condition, the intensity of lipid peroxidation increases. It is believed that in type 2 diabetes mellitus, hyperhomocysteinemia can aggravate endothelial dysfunction, accelerate the development of atherosclerotic processes and increase platelet aggregation and their adhesive properties. As a result, high homocysteine concentrations in patients with type 2 diabetes mellitus contribute to the development of micro- and macroangiopathies and hypertension. Carriers of the CT and TT genotypes of the *MTHFR* gene had higher levels of cholesterol, low-density lipoproteins and triglycerides and lower levels of vitamin B_6 and folate ($p < 0.05$) [95].

Oncological diseases. It was previously mentioned that the *MTHFR* rs1801133 gene polymorphism can lead to changes in the epigenetic regulation of other genes, which may be associated with cancer, but in this case, according to a number of studies, the mutation has a positive effect. Huang Y et al., when studying colorectal cancer, noted that the frequency of the *MTHFR* CC genotype and C allele in patients with colorectal cancer was significantly higher than in healthy people ($p = 0.006$ and $p =$

0.001, respectively): the frequencies of the *MTHFR* rs1801133 CC, CT and TT alleles in patients were 50%, 38.57% and 11.43%, respectively, and in healthy people – 37.8%, 44.86% and 17.84%, respectively. Thus, individuals with the *MTHFR* rs1801133 CC genotype had a higher risk of developing colorectal cancer, and *MTHFR* rs1801133 TT carriers were more susceptible to methylation of the Septin 9 gene, a marker of colorectal cancer risk [81]. In relation to gastric cancer, data on the protective effect of the *MTHFR* rs1801133 gene polymorphism have also been obtained. The following results were obtained in the study by Han Z et al.: the observed frequency of the T allele of rs1801133 in the control group was 43.9%, which corresponds to the frequency of the T allele in the Han population in Beijing (44.4%). Compared with the control group, the frequency of the T allele in patients with gastric cancer was significantly lower (35.83%). The frequency of the rs1801133 genotypes was 29.3% (CC), 52.7% (CT), and 18% (TT) in the control group and 41.4% (CC), 45.6% (CT), and 13% (TT) in the main group, respectively [96]. However, the influence of polymorphism on predisposition to cancer is ambiguous; when examining the genetic profile of patients with breast cancer, it was found that the T allele rs1801133 is associated with an increased risk of developing breast cancer [97].

Methylenetetrahydrofolate reductase (MTHFR) gene A1298C (Glu429Ala, rs1801131) polymorphism

There is another studied polymorphism in the methylenetetrahydrofolate reductase gene. The A1298C variant results in the substitution of glutamate for alanine at codon 429 in the SAM regulatory domain of the MTHFR protein [85,86]. This single nucleotide substitution variant also results in a decrease in the activity of the methylenetetrahydrofolate reductase enzyme, but not as strong. In carriers of the homozygous CC genotype, the activity decreases by approximately 40% [98,99]. The prevalence of the mutant C allele in the world is 30.24%, in the European population – 31.41%, and in Asia – 24.7% [100].

The influence of the *MTHFR* A1298C gene polymorphism on the development of pathological conditions has also been studied, but the results are fewer and more controversial in comparison with other polymorphisms of this gene.

Hypertension. *MTHFR* plays an important role in folate metabolism and maintaining homocysteine levels. *MTHFR* rs1801131 polymorphism can lead to persistently elevated homocysteine levels, which is involved in many pathophysiological processes such as vascular endothelial injury, oxidative stress, and inflammatory response, and can ultimately lead to hypertension. A study by Liu Y's team on the Bai people of Yunnan Province, China, assessed the effect of rs1801131 on the development of hypertension. Among patients with hypertension, the unfavorable CC genotype was common with a frequency of 3.22%, and in the control group – with 0.42%. Risk assessment showed that the CC *MTHFR* A1298C genotype was associated with an increased predisposition to hypertension and increased its risk by 8.442 times ($p = 0.015$) [101].

Ischemic heart disease. MTHFR A1298C is also associated with coronary heart disease. In a study by Shivkar RR et al. on a sample of young patients with coronary heart disease, statistically significant differences in the distribution of allelic variants of the polymorphism in the patient group and in the control group were found ($p = 0.05$). For the *MTHFR* 1298 A/C (rs1801133) variant, the frequency of AA, AC, and CC was 8.9% versus 13.3%, 62.2% versus 77.8%, and 28.9% versus 8.9% in the group of young patients with coronary heart disease compared to the control group, respectively. The most common genotype in both groups was the heterozygous AC variant. However, when genotyping *MTHFR* 1298 A/C in the control group, a significant association was noted between the serum homocysteine level and the three genotypes ($p = 0.003$), while in the patient group this association was not statistically significant ($p = 0.82$), although the serum homocysteine concentration in patients was higher than in the control group [102].

Type 2 diabetes. The *MTHFR* rs1801131 gene polymorphism, like rs1801133, affects the folate cycle and homocysteine metabolism, which may indirectly affect all types of metabolism. Thus, in a study by Liu Y et al. on residents of Dali, Yunnan Province, China, it was found that the CC genotype (OR = 3.132, $p = 0.032$) in the *MTHFR* A1298C locus significantly increased the likelihood of developing type 2 diabetes. The level of fibrinogen and homocysteine was significantly higher in patients with type 2 diabetes mellitus with the CC or AC *MTHFR* A1298C genotype than in patients with the AA genotype. However, no significant association was

found between the *MTHFR* polymorphism and the risk of cardiovascular disease in type 2 diabetes [103].

Oncological diseases. When studying the effect of the rs1801131 polymorphism on the predisposition to urinary tract tumors, heterogeneous results were obtained: in some nationalities, the mutation led to a decrease in the risk of cancer, while in others, on the contrary, it increased. On the one hand, Xu S et al. found that the *MTHFR* rs1801131 A/C polymorphism was associated with an increased risk of developing urinary tract cancer (AC vs. AA: OR = 1.12, 95% CI = 1.01–1.24). On the other hand, the authors found that MTHFR rs1801131 A/C polymorphism may increase the risk of bladder cancer in both Asian (C allele vs. A allele: OR = 1.35, 95% CI = 1.15–1.60) and African populations (AC vs. AA: OR = 1.63, 95% CI = 1.17–2.25). However, no significant association was found between *MTHFR* rs1801131 A/C carriage and the risk of prostate cancer and renal cell carcinoma [98].

A meta-analysis assessing the associations between the *MTHFR* rs1801131 polymorphism and liver cancer risk from a total of seven studies showed that the homozygous CC genotype of the *MTHFR* rs1801131 polymorphism was significantly associated with a reduced risk of liver cancer (for CC vs. AA: odds ratio (OR) = 0.65, 95% CI 0.47–0.89, p = 0.007; for CC vs. AA + AC: OR = 0.65, 95% CI 0.48–0.89, p = 0.006) [104].

The impact of *MTHFR* rs1801131 polymorphism on predisposition to breast cancer has a negative effect. The wild-type AA variant was identified in 21% of the control group compared to 7.8% among women with breast cancer. In addition, the heterozygous AC genotype was observed in 65% of healthy controls compared to 57% of patients with cancer (p = 0.033). In the healthy control group, the homozygous CC genotype was less common (14% of cases) compared to 35.2% of cases in women with cancer (p < 0.001). Looking at the allele distribution, the A allele was detected in 43.4% of all participants, and was more common in the healthy control group (53.5%) than in women with cancer (36.3%). However, the C allele was the dominant allele, observed in 56.6% of participants in the overall study, 46.5% among the healthy control group, and 63.7% among the cancer cohort (p < 0.001). In women with the CC genotype of rs1801131, the adjusted odds ratio was 6.65 with a 95% confidence interval of 2.59–17.1 and a p-value of less than 0.001 when comparing homozygotes, indicating

a significant difference in the increased risk of developing breast cancer compared to women with the AA genotype [97].

Thus, the influence of the *MTHFR* rs1801131 polymorphism on the predisposition to cancer is controversial, since the data varies depending on the localization of the tumor process and the race of the study participants: the polymorphism has a protective effect in some, and a provocative effect in others.

Summarizing the results of studies on the polymorphism of the *MTHFR* rs1801131 and rs1801133 gene, we can conclude that mutant alleles C and T are associated with a predisposition to cardiovascular diseases, type 2 diabetes mellitus, but the effect on the predisposition to cancer is variable.

Methionine synthase (MTR) gene A2756G (Asp919Gly, rs1805087) polymorphism

A point mutation has been found in the methionine synthase gene located on the long arm of chromosome 1: adenine is replaced by guanine at position 2756 [105]. This mutation results in decreased enzyme activity due to the replacement of aspartic acid by glycine at position 919 [106] (in the protein-binding region of methionine synthase [107]) and accumulation of homocysteine in the blood [108]. The prevalence of the mutant allele G in the world is 19.19%, in Europe – 18.84% and in Asia – 11.98% [107].

Intrauterine development of the fetus. The effect of polymorphism on intrauterine fetal development is being studied. The case-control study by Efremova et al. involved 122 pregnant women with intrauterine growth retardation and 243 pregnant women with normal birth weight. The results showed that there is a connection between the G allele of rs1805087 of the *MTR* gene and the development of intrauterine growth retardation in accordance with the recessive model (OR = 3.01, 95% CI 1.05-8.68, p = 0.04) [109]. Thus, it is possible to suggest including this genetic test when planning pregnancy. And in the case-control study by Liu Y et al. involving 620 patients with congenital heart defects and 620 healthy individuals, 18 single nucleotide polymorphisms were identified and analyzed, including MTR A2756G. The analysis showed that the genetic polymorphism of the MTR gene in rs1805087 (GG vs. AA: OR = 6.85, 95% CI 2.94-15.96; dominant model: OR = 1.77, 95% CI 1.35-2.32; recessive model: OR = 6.26, 95% CI 2.69-14.54; addictive model: OR = 1.81, 95% CI 1.44-2.29)

was significantly associated with a higher risk of congenital heart disease [110].

Autism. The association of methionine synthase gene polymorphism with mental disorders is also observed. Thus, in the study by Haghiri R et al., the prevalence of *MTR* A2756G polymorphism was estimated in 108 children with autism and 130 children from the control group in northern Iran. The frequency of AA, AG and GG genotypes in children with autism was 57.41%, 22.22% and 20.37%, respectively, while in the control group it was 61.54%, 32.31% and 6.15%, respectively. A significant difference in the distribution of *MTR* gene polymorphism was found in the control group and the group of patients. The frequency of A and G alleles in children with autism was 0.69 and 0.31, while in the control group it was 0.78 and 0.22, respectively ($p = 0.03$). Carrying the *MTR* G allele increased the risk of developing autism by 1.6 times compared to the A allele (95% CI = 1.06–2.41, $p = 0.02$). The present study shows that the G allele of the *MTR* A2756G polymorphism is associated with an increased risk of developing autism [111].

Caries. Of interest are also studies in the field of dentistry that assess the relationship between the risk of caries and the MTR gene polymorphism. In a study by Chinese scientists, genotyping was performed using buccal mucosa cells from 150 healthy children and 150 children with caries in early childhood. The distribution of the AG genotype of the MTR rs1805087 gene in the caries group was significantly different from the control group ($p = 0.029$), and the risk of caries in patients with the AG genotype was 0.525 times lower than in patients with the AA genotype (95% CI = 0.292–0.942). Logistic regression analysis taking into account other clinical parameters showed that the rs1805087 AG genotype of the MTR gene remains closely associated with caries susceptibility (OR = 0.499, 95% CI = 0.273–0.913, $p = 0.024$) [112].

Oncological diseases. Since methionine synthase is associated with the methylation profile, its polymorphisms are considered as protective/provoking factors for oncological diseases. In the study by Zara-Lopes T et al., the association of the *MTR* rs1805087 polymorphism with the risk of developing thyroid cancer was assessed. According to the results of statistical analysis, no association of SNP with the risk of developing the disease was found, but as for clinical and histopathological parameters, the *MTR*

A2756G polymorphism was associated with tumor size (T) (OR = 2.69; 95% CI: 1.27–5.71; $p < 0.01$) and aggressiveness (OR = 4.51; 95% CI: 1.67–12.1; $p < 0.01$). No significant differences in regional lymph node (N) involvement and extrathyroidal spread were found depending on the presence of a particular *MTR* allele [113]. The functional impact of the A>G transition at position 2756 of the *MTR* gene was investigated by Galbiatti AL's team as a risk factor for head and neck squamous cell carcinoma. The frequency of the *MTR* A2756G (rs1805087) polymorphism was compared in patients with head and neck squamous cell carcinoma and in individuals without a history of cancer. Using univariate analysis, the results showed no significant differences in the distribution of alleles or genotypes. And multivariate analysis showed that tobacco and alcohol consumption ($p < 0.05$), AG genotype ($p = 0.019$) and G allele ($p = 0.028$) could be predictors of the disease, and a higher frequency of the G polymorphism was found in men with head and neck squamous cell carcinoma compared with men from the control group ($p = 0.008$). Analysis of polymorphisms in relation to clinical histopathological parameters did not reveal any association with primary localization, aggressiveness, lymph node involvement or tumor spread [114].

Methionine synthase reductase (MTRR) gene polymorphism A66G (Ile22Met, rs1801394)

Polymorphism of the methionine synthase reductase gene located on the short arm of chromosome 5 leads to post-translational modifications of the enzyme: replacement of isoleucine with methionine in codon 22 [107]. The presence of the mutant allele G causes a decrease in the activity of this enzyme by 4 times, which leads to hyperhomocysteinemia [115,116]. The G allele *MTRR* rs1801394 reduces the availability of S-adenosylmethionine by decreasing the level of active methionine synthase. The prevalence of the mutant variant G is quite high and is 51.51% in the world population and 54.4% in the European population, but only 26.94% in the Asian population [117].

Oncological diseases. The G allele of *MTRR* rs1801394 induces DNA hypomethylation by reducing the level of reduced methionine synthase, thereby modulating the risk of cancer. In a study of patients with colorectal cancer, Gunathilake M and team found that higher vitamin B_2 intake was

associated with a significantly lower risk of colorectal cancer (OR: 0.65; 95% CI: 0.51 – 0.82; $p < 0.001$). Carriers of at least 1 minor allele of *MTRR* rs1801394 showed a significantly higher risk of colorectal cancer (OR: 1.43; 95% CI: 1.12 – 1.83, $p = 0.02$). In men homozygous for the major allele A of *MTRR* rs1801394 and consuming more vitamin B_2, the risk of colorectal cancer was significantly lower (OR: 0.31; 95% CI: 0.18 – 0.54; $p = 0.02$) [107]. Kumawat R et al., when studying the association of single nucleotide polymorphisms in genes encoding enzymes involved in folate metabolism with glioma and meningioma in representatives of the Indian population, revealed a significant effect of the *MTRR* rs1801394 polymorphism on the development of the above-mentioned conditions. For *MTRR* A66G SNP, the main genotype in the control group was AG (47.08%), followed by AA (44.46%) and GG (12.46%). However, in patients, the main genotype was AA (58.3% in glioma, 57.9% in meningioma), followed by the AG genotype (40.7% in glioma and 42.1% in meningioma) and the GG genotype (0.9% in glioma and no cases in meningioma). In patients with glioma, there was a significant difference in the distribution of genotypes and allele frequencies compared to the control group for *MTRR* A66G (95% CI: 0.32–0.97, p=0.039). The probability of developing glioma with the AG genotype was 44% lower compared to the AA genotype, and the probability of developing glioma with the G allele was 37% lower compared to the A allele (95% CI: 0.41–0.99, p=0.045) [118].

Intrauterine development of the fetus. Elevated homocysteine levels in the mother's blood or amniotic fluid caused by folate cycle disorders have been shown to correlate with the development of congenital heart defects in the embryo. In a study by Guo QN et al., parents who had congenital heart defects in their fetuses diagnosed during pregnancy were divided into two subgroups: those with ventricular septal defects ($n = 21$) and those without ($n = 78$). The *MTRR* 66G allele frequency was significantly higher in parents with ventricular septal defects in the fetus than in parents in the control group (mothers: 40.5% vs. controls 21.9%, $p < 0.05$; fathers: 38.1% vs. controls 21.1%, $p < 0.05$). Thus, the *MTRR* 66 G allele in both mother and father was an independent risk factor for fetal congenital heart defects (mother: OR = 2.421, 95% CI: 1.213–4.833; father: OR = 2.308, 95% CI: 1.147–4.645; $p < 0.05$). The frequency of the *MTRR* 66 GG genotype was significantly higher in mothers with fetal congenital heart defects

(14.3% vs. 7.7% in the control group, $p < 0.05$), and the GG genotype increased the risk by 9.571 times (OR = 9.571, 95% CI: 1.615–56.736, $p < 0.05$) of fetal congenital heart defects compared to the CC genotype [119].

Ischemic heart disease. According to the results of the Timizheva KB team, heterozygotes AG of the *MTRR* rs1801394 polymorphism were more common among patients with coronary artery disease under 65 years of age ($p = 0.003$), and homozygotes GG were more common in the group of patients with late restenosis (12 months after balloon angioplasty and stent placement). Young people are predisposed to restenosis if they are carriers of the *DNMT3B* rs1569686 TT or *MTRR* rs1801394 AG genotypes. Early restenosis is associated with the homozygous genotype *MTHFR* rs1801131 CC; late restenosis is more common in homozygotes *MTR* rs1805087 GG and *DNMT3B* rs1569686 TT. The results of this study can be used for a comprehensive assessment of the risk of developing ischemic stroke, determining the optimal tactics and individual approach to the treatment of patients with coronary heart disease before or after percutaneous coronary interventions, including treatment that lowers homocysteine levels in patients with hyperhomocysteinemia and a high risk of developing ischemic stroke [108].

Folate transporter gene polymorphism (SLC19A1) A80G (His27Arg, rs1051266)

The *SLC19A1* gene (*RFC1, IFC1*) encodes a protein that is a folate transporter and regulates the intracellular concentration of folate. The *SLC19A1* gene is located on chromosome 21 at position 21q22.3 and contains six exons. The protein encoded by the *SLC19A1* gene is called folate transporter 1 and contains 591 amino acids. It normally regulates the absorption and distribution of folate by capturing it using membrane vesicles (receptor-mediated endocytosis mechanism). This mechanism functionally links three components: the folate receptor, folate transporter 1, and the V-type proton pump [120]. The *SLC19A1* transporter is mainly localized on the apical membrane of the brush border of the jejunum and colon and is considered the main pathway for folate absorption in mammalian cells [121]. *RFC1* rs1051266 contains a base substitution of A at position 80 to G, resulting in a histidine to arginine amino acid substitution at position 27, which may affect splicing regulation, transcriptional regulation, and post-translational modification [122].

Folate transporter polymorphism reduces intracellular folate concentration, which is generally negative, but may be beneficial in certain cases. For example, *SLC19A1* rs1051266 promotes a more rapid response to antimetabolite chemotherapy (methotrexate, fluorouracil). Low folate levels may enhance the ability to form a complex that inhibits thymidylate synthase and cell division. The study by Huang L et al. showed that people with the GG rs1051266 genotype have lower plasma folate levels, which contributes to better drug performance. In the rs1051266 GG group, the relapse rate was 45.8% (11 of 24 patients) compared to 19.4% (12 of 62 patients) in the rs1051266 (GA/AA) group, $p = 0.013$. The authors also found that the common *SLC19A1* rs1051266 GG single nucleotide polymorphism, which was present in 29.9% of the sample participants, was associated with a higher rate of rapid response to the FOLFIRI/mCapeIRI regimen ($p = 0.019$) [121].

In a study by Chatterjee M et al., the influence of the rs1051266 80A>G gene variant in the development of Down syndrome in the population of East India was investigated. It was noted that in patients with Down syndrome, a more frequent occurrence of the mutant allele rs1051266 G was observed ($p = 0.05$) [122].

To summarize this section, it is important to say that the presence of unfavorable gene variants does not mean 100% development of pathological conditions. In the absence of other provoking risk factors, such as physical inactivity, unbalanced diet, smoking, alcohol abuse, etc., these polymorphisms may not manifest themselves, since the nature of diseases is often multifactorial.

Situational tasks

Task 1. Patient A, 35 years old with the diagnosis of usual pregnancy failure, has applied to gynecologist about premarital training. Previously, the patient was performed a molecular genetic study to identify polymorphism of folate exchange genes.

Results of genetic research: MTHFR-677TT, MTHFR-1298AA, MTR-2756AG, MTRR-66GG.

1. What additional studies should be ordered?

2. What recommendations should be given to the patient for the correction of folate exchange?

Task 2. Patient G. 72 years old, went to the polyclinic to a physician with complaints about general weakness, increased fatigue, dizziness, shortness of breath, heart palpitations during physical exertion, reduced appetite, nausea, abdominal pain. During the examination, there is a lack of skin tissue and skin damage, and when palpation occurs, the lower edge of the liver emerges from under the rib arch by 2 cm. body temperature up to 37.2C.

Clinical blood analysis: Hgb 78 g/l, RBC 2.8 mln/µl, MCV 180 fl. The blood smear shows a basophilic puncture, a Zholli and a Keaton ring.

Biochemical blood test: total bilirubin 45.8 µmol/l, direct bilirubin 22.8 µmol/l, LDH 650 U/l, AST 80 U/l, ALT 75 U/l. Homocysteine 38.4 µmol/l, vitamin B12 600 pg/ml, folic acid 1.2 ng/ml

1. The patient should be diagnosed and treated.

Task 3. Patient S, 30 years old. Admitted to the neuroreanimation department with a diagnosis of acute cerebrovascular accident. History of smoking, 15 years of smoking, 20 cigarettes per day. History of deep vein thrombosis of the lower extremities.

Coagulogram: APTT 26 sec, fibrinogen 6.2 g/l, INR 0.9.

Biochemical blood test: ALT 40 U/L, AST 38 U/L, bilirubin 19 µmol/L, total cholesterol 7.38 mmol/L, HDL cholesterol 1.0 mmol/L, LDL cholesterol 4.7 mmol/L, triglycerides 2.0 mmol/L, homocysteine 69.7 µmol/L, folic acid 3.0 ng/ml.

1. What studies in personalized medicine should be prescribed to the patient?

Task 4. During the preventive examination, the patient was found to have erythrocyte macrocytes in the general blood test.

1. What additional studies can be recommended for diagnosis?

Task 5. Name the anolyte that causes the most damage compared to homocysteine. The process leading to cassava-independent death of endothelial cells, formation of neoantigens.

Task 6. Describe a possible variant of the eczyotoxic effect of homocysteine on leukocytes and neurons.

Test assignments

1. The following changes in the general blood analysis are characteristic of megalobular anaemia.

A. Macrocytosis, anisocytosis, pocketocytosis, hyperchromia, basophilic erythrocyte puncture, Jolli cell and Keaton ring,
B. Microcytosis, hypochromia, decreased concentration of pherin, serum iron,
C. Microcytosis, Normochromia, Reticulocytosis.

2. Causes of folate metabolism disorders.

A. Nutritional deficiency,
B. Polymorphism of folate exchange genes,
C. Violation of absorption of vitamins of group B,
D. Taking drugs that affect the metabolism of group B vitamins,
E. All of the above.

3. Polymorphism of which genes affects folate exchange

A. Acetaldehyde dehydrogenase
B. Methylenetetrahydrofolate reductase
C. N-acetyltransferase
D. Alcohol dehydrogenase

4. The frequent manifestation of hyperhomocysteinemia is:

A. Habitual pregnancy failure
B. Megaloblastic anemia
C. Vascular disorders
D. All of the above

5. Homocysteine is deactivated during the reaction:

A. Remethylation
B. Oxidation
C. Reduction
D. Conjugation

6. Folate metabolism disorders can occur when:

A. Vitamin deficiency
B. Enzyme polymorphism
C. Disruption of transport proteins
D. All of the above

7. What the polymorphism of folate exchange genes leads to:

A. Alcoholism
B. Reduced activity of the folate cycle enzymes
C. Renal failure
D. Dementia

8. Hyperhomocysteinemia is

A. increase in the concentration of total homocysteine in blood plasma above the permissible limits
B. increase in the concentration of mutant homocysteine in blood plasma above the permissible limits
C. increase in the ratio of free homocysteine to bound
D. increase in the ratio of bound homocysteine to free

9. Acceptable homocysteine levels in children:

A. Less than 5 μmol/L
B. Less than 10 μmol/L
C. Less than 15 μmol/L
D. Less than 20 μmol/L

10. Acceptable levels of homocysteine in adults:

A. Less than 5 μmol/L
B. Less than 10 μmol/L
C. Less than 15 μmol/L
D. Less than 20 μmol/L

11. Men have an average plasma homocysteine level of ... μmol/L higher than women of reproductive age:

A. 1
B. 2

C. 5
D. 10

12. Mild hyperhomocysteinemia is characterized by homocysteine levels:

A. up to 10 μmol/l
B. from 11 to 30 μmol/l
C. 31-100 μmol/l
D. more than 100 μmol/l

13. Moderate-severity hyperhomocysteinemia is characterized by the level of homocysteine:

A. up to 10 μmol/l
B. from 11 to 30 μmol/l
C. 31-100 μmol/l
D. more than 100 μmol/l

14. Severe hyperhomocysteinemia is characterized by the level of homocysteine:

A. up to 10 μmol/l
B. from 11 to 30 μmol/l
C. 31-100 μmol/l
D. more than 100 μmol/l

15. Normal level of homocysteine:

A. up to 10 μmol/l
B. from 11 to 30 μmol/l
C. 31-100 μmol/l
D. more than 100 μmol/l

16. Causes of hyperhomocysteinemia:

A. Hereditary defect of cystathionine synthase
B. Vitamin K deficiency
C. Iron deficiency
D. Hereditary defect of homogene transferase

17. Choose the correct statement:

A. Homocysteine enters the human body with thermally unprocessed food
B. Homocysteine is biosynthesized from methionine in a multi-stage process
C. Homocysteine levels gradually decrease throughout life
D. Homocysteine levels are usually lower in men than in women

18. Which enzyme is involved in the synthesis of homocysteine from methionine?

A. Adenosylhomocysteinase
B. Adenosine deaminase
B. Nucleotidase
G. Thymidine synthase

19. Which enzyme is involved in the remethylation of homocysteine into methionine?

A. Methionine synthase
B. Ubiquitinase
C. Homocysteine transferase
D. Reductase

20. Which pregnancy disorders are caused by hyperhomocysteinemia?

A. Preeclampsia
B. Edwards syndrome
C. Patau syndrome
D. Parkinson's disease

21. For the prevention of defects in the neural tube development of the fetus is used:

A. Prescription of folic acid
B. Sunbathing
C. Prescription of heparins
D. Routine prophylaxis is not indicated

22. Methylation of DNA in the promoter area of the gene leads to:

A. Suppression of gene expression

B. Increased gene expression
C. No effect on gene expression level

23. Epigenetic regulation of the genome includes
A. Nonsense mutations
B. DNA methylation
C. Aberrations
D. Frame shift mutations

24. «Foam cells» is it?
A. Macrophage filled with fatty inclusions.
B. Epithelial cell with bacteria attached to its surface
C. Mature neutrophil

25. Signs of megalobular anemia
A. Microcytosis
B. Reduced RBC
C. Increased serum B_{12}

26. Signs of iron deficiency anemia
A. Macroovalocytes
B. Increased RBC
C. Increased serum folate
D. Microcytosis

27. The chronic disease anemia marker is
A. Hepcidin
B. Homocysteine
B. Methionine
G. Cysteine

28. Homocysteine level in blood serum can be determined
A. ELISA method
B. Real-time PCR
C. Bacteriological method
C. The method of hookah plates

29. In which cases it is preferable to prescribe active metabolite folate rather than folic acid monohydrate.

A. FGB gene polymorphism: 455 G>A (A/A homozygous variant)
B. polymorphism of the gene MTHFR 677 C>T gene (T/T homozygous variant)
B. ITGB3 gene polymorphism: 1565 T>C (C/C homozygous variant)

30. The state of hyperhomocysteinemia causes

A. hypocoagulation
B. hypercoagulation
C. does not affect the coagulation capacity of the blood

31. The main form of homocysteine present in blood flow:

a) free,
b) albumin-bound,
c) cysteinyl homocysteine disulfide,
d) oxidized form.

32. Which cells are the most active blood plasma «suppliers» of homocysteine?

a) neurons,
b) epithelial cells,
c) enterocytes,
d) actively proliferating cells.

33. Transsulfation of homocysteine occurs in

a) pancreas and small intestine,
b) kidneys,
c) liver,
d) lungs.

34. Which enzyme is involved in the formation of homocysteine?

a) methionine adenosyltransferase,
b) adenosylhomocysteinase,
c) cytinosyl-5-methyl transferase,
d) methionine synthase.

35. In which cells can folate-independent remethylation and homocysteine transsulfuration occur?

a) endothelial cells,
b) thyrocytes,
c) enterocytes,
d) hepatocytes and kidney cells.

36. Oxidative stress does not cause development

a) endothelial dysfunction,
b) increased synthesis of adhesion molecules and growth factors,
c) inactivation of apoptosis,
d) increased platelet aggregation and thrombus formation.

37. Process of homogenization for lysine residues is carried out

a) cystathione,
b) dimethylarginine,
c) cysteinyl homocysteine,
d) homocysteine thiolactone.

38. The presence of autoantibodies to N-homocysteine albumin is an independent predictor

a) early development of coronary heart disease,
b) thyroiditis,
c) tumor process,
d) ulcerative colitis.

39. Hyperhomocysteinemia increases the procoagulant activity of blood plasma due to

a) increasing the activity of antithrombin III,
b) suppressing the activation of factor V,
c) increasing the number of receptors for tissue plasminogen activator,
d) suppressing the expression of surface thrombomodulin and activation of protein C.

40. Endothelial dysfunction is associated with

a) disruption of the transsulfuration process,
b) decreased production and/or bioavailability of NO,

c) decreased synthesis of endothelin-1,
d) depression of NMDA-receptors.

41. Mechanism of the harmful action of homocysteine on brain functions:

a) DNA hypomethylation,
b) NMDA-receptor depression,
c) hypophosphorylation,
d) apoptosis blocking.

42. The main symptoms of vitamin B_6 deficiency do not include:

a) immunodeficiency,
b) cognitive decline,
c) dementia,
d) autonomic dysfunction.

43. For the activity of the enzyme methioninthase is necessary

a) tetrahydrofolate,
b) 5-methyltetrahydrofolate,
c) methenyltetrahydrofolate,
d) folacin.

44. What is the role of B_{12} in homocysteine metabolism?

a) is a methyl group donor,
b) is a sulfide group donor,
c) is a cofactor for the enzyme methionine synthase,
d) is a carrier of a one-carbon fragment.

45. What forms of vitamin B_{12} are used in pharmaceuticals?

a) dimethylbenzimidazole,
b) corrin,
c) adenosylcobalamin,
d) cyanocobalamin.

46. The cause of macrocytosis is

a) blocking NMDA- receptors,
b) free radical oxidation,

c) activation of procaspase 8,
d) disruption of DNA synthesis.

47. The most effective vitamin B_{12} deficiency assessment test is
a) total serum vitamin content,
b) plasma homocysteine level,
c) complete blood count,
d) transcobalamin level.

48. The main food sources of methionine are
a) meat, fish, eggs, dairy products,
b) legumes, dried fruits, peanuts,
c) spinach, lettuce, mustard greens,
d) bananas, raisins, mango.

49. Why does choline deficiency lead to hyperhomocysteinemia?
a) the transsulfuration process is disrupted,
b) choline is a methyl donor,
c) choline is a cofactor for the enzyme cystathionine synthase,
d) the enzyme methylenetetrahydrofolate reductase is blocked.

50. The coenzyme of cystathionine synthase is
a) choline,
b) betaine,
c) vitamin B_6,
d) niacin.

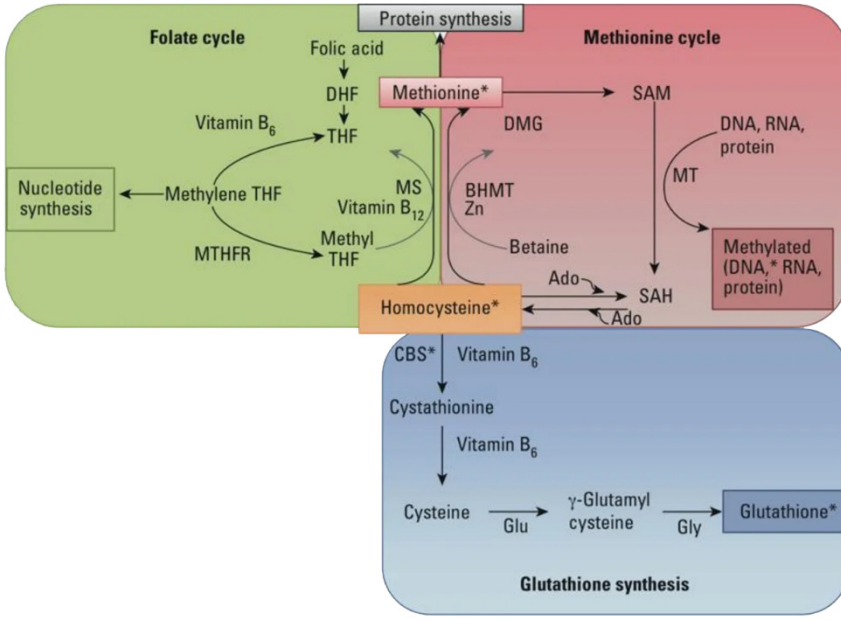

Fig. 7. Cycle production of homocysteine (https://www.semanticscholar.org/paper/FOLLICULAR-FLUID-HOMOCYSTEINE-WITH-ACETYL-CYSTEINE-Hebisha-Mahmoud/beda5e400df87ee5c7038ca86a4dea9b292ba96d/figure/0)

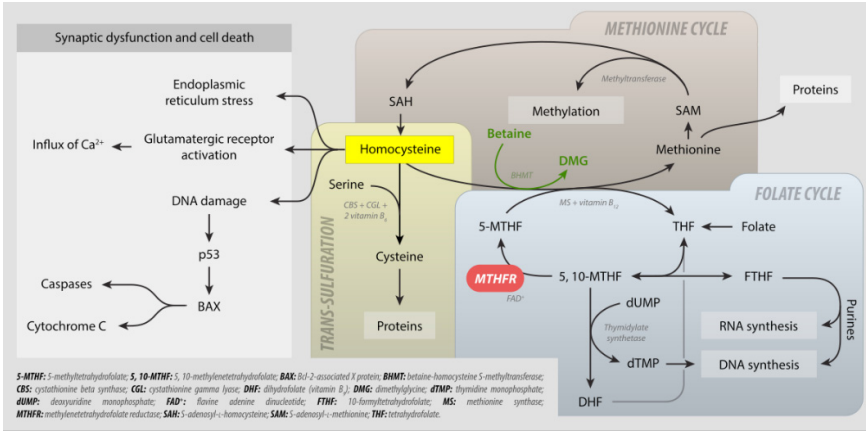

Fig. 8. Folate cycle and homocysteine metabolism (https://ru.wikipedia.org/wiki/Файл:MTHFR_metabolism.svg)

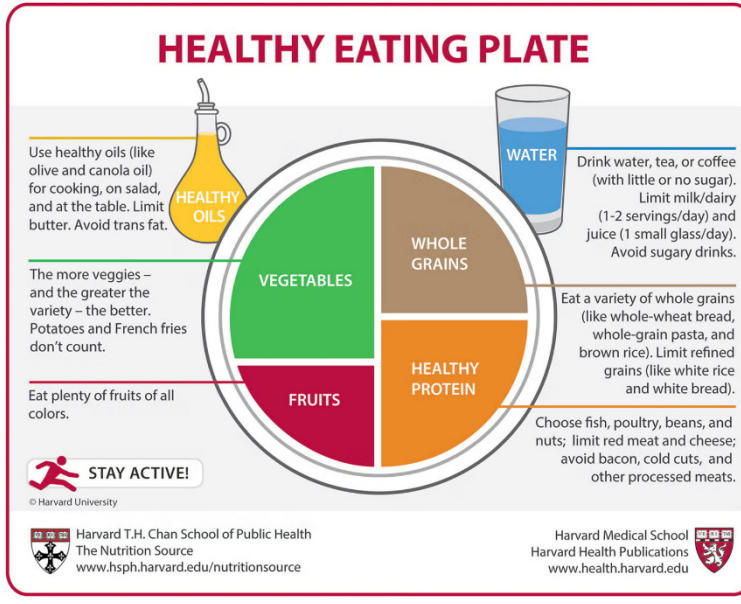

Fig. 9. Healthy eating plate (https://nutritionsource.hsph.harvard.edu/healthy-eating-plate/)

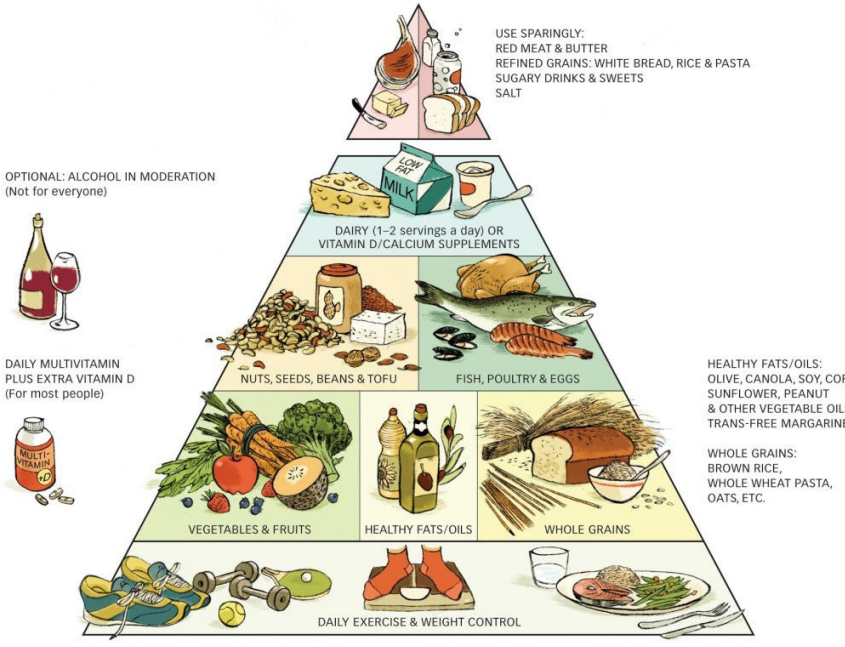

Fig. 10. Healthy eating pyramid (https://nutritionsource.hsph.harvard.edu/healthy-eating-pyramid/)

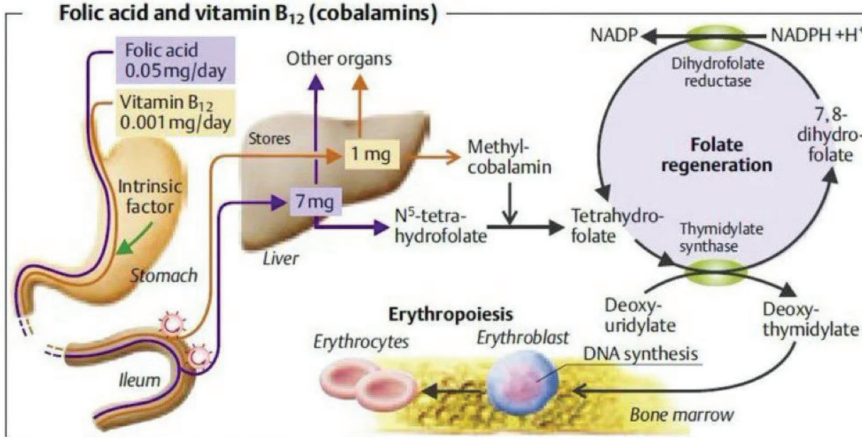

Fig. 11. The Importance of Folic Acid and Cobalamin in the Body (https://dzen.ru/a/XFGEzsRzuACtInhC)

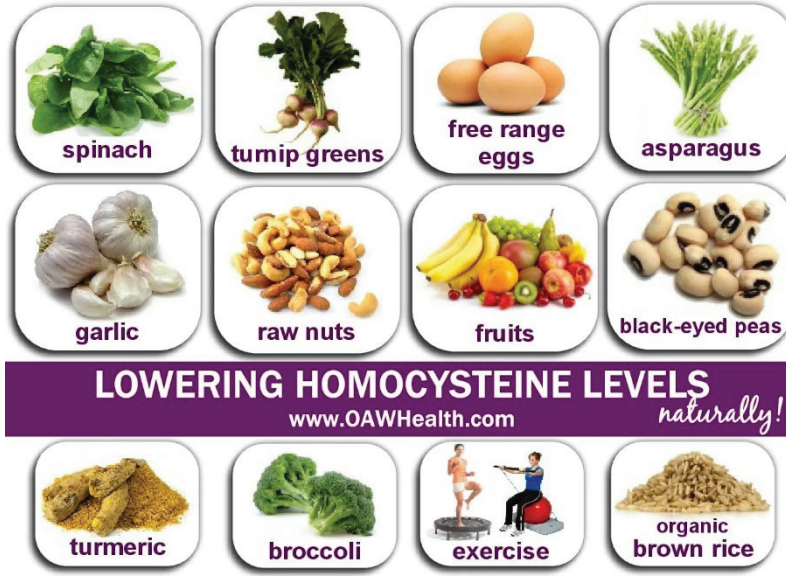

Fig. 12. Products recommended for elevated homocysteine levels (https://www.pinterest.com/pin/methionine--676243700273977590/)

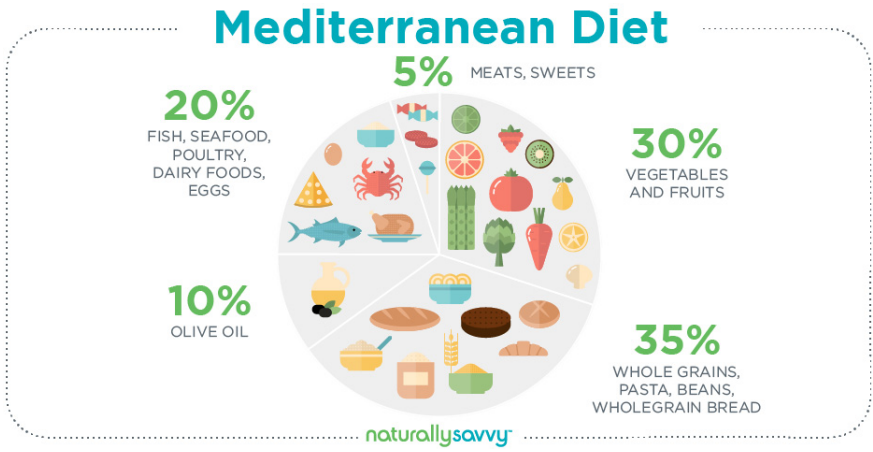

Fig. 13. Mediterranean diet rich in folate (https://elispot.biz/about-all/healthy-a1c-number-understanding-a1c-ada.html)

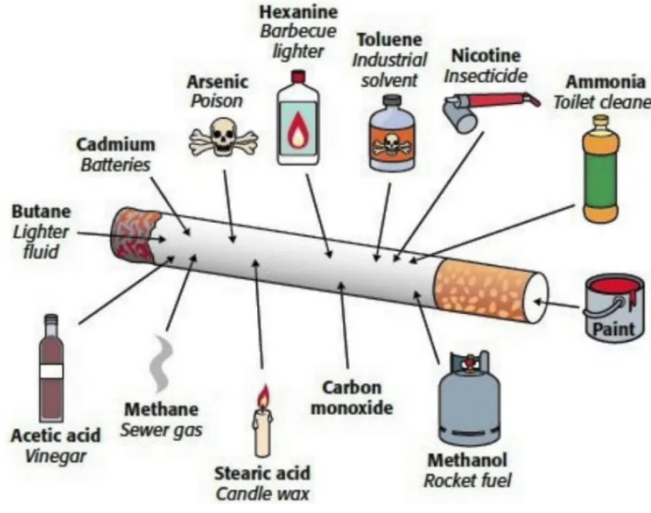

Fig. 14. Toxic substances found in cigarettes (https://storage.googleapis.com/dadgmpfirepvve/are-cigarettes-more-dangerous-now.html)

Fig. 15. Methods of delivering nicotine to the body (smoking options) (https://vk.com/wall-117728204_1969)

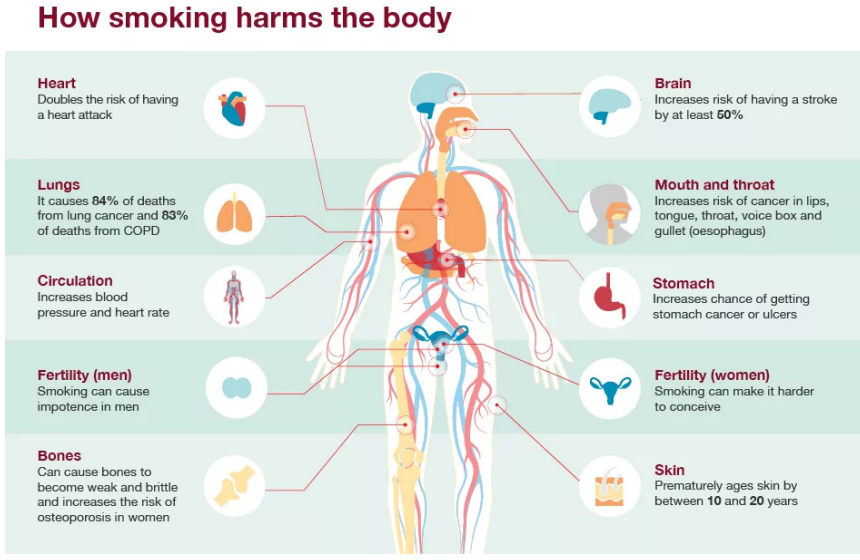

Fig. 16. The impact of smoking on the human body (https://ru.pinterest.com/pin/632474341402316590/)

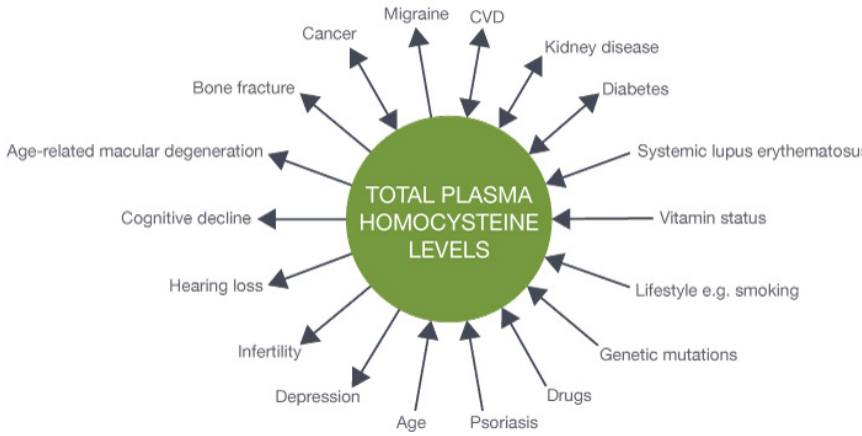

Fig. 17. The impact of hyperhomocysteinemia on the human body (https://elispot.biz/about-all/homocysteine-levels-range-reference-range-interpretation-collection-and-panels.html)

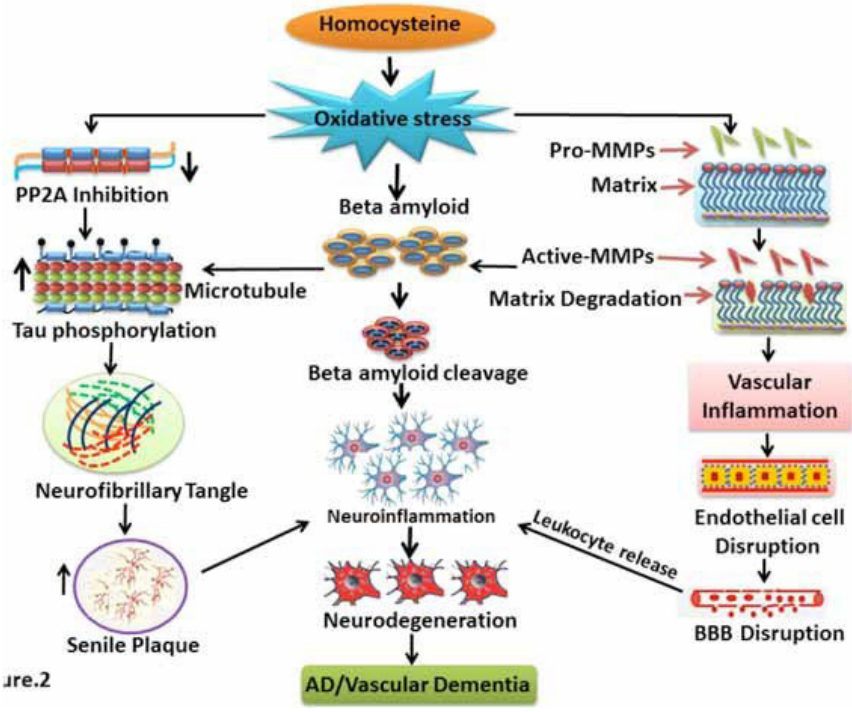

Fig. 18. Homocysteine and vascular dementia (https://www.researchgate.net/figure/Fig-2-Homocysteine-and-Vascular-Dementia-Flow-diagram-showing-the-mechanism-of_fig8_8570461)

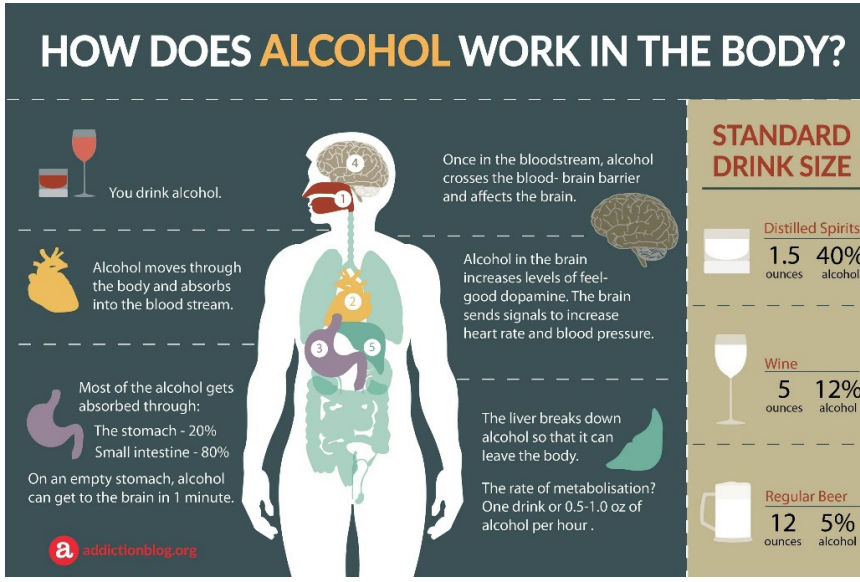

Fig. 19. How does alcohol work in the body? (https://mavink.com/post/CB77141E50D080D3F6687076BD830390BCAM89CC57/Alcohol-in-the-body:-How-drinking-affects-the-body-and-brain-(INFOGRAPHIC))

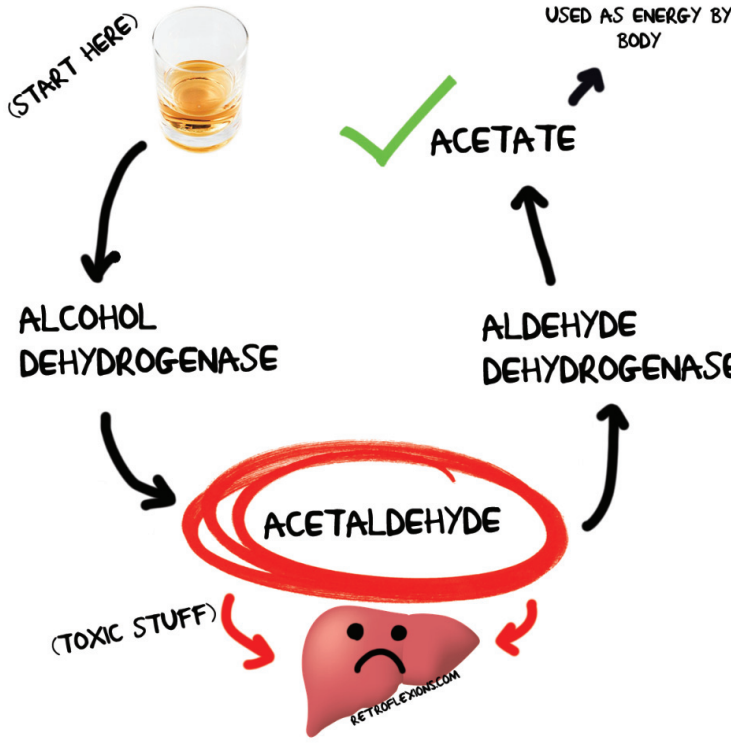

Fig. 20. Alcohol metabolism (https://storage.googleapis.com/dtveqahzomnove/digestive-enzymes-liver-damage.html)

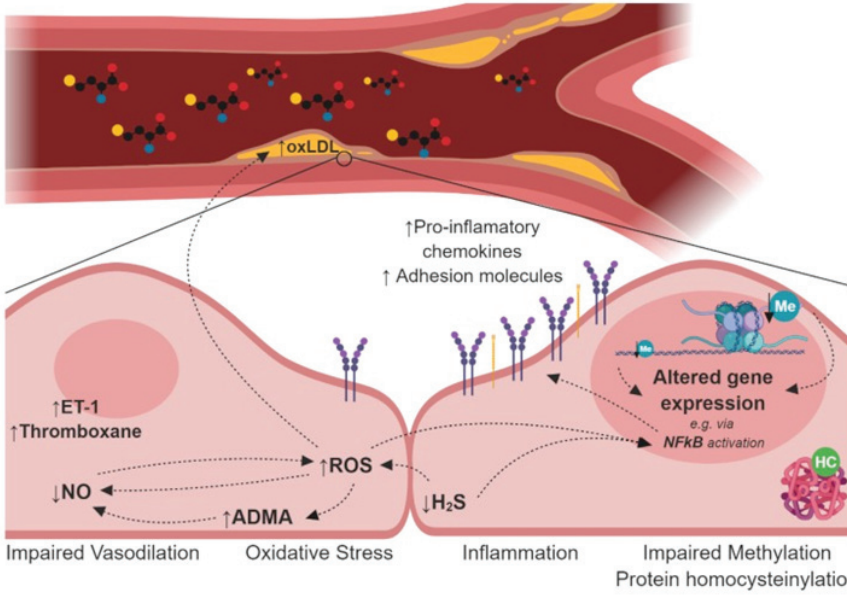

Fig. 21. The relationship between hyperhomocysteinemia and atherosclerosis (https://www.researchgate.net/figure/Schematic-representation-of-the-leading-mechanisms-proposed-to-underlie-the-implication_fig2_331173007)

Fig. 22. The Impact of MTHFR Gene Polymorphism on Pregnancy (https://womanhall.ru/polimorfizm/gena/mthfr/)

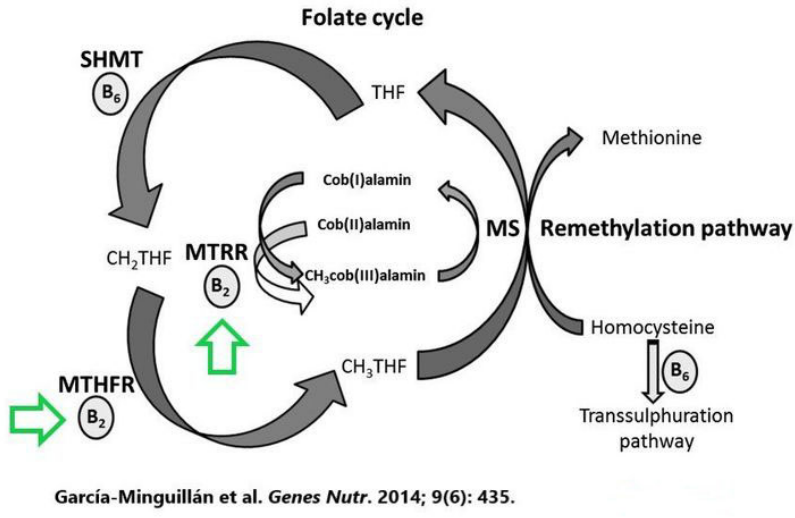

Fig. 22 Riboflavin Can Fix The MTHFR Polymorphism (https://yandex.ru/images/search?from=tabbar&img_url=https%3A%2F%2Fwww.orthokennis.nl%2FUploads%2Forthokennis%2Fafb-artikel-5-MTHF.)

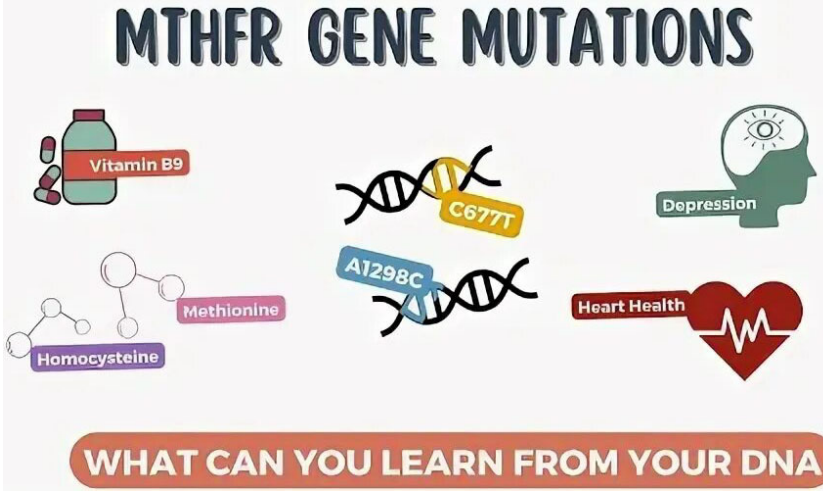

Fig. 23. MTHFR gene mutations (https://www.youtube.com/watch?v=5fP_IhddSdQ)

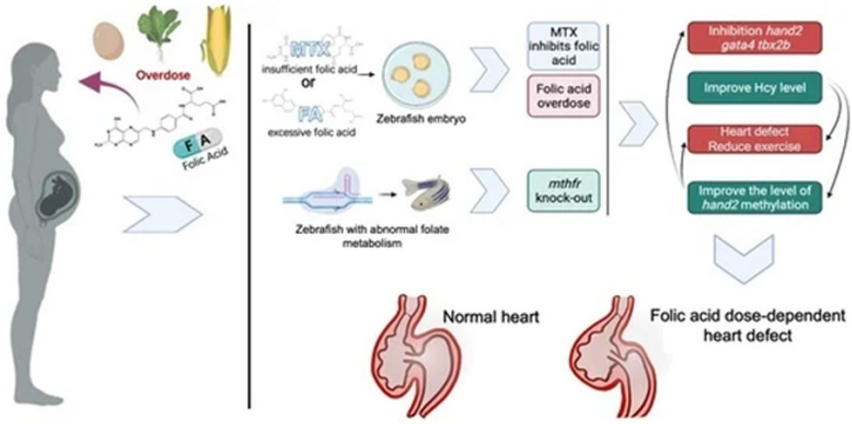

Fig. 24 The effect of maternal folate levels on fetal heart development https://g-academy.ru/news/vliyanie-urovnya-folatov-u-materi-na-razvitie-serdca-ploda

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Educational edition

Vorobyeva Nadezda Aleksandrovna
Vorontsova Alexandra Sergeevna
Murashkina Alexandra Alekseevna

HEALTHY LIFESTYLE AS A PREVENTIVE MEASURE FOLATE METABOLISM DISORDERS

Study guide

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«Северный государственный медицинский университет»
Министерства здравоохранения Российской Федерации
163000, г. Архангельск, пр. Троицкий, 51
Телефон (8182) 20-61-90. E-mail: izdatelnsmu@nsmu.ru