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ADVERSE EFFECTS OF ANTIOXIDATIVE VITAMINS

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Abstract
High doses of synthetic antioxidative vitamins: A, E, C and β-carotene are often used on long-term basis in numerous preventive and therapeutic medical applications. Instead of expected health effects, the use of those vitamins may however lead to cases of hypervitaminosis and even to intoxication. The article points out main principles of safety which are to be observed during supplementation with antioxidative vitamins. Toxic effects resulting from erroneous administration of high doses of those substances on organs and systems of the organism are also discussed. Attention is drawn to interactions of antioxidative vitamins with concomitantly used drugs, as well as intensification of adverse effects caused by various exogenous chemical factors. Moreover, the article presents the evaluation of supplementation with these vitamins, which was performed in large studies.

Key words:
Vitamins A, E and C, β-carotene, Supplementation, Side actions, Toxic effects

INTRODUCTION

Supplementation with synthetic antioxidative vitamins: A, β-carotene (provitamin A), E and C, is widely propagated to-day, both as an adjunct to the applied pharmacotherapy and as one of factors to provide ”health and beauty”. Common use of additional intake of the above mentioned supplements is well-known [1,2].

Such popularised usage of antioxidative vitamins stems mainly from the fact they are treated as natural substances (even though today they are mostly products of organic synthesis) and regarded as safe and free from any significant toxicity. That is why they do not have pharmaceutical status of medicines, but are included only among auxiliary agents as ”dietary supplements”. As a result, they are not subject to any rigorous safety tests specific for pharmaceutical drugs, and their preparations are generally sold over the counter. However, it is commonly disregarded that the excessive intake of antioxidative vitamins may be in fact harmful. Enthusiasts of at-home vitamin supplementation, who blindly believe in newspaper revelations and advertisements, are therefore vulnerable to overdose and – as a result – may experience concealed adverse effects.

The problem of those effects emerged on a large scale during medical trials of vitamin supplementation, after discovering the disorders of oxygenic metabolism in pathogenesis of numerous diseases and introducing vitamins A, E C and β-carotene as antioxidants in adjunctive therapy. In order to achieve the assumed efficiency of antioxidative effects, these vitamins were applied on long-term basis in arbitrarily set high doses [3–6]

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in accordance with the motto "more means better". As a result, their bodily concentrations many times exceeded the physiological values. Large-scale research testing the effectiveness of such vitamin supplementation revealed, however, that in most cases the hopes were not fulfilled, while – surprisingly enough – multiple adverse effects appeared. Therefore, authors of some publications started to warn against supplementation conducted in this way [e.g. 7].

Data on adverse effects of antioxidative vitamins, so important for the safety of planned supplementation trials on selected groups of patients, are dispersed in literature and very often fragmentary or only superficial (as was already noted by some other authors [8,9]). This is the rationale behind this article – its aim is to gather and systematise possibly the most complete data to present the current state of knowledge. We hope this article will go a long way towards paying more attention to the discussed and sometimes overlooked issues connected with vitamin therapy – nowadays common, but often applied without due prudence.

To proceed with our discussion, please note that the data presented below is based on various isolated and often incidental observations. Only few data come from fragmentary research on animals (tests of vitamins A and E on pregnant rats) or healthy volunteers (tests on the influence of high doses of vitamin C on the state of health). Information on acute vitamin A toxicity was provided by incidents of unintentional intake of excessive amount of this vitamin. Other highly harmful effects were consequences of random mistakes in medically applied doses of particular vitamins. Data regarding chronic outcomes was gathered as a side effect of a long-term supplementation with antioxidative vitamins, applied in diseases described in the text below. The authors used also some information adduced by the quoted vitamin experts.

VITAMIN A

Biomedical action

Vitamin A (retinol) performs many important physiological functions in human body [1,2]. It accelerates the synthesis of collagen and elastin fibres by fibroblasts, as well as the processes of cell division, thanks to which it stimulates growth of foetus and young organisms. It ensures proper proliferation and differentiation of epithelial cells, contributing also to their regeneration and, owing to this, it significantly influences proper functioning of skin and mucous membranes. Vitamin A positively influences the development of skeleton by regulating activities of osteoblasts and osteoclasts. It participates in hormonal regulation of the body by decreasing secretion of thyroxin (one of thyroid hormones) by means of suppressing production of thyrotropin by the pituitary gland. It is also essential for the process of seeing, as it is needed (in a form of 11-cis-retinal) for synthesis of rhodopsin, also known as visual purple, in the retina of the eye [2,10]. Furthermore, vitamin A stimulates functioning of the immune system in the body and increases its resistance to infections [11].

A vital property of vitamin A is also its antioxidative action, including both prevention against reactive oxygen species (ROS) and termination of reactions taking place with their participation [12–14]. Very crucial is this vitamin's ability to quench singlet oxygen $^1O_2$, as well as trap and neutralize free radicals [12,15]. The above described action arises from the presence of the conjugated C = C bonds in the side chains of its molecules. These bonds compete in addition reactions of ROS and free radicals to polyunsaturated fatty acids contained in lipids of cell membranes, and in this way protect the cells against peroxidative damage. At the same time vitamin A suppresses the activity of enzymes participating in propagation of peroxidation of these lipids, as well as prevents oxidative disorders of protein glycosylation in cell membranes [13,14]. The presented antioxidative mechanisms have their significant contribution to the previously mentioned impact

of vitamin A on proliferation and differentiation of epithelial cells, as they prevent their neoplastic transformation (promotion stage) induced by processes of uncontrolled oxidation. This concerns especially incorrect glycosylation of proteins in cell membranes, which influences their surface properties – transformed cells have fewer surface glycoproteins than regular ones [16–18]. Vitamin A stabilizes also thiol groups (–SH) of membrane proteins and suppresses oxidatively stimulated expression of c-myc oncogene on chromosome 8. It enables also the recovery of intracellular communications via the already transformed cells [16,18]. Due to the fact that around 90% of neoplasms are developed in epithelial tissues, the discussed vitamin has particular significance for these tissues in terms of its preventative and suppressive effect [17–19]. This concerns various parts of the body containing epithelial tissues, including: nasal and throat cavity, oesophagus, stomach, intestines, respiratory tract, bladder, as well as prostate (suppression of its overgrowth).

Potential risk

Therapeutic intake of large amounts of vitamin A, exceeding the limited storage capacity of the liver, may lead to the supersaturation of the body with this vitamin and the poisoning referred to as hypervitaminosis A – the blood concentration of the a/m vitamin > 1.0 mg/l (3.5 μmol/l) is indicative of that condition [20,21]. Toxic effects appear when the ability of plasma RBP (retinol binding protein – the carrier for vitamin A in blood) to bind retinol and to conjugate its excess with glucuronate is exceeded, which leads to harmful exposure of the cells to free vitamin A [14,22,23]. Patients with hepatic and renal diseases and children are especially susceptible to the adverse effects of this vitamin. The supplementation with provitamin A, i.e. β-carotene, is safer [2,24].

Hypervitaminosis A can take the form of acute or chronic poisoning. The first of these two forms appears after the intake of 150–1200 mg (in International Units: 500 000––4 000 000 IU) of vitamin A over two days by adults, after the single dose about of 45 mg (150 000 IU) by school-age children, or after the intake about of 22 mg (75 000 IU) by small children. This kind of poisoning appeared, for example, in preschool children (supplemented with this vitamin for prevention against nyctalopia), who were mistakenly given in single doses as much as 500 000 IU of vitamin A. As a result, over 20 of these children died [25]. An extreme case was a fatal poisoning of polar explorers after the consumption of polar bear liver (where vitamin A content is approx. 10 mg/g) [3,26,27]. The chronic poisoning results from the long-term intake of this vitamin (ranging from several weeks to more than 3 years) at the doses > 30 mg (100 000 IU)/d by adults, or for 2 to 6 months at the doses of 3–9 mg (10 000–30 000 IU)/d by children, and for 1–3 months at the dose of 3 mg (10 000 IU)/d by infants.

The symptoms of acute poisoning are: general weakness, dizziness and a severe headache (in the occipital part), an increase in intracranial pressure, nausea, vomiting, sitophobia, hepatosplenomegalia, and after a few days, skin changes (itching, erythema, and desquamation). The chronic poisoning symptoms include: feeling of chronic fatigue, irritability, double vision, nystagmus, sleep disturbances, cracking and bleeding lips and gums, shedding of hair, rashes and ulceration of the skin, muscular coordination disturbances, renal dysfunction (haematuria), and even oedema of the optic disc and other symptoms that could suggest a brain tumor. Hypervitaminosis A can also lead to auditory sensations which are not caused by any sound stimulus (parestesia) [22,23,28,29].

The excessive intake of vitamin A affects harmfully the haematopoietic system, leading to aplastic anemia. It accelerates erythrocyte sedimentation rate and prolongs the prothrombin time. It also causes leukopaenia, weakening at the same time the defense ability of the body [14,24,27]. Another consequence of the excessive supply of vitamin A

is the disruption of absorption of β-carotene and mineral salts from food [1,14].

Taking excessive amounts of vitamin A is also harmful for the osteoarticular system. It leads to joint and spine pains, as well as decalcification of bones (with the increase of calcium concentration in blood), which causes their brittleness and increases the risk of osteoporosis, and – as a result – also femoral neck fractures [30,31]. Some researchers have posited also that another result of vitamin A overdose is the decrease of bone mineral density (BMD). In a Swedish research [32] it was found out that the effect of the four-year intake of the discussed vitamin by women at doses > 1.5 mg (5000 IU) per day was a decrease of BMD by: 10% at the femoral neck, 13% at the Ward triangle, 9% at the trochanter region of the proximal femur, 14% at the lumbar spine, and 6% for the total body. Similar results were achieved in the United States [33], where it was also noted that the decrease of BMD appeared both in women (W) and men (M), annually amounting to: at the hip 1.6% (W), 1.2% (M); at the femoral neck 2% (W), 1.4% (M); at the spine 1.3% (W), 1.2% (M). However, another research from the US [34] did not reveal any connection between high concentrations of vitamin A in blood and the decrease of BMD.

The adverse effects of vitamin A recede after its withdrawal or the decrease in the applied dose (in children, however, growth inhibition may occur, as a result of premature ossification of the epiphyses of long bones) [28,29]. The above-mentioned effects are exacerbated by alcohol consumption [35].

High doses of vitamin A must not be administered in liver failure and cirrhosis or in bile ducts obstruction, due to the increase in the risk of toxic effects described above [20,26,36]. These high doses should be avoided during pregnancy, due to their contribution to fetal development anomalies (teratogenic activity). Pregnant women should not take more than approx. 2 mg (6000 IU) of this vitamin D (including its content in nourishment) and it should be administered under strict medical control (it is advisable to eliminate animal liver from the diet) [3,37,38]. High-dose vitamin A therapy in breast-feeding women, which could cause symptoms of hypervitaminosis in infants, is contraindicated [20,21].

Many dose-dependent, adverse effects are also caused by synthetic retinoids (e.g. by isotretinoin) [29]. These potentiate the action of vitamin A and hence should not be administered during this high-dose vitamin therapy. Synthetic retinoids irritate skin and mucosa, causing erythema, rash, contact dermatitis and loss of hair, and exacerbate the symptoms in patients with psoriasis. They impair liver functions, what may be manifested in blood by increased activities of various enzymes, e.g. ALAT (alanine aminotransferase), AspAT (aspartate aminotransferase) and LDH (lactate dehydrogenase), increased lipid profile with the decrease of the level of HDL (high-density lipoprotein), as well as increased concentrations of metabolites such as bilirubin and uric acid. The resultant toxic effects disrupt also composition and properties of the blood itself, leading to anaemia, neutropenia, acceleration of erythrocyte sedimentation rate (ESR) and coagulability disorders. Like vitamin A, synthetic retinoids increase the risk of osteoporosis. Also, they show embryotoxic and teratogenic activity, so they must not be used during pregnancy and lactation, even at low doses [23,30,31,37,38].

β-CAROTENE

Biomedical action

In the human body, β-carotene supports intercellular communication, inhibits proliferation of cells and modulates their differentiating. It also strengthens immune system, among others by activating interferons [39,40]. Part of β-carotene stored in the fatty tissue plays the role of stock form of vitamin A and in case of deficiencies can be cleaved into this vitamin [41].

Except these physiological functions, owing to the polyene's molecular structure, β-carotene demonstrates an effective antioxidative action. Just like vitamin A it quenches $^1O_2$, as well as traps and neutralizes various free radicals [40,42]. Besides, β-carotene deactivates the earlier formed peroxy radicals of the body's lipids, preventing their propagation and participation in peroxidative processes. Therefore, its action involves prevention and termination of the course of ROS-mediated reactions [12,15,43]. What is especially important, β-carotene is antioxidatively active in low oxygen partial pressure (pO_2) that is prevalent in peripheral tissues, and in this way it completes the further described antioxidative effects of vitamin E, which are strongest for the highest values of pO_2 (in pulmonary alveoli) [40,43].

As a result of the above mentioned reactions, β-carotene effectively participates in prevention of the oxidative stress – that is the shifting of the pro-/antioxidative systemic balance towards the processes of oxidation. It is used so in prevention and therapy of many diseases developing with participation of this stress, mainly neoplasms and cardiovascular abnormalities [36,44–46].

Potential risk

For a long time β-carotene, considered only as provitamin A, was believed to be practically harmless. It was stressed that its enzymatic conversion to retinol (in liver and small intestine wall), thanks to homeostatic control, provided only as much vitamin A as required by the body due to a temporary deficiency, and that β-carotene excess was expelled in faeces [41,47]. This opinion was confirmed by an observation that even a long-standing supplementation with β-carotene (25 mg/d) did not cause hypervitaminosis A with its effects as described above, provided that no preparations of vitamin A were taken at the same time [6,21,48].

Research has shown that β-carotene is not embryotoxic, mutagenic or carcinogenic [49]. One harmless result of its prolonged intake in large doses is a yellowish skin tinge, known as carotenemia. This is caused by deposition of β-carotene in the subcutaneous fatty tissue and recedes within a few days after decreasing the dose. Especially susceptible to this condition are people with hyperthyroidism, cirrhosis, hypercholesterolemia, diabetes and nephrosis (this discolouration is different from jaundice because of no yellow tinge of sclera) [2,29,40]. Another harmless consequence of supplementation with β-carotene may be slight diarrhoea which, however, may affect only those people who are oversensitive to this substance due to an innate metabolic defect [50].

In view of the data presented above that β-carotene does not cause any significant health threats, the effect of supplementation with this substance (20 mg/d) in smokers was surprising, with 18%-increase in lung cancer incidence and the increased mortality caused by this compound [51–54]. Similar results were noted for the supplementation of patients occupationally exposed to asbestos [39,55]. An increase in the frequency of intracerebral haemorrhage cases in supplemented alcoholics was also found [36]. These facts lead to the conclusion that cigarette smoke, asbestos and alcohol, which all exacerbate oxidative stress [15], contribute to the prooxidant activity of β-carotene, potentiating the damage to body cells [28,35,42,49].

It indicates that β-carotene, which was antioxidatively active at low pO_2, at high values of pO_2 was not only antioxidatively passive, but became a prooxidant [39,42,43]. Beta-carotene can even lead to the increase in DNA (deoxyribonucleic acid) damage when its concentration reaches about 5.5 mg/l (10 μmol/l), which results in the loss of its activity characteristic of low concentrations protecting the cells from DNA damage [39,50].

The products of β-carotene reactions with ROS and free oxygen radicals formed in the organisms of people exposed to oxidative stress and supplemented with that compound also contribute to prooxidant effects. The majority of those products are prooxidants and they cause

cellular damage or can be even carcinogenic during selective supplementation with β-carotene [22,40,49]. In turn, β-apo-carotenals, formed from peroxide radical-induced asymmetric breakdown of β-carotene, produce specific isoforms of cytochrome P-450 which convert procarcinogens present in the cigarette smoke into ultimate carcinogens, thereby enhancing progression of lung neoplasms in smokers. Those effects are eliminated by the parallel administration of vitamin E, which prevents β-apo-carotenal generation [43,54].

Selective supplementation with β-carotene also affects the content of various other antioxidants in the organism. The intake of β-carotene at high doses increases plasma concentrations of α-carotene, lycopene and vitamin E, and decreases simultaneously luthein and zeaxanthin concentrations [28,40,56].

VITAMIN E

Biomedical action

Vitamin E has eight forms: four tocopherols (α, β, γ, δ) and four tocotrienols (also α, β, γ, δ), while α-tocopherol constitutes some 90% of them and shows also the strongest biological activity [57,58]. The terms α-tocopherol and vitamin E are often used alternatively. The latter term has been used in this sense in subsequent chapters of this text.

The physiological functions of vitamin E comprise: stimulation of fertility (including prevention of miscarriages), participation in biosynthesis of collagen (crucial for the proper structure of muscles and walls of blood vessels), as well as activation of immune system [1,2,58]. Vitamin E participates also in tissue respiration as it may substitute for ubiquinones in electron transport [1,15].

Vitamin E acts also as an antioxidant and deactivates ROS, including $^1O_2$, $O_2^{\bullet -}$ (superoxide radical anion) and $^{\bullet}OH$ (hydroxyl radical). However, this is true only about the free, i.e. non-esterified vitamin E [59,60], while the majority of its preparations contain α-tocopherol acetate. The antioxidative action of vitamin E is mostly directed at lipids of cellular membranes, protecting them at the stage of prevention and termination against ROS-mediated reactions, which generate peroxides acting as the secondary ROS. Vitamin E also regenerates the antioxidatively used up β-carotene and protects vitamin A against oxidation [12,13,59]. Generally it plays such an important role in oxidative stress counteraction that it is considered to be the main lipophilic antioxidant of the body [59,60].

Because of these properties, the synthetic vitamin E is applied as an antioxidant in prevention and support treatment of various diseases involving defects in oxygenic metabolism, usually combined also with other antioxidants. In the above mentioned applications, patients are given high doses of this vitamin of several hundreds mg/d, which many times exceed the RDA (Recommended Dietary Allowance) values [36,45,59,61,62].

Potential risk

The synthetic vitamin E doses used in antioxidant therapy may arouse justified concerns because, for example in the United States, 300 mg/d is the maximum tolerated daily dose of this vitamin, and 2 g/d is already considered to be toxic [5,21,24,48]. In fact, the data coming from popular handbooks and lexicons about good tolerance of this vitamin and no hypervitaminosis or toxic effects, refer to its classic (i.e. non-antioxidant) applications and thus the doses not much exceeding the daily requirements.

Vitamin E is only seemingly safe, i.e. moderately non-toxic compared with vitamins A or D [22,63]. When used at high doses, it can lead to harmful effects (mainly during injections) due to the inhibition of 5-lipoxygenase in blood platelets and leucocytes (which results in insufficient synthesis of thromboxane and leucotrienes), decreased blood coagulability (resulting from the excessive suppression of platelet aggregation), and disruption of granulocyte and

phagocyte anti-infective function based on the generation of "useful" ROS [23,59,64].

In the case of too low systemic concentrations of α-tocopheryl radical-regenerating substances, large received doses of vitamin E act prooxidatively [65]. They cause excessive generation of those radicals which initiate lipid peroxidation in cellular membranes and LDL (low-density lipoproteins) [15,44,59,61] – the bodily structures which bind the above vitamin. Such doses may amplify ROS generation in the presence of iron and copper [24,28,66].

High doses of vitamin E decrease systemic vitamin A stores [29], and, when administered in the α-tocopherol or its acetate form, they limit the dietary absorption of therapeutically important γ-tocopherol, present only in the diet [57,67]. Vitamin E anticoagulant activity causes its antagonism with vitamin K, which can lead to bleeding when the latter is deficient, especially in patients treated with anticoagulants (e.g. with acenocoumarol or warfarin) or estrogens – both groups should not be given more than 40 mg/d of vitamin E [28,56,64].

The harmful effects of vitamin E were also observed after it had been applied in the therapy of coronary artery disease treated with synvastatin and niacin (i.e. nicotinic acid or vitamin PP). As a result, attenuation of those two lipid disturbance-correcting drug-mediated positive effects was noted along with atherosclerosis progression in the coronary vessels [68].

The adverse effects of vitamin E were confirmed in the long-term intake of this substance at doses over 240–480 mg/d [21,24]. These include the so-called influenza symptoms and also the alimentary tract symptoms (gastric pain, vomiting and diarrhea), muscular weakness, skin inflammations and rashes; more severe forms include double vision, liver enlargement, sexual dysfunction, hyperglycemia and hyperlipidemia [22,23,29]. These symptoms generally subside shortly after reduction of the doses. It is should be emphasized that the above described harmful effects of long-term antioxidative therapy with use of high doses of vitamin E are not inevitable. For example they were not observed in patients who were subject to such treatment in disease cases mentioned in the last part of this article.

Special caution is necessary, however, when applying this vitamin to neonates and babies. For them, the safe dose is only 2.8–3.5 mg/kg BM (there were cases of deaths of premature infants caused by liver damages after oxygen therapy, when vitamin E was applied at 20 mg/kg BM for protection of their alveoli, lenses and retina against ROS intensively generated in these conditions [69]). Large doses of vitamin E are also not recommended in pregnant and breast-feeding women, due to lack of safety data [1,5,21,48]. Other groups of people can be given large doses of this vitamin, but with concomitant monitoring of its plasma concentration along with vitamin A, total lipids and iron concentrations (monitoring of vitamin C, selenium, and total cholesterol would be also advantageous) [57,59,63]. This is the prerequisite for treatment efficacy and will protect from the development of adverse effects.

VITAMIN C

Biomedical action

Physiological functions of vitamin C (L-ascorbic acid) include participation in hydroxylation reactions while producing: hydroxyproline and hydroxylysine (crucial for production of collagen), tyrosine, noradrenalin and serotonin, as well as L-carnitine, steroid hormones of the adrenal glands and bile acids [1,70,71]. As a reducer, it participates in biosynthesis of tetrahydrofolic acid, hyaluronic acid and prostaglandins [2,70]. It also modulates the body's immunity by stimulating the production of immunoglobulins and interferons [1,70].

Vitamin C is easily and reversibly oxidized into dehydro-L-ascorbic acid, creating a redox system with the potential of 0.08 V, which allows it to act as an antioxidant.

It deactivates multiple ROS: $^1O_2$, $O_2^{\bullet-}$, $^\bullet OH$, H_2O_2 (hydrogen peroxide), $HO_2^\bullet$ (hydroperoxyl radical) as well as peroxides and free radicals produced with their participation [12,15,72,73]. Thanks to this it prevents reactions of these ROS with biomolecules at the stage of prevention, termination and even repair of some damages. It also regenerates vitamin E used up during similar processes [71–74]. Thus, vitamin C plays an important role in eliminating oxidative stress which, in combination with its water solubility, makes it the main antioxidant of extracellular fluids [46,71].

The above described processes constitute grounds for using vitamin C in prevention and therapy of diseases resulting from, or occurring with, oxidative stress. These are the diseases listed in the description of vitamin E (both vitamins cooperate with each other [74]) and additionally also cataract, rheumatoid arthritis, accelerated ageing syndrome and others [4,18,45,72,75]. Vitamin C also takes part in detoxification of xenobiotics contributing to the production of ROS [76].

Potential risk

Vitamin C is practically nontoxic [22–24], however, its large doses (500 mg/d or more) can cause alimentary tract disturbances (nausea, pyrosis and diarrhea), enhanced urination with a feeling of burning, and also erythrocyte hemolysis during glucose-6-phosphate dehydrogenase (G-6-PD) and vitamin B_{12} deficiency [4,77]. By inducing severe urine acidification, such doses impair the excretion of weak acids and bases, which may result in the precipitation of cystinate and urate depositions in the urinary tract, leading to the formation of renal calculi [29,73,78,79]. Vitamin C doses > 1g/d elevate blood and urinary oxalic acid concentrations to a degree increasing the risk of calculi formation from calcium oxalate [2,79,80]. Thus, such large doses should not be administered in chronic renal failure, cystinuria, predisposition to gout, urate and oxalate calculosis.

Therapeutically applied vitamin C increases blood sodium concentration and decreases potassium concentration which can lead to deficiency of the latter [21,73]. Also, it negatively impacts on some drugs taken, causing vitamin B_{12} destruction (therefore, the two vitamins should not be administered in combination), and augments amphetamine derivative and tricyclic antidepressant drug elimination by inhibiting their reabsorption in renal tubules [28,29,56]. Due to its chemical incompatibility, vitamin C should not be administered with drugs which have oxidizing properties [44]. By acidifying the urine, it can lead to the crystallization of urine excreted p-aminosalicylic acid and sulphonamides [77,80]. The use of high-dose vitamin C yields false results in body fluid analyses performed with the use of methods based on redox reactions, e.g. determinations of bilirubin, glucose, or creatinine concentrations, and LDH or AspAT activity [1,29,73].

High doses of vitamin C hamper copper absorption and inhibit copper-containing ceruloplasmin and superoxide dismutase (Cu,Zn-SOD) activities [15,73]. In contrast, they enhance iron absorption to the harmful degree in individuals with its excessive intestinal absorption, and also in those with increased blood iron concentration, and with hemochromatosis, sideroblastic anemia or thalassemia (where the symptoms exacerbate). Such doses can also lead to the release of that element from its tissue resources, i.e. stored in ferritin [1,4,77]. The excess unbound iron accumulates in the tissues and skin, irritates gastrointestinal mucosa, causes intoxication, and contributes to the prooxidant effects of vitamin C [81,82].

Vitamin C-induced prooxidant effects can occur mainly due to the transition ion metal-reducing abilities of the vitamin, and the affected metals include among others iron and copper. In fact, reduced ions of the metals contribute to generating $^\bullet OH$, which is a ROS particularly dangerous to the cells, and also of $O_2^{\bullet-}$, H_2O_2, and $HO_2^\bullet$ [15,73,77]. Those reactions can repeat cyclically; they lead to the generation of large concentrations of the mentioned

ROS which stimulate free radical reactions and show i.a. a mutagenic and neurotoxic activity. This is the reason why this vitamin should not be used along with iron and copper salts [24,28,77]. However, it has been found that vitamin C is able to act as prooxidant only at its low concentrations [44,82].

The adverse effects of vitamin C can appear during the selective use of the vitamin in the prevention of lipid peroxidation-mediated diseases. The above vitamin prevents this process, especially with respect to LDL lipoproteins, but also decomposes previously formed lipid peroxides to more reactive 8- and 10-carbon aldehydes. These compounds act i.a. as mutagens, e.g. promote the reaction of 4,5-epoxy-2(E)-decenal with DNA, generating very mutagenic ethene-2'-deoxyadenosine residues [83]. Lipid peroxide formation is prevented by vitamin E, which interrupts free radical-catalyzed peroxidative chain reactions. Therefore, the use of vitamins C and E together is not only important for their combined actions in lipid environment, but also makes the course of the aforementioned process impossible to take place [74].

Taking large doses of vitamin C may lead to vitamin C tolerance or even dependency (including pregnant woman-mediated effect on fetus). The instantaneous withdrawal from such doses may result in deficiency symptoms, including scurvy; therefore, the doses must be lowered gradually to allow the body to accommodate the changes in delivery [1,21,29]. Also, vitamin C has a slightly stimulating activity, so its night-time taking is not recommended [2,4].

A range of adverse effects are induced by the oxidized form of vitamin C (formed in high concentrations when the reduced form of this vitamin is intensively, oxidative stress-dependently expended) [15,44,84]. It can lead to the inhibition of the transport of different substances and the impairment of some metabolic processes. This is due to its reactions with enzyme –SH groups (e.g. present in hexokinase, a key enzyme of glucose metabolism) and with other proteins [77,85]. The oxidized form of vitamin C is also able to damage erythrocytes (leading to hemolysis) and pancreatic β-cells, similarly as alloxan, well-known for that capability [73,77,80].

CONTROVERSIALITY OF RESULTS OF LARGE STUDIES EVALUATING THE HEALTH EFFECTS OF SUPPLEMENTATION WITH ANTIOXIDATIVE VITAMINS

Numerous experimental studies on the action of antioxidative vitamins, performed on cell cultures and animals, as well as small short-term clinical trials relating to this action suggested that vitamins A, E, C and β-carotene have a beneficial effect on pathological states associated with oxidative stress. Based on those findings, optimistic implications were drawn that antioxidative vitamins used in high doses might play an important role in prevention and supportive treatment of diseases in which ROS and free radicals are involved [3–6].

This hypothesis, however, has not been confirmed by large-scale studies evaluating the health effects of long-term and high-dose antioxidative vitamin supplementation. These have been carried out since the 1990s on thousands of subjects from various risk groups, as well as potentially healthy volunteers, although less frequently on large populations of patients. The results of these investigations have turned out to be clearly controversial: contradictory in relation to results of experimental studies and small clinical trials, considerably differentiated and sometimes outright divergent. The expected favourable effects of vitamin supplementation has been dominated by the lack of ascertainment of these effects, and even increase in morbidity and/or mortality risk; also some surprising adverse effects appeared.

Presented below are the principal kinds of large studies which led to obtaining these results – ambiguous and distant from expectations. Due to a very great number of such investigations resulting, among others, from their various

medical profiling, the scope of this presentation has been limited to two profiles connected with the most common and most severe diseases of current times: cancer of various organs and cardiovascular diseases.

Selected studies targeted at cancer of various organs

The large studies concerning this area are illustrated in the listing below based on a review by Bardia et al. [86], from which their most representative kinds were chosen; the listing comprises additionally three important studies not included in the Bardia et al. review. The descriptions are presented in a compact form, each incorporating the study cryptonym, country in which it was performed and the year of publication, number of participants, their sex (M – men, W – women) and threats to their health, antioxidants used for supplementation, its duration, and the results (literature references to research descriptions by Bardia are presented in his quoted review). It should be added that the participants of these studies were receiving large but still non-toxic doses of antioxidative vitamins specified in subsequent descriptions. The prepared listing allows perceive only rare cases of favourable effects of vitamin supplementation.

— CCPS (China 1993): 29 584 M+W with increased risk of various health disorders (a result of nutritional deficiency); vit. A, E, C, β-carotene, selenium, molybdenum, zinc (in various combinations) – 5 years; taking vit. E, β-carotene and selenium decreased mortality from stomach cancer by 21% and from cancer overall by 13%, as well as from cardiovascular diseases by 10%; the joint effect was a decrease of all-cause mortality by 9% (other applied variants of supplementation were preventively ineffective);
— ATBC (Finland 1994): 29 133 M with increased risk of cancer and/or cardiovascular diseases (smokers); vit. E or β-carotene – 6 years; taking vit. E reduced cancer frequency of prostate by 34% and of large intestine by 16%, but it increased by 25% for stomach cancer and by 9.5% for bladder cancer (also mortality from ischemic stroke dropped by 16% and from coronary artery disease by 5.5%, but for hemorrhagic stroke it increased by 50%); taking β-carotene increased frequency of stomach cancer by 25%, of prostate cancer by 23% and of lung cancer by 18% (there were no effects for cardiovascular diseases);
— PHS I (US 1996): 22 071 M with average risk of cancer; β-carotene – 12 years; there were no preventive effects for cancer overall, but there were no harmful effects either (the same was found for cardiovascular diseases);
— WHS (US 1999): 39 876 W with average risk of cancer; β-carotene – 2 years, continuation with vit. E (2005) – 10 years; for both applied antioxidants the results were analogous to the PHS I study (without cardiovascular diseases, which were not taken into account);
— SU.VI.MAX (France 2004): 13 017 M+W with average risk of cancer; vit. E, C, β-carotene, selenium and zinc (taken together) – 7.5 years; decreased frequency of cancer overall by 27.5% and all-cause mortality by 35%, but only in M and not in W;
— PLCO (US 2006) [87]: 29 361 M with intensified risk of prostate cancer; vit. E – 8 years; decreased risk of development of advanced prostate cancer by 70%;
— PHS II (US 2009) [88]: 14 641 M with intensified risk of prostate cancer; vit. E and C (taken together) – 10 years; for both antioxidants there were no preventive effects;
— SELECT (US, Canada, Puerto Rico 2009) [89]: 35 533 M with intensified risk of prostate cancer; vit. E and/or selenium – 7 years; both antioxidants were preventively ineffective.

Selected studies targeted at cardiovascular diseases

The listing of relevant large studies presented below is based on review by Ueda and Yasunari [90]; our listing

includes additionally two important studies not included in the Ueda and Yasunari review. As in the previous subchapter, the descriptions are given a compact form, with the same arrangement also with addition of the abbreviations of diseases present in the descriptions: CAD – coronary artery disease, CVD – cardiovascular disease, MI – myocardial infarction (literature references to research descriptions by Ueda and Yasunari are presented in their quoted review). The participants in these studies were receiving large but still non-toxic doses of antioxidative vitamins specified in the subsequent descriptions. As it can be seen from the presented listing, the observed effects of the supplementation were seldom entirely favourable.

— HPS (US 1993): 39 910 M with risk of CAD; vit. E or C or β-carotene – 4 years; taking vit. E decreased risk of CAD by 37% (vit. C and β-carotene were preventively ineffective);
— NHS (US 1993): 87 245 W with risk of CAD; vit. E – 8 years; drop of CAD risk by 41%;
— CHAOS (UK 1996): 2002 M+W with CAD; vit. E – 1.4 years; drop of nonfatal MI frequency by 77% without changes for fatal MI, but increase (though insignificant) of mortality from CVD;
— EPESE (US 1996): 11 178 M+W with risk of CAD; multivitamins + vit. E and C – 9 years; decrease of mortality from CAD by 47% and all-cause mortality by 37%;
— IWHS (US 1996): 34 387 W with risk of CAD; vit. E, C and carotenoids (taken together) – 6 years; drop of CAD risk by 62%, but increase (though insignificant) of breast cancer risk;
— GISSI (Italy 1999): 11 324 M+W with history of MI; vit. E and/or n-3 polyunsaturated fatty acids (PUFA) – 3.5 years; vit. E did not bring health benefits (as opposed to positive effect of PUFA);
— CARET (US 1996) [91]: 18 314 M+W with increased risk of CVD and/or cancer (smokers or working with asbestos); vit. E and β-carotene – 1.75 years; increase of MI risk by 26% and death from CVD by 55%, as well as increased incidence of lung cancer by 28%; joint effect was an increase of all-cause mortality by 17% (for ethical reasons, the study was stopped);
— WACS (US 2007) [92]: 8171 W with risk of CVD; vit. E, C and β-carotene (taken separately or together) – 9.4 years; none of the supplementation variants brought the preventive effects.

In the above listing, three large studies were omitted: HOPE, PPP and MRC/BHF, as their aim was not to test the effects of supplementation solely with antioxidative vitamins, but to control the potential for their administration in supporting the action of some medicines used in cardiovascular diseases therapy: ramipril, aspirin and simvastatin, in order of the studies mentioned above (no favourable effect of the applied vitamins was noted).

Meta-analysis of results of studies on the effectiveness of antioxidative vitamin administration in the prevention of various diseases

The lack of unequivocal results from numerous studies evaluating the health effectiveness of long-term supplementation with large doses of antioxidative vitamins (*vide* examples given above) was the inspiration for this summary through the meta-analyses. However, there were considerable differences in the choices of source data and their modes of processing by particular authors. Owing to this, the results were found to be largely incoherent; they were not only insufficient to eliminate the doubts connected with vitamin supplementation, but even increased them.

The largest number of these meta-analyses were performed by Bjelakovic and Gluun with their team. Based on the results of the studies selected from databases and literature, they evaluated the health effectiveness of the administration of vitamins A, E, C, β-carotene, and selenium (in combinations) in prevention of various diseases.

The most attention was paid to digestive system disorders: stomach and intestine cancer [93–96], colorectal adenoma [97], various other gastrointestinal disorders [98,99], and liver diseases [100]. However, the range of works included also other types of diseases: cardiovascular, nervous, osteoarticular, renal, endocrine, ocular, dermal, and non-organ-specific [98,99].

The resulting whole of their meta-analyses delivered only negative findings. With respect to digestive system diseases, the researchers did not find evidence of any preventive action by antioxidative vitamins, and regarding stomach and intestine cancer they even suggested an increase of all-cause mortality caused by intake of these vitamins (selenium was regarded as not sufficiently examined). For all other diseases they also found lack of evidence for preventive effectiveness of the above listed antioxidants and came to the same conclusions concerning the increase of all-cause mortality: from vitamin A by 16%, from vitamin E by 4–7% and from β-carotene by 7% [99]. According to these authors, the usage of vitamin C or selenium instead does not have either a favourable or a harmful effect, as can be seen from the data.

The meta-analyses by Bjelakovic et al. leading to such disturbing conclusions received a number of negative appraisals, based on the opinions of various experts. A multidirectional criticism of their meta-analyses was given by Levin [101], referring to Stampfer (nutrition and epidemiology expert) and Barry (biostatistics expert). His main objections included: during the selection of source data, the omission of over 400 studies with the use of the a/m antioxidants where no deaths occurred; equivalent treating of studies including potentially healthy subjects with those including sick persons with various chronic diseases (taking medicines used in their therapy), with large differences between doses of applied antioxidants and incomparable times of administration – from one month to 12 years; non-consideration of death causes in the selected studies, with lack of evidence for antioxidants being the cause of death (what is supported by the fact that only 4 cases of death caused by vitamin overdose were noted in the US in 2003 [102]). Nearly the same objections were raised by Wilson [103], referring also to Stampfer as well as Shao (biochemistry of nutrition expert), with addition further critical remarks: ignoring in the meta-analyzes those studies which confirmed the benefit of applying the antioxidative supplements, and focusing on mortality and secondary prevention (in the sick persons) instead of on primary prevention (in potentially healthy subjects).

Completely different from Bjelakovic et al. were the results of thematically analogous meta-analyses made by other researchers. Indeed, Knekt et al. [104] found vitamin E and carotenoids lacked efficacy in the prevention of cardiovascular diseases, but they noted a significant decrease in frequency of coronary artery disease and other serious cardiac events in sick persons supplemented with large doses of vitamin C (> 700 mg/d). A meta-analysis by Wright et al. [105] showed, however, that a long-term intake rich in dietary and/or supplemental vitamin E significantly reduces mortality from coronary artery disease and from ischemic stroke, as well as all-cause mortality. Ye and Song [106] noted a decreased risk of coronary artery disease through a regular intake of dietary and/or supplemental vitamin E, C and β-carotene. Regarding oncology, a meta-analysis by Myung et al. [107] affirmed the preventive effects of supplementation with vitamins E, C and β-carotene against cervical neoplasms, including invasive cancer.

At the end of this review of meta-analyses, it is worth referring to a recently published work, which questions credibility of the aforementioned alarmist results that have been earlier reported by Bjelakovic et al. It is a reanalysis of two meta-analyses by these researchers [98,99] made by Biesalski et al. [108] using the same dataset. This reanalysis has led to statement contradictory to the above results that 36% of source studies revealed beneficial effects of supplementation with antioxidants, 60% null

effects (neither positive, not negative), and only 4% harmful effects. The above example proves the necessity of care when interpreting meta-analyses, because they are purely secondary statistical studies, with the quality of the results being dependent on reliability of their authors. Therefore, it is indispensable to continue large studies, but carefully designed and standardized, evaluating the health effectiveness of long-term supplementation based around the use of well-chosen doses of various combinations of antioxidative vitamins.

SUMMARY

In summary, it should be emphasized that antioxidant vitamins do not act as independent factors, but they constitute a part of a complex system called the systemic antioxidant barrier [15]. Therefore, additional substances with similar action profile may be essential to achieve the desired therapeutic goals. In the body, vitamin C closely cooperates e.g. with vitamin E. The latter protects vitamin A from oxidation in the alimentary tract and co-works with β-carotene and selenium [1,39,74]. Antioxidant vitamins also act synergistically with different bioflavonoids [44,56]. Thus, large doses of individual antioxidant vitamins with the use of their synthetic preparations can lead to the disruption or abrogation of other ROS- and free radical-neutralizing mechanisms. As a result, antioxidant vitamin-induced damage is sometimes more serious. It might be less harmful if this-type vitamin therapy was not ever applied [15].

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