Palestrica of the third millennium – Civilization and Sport
Vol. 19, no. 2, April-June 2018, 92–97

Risks associated with sub- and overdosing of water-soluble vitamins in professional or amateur athletes and the quality of dietary supplements

Riscurile asociate subdozării/excesului unor vitamine hidrosolubile la sportivii de performanță sau amatori şi calitatea suplimentelor alimentare

Amelia Tero-Vescan [1], Silvia Imre [2*], Camil-Eugen Vari [3], Anamaria Toma [2], Bianca-Eugenia Ősz [3]
Faculty of Pharmacy, University of Medicine and Pharmacy Tîrgu Mureş, Romania
[1] Department of Biochemistry. Chemistry of Environmental Factors
[2] Department of Analytical Chemistry and Drug Analysis
[3] Department of Pharmacology and Clinical Pharmacy

Abstract

Background. Vitamins are the most commonly used active ingredients in dietary supplements (DS), and the quality of DS is subject to highly permissive legislation.

Aims. The purpose of this study was to underline the risks associated with sub- and overdosing of water-soluble vitamins in the case of performance and amateur athletes who use vitamins as dietary supplements without professional counseling and to test the quality of vitamins as DSs or drugs marketed in pharmacies through a new simple and rapid HPLC method.

Methods. Vitamins were analyzed by HPLC using a Phenomenex Luna C_{18} column, 3 µm, 150 x 4.6 mm, a mobile phase with 100 mM ammonium acetate (pH = 5.8) - acetonitrile - methanol in a concentration gradient from 88:5:7 to 68:25:7 in 10 minutes with 1 ml/min flow rate. Sample processing: the vitamins from the tablets were extracted in 15 mM phosphoric acid and the injectable solutions diluted with 15 mM phosphoric acid prior to chromatographic analysis.

Results. The risk of overdosing water-soluble vitamins is highly questionable, but the under-dose intake is related to their use in the case of athletes, these vitamins being involved in energy metabolism. Hence the importance of the quality of commercial products with these vitamins. The proposed HPLC method allows the separation of ascorbic acid, folic acid, thiamine, pyridoxine, nicotinamide, cyanocobalamin and riboflavin. The linearity of the analytical method was tested and applied for five vitamin assays in tablets and injectable solutions purchased from the pharmacy, which were quantitatively analyzed, and the results were compared with those declared by the manufacturer.

Conclusions. Permissive legislation on DS implicates acquiring them from "safe" sources such as community pharmacies, especially for amateur athletes who do not benefit from specialized nutritional counseling.

Keywords: water-soluble vitamins, dietary supplements, HPLC method, quality control

Rezumat

Premize. Vitaminele sunt cele mai utilizate substanțe active în compoziția suplimentelor alimentare, iar calitatea suplimentelor alimentare este supusă unor norme legislative extrem de permisive.

Obiective. Scopul acestui studiu a fost de a evidenția efectele negative pe care le are subdozarea/excesul de vitamine hidrosolubile în cazul sportivilor de performanță și amatori care utilizează vitamine fără consiliere profesională și de a testa calitatea unor suplimente alimentare şi medicamente cu vitamine pe baza unei noi metode HPLC, simplă și rapidă.

Metodă. Vitaminele au fost analizate prin metoda HPLC, pe o coloană Phenomenex Luna C_{18}, 3 µm, 150 X 4.6 mm, folosind ca fază mobilă un amestec de acetat de amoniu 100 mM (pH=5,8) – acetonitril - metanol, în gradient de concentrație de la un raport 88:5:7 la 68:25:7 în 10 minute, cu un debit de 1 ml/min. Prelucrarea probelor: tabletele au fost triturate în mojar și vitaminele au fost extrase în soluție de acid fosforic 15 mM, iar soluțiile injectabile au fost diluate cu acid fosforic 15 mM înaintea analizei cromatografice.

Rezultate. Riscul supradozării cu vitamine hidrosolubile este interpretabil, dar aportul lor insuficient este important mai ales în cazul sportivilor, aceste vitamine fiind implicate în metabolismul energetic. De aici şi importanţa calităţii produselor comerciale cu aceste vitamine. Metoda HPLC propusă permite separarea vitaminelor acid ascorbic, acid folic, tiamină, piridoxină,

Received: 2018, March 25; *Accepted for publication:* 2018, April 20
Address for correspondence: Faculty of Pharmacy, University of Medicine and Pharmacy Tîrgu Mureş, 38 Gheorghe Marinescu Street, 540139 Tîrgu Mureş, Romania
E-mail: silviaimre@umftgm.ro
Corresponding author: Silvia Imre; silviaimre@umftgm.ro
https://doi.org/10.26659/pm3.2018.19.2.92

nicotinamidă, cianocobalamină și riboflavină. A fost verificată linearitatea metodei analitice și apoi metoda a fost aplicată la analiza a cinci vitamine din tablete de suplimente alimentare și soluții injectabile cu vitamine, achiziționate din farmacie, acestea corespunzând din punct de vedere cantitativ cu conținutul declarat de producător.

Concluzii. Legislația permisivă din domeniul suplimentelor alimentare ridică problema achiziționării acestora din surse "sigure" precum farmaciile comunitare, mai ales în cazul sportivilor amatori care nu dispun de o consiliere nutrițională specializată.

Cuvinte cheie: vitamine hidrosolubile, suplimente alimentare, metodă HPLC, controlul calității.

Introduction

Vitamins are indispensable substances, essential to humans, and intake of vitamins, especially from the water-soluble group, is practically associated in biochemistry with the proper development of energy processes (Schenk et al., 2018). Legislation in the field of dietary supplements (DS) raises serious problems regarding their quality, the variation limits of their composition in active compounds (***, 2007), therefore the purchase source of such products is extremely important (pharmacies, naturist shops, Internet, etc.). The American Association of Poison Control Centers reported in 2016 that vitamin intoxications are rare, unintentional, especially in children, and in the literature, there are no cases of intoxications with water-soluble vitamins (Gummin et al., 2017). A study from 2010 shows that over 60% of performance athletes use dietary supplements, most of which contain multivitamins (52.2%) (Knez & Peake, 2010). If the risk of overdosing water-soluble vitamins is highly questionable, instead, there is the issue of under-dosed DS with vitamins, especially in the case of amateur athletes who, in the absence of pharmaceutical advice, use products purchased from sources that do not offer a guarantee for their quality.

Hypothesis

Dietary supplements are not subjected to routine qualitative/quantitative control of their declared composition like medicinal products. Therefore, the purpose of this study was to analyze the water-soluble vitamin content of drugs and multivitamin DS purchased from the pharmacy based on the particular importance of these vitamins in energy metabolism of amateur athletes and the demand for high quality commercial products. Many analytical methods for the determination of the vitamin content of various drugs and supplements are described in the literature, the most widely used method being high performance liquid chromatography.

Materials and methods

The quality control of the commercial products was performed with the aid of an HPLC system, Agilent Technologies 1100 series, USA, consisting of: single monitoring wavelength detector UV G1314A; column thermostat G1316A; autosampler G1329A; autosampler thermostat G1330B; quaternary pump G1311A; mobile phase degasser G1379A.

The chromatographic parameters were: chromatographic column - Phenomenex Luna C_{18}, 3 µm, 150 X 4.6 mm; the mobile phase was delivered at a flow rate of 1 ml/min and the concentration gradient started with the initial composition ammonium acetate 100 mM pH 5.8 - acetonitrile – methanol 88:5:7; the composition was linearly changed in 10 minutes to 68:25:7, followed by a short washing step and reequilibration; the detector was set at 260 nm; the column temperature was 40°C; the sample injection volume - 10 µl.

Reference substances, ascorbic acid, thiamine, riboflavin, nicotinamide, pyridoxine and folic acid, were working standards provided by Ferrosan, Romania. For cyanocobalamin resolution, an injectable commercial pharmaceutical product was used.

The 5-level calibration curves were prepared in the following concentration ranges: 30-100 µg/ml thiamine, 13.5-45 µg/ml riboflavin, 15-50 µg /ml nicotinamide, 7.5-25 µg /ml pyridoxine and 30-100 µg /ml ascorbic acid.

DS and drug samples: DS tablets with the following composition: thiamine 2 mg, riboflavin 1 mg, pyridoxine 0.5 mg, ascorbic acid 30 mg, nicotinamide 1 mg, calcium pantothenate 1 mg; thiamine solution 50 mg/ml for injection containing thiamine hydrochloride; pyridoxine injection 25 mg/ml containing pyridoxine hydrochloride.

Sample processing was done as follows: 10 DS tablets were triturated in the mortar and an amount of powder corresponding to one tablet was dissolved by stirring in the ultrasonic bath in 15 mM phosphoric acid and filtered for analysis prior to HPLC analysis. Injectable solutions were diluted with 15 mM phosphoric acid to the proper concentration.

Results

In the proposed chromatographic conditions, the substances in the standard calibration mixture were separated at the baseline with an experimentally determined dead time value t_0 = 1.34 min (Fig. 1). Under the same conditions, seven water-soluble vitamins were separated at the baseline (Fig. 2).

In order to verify the linearity of the method, four calibration series were obtained and analyzed, and the obtained calibration curves are shown in Table I.

The results obtained for the determination of vitamins from DS and injectable solutions by the HPLC method are shown in Table II. The chromatogram of a tested commercial product is presented in Fig. 3.

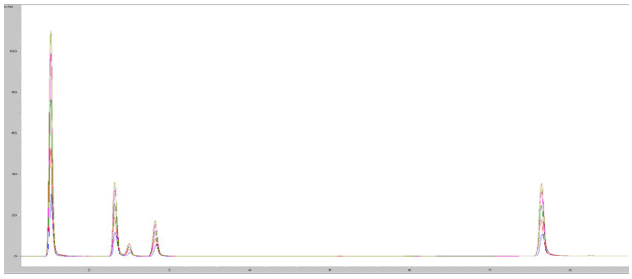

Fig. 1 – Chromatograms of a series of standard calibration solutions with the following order of elution: ascorbic acid (1.6 min), thiamine (2.3 min), pyridoxine (2.5 min), nicotinamide (2.8 min), riboflavin (6 min)

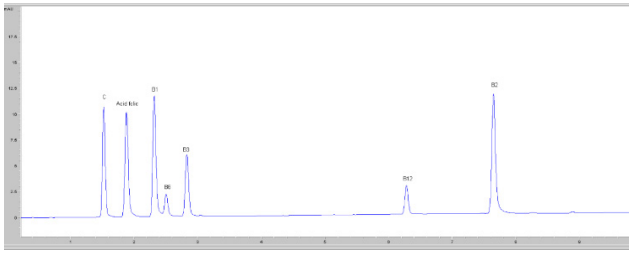

Fig. 2 – Chromatogram of a mixture of 7 water-soluble vitamins under optimized HPLC conditions. The order of elution: C ascorbic acid (1.6 min), folic acid (1.9 min), B_1 - thiamine (2.3 min), B_6 - pyridoxine (2.5 min), B_3 - nicotinamide (2.8 min), B_{12} - cyanocobalamin (6.4 min), B_2 - riboflavin (7.6 min)

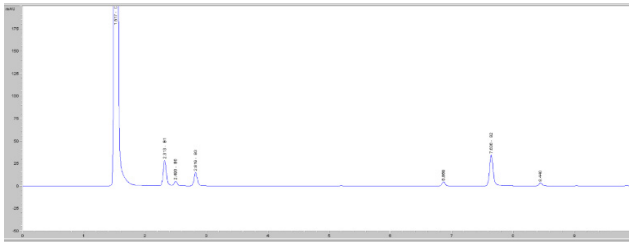

Fig. 3 – Chromatogram of the DS sample

Table I

The average calibration curves of the determined substances

Substance	Average calibration curve (N=4) ± SD	R^2
Ascorbic acid	Area =3.371 (±0.037) x c – 9.976 (±1.410)	>0.999
Thiamine	Area =1.214 (±0.014) x c + 3.894 (±0.775)	>0.999
Riboflavin	Area =3.348 (±0.024) x c + 0.617 (±0.404)	>0.999
Nicotinamide	Area =1.336 (±0.010) x c + 1.662 (±0.181)	>0.999
Pyridoxine	Area =1.005 (±0.011) x c + 0.263 (±0.141)	>0.999

Table II

Water-soluble vitamin content of DS and injectable solutions determined by the HPLC method

Vitamin	Declared concentration (mg/tablet)	Obtained concentration (mg/tablet)	Recovery (%)	Limits allowed (%)	Quality standard
Dietary supplement					
Thiamine	2	2.05	102.5	90-110	Checked
Riboflavin	1	1.071	107.1	90-110	Checked
Nicotinamide	1	0.995	99.5	90-110	Checked
Pyridoxine	0.5	0.45	90.6	90-110	Checked
Ascorbic acid	30	31.5	105.1	92.5-107.5	Checked
Injection solutions					
Vitamin	Declared concentration (mg/ml)	Obtained concentration (mg/ml)	Recovery (%)	Limits allowed (%)	Quality standard
Thiamine	50	50	100	95-105	Checked
Pyridoxine	25	23.9	95.6	95-105	Checked

Discussion

The relevance of water-soluble vitamin intake

The quality of DS is extremely important, and in the case of athletes it can make a difference not by excess (especially for water-soluble vitamins) but by subdosing; the intensity of energy processes is much higher in athletes, hence the daily requirement of these vitamins. It is known that the rate of basal metabolism depends on a number of factors such as gender, height, body surface, thyroid function, decreases with age and depends on the level of physical activity (Zemirli et al., 2018). Therefore, in the case of physical effort, the need for water-soluble vitamins increases, as they are involved in catabolic processes of important energy substrates. Particular attention should be paid to endurance athletes who are exposed to high oxidative stress, vitamin and mineral supplementation being crucial (Brisswalter & Louis, 2014).

Thiamine in its active form, thiamine pyrophosphate (TPP), is involved in carbohydrate metabolism, so practically it is synonymous with energy production. TPP is part of the multienzymatic complex of *pyruvate dehydrogenase* involved in the oxidative decarboxylation of α-ketoacids, therefore aerobic metabolism (aerobic glycolysis) is promoted by transforming pyruvate into acetyl-coenzyme A with a much better energy efficiency. A study on an additional 10 mg/kg body weight thiamine intake for 4 weeks showed a reduction in fatigue, lactate concentration and ammonium ions during aerobic exercise (bicycle, 60 minutes at 50 rpm) (Choi et al., 2013). A supplementary intake of thiamine during a carbohydrate rich diet is beneficial.

Pyridoxine in the form of pyridoxal phosphate (PLP) is a cofactor of over 100 enzymes, most of which are involved in the metabolism of amino acids (transamination, decarboxylation, deamination, etc.). Besides the amino acid catabolism processes, PLP is involved in the synthesis of important muscle peptides for sports performance, such as anserine and carnosine, and its influence in beta-alanine production is the limiting rate step in carnosine synthesis (Suidasari et al., 2016). Pyridoxine has an inhibitory effect on digestive enzymes involved in the metabolism of carbohydrates (*alpha-glucosidases, sucrase, maltase* and *glucoamylase*). Therefore, the administration of pyridoxine during a meal favors protein digestion and absorption of amino acids on the one hand, and prevents polysaccharide digestion and simple carbohydrate uptake on the other hand (Kim et al., 2018). Although pyridoxine is a water-soluble vitamin and therefore presents a low risk of accumulated toxicity, the literature presents the case of an 8-year-old child with chronic pyridoxine treatment for cerebral PLP dependent epilepsy, who developed hemophilia A following increased doses of pyridoxine with aging (Lheureux et al., 2005). Older studies show that PLP inhibits adenosine diphosphate (ADP)-induced platelet aggregation, therefore administration of higher doses to athletes to favor protein metabolism should be performed with caution, especially in sports where there is a risk of injury (Kornecki & Feinberg, 1980). Pyridoxine deficiency/overdose is associated with the occurrence of neuropathies by preventing transmission of the nervous impulse. Clinical manifestations of this pathology include: pain, muscle cramps, muscle weakness and tremor, all of which may affect performance. These modifications are reversible by optimizing vitamin intake (Chaudary et al., 2003). In high protein diets, the pyridoxine requirement is increased but remains within limits of 10 mg/day. Long-time treatment with high doses can cause neuropathy (also encountered in case of deficiency).

Niacin is converted into two coenzyme forms: NAD^+/NADH, involved in energy metabolism by collecting hydrogen from different compounds and oxidation in the mitochondrial respiratory chain, and $NADP^+$/NADPH, involved in reductive biosynthesis reactions in fatty

acids, nucleic acids, cholesterol metabolism (Chawla & Kvarnberg, 2014). According to the Framingham score, increases of LDL and decreases of HDL lipoprotein fraction are independent risk factors for coronary artery disease and, on the other hand, it is known that the risk of sudden cardiac death is higher for athletes than in the general population. Niacin is the oldest compound used to increase the HDL fraction of total cholesterol and reduce the fraction of LDL and serum triglycerides (O'Riordan et al., 2018). Niacin prevents lipolysis in the adipose tissue, therefore decreases the fraction of free fatty acids and plasma triglycerides and increases insulin sensitivity (Nelson et al., 2012), which leads to better use of carbohydrates as an energy substrate (Torrens et al., 2016). Niacin is degraded in the body by methylation, therefore excess niacin will deplete the reserves of methyl group donors such as betaine or methionine and, at the same time, there is a competition for methylation with catecholamines via *catechol ortho-methyl transferase* inducing noradrenaline plasma accumulation. Niacin fortification of grain in the US resulted, due to its chemical composition - starch (glucose source) and niacin, in increased insulin release, decreased glucose tolerance, and ultimately insulin resistance and at the same time obesity by increasing appetite (Zhou et al., 2015). Food fortification, especially with niacin, in order to reduce pelagic risk, resulted in daily doses of at least 33 mg niacin, a recommended dietary allowance (RDA) of 14-16 mg/day in adults being exceeded at least twice (Li et al., 2010).

The antioxidant effect of ascorbic acid is undeniable, and studies show that over 90% of vitamin DS contain ascorbic acid. Besides its antioxidant effect, ascorbic acid is involved in iron absorption, synthesis of collagen and catecholamines, all of these effects being important to athletes. With aging, muscle sensitivity to damage increases and recovery after exercise is much more difficult because the higher number of type 1 muscle fibers involves increased oxygen consumption and an increased rate of free radical production (Brisswalter & Louis, 2014). Ascorbic acid directly reacts with superoxide ion, and skeletal muscles in humans are extremely sensitive to ascorbic acid supplementation (Cobley et al., 2015). Although it has clear long-term beneficial effects on health, in the short-term, ascorbic acid can reduce sports performance by reducing muscle mitochondrial biosynthesis. Studies on experimental animals receiving ascorbic acid overdoses show a net reduction in athletic performance, but extrapolation of these data to the human species should take into account the fact that other animals, except humans, guinea pigs and monkeys, can synthesize ascorbic acid from glucose (Braakhuis, 2012). The chemical sensitivity of ascorbic acid, its conversion to dehydroascorbic acid, raises the issue of the quality of DS and, for analysts, of finding a method for extraction and determination of ascorbic acid in the reduced form (Spínola et al., 2014).

A rare deficiency in athletes who consume raw egg white (a myth imposed on athletes by the media) is biotin deficiency (avidin, a protein contained in raw egg white, links biotin in a non-absorbable form, heat treatment of eggs denatures biotin).

Oxidative stress directly correlated with physical activity, lifestyle, environmental factors and diet is known to be involved in many acute and chronic diseases and is caused by changing the redox state of the cell through an imbalance between prooxidants and antioxidant defense. The level of physical activity directly influences this balance, paradoxically sedentariness as well as high intensity physical exercise favors the formation of reactive oxygen species (ROS), while during moderate intensity effort, exercise-induced redox adaptations of the cell are involved and ROS are degraded as they are formed (Pittaluga et al., 2006). During intense exercise, aerobic metabolism increases the flow of oxygen in the cell mitochondria, increasing it up to 200 times in the striated muscle and leading to ROS formation. The antioxidant defense of the body includes a number of enzymes (superoxide dismutase, catalase or peroxidase) as well as other substances such as glutathione and antioxidant vitamins such as fat-soluble vitamins A, E, D or omega 3 fatty acids (Garcia-Bailo et al., 2011) and water-soluble vitamins thiamine, riboflavin, niacin, pyridoxine, folic acid, cobalamin, pantothenic acid, biotin, and ascorbic acid which is the most important hydrophilic antioxidant in human plasma.

Vitamin deficiency, types of anemia, consequences for athletes

Anemia is frequently reported in performance athletes and it may have different causes. The diagnosis of anemia should be made with caution, athletes often having pseudoanemia (or athletic anemia), and refers to an insufficient number of red blood cells correlated with the intensity of effort. "Sports anemia" is usually encountered in vegetarian athletes - in this case anemia is caused by diet, or in endurance athletes - in this case it is caused by iron loss through intestinal hemorrhage, urine or intravascular hemolysis (due to dehydration) (Latunde-Dada, 2013). However, there are situations where anemia can be explained by vitamin deficiency. The vitamin B group has an important role in the synthesis of red blood cells. Vitamin B6 is involved in heme synthesis, PLP being the co-enzyme of *aminolevulinic acid synthase*, an enzyme catalyzing the limiting step in heme synthesis. Vitamin deficiency may contribute to sideroblastic anemia (Cazzola & Malcovati, 2015). Vitamins B_{12} (cobalamin) and B_9 (folic acid) are erythrocyte maturation factors, vitamin deficiency causing megaloblastic anemia (Green, 2017).

Although the role of hemoglobin is well known, especially in aerobic sports, it is very difficult to determine how the ideal profile of the main hematological parameters should look like for athletes. Red blood cells are essential for the transport of oxygen from the lungs to tissues, but also contribute to maintaining optimal blood pH (7.4) as a component of the blood buffer system, to carrying carbon dioxide, binding protons H^+ and removing excess lactate released from the striated muscles during intense workout. Also, ATP and NO released from the red blood cells have vasodilator action, this action contributing to a better oxygenation of the tissues (Mairbäurl, 2013). An increase in hematocrit values has been observed especially in athletes practicing strength sports and less in those practicing endurance sports. This change in hematocrit

is associated with an increase in blood viscosity and is considered a cardiovascular risk factor. On the other hand, an increase in plasma homocysteine, another cardiovascular risk factor (homocysteine influences vascular endothelium and vascular muscle cells), was observed in the case of endurance athletes as a consequence of alteration of vascular structure and function (König et al., 2003; Herrmann et al., 2003; Ganguly & Alam, 2015). Hyperhomocysteinemia was negatively correlated with plasma levels of vitamin B_{12}, ferritin and hemoglobin, hematological biomarkers of iron deficiency anemia (Sirdah et al., 2014). Since hyperhomocysteinemia may occur due to vitamin B_6 and B_{12} deficiency, vitamin supplementation is effective in reducing plasma homocysteine levels and in reducing cardiovascular risk (Kumar et al., 2017).

The quality of tested commercial products and regulatory issues

Regarding the analytical experiment, in order to develop a simple, rapid and highly robust HPLC reversed-phase method for vitamin assay, structural issues were taken into consideration. The analyzed substances have structural heterogeneity with consequences on the dispersion of interaction with the stationary phase. The following physico-chemical properties were evaluated: the high hydrophilicity of ascorbic acid (log $P_{octanol/water}$ = -1.9) and thiamine (log $P_{octanol/water}$ = -2.1) in comparison with the other studied vitamins (riboflavin log $P_{octanol/water}$ = -1.46, pyridoxine log $P_{octanol/water}$ = -0.77, nicotinamide log $P_{octanol/water}$ = -0.37) (Drug Bank, 2018); the differences of their acid-basic properties and molecular masses. The proposed reversed-phase HPLC method proved to be suitable for specific separation and quantification of five hydrosoluble vitamins in less than 8 minutes. The monitoring wavelength was selected taking into consideration the absorptivity of the analytes and a high specificity against any kind of interference from the samples. The presence of methanol as part of the organic modifier in the mobile phase finally allows the specific separation of seven vitamins. As it was demonstrated (Fig. 2), the method is specific against two other hydrosoluble vitamins, folic acid and cyanocobalamin, which were not quantified as they were not present in the studied products. However, the DS contains calcium pantothenate and three other lipophilic vitamins, A, D and E. Pantothenate does not have absorptivity at 260 nm and we were not able to quantify this substance with the proposed method on the same HPLC system with a single wavelength detector (Wang et al., 2004; Hudson & Allen, 1984). On the other hand, the lipophilic vitamins from DS were not extracted by the proposed hydrophilic extraction method. Thus, specificity against the other vitamins from the analyzed DS was guaranteed. The proposed analytical method had good linearity in the chosen concentration ranges. Regarding the quality of commercial products, both DS and medicinal products had the concentrations of the studied vitamins in agreement with Pharmacopoeia provisions (Table II) (***, 2016).

The results confirmed that if the DS is a simple pharmaceutical "cocktail" of vitamins, quality could be easily verified by simple and valid analytical methods and these products could obey drug regulations. However, it is difficult to achieve the same quality for all types of DSs as for medicinal products, especially for DS with natural complex ingredients. As it was previously mentioned, regulations in many countries are permissive. There is an international debate regarding the necessity for a real, constructive cooperation between scientists and regulators to harmonize at global level their approaches in order to guarantee both safety and efficacy of DSs (Dwyer et al., 2018). In this case, skepticism regarding the quality of DSs will be no longer realistic.

Conclusions

1. Vitamin supplementation for performance athletes is justified by several reasons: vitamins (pyridoxine, thiamine, niacin) are involved in the catabolic processes of important energy substrates, have antioxidant properties (ascorbic acid), and are involved in the synthesis of red blood cells (pyridoxine, cobalamin, folic acid).

2. Most of the athletes use vitamin DS, but there is a risk that the products do not contain exactly the quantity declared by the manufacturer.

3. The tested products subjected to HPLC analysis contained the declared quantity of vitamins in agreement with the provisions of the European Pharmacopoeia.

Conflict of interest

There are no conflicts of interest.

References

Braakhuis AJ. Effect of vitamin C supplements on physical performance. Curr Sports Med Rep. 2012;11(4):180-184. doi: 10.1249/JSR.0b013e31825e19cd.

Brisswalter J, Louis J. Vitamin supplementation benefits in master athletes. Sports Med. 2014; 44(3):311-318. doi: 10.1007/s40279-013-0126-x.

Cazzola M, Malcovati L. Diagnosis and treatment of sideroblastic anemias: from defective heme synthesis to abnormal RNA splicing. Hematology Am Soc Hematol Educ Program. 2015; 2015:19-25. doi: 10.1182/asheducation-2015.1.19.

Chaudary A, Porter-Blake A, Holford P. Indices of pyridoxine levels on symptoms associated with toxicity: a retrospective study. J Orthomolecular Med. 2003;18(2):65-76.

Chawla J, Kvarnberg D. Hydrosoluble vitamins. Handb Clin Neurol. 2014;120:891-914. doi: 10.1016/B978-0-7020-4087-0.00059-0.

Choi SK, Baek SH, Choi SW. The effects of endurance training and thiamine supplementation on anti-fatigue during exercise. J Exerc Nutrition Biochem. 2013;17(4):189-198. doi: 10.5717/jenb.2013.17.4.189.

Cobley JN, McHardy H, Morton JP, Nikolaidis MG, Close GL. Influence of vitamin C and vitamin E on redox signaling: Implications for exercise adaptations. Free Radic Biol Med. 2015;84:65-76. doi: 10.1016/j.freeradbiomed.2015.03.018.

Dwyer JT, Coates PM, Smith MJ. Dietary Supplements: Regulatory Challenges and Research Resources. Nutrients. 2018 Jan 4;10(1). pii: E41. doi:10.3390/nu10010041.

Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J. 2015; 14:6. https://doi.org/10.1186/1475-2891-14-6.

Garcia-Bailo B, El-Sohemy A, Haddad PS, Arora P, Benzaied F, Karmali M, Badawi A. Vitamins D, C, and E in the prevention

of type 2 diabetes mellitus: modulation of inflammation and oxidative stress. Biologics. 2011;5:7-19. doi: 10.2147/BTT.S14417.

Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood. 2017; 129(19):2603-2611. doi: 10.1182/blood-2016-10-569186.

Gummin DD, Mowry JB, Spyker DA, Brooks DE, Fraser MO, Banner W. 2016 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 34th Annual Report. Clin Toxicol (Phila). 2017; 55(10):1072-252. doi: 10.1080/15563650.2017.1388087.

Herrmann M, Schorr H, Obeid R, Scharhag J, Urhausen A, Kindermann W, Herrmann W. Homocysteine increases during endurance exercise. Clin Chem Lab Med. 2003; 41(11):1518-1524.

Hudson TJ, Allen RJ. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid-chromatography, J Pharm Sci. 1984;73(1):113-115.

Kim HH, Kang YR, Lee JY, Chang HB, Lee KW, Apostolidis E, Kwon YI. The Postprandial Anti-Hyperglycemic Effect of Pyridoxine and Its Derivatives Using In Vitro and In Vivo Animal Models. Nutrients. 2018 Feb 28;10(285):1-11. doi: 10.3390/nu10030285.

Knez WL, Peake JM. The prevalence of vitamin supplementation in ultraendurance triathletes. Int J Sport Nutr Exerc Metab. 2010;20(6):507-514.

König D, Bissé E, Deibert P, Müller HM, Wieland H, Berg A. Influence of training volume and acute physical exercise on the homocysteine levels in endurance-trained men: interactions with plasma folate and vitamin B12. Ann Nutr Metab. 2003;47(3-4):114-118.

Kornecki E, Feinberg H. Pyridoxal phosphate inhibition of platelet function. Am J Physiol. 1980; 238(1):H54-H60. doi:10.1152/ajpheart.1980.238.1.H54.

Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. Nutr Metab (Lond). 2017;14:78. doi: 10.1186/s12986-017-0233-z.

Li D, Sun WP, Zhou YM, Liu QG, Zhou SS, Luo N, Bian FN, Zhao ZG, Guo M. Chronic niacin overload may be involved in the increased prevalence of obesity in US children. World J Gastroenterol. 2010;16(19):2378-2387.

Latunde-Dada GO. Iron metabolism in athletes–achieving a gold standard. Eur J Haematol. 2013;90(1):10-15. doi: 10.1111/ejh.12026.

Lheureux P, Penaloza A, Gris M. Pyridoxine in clinical toxicology: a review. Eur J Emerg Med. 2005;12(2):78–85.

Mairbäurl H. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. Front Physiol. 2013;4:332. doi: 10.3389/fphys.2013.00332.

Nelson RH, Vlazny D, Smailovic A, Miles JM. Intravenous Niacin Acutely Improves the Efficiency of Dietary Fat Storage in Lean and Obese Humans. Diabetes 2012;61(12):3172-3175. doi: 10.2337/db12-0236.

O'Riordan CN, Newell M, Flaherty G. Cardiovascular disease risk factor profile of male Gaelic Athletic Association sports referees. J Med Sci. 2018 Mar 27; doi: 10.1007/s11845-018-1792-3.

Pittaluga M, Parisi P, Sabatini S, Ceci R, Caporossi D, Valeria Catani M, Savini I, Avigliano L. Cellular and biochemical parameters of exercise-induced oxidative stress: relationship with training levels. Free Radic Res. 2006;40(6):607-614.

Schenk K, Bizzini M, Gatterer H. Exercise physiology and nutritional perspectives of elite soccer refereeing. Scand J Med Sci Sports. 2018 Mar; 28(3):782-793. doi: 10.1111/sms.12989.

Sirdah MM, Yassin MM, El Shekhi S, Lubbad AM. Homocysteine and vitamin B12 status and iron deficiency anemia in female university students from Gaza Strip, Palestine. Rev Bras Hematol Hemoter. 2014;36(3):208-212. doi: 10.1016/j.bjhh.2014.03.005.

Spínola V, Llorent-Martínez EJ, Castilho PC. Determination of vitamin C in foods: current state of method validation. J Chromatogr A. 2014;1369:2-17. doi: 10.1016/j.chroma.2014.09.087.

Suidasari S, Stautemas J, Uragami S, Yanaka N, Derave W, Kato N. Carnosine Content in Skeletal Muscle Is Dependent on Vitamin B6 Status in Rats. Front Nutr. 2016;2:39. doi: 10.3389/fnut.2015.00039.

Torrens SL, Areta JL, Parr EB, Hawley JA. Carbohydrate dependence during prolonged simulated cycling time trials. Eur J Appl Physiol. 2016;116(4):781-790. doi: 10.1007/s00421-016-3333-y.

Wang TM, Chiu YM, LC L, Hsu MC. Liquid chromatographic method for determination of calcium pantothenate preparations and related stability studies. J Food Drug Anal. 2004;12(1):1-6.

Zemirli N, Morel E, Molino D. Mitochondrial Dynamics in Basal and Stressful Conditions. Int J Mol Sci. 2018;19(2):564. doi: 10.3390/ijms19020564.

Zhou SS, Li D, Chen NN, Zhou Y. Vitamin paradox in obesity: Deficiency or excess? World J Diabetes. 2015;6(10):1158-1167. doi: 10.4239/wjd.v6.i10.1158.

***. Drug Bank. Available online at: https://www.drugbank.ca/, DB00126, DB00152, DB00140, DB00165, DB00158, DB02701, DB00115. Accessed in February 2018.

***. European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines (EDQM). European Pharmacopoeia, 9th edition, 2016.

***. Order 1069/2007 of the Ministry of Public Health – for the approval of Norms regarding dietary supplements. Official Monitor No. 455/ 05.07.2007.