

Research Article

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Role of water soluble vitamins in the reduction diet of an amateur sportsman

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Abstract: This study is aimed at determining the content of water soluble vitamins in amateur sportsmen before and after the use of reduction diet. Twenty adult male amateur sportsmen aged between 20 and 43 qualified for this study. The participants adhered to individually adjusted reduction diets for 3 months. Diet supplements were excluded from the diet during that time. Vitamins were analyzed before and after the reduction diet using HPLC. The following vitamins were analyzed: B1, B2, B3, PP, B5, B6, B7, B9, B12 and vitamin C. Statistically significant increase of riboflavin in blood plasma ($p=0.0254$) and statistically significant ($p=0.048$) decrease in the concentration of niacin was found when compared to the period before the diet. Appropriate level of riboflavin can be obtained by the consumption of proper amount of dairy in the diet. Supplementation with PP is advised and, in case of improper diet, the supplementation of B1, B7 and B12 may also be considered.

Keywords: vitamins group B, ascorbic acid, physical activity, reduction of adipose tissue, nutrition, sport

1 Introduction

It is generally known that to achieve better results in sport it is necessary to reduce fatty tissue, which can be done

through increased physical activity and/or reduction diet. In endurance sports, such as long-distance running, in addition to the reduction of fatty tissue it is also important to reduce total body mass [1]. To achieve this goal, sportsmen often undertake risky actions, which, if not properly controlled, can lead to nutritional deficiencies and serious health issues. In this study we are interested in water soluble vitamins, especially riboflavin and folic acid.

Riboflavin is a basic component of two coenzymes: flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). These coenzymes are involved in the transfer of electrons in energy and amino-acids metabolism and in the production of steroid hormones. This vitamin is crucial, especially for physically active people, due to its function in transferring the electrons to respiratory chain (electron transfer chain) to form ATP and in conversion of vitamin B₆ to its active form [2].

The latest FAO (Food and Agriculture Organization of the United Nations) / WHO (World Health Organization) standards regarding recommended riboflavin daily intake for adult men and women (at the age 19+) determined the same RDA levels as for population in Poland [3]. They are: 1.1 mg/day for women and 1.3 mg/day for men aged 19 and above. However, in the study by Szczuko et al. it was observed that currently recommended intake of riboflavin for Polish population may be insufficient [4].

Major sources of riboflavin in food are: dairy products, eggs, wholegrain products, green leafy vegetables, giblets, lentils and almonds, but dairy products are the richest in vitamin B₂. Some researchers claim that the inability to store the vitamin in large quantities in requires constant supplementation of riboflavin through diet [5]. Reduction diet used by examined sportsmen contained more dairy products (at least 3 portions a day), wholegrain products and leafy vegetables, which mainly contributed to higher amount of riboflavin in the diet – on average 2.68 and 2.89 mg/day (Table 2).

Recommended daily allowance (RDA) for vitamin B₂, according to standards for Polish population, both male

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and female, at the age ≥ 19 is 400 $\mu\text{g}/\text{day}$ [6]. The diets used by people participating in this study supplied, on average, 694 μg of vitamin B₉ per day. Folic acid is involved in the synthesis of purine and pyrimidine bases (methionine, histidine, choline), plays a crucial role in synthesis and methylation of DNA and in maintaining the integrity, stability and repair of deoxyribonucleic acid. It is also involved in amino acid metabolism and is an important compound in the synthesis of new cells, including red blood cells, and in repair of damaged cells and tissues. Because increased physical activity contributes to muscle tissue damage, the demand on folates may be higher. Folic acid together with vitamin B₁₂ and vitamin B₆ are closely interrelated in methionine metabolism (an essential amino acid). If any of these crucial vitamins is unavailable, the level of homocysteine, as an indirect metabolite in methionine metabolism, is growing. Elevated level of homocysteine potentiates cardio-vascular problems [2, 7]. Increase in homocysteine concentration in blood plasma is directly connected to damage and dysfunction of endothelial cells, leads to decreased concentration of nitric oxide (NO) and affects reduced elasticity of arteries. Moreover, hyperhomocysteinemia increases the peroxidation of LDL lipids, migration and infiltration of leukocytes into vessels' walls and accelerates the differentiation of monocytes to macrophages containing cholesterol – foam cells, thus contributing to increased oxidative stress.

Most common causes of deficiencies of folic acid in a diet are absorption disorders, culinary losses and improper dietary choices [2, 8]. The latest reports also suggest distorted methylation and effect of methylenetetrahydrofolate reductase (MTHFR) polymorphisms in improper absorption of folic acid and diet supplements. In their study Cabo *et al.* presented the effect of genetic polymorphisms on the concentration of folic acid in blood plasma before and after the introduction of supplementation with vitamin B₉ in diet [9].

Main source of folic acid in the diet are green vegetables such as spinach, lettuce, kale, broccoli, peas, beans, and also citrus fruits, yeast, whole grains and parsley roots. Folate belongs to vitamins especially susceptible to culinary processes leading to substantial losses during thermal treatment. Due to the fact that only half of the folic acid available in food is absorbed by our body, it becomes more and more attractive to enrich food products with folates, e.g. breakfast cereals and fruit juices, and introduce additional supplementation in everyday diet [10].

In our study, we also focused on proper supply of other vitamins and nutrients. The following vitamins were also analyzed in blood plasma: - thiamine, which is involved in the

metabolism of carbohydrates, fats and proteins, especially branched-chain amino acids (BCAA), - pantothenic acid that are essential in the Krebs cycle, - pyridoxin, mainly linked to amino acids metabolism and synthesis of neurotransmitters, - biotin, playing an important role in proper function of mitochondria, - cobalamin, essential in metabolism of methionine and formation of blood cells, - ascorbic acid, being a cofactor in collagen synthesis and playing a role in antioxidative defense of the organism.

The aim of this study was to compare the supply status of water soluble vitamins in amateur sportsmen before and after the reduction diet. It was assumed that using a properly balanced low-energy diet would contribute to increase in the concentration of all vitamins in blood plasma (without additional supplementation) in comparison to previous period comprising of regular diet. Undoubtedly, professional sportsmen should be supplemented, but we were interested to know whether an amateur sportsman requires such intervention if the diet is properly composed.

2 Materials and methods

2.1 Participants

The study involved 20 male amateur sportsmen at the age between 20 and 42 (average age 27.5), who reported to devote 4-6 hours a week to physical activity. The assumed physical activity index was at the level of 1.6 for training days, and 1.4 for other days. The study participants represented the following sport disciplines: canoeing, rowing and swimming. At the time of the analyses the average Body Mass Index (BMI) was 27.47 kg/m^2 (SD \pm 4.10). The measurements of body composition and basal metabolic rate were performed using bioimpedance method on the device BIA 101 (Akern, Italy). Essential criteria, qualifying for the study, were: the adherence to reduction diet for 3 months, not using any diet supplements during that period of time and body mass reduction by > 2.5 kg. The lack of fulfilling these criteria disqualified 8 people from the test group.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by Pomeranian Medical University Ethics Committee.

2.2 Analyses of water soluble vitamins with HPLC

All the HPLC grade reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). The following reagents were used in the analyses: NaHCO_3 , NaOH , HK_2PO_4 , methanol and acetonitrile. The buffers were prepared with Millipore grade water (Millipore, Billerica, MA, USA). To isolate the vitamins, amber-coloured Eppendorf tubes were used. The room for vitamins isolation was darkened to prevent photooxidation of vitamins.

For the analyses, 400 μl of plasma was collected and then the same amount of acetonitrile was added, as well as 100 μl of internal standard (Theobromine at the concentration of 100 ng/ml). All the reagents were mixed thoroughly for 2 minutes and then centrifuged for 15 minutes at 4000 rpm. Supernatant was collected in new tubes to evaporate acetonitrile. Water phase was transported onto solid-phase extraction (SPE) columns containing silica C-18 cartridge (Thermo Scientific) – activated beforehand with 1 ml of methanol and 1 ml of ultra-pure water. The compounds on the columns were eluted using 85% methanol with 1.5 ml of water. Obtained solution was further vacuum dried and solved directly before HPLC analysis in 100 μl of buffer A (25 mM HK_2PO_4) [11, 12].

Conditions of separation: The analysis was performed using HPLC chromatograph Infinity1260 or Binary LC (Agilent Technologies, Waldbronn, Germany). To separate the vitamins a gradient method was used with 25 mM HK_2PO_4 buffer, pH 7.0 – buffer A, and 100% methanol – buffer B, according to defined proportions presented in Table 1.

The separation of vitamins was performed on BDB Hypersil C – 18 column (Thermo Scientific) at the temperature of 35°C. The flow rate of the buffers was 0.9 ml/min and the injection volume was 10 μl [13]. Particular vitamins in analyzed samples were identified based on retention time of standard peaks. The analysis of the data was performed using ChemStation software, where the amount of each compound was calculated automatically

on the basis of previously obtained standard curves taking into consideration the correction for the internal standard (theobromine at the concentration of 100 mg/ml). Vitamins pattern shown in the chromatogram (Figure 1).

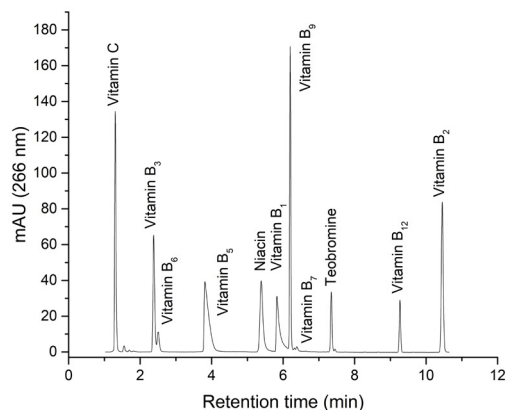


Figure 1. The pattern of water-soluble vitamins

2.3 Dietary intervention

For this study, two six-day reduction diets were prepared. They were individually assigned to the participants of the study depending on the type of physical activity. The calorificity of the diets was determined based on the needs to 2500 and 2800 kcal. The calories in the diets was dependent on individual anthropometric measurements. The diets were composed using the software „Diary of Meals MR BIG” ver. 4.92. The content of nutrients in the reduction diets is presented below in Table 2.

2.4 Statistical analysis

The analysis of obtained results was performed using Statistica 12.5 (StatSoft Polska). Mean values and standard deviation were calculated. The average contents of vitamins before and after the reduction diet were compared and $p \leq 0,05$ was regarded as statistically significant value.

Table 1. Separation of vitamins – proportions of buffers with respect to time.

Time [min]	Amount of buffer A [%]	Amount of buffer B [%]
0.0	97	3
2.5	97	3
7.2	70	30
14.0	70	30
16.0	97	3

Table 2. Nutritional content in reduction diets in comparison with current standards [6].

Nutrient	Standard	Mean±SD in 2500 kcal diet	% of norm realization	Mean±SD in 2800 kcal diet	% of norm realization
Protein [g]	45-81	104±47	128.4	118±43.7	145.7
Fat [g]	77-100	87±35.3	+	105±46.3	+
Digestible carbohydrates [g]	130	321±141.8	246.9	352±141	270.8
Dietary fibre[g]	25	40±15.6	160	46±17	184
FA SFA [g]		20±5.9		23±6.7	
MUFA[g]		42±21.5		46±19.3	
PUFA [g]		17±9.1		27±27.7	
Cholesterol [mg]	300	189±140.7	63	234±139.3	78
Sucrose [g]		35±7.2		34±7	
Lactose [g]		33±17.8		37±23.3	
β – carotene [µg]		11467 ±11178		10062 ±12338	
Vitamin A [µg]	900	2098 ±1865	233.1	1910 ±2021	212.2
Vitamin D [µg]	15	3.27±4.2	20	4.26±5.4	27
Vitamin E [mg]	10	19±9.6	190	20±10.6	200
Vitamin B1 [mg]	1.3	1.88±0.9	153.8	2.12±0.9	153.8
Vitamin B2 [mg]	1.3	2.68±0.6	230.8	2.98±0.8	230.8
Vitamin B3 [mg]	16	25±22.2	156.3	28±22.5	175
Vitamin B6 [mg]	1.3	3.4±2.3	153.8	3.88±2.3	307.7
Vitamin B9 [µg]	400	667±420.7	166.8	721±404	180.3
Vitamin B12 [µg]	2.4	5.38±1.2	208.3	6.21±2.2	250
Vitamin C [mg]	90	576±199.1	640	630±159.1	700
Sodium [mg]	1500	1917±1440.9	127.8	2189±1592.2	145.9
Potassium [mg]	4700	5633±2374.5	119.9	5995±2121.9	127.6
Calcium [mg]	1000	1288±437.5	128.8	1455±643.9	145.5
Phosphorus [mg]	700	2147±596.8	306.7	2479±663.4	354.1
Magnesium [mg]	400 - 420	586±191.4	139.5	644±190.2	153.3
Iron [mg]	10	15.91±7	160	17.63±6.6	180
Zinc [mg]	11	15.46±5.9	136.4	17.52±6.1	163.6
Copper [mg]	0.9	2.31±0.7	222.2	2.64±1.1	333.3
Manganese [mg]	-	9.67±4.3	-	12.07±4.2	-
Energy from proteins [%]	10-20	16.6		16.6	
Energy from fat [%]	20-35	31.3		33.3	
Energy from carbohydrates [%]	45-65	52.1		50.1	

3 Results

It was observed that the concentrations of niacin (vitamin PP) and riboflavin (vitamin B₂) were different before and after the reduction diet and this difference is statistically significant. The content of riboflavin, however, was

significantly increased by 0.015 ng/µl, and the content of niacin was significantly reduced by 0.01 ng/µl. The changes in the concentration of other vitamins in blood plasma of the participants of the study were determined as statistically insignificant (Table 3). However, insignificant increase in folic acid and pyridoxine and insignificant decrease in thiamine and biotin were also noted.

Table 3. Average concentration of vitamins in blood plasma before and after the reduction diet.

Vitamin [ng/ μ l]	Before reduction diet	After reduction diet	<i>p.</i> values
B ₁ – Thiamine	679.3 \pm 188.7	618.53 \pm 406.7	0.626
B ₂ - Riboflavin	0.033 \pm 0.018	0.048 \pm 0.022	0.025*
B ₃ – Nicotinic acid	0.260 \pm 0.053	0.257 \pm 0.052	0.884
PP – Niacin	0.027 \pm 0.019	0.017 \pm 0.012	0.048*
B ₅ - Calcium pantothenate	0.140 \pm 0.027	0.150 \pm 0.058	0.600
B ₆ - Pyridoxine	0.368 \pm 0.201	0.433 \pm 0.261	0.429
B ₇ – Biotin	0.161 \pm 0.559	0.100 \pm 0.000	0.339
B ₉ – Folic acid	0.410 \pm 0.143	0.573 \pm 0.910	0.532
B ₁₂ – Cobalamin	0.405 \pm 0.291	0.381 \pm 0.377	0.846
C – Ascorbic acid	0.977 \pm 0.086	0.997 \pm 0.136	0.743

4 Discussion

The supply of daily demand of required vitamins in case of physically active people requires a rational approach. Some of the vitamins play the role of biocatalysts for biochemical reactions in an organism. One of the most important roles of vitamins, especially antioxidative ones, is their protective function against the harmful effects of free radicals and reactive oxygen species, which damage cells, tissues, proteins, fats or nucleic acids, that leads to many diseases including those of digestive system, cardiovascular system, neurological disorders or tumors [8]. Such situation requires corrective measures to rationalize the diet by selecting appropriate products. This, however, might not be always the most successful way especially in the case of reduction diet with lowered calorificity. It seems that the best and fastest strategy to eliminate the deficiencies in vitamins and mineral compounds in sportsmen is to introduce enriched products and supplements to their diet [14]. Referring to the demand on vitamins in sportsmen one should consider mainly the type, intensity and duration of physical activity. The demand on energy in such people is usually between 3500 and 5000 kcal per day, which is a challenge due to increased demands on nutrients, including vitamins and minerals [15]. For professional sportsmen, the type of diet and obtained results are interconnected. Thus, it is not sufficient to adjust the energy supply only, but it is also crucial to supply the demands on vitamins and mineral by diversification of food products. A diet supplying higher amount of energy, protein, B group vitamins, antioxidative vitamins, mineral salts (being lost at higher amounts with water), and electrolytes increases health potential in a sportsman, favors optimal results of physical efforts and

contributes to increased efficiency [16]. Malnutrition and dehydration, which are common metabolic consequence of caloric restrictions and are also connected with more frequent occurrence of sports injuries and contusions [17].

4.1 Riboflavin

Analyzing the supply of riboflavin, we would like to refer to the study of Hustad et al., who performed dietary intervention connected with this vitamin [18]. The study lasted for 12 weeks, in two examined groups a diet containing 1.6 mg of riboflavin per day was used, and the caloric load of the diets did not significantly differ between the groups. One of the group was additionally given a supplementation of 1.6 mg riboflavin per day, the other group obtained placebo. Additional supplementation did not contribute to increased activity of glutathione reductase (EGRAC), which signifies that there were no riboflavin deficiencies with average content of this vitamin in the diet at the level of 1.6 mg/day. However, the diet containing in total 3mg of riboflavin per day increased the concentration of vitamin B₂ in blood plasma [18].

In another study by Szczuko et al. the nutritional status with respect to riboflavin was assessed among 120 people at the age between 22-25 years. The analysis was also performed based on EGRAC. In that study, EGRAC levels were compared against riboflavin content in 7-day food records. The average riboflavin consumption in female group, where biochemical deficiencies in vitamin B₂ were observed, amounted to 1.05 mg/day, whereas in male group – 1.39 mg/day. In groups where biochemical deficiencies of vitamin B₂ were not observed, the average consumption of riboflavin was 1.43 \pm 0.25 mg in female and 1.8 \pm 0.30 mg in male groups per day [4]. Analysis of

the two above mentioned studies suggests that riboflavin consumption should be higher than current RDA. In the study by Malary et al. the nutritional status with respect to the vitamins B₁, B₂ and B₆ was assessed and compared against the consumption of these vitamins with diet in the group of women and men characterized by lower and higher physical activity. Higher riboflavin consumption was determined in all the groups and it amounted to 1.63 ± 0.33 and 1.83 ± 0.27 for women and 2.22 ± 0.37 and 2.42 ± 0.54 for men [19]. On the other hand, in the study by Frank et al., which concerned race-walkers, after the physical effort it was observed that the level of riboflavin in blood plasma increased and, simultaneously, the activity of glutathione reductase (EGRAC) decreased [20]. Roghead and McCormick reported in their study that the doses of riboflavin of 1.7 mg/day in healthy adult people (physical activity was not mentioned) increase the excretion of vitamin B₂ with urine [21]. It indicates that for an average healthy person the daily demand is lower than 1.7 mg.

The fact that riboflavin plays a role in many biochemical and energetic processes in a human body makes it especially vital for sportsmen. Occurring mainly in the form of FMN and FAD, the vitamin takes part in redox reactions, such as β -oxidation of fatty acids, citric acid cycle, amino acids metabolism, steroid hormones production and mitochondrial electrons transport. An attention should be put on the significance of riboflavin in glutathione oxidation. Glutathione plays a crucial role in maintaining proper structure of erythrocytes and the stabilization of hemoglobin being in reduced state capable of binding oxygen by Hb-Fe²⁺. The physiological level of glutathione and appropriate activity of glutathione peroxidase (GSH-Px) are responsible for antioxidative defense in erythrocytes. Reduced levels of glutathione and GSH-Px are linked to increased inflammation processes connected with oxidative modification in lipids and proteins of the membrane, leading in consequence to destabilization and decreased survival among erythrocytes [22]. An enzyme- glutathione reductase, related to glutathione, is present in cytosol and mitochondria. The enzyme is a flavoprotein, whose role is to keep a proper concentration of GSH (glutathione, reduced form) in the cells. The process is based on the conversion of glutathione disulphide, GSSG (oxidized glutathione) to GSH [23]. The concentration of GSH depends, among others, on the regeneration rate of GSSG in the cells. This in turn is dependent on the activity of glutathione reductase and the availability of NADPH, which is connected to the amount of riboflavin present in the tissues [22]. Based on this information it can be indicated that vitamin B₂ indirectly contributes to the prevention of blood cells hemolysis and

thus to efficient transport of the oxygen by erythrocytes. It is very important because the following factors facilitate the adaptation of respiratory system to higher physical activity: increased lung diffusing capacity, higher oxygen transport to tissues and much increased respiratory minute volume. Our studies show that 3 portions of dairy in the diet are sufficient to cover the demand on riboflavin in amateur sportsman. An additional advantage of dairy products is the content of calcium, which favorably affects pH balance and alkalisation of the organism.

4.2 Niacin

The recommendations of FAO/WHO regarding niacin indicate that for men at the age ≥ 19 the daily allowance should be at the level of 16 mg, and for women – 14 mg. In the reduction diets the average content of niacin was 25 and 28 mg/day and despite that the concentration of niacin in blood plasma from the participants of the study significantly decreased. People obtain niacin from exogenous and endogenous sources. The latter is the result of metabolic conversion of tryptophan to niacin [24]. Niacin is present in food products such as: meat, poultry, fish, yeast, enriched and whole meal bread, grains, peanuts, milk, eggs and green vegetables [5]. Both NADP⁺ and tryptophan in diet are the main sources of nicotinamide or niacin with the conversion rate of 60:1 (1mg of niacin = 60mg of tryptophan) [25]. In the study of Wierniuk and Włodarek involving 25 men training aerobic sports and not using any supplementation, the deficiencies in energy were observed but did not contribute to deficiencies in niacin [26].

Niacin is crucial for proper function of our body and is especially important for sportsmen. It is a compound of two important enzymes – NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate). These coenzymes are engaged in more than 500 enzymatic reactions in the human body. The whole energy production in our bodies, including oxidative phosphorylation in mitochondria, Krebs cycle and cytosol glycolysis, depend on these enzymes. NADP and NADH, biomolecules also dependent on niacin, are important for the synthesis of nucleic acid, fatty acids and cholesterol, which is vital for the repair of DNA and production of steroid hormones. Moreover, it has a huge influence on the reduction of the risk of cardiovascular diseases and cancers [5, 25]. Due to the increase in the concentration of NADPH in cells, an increased amount of glutathione reductase is observed (GSH), the activity of which requires vitamin B₂. As a result, this improves the

ability of the cells to detoxify H_2O_2 and shows that niacin has an antioxidative activity [8].

Reduced form of NAD^+ , i.e. NADH, is oxidized on complex I in oxidation chain. Because of electron transport system in electron transport chain, the oxidized form of nucleotide is responsible for the synthesis of three ATP molecules [25]. The basic task for our body is to obtain the energy stored in the form of high-energy compounds, mostly in the form of adenosine triphosphate (ATP). Muscle contraction causes the ATP molecule to break up, and the formation of the next ATP molecule allows for another such contraction. The muscle may produce ATP through aerobic and anaerobic processes. Initial physical effort is connected to anaerobic processes, where the energy is obtained from phosphocreatine or glycolysis. As physical activity continues, the energy is gradually taken from aerobic processes, where ATP molecule is subjected to cellular oxidation [27]. Aerobic glycolysis enables to obtain one mole of glucose from 38 moles of ATP, oxidation of one molecule of palmitic acid (major representative of free fatty acids – FFA) enables to obtain 129 moles of ATP, one mole of glucose in the process of anaerobic glycolysis enables to obtain 2 moles of ATP.

As can be observed, the above values have a substantial influence on the use of energetic substrates from the point of view of contraction activity, the period and maximal power developed during the activity performed by muscles. The efficiency of physical activity depends on appropriate course of the energetic changes mentioned above. Involvement of niacin in energy production makes it very crucial for a sportsman. However, too high supplementation (280 mg/day) may contribute to the inhibition of the oxidation of free fatty acids through decreased lipolysis. This, as a result, would reduce the availability of the main source of fuel, forcing the muscles to base their activity on stored glycogen. This process may be disadvantageous for long-lasting, intensive physical activity [28]. Niacin is deeply rooted in energetic metabolism, so the demands on this vitamin seem to be substantially - perhaps several times - higher in sportsman. We think that the decrease in niacin in the participants of the study after the reduction diet might be also caused by higher amount of carbohydrates in the diet in comparison to the amount they consumed before [29].

4.3 Thiamine

The recommended daily allowance (RDA) on vitamin B_1 for Polish population at the age ≥ 19 is 1.3 mg for men and 1.1 mg for women. Even though the average consumption

of vitamin B_1 with the diet was 1.88 ± 0.9 and 2.12 ± 0.9 mg/day, the level of its concentration in blood plasma insignificantly statistically decreased by 60.8 ng/ μ l. Thiamine plays an important role in the metabolism of carbohydrates, fats and proteins, especially branched-chain amino acids (BCAA) – isoleucine, leucine and valine. An active form of thiamine (thiamine pyrophosphate (TPP) is a coenzyme in many key reactions in energy production, which are extensively utilized during physical activity. For example, TPP is needed to convert pyruvate into acetyl-CoA and for α -ketoglutarate dehydrogenase in citric acid cycle (Krebs cycle). It means that thiamine plays a crucial role in aerobic metabolism of carbohydrates, fats, as well as BCAA metabolism, which increases especially with endurance exercises [2]. It was also proved that thiamine has an antioxidative function. Reduction of the level of vitamin B_1 in blood may affect proper course of abovementioned processes, and hence its deficiency may have a negative effect on a sportsman organism. Thus it seems that in case of vitamin B_1 we may consider additional supplementation for amateur sportsmen.

4.4 Pantothenic acid

The daily demand on pantothenic acid in the group of men and women aged 19 and above is 5mg/day [6]. The active form of pantothenic acid is coenzyme A, which plays an important role in our organism. It is a major cofactor in oxidation of fatty acids, lipids elongation and fatty acids synthesis. It participates in Krebs cycle (CoA is part of a catalyzing process leading to the formation of acetyl-CoA in mitochondria), is engaged in the formation of many secondary metabolites such as ubiquinone, squalene, cholesterol, steroid molecules (steroid hormones, vitamin D and bile acids) and in the synthesis of porphyrin ring structures of hemoglobin, and takes part in the acetylation of compounds, e.g. neurotransmitters, forming acetylcholine and acetylserotonin. It also participates in the biosynthesis of phospholipids, including phosphatidylcholine, serine or inositol [5, 25, 30]. The above information show that the involvement of pantothenic acid in many processes in human body makes this vitamin indispensable, especially for a person with higher physical activity, in whom the activity of metabolic pathways, including energetic ones, is increased. The diet rich in pantothenic acid influences the increase of the level of glutathione (GSH) in human organism. The balance between the pro-oxidants and anti-oxidants is mainly dependent on glutathione [14]. Pantothenic acid and its derivatives have a favourable

effect by promoting the synthesis of GSH and preventing its degradation. Physical activity is linked to intensified production of reactive oxygen species (ROS), mainly in mitochondria, leading to increased prooxidative activity, which can thus result in oxidative stress, cellular damage and inflammatory processes in a sportsman organism [14, 31]. According to the above information, it can be claimed that due to increased physical activity, which is connected to intensified production of pro-oxidants, the supply of large enough amount of vitamin B₅ is very important, as it indirectly causes the reduction of ROS in an organism. Interestingly, harmful effects of pantothenic acid were not observed even with oral supplementation at the level of 10 g per day. It is caused by effective excretion of the excess of vitamin B₅ with urine. The doses of >10 g /day may lead to mild intestinal problems and diarrhoea [5, 14]. In the diet, pantothenic acid is present mainly in the form of coenzyme-A, which undergoes hydrolysis to free pantothenic acid before absorption in intestines. The mechanism of pantothenic acid absorption in the intestines is mediated by sodium dependent multivitamin transporter (SMVT). However, there are no information on how pantothenic acid leaves the absorption cells by bulk liquid membrane (BLM) [32]. The fact that the concentration of pantothenic acid in blood of the participants of the study increased after using the reduction diet may show that its amount in appropriately composed reduction diet is sufficient.

4.5 Pyridoxin

Both men and women between 9 and 50 year of age should consume 1.3 mg of vitamin B₆ daily. Above 50 year of age the norm for men is 1.7 mg/day and for women – 1.5 mg/day [6]. The average concentration of vitamin B₆ in the diets of participants of this study was 3.637 mg/day and fully realized the demand.

Vitamin B₆ is available in products of both plant and animal origin. The following products are good sources of this vitamin: meat, poultry, fish, chickpeas, wholemeal products, nuts, potatoes, spinach, bananas, avocado, baking yeast and sunflower seeds. The most commonly present form of vitamin B₆ in food are: pyridoxin, pyridoxal and pyridoxamine [6, 32].

Vitamin B₆ (pyridoxin, pyridoxal, pyridoxamine and their phosphate derivatives) has primarily a function as an essential cofactor of more than 100 enzymes, which are mainly linked to amino acids metabolism (transamination, deamination, decarboxylation), glycolysis and gluconeogenesis (during exercises pyridoxal phosphate

is necessary for gluconeogenesis and glycogenolysis, where it serves as a cofactor for glycogen phosphorylase). Vitamin B₆ is also used in decarboxylation of some amino acids to neurotransmitters. Examples of such conversions are: histidine to histamine, tryptophan to serotonin, glutamate to γ -aminobutyric acid (GABA) and dihydroxyphenylalanine to dopamine. Oxydoxin is also needed in the synthesis of sphingolipids, hemoglobin and for genes expression. Due to its stable level before and after the reduction diet it doesn't seem that its supplementation is necessary in amateur sportsman.

4.6 Biotin

Both men and women at the age ≥ 19 need to consume 30 μg of biotin per day. Biotin deficiency is extremely rare. It is most commonly justified by the presence of avidin (in raw eggs), which forms a stable complex with biotin disabling the absorption of the vitamin. The most important symptoms of deficiency, from the point of view of a person with increased physical activity, is drowsiness, apathy, anxiety, muscle pains or tactile hypersensitivity. Another cause of vitamin B₇ deficiency is shortage in holocarboxylase synthetase linking biotin to lysine. This vitamin is a coenzyme for five mitochondrial carboxylases and plays an essential role in growth, development and proper function of mitochondria and cells. Carboxylases take part in the process of gluconeogenesis, amino acids and fatty acids metabolism [25, 33].

Biotin is present mainly in products of animal origin and in smaller amounts in plants. Reduction diets used by the participants in this study, were richer in products of plant origin. Therefore, it seems that strictly vegetarian diet is not a good solution for an amateur sportsman.

4.7 Folic acid

Diet rich in vegetables and fruits allows to easily supply vitamin B₉ at RDA level (400 $\mu\text{g}/\text{day}$). Despite low absorbability of folic acid from food products and potentially large losses of this vitamin during thermal treatment, the decrease of the concentration of vitamin B₉ in blood was not observed. Increased level of vitamin B₉ in blood plasma of people adhering to reduction diet, in comparison to previous period, may indicate that higher physical activity does not contribute to vitamin B₉ deficiency in case of significantly exceeded RDA level. In such situation it does not seem necessary to supplement vitamin B₉ with a separate preparation containing folic

acid only.

4.8 Cobalamin

After the diet, an insignificant (0.0238 ng/ μ l) decrease in cobalamin concentration was observed in blood plasma in comparison to the level of vitamin B₁₂ from the period before using reduction diets.

Vitamin B₁₂ helps in DNA synthesis, is necessary for the formation of red blood cells and its deficiency leads to megaloblastic anemia [2]. Disturbed cobalamin absorption may be the result of e.g. gene mutations or atrophic gastritis, which contributes to reduced secretion of Castle's intrinsic factor [24]. Cobalamin is essential for proper function of nervous system. By forming layers protecting nerves it guards nerve cells from their damage, which may lead to impaired transmission of nerve impulses and cause neurological problems. Vitamin B₁₂ is also involved in methionine metabolism, mentioned before in case of folic acid. The vitamin deficiency increasing the risk of cardiovascular diseases [2].

Cobalamin comes from endogenous and exogenous sources. Human microbiota is able to produce this vitamin, but its main sources are animal products consumed with the diet.

The study of Herrmann et al. aimed at analyzing the changes of vitamin B12 levels in recreational endurance sportsmen. All the people consumed food products of plant and animal origin and did not use supplementation. The reference level for vitamin B12 in blood plasma was determined at the level 211-911 pg/mL. In the study it was observed that groups of physically active and not active people had similar levels of vitamin B₁₂ in blood (average for athletes – 343 pg/mL, control – 350 pg/mL) [34]. It may suggest that physical activity does not increase the demand on cobalamin. Similar conclusions were drawn by other authors. These results correspond with our observations.

4.9 Ascorbic acid

According to RDA for Polish population, the daily demand for ascorbic acid in men aged ≥ 19 is 90 mg/day. Women at the same age should supply 75 mg/day [6]. The average consumption of this vitamin in the diets of the participants of this study was 603 mg/day. It is an almost 6 times higher amount than the RDA mentioned above.

Ascorbic acid is an important vitamin for humans. It is needed as an enzymatic cofactor in collagen synthesis

and thus it is used in production and regeneration of skin, tendons or blood vessels. It is also used by enzymes in activation of various types of neuropeptides and to release hormonal factors. It participates in carnitine synthesis and in liner – in glucose, cholesterol and lipids metabolism. Deficiencies may contribute to problems in nervous and immune systems. In central nervous system the vitamin is involved in antioxidative protection (as a first line of defense), amidation of peptides, formation of myelin, protection of synapses and against glutamate-induced toxicity. Because our organism stores only a small amount of vitamin C and the stock is quickly depleted, it is necessary to supply this vitamin with food [5, 35].

Physical activity increases the production of free radicals and lipids peroxidation. Hard exercises done by untrained people may cause oxidative damage leading to muscle injuries. Aerobic training strengthens antioxidative defense system through increasing the level of superoxide dismutase. Vitamin C affects the reduction of the speed of lipid peroxidation induced by physical effort [36]. The vitamin is susceptible to thermal treatment. The losses may even exceed 80% in comparison with fresh raw produce. It is also affected by the presence of oxygen, trace amounts of copper and iron and enzymes.

The study of Bryer and Goldfarb aimed at assessing the effect of high doses of vitamin C on functions, fatigue, muscle damage and oxidative stress in exercises of eccentric character. The participants of the study were divided into two groups – one of them was given a supplementation with 3g of vitamin C per day, the other was given placebo. After analyzing all the data, it was determined that ascorbic acid may alleviate muscle pain, delay the increase of creatine kinase and prevent the oxidation of glutathione in blood with little effect on muscle function loss [37]. Zoppi et al. in their study tested the effect of vitamins C and E supplemented in professional football players who regularly attended the training sessions. The data showed that the supplementation of antioxidative vitamins (C and E) may reduce lipids peroxidation and muscle injuries during high intensity exercises but it does not influence higher efficiency [38].

Appropriate concentration of ascorbic acid in blood plasma was established at 23 – 84 μ mol/L [35]. However, in the study by Szczuko et al. people consuming 60 mg of ascorbic acid per day had an average concentration of this acid in their blood plasma at the level above 55 μ mol/L [40]. In Peake's study, the average concentration of vitamin C in blood plasma in the participants of the study were: before the test – 97 μ mol/L, after the test – 99 μ mol/L. Both levels exceeded reference levels mentioned in this work [35]. Based on the results obtained by Szczuko

et al. it may be claimed that the participants of Peake's study consumed much more vitamin C than 60 mg/day. As the study of Jonathan M. Peake reports, endurance sports (running) demand higher intake of ascorbic acid but high doses are excreted from the organism and do not significantly affect the increase of the level of ascorbic acid in blood plasma.

Analyzing the information, the level of ascorbic acid in blood is a parameter mainly dependent on current intake of vitamin C with the diet [5]. Therefore, with appropriate consumption of vegetables and fruits in the diet the supplementation with vitamin C does not seem to be necessary.

5 Conclusions

The diet containing at least 3 portions of dairy products per day fulfils daily demand on vitamin B₂ and results in the increase of the concentration of this vitamin in blood plasma, even in amateur sportsmen.

Increased physical activity does not contribute to the reduction of riboflavin concentration in blood plasma if its average consumption is at the level of 2.830 mg/day.

Consumption of niacin at the level of 27 mg per day in amateur sportsmen, with simultaneous increased physical activity and increased amount of carbohydrates in the diet, is insufficient. Thus, the supplementation with vitamin PP for sportsmen using a reduction diet seems to be necessary.

In case of vitamins B₁, B₇ and B₁₂ the realization of RDA norms for Polish population is insufficient for amateur sportsmen and the introduction of supplementation with these vitamins should be considered, especially if the diet is not properly composed.

Supplementation with vitamins B₅, B₆, B₉ and C does not seem necessary in case of properly composed reduction diet for amateur sportsmen.

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