ASSESSMENT OF INTAKE AND NUTRITIONAL STATUS OF VITAMIN $B_1$, $B_2$, AND $B_6$ IN MEN AND WOMEN WITH DIFFERENT PHYSICAL ACTIVITY LEVELS

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ABSTRACT: The purpose of the present study was to examine the nutritional status of vitamin $B_1$, $B_2$, and $B_6$ in respect to dietary intake of these vitamins and activity coefficients of the erythrocyte enzymes transketolase, glutathione reductase, and aspartic aminotransferase in young men and women with different physical activity levels. The participants of this study were 20 women and 20 men with high physical activity (groups HAW and HAM, respectively), and 20 women and 20 men with low physical activity (groups LAW and LAM, respectively). The intake of vitamins $B_1$, $B_2$, $B_6$, proteins, and calorie content of the diet was based on the average of the 4-day dietary recalls. To assess nutritional status of vitamin $B_1$, $B_2$, and $B_6$, the activity coefficients ($a$) of erythrocyte transketolase (ETK), erythrocyte glutathione reductase (EGR), and erythrocyte aspartic aminotransferase (EAST) were estimated in blood hemolysates. The intake of the studied vitamins in the diet was statistically significantly lower in the female groups compared with the respective male groups. Deficiency of vitamin $B_6$ in the diet was present more often in women than in men (in terms of the recommended dietary allowances [RDA]). Values of the activity coefficient $a_{ETK}$ indicated that none of the groups in this study suffered the risk of vitamin $B_1$ deficiency. The value of the activity coefficient $a_{EGR}$ indicated that the groups of women and men with low physical activity were more prone to vitamin $B_2$ deficiency compared with the high physical activity groups. The risk of vitamin $B_6$ deficiency ($a_{EAST}$) in both male groups was higher than in both female groups. The obtained results do not allow for unequivocal determination of the impact of sex and the level of physical activity on intake and nutritional status of vitamin $B_1$, $B_2$, and $B_6$. Independently of sex and the level of physical activity, the women and men consumed insufficient quantities of vitamins $B_1$ and $B_6$, although this was not always related to increased values of corresponding activity coefficients.

KEY WORDS: Vitamin intake, vitamin nutritional status, physical activity, women, men

INTRODUCTION

Vitamin $B_1$, present in the human body in the form of thiamine pyrophosphate, is a coenzyme of numerous enzymes of oxidative decarboxylation of alpha-keto acids, such as pyruvic and alpha-ketoglutaric acids, as well as synthesis of keto acids from branched-chain amino acids. These enzymes are part of an enzymatic complex which plays a key role in processes related to mitochondrial energetic metabolism [1]. Moreover, as a transketolase coenzyme, it participates in the pentose cycle as well as haemoglobin synthesis [7]. It is well known that thiamine plays a role in the control of cell metabolism in allosteric regulation of enzyme activities responsible for energy metabolism, in transmitting nerve signals in synapses, and in environmental signal transduction [27]. Thiamine deficiency, demonstrated by its decrease in urine as well as the increase of transketolase activity coefficient, leads to beriberi disease. Considering the limited ability of absorbing thiamine in the digestive system and the ease of its excretion in urine, it is extremely rare to observe undesirable effects related to its excessive intake [20].

Vitamin $B_2$ (riboflavin) appears mostly in the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These nucleotides take part in redox reactions, such as $\beta$-oxidation of fatty acids, the tricarboxylic acid cycle, amino acid metabolism, production of steroid hormones, and mitochondrial electron transport [19]. In addition, riboflavin participates in vitamin $B_6$ and oxidized glutathione metabolism, in fatty acid and uric acid synthesis, as well as in metabolism of some medications [28]. The symptoms of vitamin $B_2$ deficiency are local inflammation of mucosal membranes and disturbances in the functioning of the nervous system. The risk of adverse effects caused by excessive intake of riboflavin, because of the limited ability of its absorption, is practically nonexistent [13].

Vitamin $B_6$, in the form of pyridoxal 5'-phosphate, is a coenzyme of numerous enzymes participating in amino acid metabolism, such as aminotransferases, decarboxylases, and dehydratases. As a coenzyme of glycogen phosphorylase, it is responsible for phosphorylation of glycosidic bonds in glycogen, which control the process of
glycogenolysis in muscles. In addition, vitamin B₆ plays a key role in metabolism of neurotransmitters, such as dopamine and serotonin, and it ensures efficient functioning of the immune system. Further, this vitamin is indispensable in homocysteine metabolism, and it participates in haem biosynthesis [5]. The symptoms of vitamin B₆ deficiency are local inflammation of the skin and dysfunction of the nervous system, while adverse neurological effects of its excessive intake can only be observed when synthetic compounds containing this vitamin are used [2,12].

Numerous published studies indicate a close relationship between inadequate nutritional status of the body, related to deficiency of vitamins and minerals, and the development of various diseases. It should be kept in mind that the level of requirement for these nutrients depends on many factors such as age, sex, body mass, lifestyle, and the physiological condition of the organism [6]. It is well known that physically active people require higher intake of vitamins compared to people with low physical activity, because of the increased losses in sweat and urine, reduced levels of absorption, and increased enzyme activity [19]. Brodney et al. [3] conducted a study of young women and men with differing levels of physical activity. Their results show that the diet of women and men with high physical activity was more balanced and was characterized by higher intake of vitamins compared with participants with low and moderate physical activity. Gacek et al. [11] found that the diet of young active women and men is often characterized by an insufficient number of meals during the day and too low intake of fish, vegetables, and fruit. In addition, the diet of physically active people includes excessive consumption of sweets, fast-food style meals, and sweetened drinks, but insufficient water. For this reason, an unbalanced diet with little variety in physically active people is related to increased risk of inadequate intake of vitamins and other nutrients [11,17,26].

The nutritional status of B vitamins dietary intake can be assessed by biochemical indicators in blood and/or urine [14,25,28]. The useful markers for nutritional status of vitamin B₁ (thiamine), B₂ (riboflavin), and B₆ (pyridoxine) are the activity coefficients (α) of erythrocyte transketolase (ETK), erythrocyte reductase (EGR), and erythrocyte aspartic aminotransferase (EAST). The activity coefficients of ETK, EGR, and EAST increase when the intake of these vitamins is inadequate [13].

The purpose of the present study was to examine of nutritional status of vitamin B₁, B₂, and B₆ in respect to dietary intake of these vitamins in young women and men with differing levels of physical activity, as well as to determine to what degree sex and active lifestyle contribute to deficiencies of these vitamins.

**MATERIALS AND METHODS**

The participants of this study were 20 women and 20 men with high physical activity (groups HAW and HAM, respectively), practicing various endurance sport disciplines, and 20 women and 20 men with low physical activity (groups LAW and LAM, respectively), who participated only in the physical exercise classes prescribed by their programme of study. The participants of the study were assigned to appropriate physical activity groups based on the International Physical Activity Questionnaire (IPAQ) [4]. People using preparations containing vitamins B₁, B₂, and B₆ were excluded.

All subjects gave their written consent to participate in the study, which commenced after the local Ethics Committee issued its approval.

The assessment of intake of vitamins B₁, B₂, B₆, protein, and calorie content of the diet was based on the average of 4-day dietary recalls in the days preceding collection of blood. The interviews took into account the menus for two weekdays and two weekend days (Saturday and Sunday). Daily food intake was estimated using the Album of Photographs of Products and Meals, which shows food types and portion sizes in grams, and then analyzed using the computer program Dieta 4.0, created by the Institute of Food and Nutrition in Warsaw. The analysis of dietary intake of vitamins B₁, B₂, and B₆, taking into account the level of estimated average requirement (EAR) and recommended dietary allowances (RDA), was conducted based on dietary requirements for humans [16]. The intake of vitamin B₁ was also expressed in terms of rate per 1000 kcal, and for vitamin B₆ in terms of rate per 1 g of dietary protein [16]. Dietary reference values for B₁, B₂, and B₆ in terms of estimated average requirement (EAR) and recommended dietary allowances (RDA) are presented in Table 1.

All participants were weighed and measured in order to enable calculation of their BMI values. Fat tissue content was determined using the method proposed by Durnin et al. [8]. The measurements of skinfolds were performed in a standing position on the left side of the body, using the Holtain caliper (United Kingdom). Each measurement of four skinfolds (at the biceps, triceps, suprailiac, and subcapular sites) was performed three times, and the mean values were used in calculating the percentage of fat content. The general characteristics of male and female subjects are shown in Table 2.

**TABLE 1. DIETARY REFERENCE VALUES FOR VITAMINS B₁, B₂, AND B₆ FOR WOMEN AND MEN [15]**

| Vitamin intake (mg·day⁻¹)* | Group | Group |
|----------------------------|-------|-------|
|                            | Women | Men   |
| B₁                         |       |       |
| EAR                        | 0.9   | 1.1   |
| RDA                        | 1.1   | 1.3   |
| B₂                         |       |       |
| EAR                        | 0.9   | 1.1   |
| RDA                        | 1.1   | 1.3   |
| B₆                         |       |       |
| EAR                        | 1.1   | 1.1   |
| RDA                        | 1.3   | 1.3   |

Note: * EAR – estimated average requirement, RDA – recommended dietary allowances.
Venous blood was collected in the morning before breakfast in the sitting position into polypropylene test tubes containing lithium heparin, and then it was frozen at -70°C until assayed. In blood hemolysates, thiamin (vitamin B₁), riboflavin (vitamin B₂), and pyridoxine (vitamin B₆) status was assayed by measuring respective enzyme activities without and with their coenzyme added in vitro. The enzyme activity coefficients (α) were calculated as the ratio of stimulated (coenzyme added) to unstimulated (no coenzyme added) activity. Erythrocyte transketolase (E.C.2.2.1.1) activity was assayed according to Hübner-Woźniak et al. [15] before and after stimulation by adding thiamine diphosphate. Erythrocyte glutathione reductase (E.C.1.6.4.2) activity, before and after stimulation by adding riboflavin and erythrocyte aspartic aminotransferase (E.C.2.6.1.1), and before and after adding pyridoxal-5’-phosphate, was measured using commercial kits by Randox (United Kingdom) and Alpha-Diagnostics (Poland), respectively. The activity coefficients were then calculated. The cut-offs of activity coefficient values of thiamine, riboflavin, and pyridoxine status are the same for women and men, and they are >1.25, >1.20 and >1.70 for αₑₜₖ, αₑᵣᵣᵣ, and αₑₐₑₐₑ, respectively [28].

All calculations were performed using the program Statistica v.6 (StatSoft, USA). The results were expressed as means ± SD. Normal distribution was analyzed using the Shapiro-Wilk test. The significance of differences between particular pairs of means was estimated using two-way analysis of variance (ANOVA) (sex x activity) and Tukey’s post hoc test. The frequency of occurrence of vitamin deficiencies was estimated using the accurate chi-square Fisher test. The differences were considered significant at p<0.05.

RESULTS

As shown in Table 2, all participants were of similar age. Women (in groups HAW and LAW) were characterized by lower body mass and height, and lower BMI compared with men in the corresponding groups (HAM and LAM). The HAM group men had considerably longer training experience than the HAW group women. Physical activity, expressed in hours·week⁻¹, was statistically significantly higher in HAW and HAM groups compared with LAW and LAM groups, respectively.

All data regarding the daily intake of vitamins B₁, B₂, and B₆ are contained in Table 3. It was established that the intake of vitamin

### TABLE 2. GENERAL CHARACTERISTICS OF SUBJECT GROUPS (MEAN ± SD)

| Variable                  | HAW (n=20) | HAM (n=20) | LAW (n=20) | LAM (n=20) |
|---------------------------|------------|------------|------------|------------|
| Age (years)               | 19.6 ± 0.7 | 19.9 ± 0.9 | 19.5 ± 0.6 | 19.8 ± 0.6 |
| Body height (cm)          | 168.8 ± 5.7 | 182.7 ± 7.2 | 168.7 ± 4.9 | 179.7 ± 7.6 |
| Body mass (kg)            | 58.9 ± 5.6 | 77.4 ± 7.2 | 58.6 ± 4.9 | 75.7 ± 7.8 |
| BMI                       | 20.5 ± 1.1 | 23.2 ± 1.9 | 20.5 ± 1.6 | 23.9 ± 3.3 |
| Training experience (years)| 7.9 ± 2.5 | 10.7 ± 3.2 | -          | -          |
| Physical activity (hours·week⁻¹)** | 11.2  | 13.9      | 6.0       | 6.0       |

Note: * HAW – women with high physical activity; HAM – men with high physical activity; LAW – women with low physical activity; LAM – men with low physical activity ** physical activity prescribed by the physical education programme of study

| Significantly lower compared with HAM group: A p<0.001; B p<0.01
| Significantly lower compared with LAM group (p<0.001)
| Significantly higher compared with LAM group (p<0.001)
| Significantly higher compared with LAW group (p<0.001)

### TABLE 3. VITAMIN B₁, B₂, AND B₆ DAILY INTAKE IN WOMEN AND MEN (MEAN ± SD)

| Variable                  | HAW (n=20) | HAM (n=20) | LAW (n=20) | LAM (n=20) |
|---------------------------|------------|------------|------------|------------|
| B₁ mg·day⁻¹               | 0.90 ± 0.20 A  | 1.16 ± 0.19 | 0.95 ± 0.16 B | 1.19 ± 0.23 |
| mg·day⁻¹·1000 kcal⁻¹      | 0.44 ± 0.12 C  | 0.36 ± 0.07 | 0.44 ± 0.09 | 0.41 ± 0.07 |
| B₂ mg·day⁻¹               | 1.83 ± 0.27 A,D | 2.42 ± 0.54 | 1.63 ± 0.33 B | 2.22 ± 0.37 |
| B₆ mg·day⁻¹               | 1.51 ± 0.28 A  | 2.38 ± 0.41 | 1.41 ± 0.24 B | 2.20 ± 0.38 |
| mg·day⁻¹·g⁻¹ protein      | 0.02 ± 0.01  | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 |

Note: * See table 2

Significant difference compared with HAM group: A p<0.001; B p<0.01
Significantly lower compared with LAM group (p<0.001)
Significantly higher compared with LAM group (p<0.001)
Significantly higher compared with LAW group (p<0.001)
Significantly higher compared with LAW group (p<0.05)
B1 (in mg·day⁻¹) in the HAW group was significantly lower, but in terms of mg·day⁻¹·1000 kcal⁻¹, it was significantly higher, compared with the HAM group. Women in the LAW group were characterized by significantly lower intake of vitamin B1 (in mg·day⁻¹), compared with the HAW group; however, there were no statistically significant differences between these groups in intake of this vitamin in terms of mg·day⁻¹·1000 kcal⁻¹. In addition, it was found that the intake of vitamin B1 (in mg·day⁻¹) was significantly lower in groups HAW and LAW compared with the respective female groups. No significant differences in vitamin B1 intake (in mg·day⁻¹·1000 kcal⁻¹·1000 kcal⁻¹) were found among the studied groups.

Women in the HAW group were characterized by significantly lower intake of vitamin B1 (in mg·day⁻¹), compared with the HAM group; however, there were no statistically significant differences between these groups in intake of this vitamin in terms of mg·day⁻¹·1000 kcal⁻¹. In addition, it was found that the intake of vitamin B1 (in mg·day⁻¹) was significantly lower in groups HAW and LAW compared with the respective female groups. No significant differences in vitamin B1 intake (in mg·day⁻¹·1000 kcal⁻¹·1000 kcal⁻¹) were found among the studied groups.

Table 4 shows data regarding the percentage of occurrence of inadequate intake of vitamins B1, B2, and B6 in the diet of the studied groups. It was demonstrated that vitamin B1 deficiency, at the average level (EAR) and recommended level (RDA), appeared respectively in 55% and 80% of group HAW women, in 30% and 65% of group HAM men, in 30% and 80% of group LAW women, and in 35% and 65% of group LAM men. None of the groups showed any deficiency in dietary intake of vitamin B2.

Vitamin B6 deficiency at the level of average (EAR) and recommended (RDA) intake was present in respectively 10% and 25% of HAW group women, and at the level of recommended intake in 40% of LAW group women (Table 4). No vitamin B6 deficiency in the diet was observed in the male groups. However, it was found that the percentage of vitamin B6 deficiency in the diet at the level of recommended intake (RDA) in groups HAW and LAW was significantly higher compared with respective groups HAM and LAM.

Mean values of activity coefficients for transketolase (αETK), glutathione reductase (αERG), and aspartate aminotransferase (αEAST) in the studied groups are shown in Table 5. There were no statistically significant differences among the studied groups for any of these coefficients.
The obtained results show that, among the studied women and men, no \( \alpha_{ETK} \) values were found to indicate substantial risk of vitamin \( B_1 \) deficiency; however, the \( \alpha_{ERG} \) coefficient value, indicating deficiency of vitamin \( B_2 \), demonstrates that 10% of group LAW women, 35% of group LAM women, and 25% of group LAM men were at risk of deficiency of this vitamin (Table 6). In addition, it was found that the percentage of occurrence of activity coefficient \( \alpha_{ERG} \), indicating deficiency of vitamin \( B_2 \), was significantly higher in the LAM group compared with the HAM group. In all studied groups, there were cases of \( \alpha_{ERG} \) values exceeding 1.70, although in a different percentage of subjects. In the HAW group, the elevated value of this coefficient was found in 35% of subjects, in the LAW group in 40% of subjects, and in the HAM and LAM groups in 55% and 60% of subjects, respectively.

DISCUSSION

Women and men participating in this study differed significantly in their body mass, height, and BMI values, which can be explained by sex differences (Table 2).

This study demonstrated that women were characterized by lower intake of vitamins \( B_1, B_2, \) and \( B_6 \) compared with men, which is related to their lower energy intake in daily food rations. However, the intake of vitamin \( B_1 \) in group HAW in terms of \( \text{mg} \cdot \text{day}^{-1} \cdot 1000 \text{kcal}^{-1} \) was significantly higher compared with group HAM, which may demonstrate that women’s diet was more balanced and included more products constituting a source of this nutrient. In addition, there is a visible tendency towards lower vitamin \( B_1 \) intake in women and men with high physical activity compared with women and men with low physical activity, which may be related to lower dietary daily energy intake in more active groups. It cannot be excluded, as confirmed by numerous studies, that unbalanced diet of young, physically active people, because of lack of diversity, may contribute to deficiencies of many nutritional compounds [3]. On the other hand, Rico-Sanza et al. [23] established that, for young soccer players, the intake of many nutrients, including group B vitamins, was higher than the recommended level (EAR). The present study found that women with high and low physical activity had inadequate intake of vitamin \( B_1 \) more often than men, especially in terms of recommended dietary allowances. Therefore, it cannot be excluded that the differences in vitamin \( B_1 \) intake in female and male groups may result in inadequate nutritional status. On the other hand, Leydon et al. [18] reported a lower than recommended intake of vitamin \( B_1 \) in the diet of active young men compared to the respective group of women, which may be related to a higher risk of deficiency of this nutrient in the male group. The present study showed that the level of physical activity did not have an unambiguous impact on dietary intake of vitamin \( B_1 \) because the frequency of occurrence of inadequate nutritional status was similar in female and male groups with both high and low physical activity.

Published reports indicate that the commonly used method for estimating the risk of inadequate vitamin \( B_1 \) nutritional status in humans is measurement of the activity coefficient for transketolase. Moreover, as is the case for many other nutrients, the level of vitamins in the body depends not only on their dietary intake, degree of absorption in the intestines and excretion in urine, but also on other factors, such as age, sex, health status, or physical activity. Sato et al. [25], studying male and female swimmers, confirmed the influence of intense training on decrease of vitamin \( B_1 \) concentration in blood. On the other hand, Fogelholm et al. [10] found that there was no risk of inadequate vitamin \( B_1 \) nutritional status in a group of male and female skiers.

The analysis of the activity coefficient for transketolase obtained in this study showed that there was no risk of inadequate vitamin \( B_1 \) nutritional status in any of the groups. However, the high frequency of occurrence of inadequate vitamin \( B_1 \) dietary intake in terms of RDA, especially in female groups, may suggest that acceptable \( \alpha_{ETK} \) values in subjects with inadequate intake were the result of increased absorption in intestines or decreased excretion in urine.

The analysis of dietary intake of vitamin \( B_2 \) in women and men did not show deficiency of this nutrient in any of the studied groups; moreover, its mean intake was higher than the estimated average requirements and recommended dietary allowances. Both women and men with high physical activity had higher dietary intake of this vitamin than the respective groups with low physical activity. Similarly, Worme et al. [29] reported higher dietary intake of vitamin \( B_2 \) for women and men practising triathlon compared with non-training subjects, while Nieman et al. [21] noted lower intake of vitamin \( B_2 \) in active female and male long distance runners. The present study shows that physical activity did not have an unambiguous impact on the dietary intake of this nutrient in men (HAM vs. LAM), but such an impact cannot be excluded in women, because the dietary intake of vitamin \( B_2 \) in women with high physical activity was statistically significantly higher compared with the LAW group (Table 4).

The value of activity coefficient for glutathione reductase, indicating inadequate nutritional status for vitamin \( B_2 \), showed that the female and male groups with low physical activity were at a greater risk compared with the respective high activity groups. This finding may be related to lower dietary intake of this vitamin in low activity groups compared with high activity groups. At the same time, vitamin \( B_2 \) intake in all studied groups was higher than both average (EAR) and recommended (RDA) intake. The results of this study do not allow for unequivocal determination of why, despite the adequate vitamin \( B_2 \) intake in diet of women and men with low physical activity, the \( \alpha_{ERG} \) values indicate the risk of deficiency of this nutrient for 35% of women in group LAW and 25% of men in group LAM. Therefore, it cannot be excluded that, despite a correctly balanced diet, the increased risk of inadequate nutritional status for vitamin \( B_2 \) found in some subjects could have been caused by increased excretion in urine or decreased absorption in intestines.

In this study, the dietary intake of vitamin \( B_6 \) for all women and men was higher than the estimated average requirement and recom-
mended dietary allowances, and at the same time, women and men with high physical activity had higher intake of this vitamin compared with the respective groups with low physical activity. Additionally, Papandreu et al. [22] reported higher dietary intake of vitamin $B_6$ in female groups compared with male basketball players and long distance runners. However, Nieman et al. [21] reported higher dietary intake of vitamin $B_6$ in male compared with female long distance runners. Faber et al. [9] obtained similar results when studying male and female javelin throwers and shot-putters. The present study showed that women, disregarding their physical activity level, are more at risk of inadequate vitamin $B_6$ nutritional status compared with men, which may be the result of lower dietary intake. This finding indicates that sex may influence the frequency of occurrence of inadequate dietary intake of vitamin $B_6$. In addition, this study showed that the diet of women with low physical activity did not ensure an adequate level of vitamin $B_6$ intake. Therefore, it cannot be excluded that the level of physical activity influenced the differences observed in the female groups.

The analysis of the activity coefficient for aspartate aminotransferase showed that women and men with low physical activity are more at risk of vitamin $B_6$ deficiency compared with the respective groups with high physical activity. In the present study, the risk of inadequate nutritional status for vitamin $B_6$ in both male groups was a little higher compared with the respective female groups, although the differences were not statistically significant. Similar results were reported by Fogelholm et al. [10], although the $\alpha_{\text{EST}}$ values obtained by them were higher than in this study. On the other hand, Rokitizki et al. [24] reported higher $\alpha_{\text{EST}}$ values in groups of female handball players compared with male basketball players and wrestlers. Therefore, it cannot be excluded that the $\alpha_{\text{EST}}$ value depends on sex. At the same time, the elevated values of this activity coefficient, despite adequate vitamin $B_6$ dietary intake in all women and men, may be caused by limited absorption of this nutrient in intestines or increased excretion with urine.

Summarizing the obtained results, it seems that no firm conclusion can be reached regarding the impact of sex and physical activity on intake and nutritional status of vitamins $B_1$, $B_2$, and $B_6$, because of the relatively small number of subjects and the possible influence of factors affecting metabolism of vitamins $B_1$, $B_2$, and $B_6$ not taken into account in this study. Independently of sex and level of physical activity, the women and men in this study had inadequate dietary intake of vitamins $B_1$ and $B_6$, although this was not always related to elevated values for respective activity coefficients.

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Conflict of interest

None declared

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