

CROSS SECTIONAL STUDY OF VITAMIN B₁₂ SUPPLEMENTATION IN SLOVAK AND CZECH VEGANS

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ABSTRACT

Background. The number of vegans in the world is growing and in Slovakia and the Czech Republic they make up 1% of the population. Vegan diet excludes all foods of animal origin and vegans who do not use vitamin B₁₂ supplements are at risk of the vitamin B₁₂ deficiency.

Objective. The aim of this study was to determine what proportion of Czech and Slovak vegans use vitamin B₁₂ supplements regularly, irregularly or not at all and what is their supplemental cobalamin intake.

Materials and methods. The research involved 1337 self-identified vegans from Slovakia and the Czech Republic who were interviewed using the CAWI (Computer-Assisted Web Interview) method. Participants were recruited by posts in veganism-themed social media groups.

Results. Out of 1337 vegans 55.5% supplemented cobalamin regularly, 32.54% irregularly and 11.97% were not supplementing. Rate of not supplementing individuals was 5.04% higher in Slovaks than in Czechs. Short-term vegans had a significantly higher rate of not supplementing individuals (17.99%) compared to medium-term (8.37%) and long-term vegans (7.50%). Mean weekly cobalamin intake from supplements was 2938.34±2566.60 µg in regularly supplementing vegans compared to 1630.31±1949.27 µg in irregularly supplementing vegans, particularly due to the lower weekly supplementation frequency among irregularly (2.93) compared to regularly supplementing vegans (5.27).

Conclusions. The rate of supplementation in Slovak and particularly Czech vegans was higher than in other countries. The number of not supplementing individuals was significantly higher among short-term vegans, indicating that there is still a need for education on the importance of adequate and regular cobalamin supplementation, especially in new vegans. Our results support the hypothesis that the reason for higher rate of cobalamin deficiency in irregularly compared to regularly supplementing vegans is the lower cobalamin intake caused by lower supplementation frequency.

Key words: *veganism, vegan, vitamin B₁₂, vitamin B₁₂ deficiency, vitamin B₁₂ supplementation, vitamin B₁₂ intake, Czech vegan, Slovak vegan*

INTRODUCTION

The number of vegans and the popularity of plant-based diets in the world are increasing [1, 2] and with them also the need to study and recognize nutritional challenges of these dietary patterns. Despite not being on the forefront of the trend, Slovakia and the Czech Republic already have a small vegan population [3]. Vegans comprise approximately 1% of all Slovaks and Czechs, with the highest rates being among young people. As the growing body of literature indicates, even exclusively plantbased diets can be healthful and nutritionally adequate, provided they are appropriately planned [4, 5]. This includes also supplementation of

the vitamin B₁₂. Due to the exclusion of all products of animal origin, such as meat, dairy, eggs and fish, the only remaining reliable non-prescription sources of vitamin B₁₂ for vegans are B₁₂ fortified foods and supplements. Despite growing availability of the fortified foods and limited evidence supporting their use as a vitamin B₁₂ source [6], regular supplementation is still recognized as the most reliable way to ensure sufficient vitamin B₁₂ intake in vegans [7].

Current data on the vitamin B₁₂ supplementation status in vegans from Slovakia are missing. Latest available data are from the year 2000, when researchers evaluated vitamin B₁₂ status of 32 Slovak vegans and 62 Slovak lacto- and lacto-ovo vegetarians, with the

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result of 78% of vegans being deficient [8]. Czech vegans were studied in the 2019 study, which involved 151 vegan participants. This research has demonstrated that the cobalamin deficiency was associated with the supplementation status of vegans and not with the duration of the vegan diet. By measuring the serum methylmalonic acid, holo-trans-cobalamin and homocysteine, researchers have found that the incidence of the cobalamin deficiency in the regularly supplementing vegans was only 5.56%, compared to 17.9% in irregularly supplementing vegans and 52.9% in not supplementing vegans [7]. We hypothesized that the reason for a higher rate of cobalamin deficiency in irregularly supplementing vegans compared to regularly supplementing vegans in the study by *Selinger et al.* has been the insufficient supplemental cobalamin intake. The aim of our study was therefore to: (i) identify, how prevalent is the regular and irregular supplementation in Slovak and Czech vegans (ii) identify their supplemental doses, frequency of supplementation and total supplemental cobalamin intake.

MATERIAL AND METHODS

Study design and participants

The study was designed as a cross-sectional study among self-reported vegans from Slovakia and Czech Republic. The sample was recruited through the social media posts in veganism-themed groups in February 2021. Data about their vitamin B₁₂ supplementation habits were collected using the CAWI (Computer-Assisted Web Interview) method. Participants received

information about the anonymity of the study, the voluntary nature and the possibility to stop their participation at any study stage.

The inclusion criteria were willingness to participate in the study and being Czech or Slovak self-identified vegan. Exclusion criteria were reported animal-based foods among vitamin B₁₂ sources and submission of the incomplete or defective questionnaire. From the total number of 1356 respondents, 1337 were included in the study. Total 19 questionnaires were excluded from the study for the reasons highlighted in Figure 1.

Questionnaire

Questionnaire consisted of 23 questions, covering basic demographic details, vitamin B₁₂ sources, supplement use and information sources. Detailed questionnaire can be found in the Annex. Questions 1. to 6. inquired participants about the duration of their vegan diet, age, education level, nationality, sex and in the case of women also about pregnancy and breastfeeding.

The main question of the research was question number 7., asking whether participants used vitamin B₁₂ supplements regularly, irregularly or not at all. This question was modeled after the research of *Selinger et al.* [7], in order to follow-up on their results. Respondents, who have selected regular or irregular use of vitamin B₁₂ supplements, were further led to the series of questions 8. - 11., focused on the type of the supplement, supplementation frequency, used dose and the cobalamin form.

Respondents who indicated that they did not use vitamin B₁₂ supplements, were instead led to the questions 12. and 13. Here they were asked about their reasons for not taking the vitamin B₁₂ supplements and if they relied on any other sources instead. All participants then answered questions 14. and 15., inquiring about medication and medical conditions, which may interfere with the vitamin B₁₂ absorption or status.

Questions 16. asked all participants if they informed their doctor about being vegan, followed up by questions 17. and 18., asking if they were ever tested for cobalamin deficiency and results of the test. Questions 19. to 23. were focused on what information sources vegans used to learn about vegan nutrition and availability and quality of information sources in Slovak and Czech languages.

Statistical analysis

We have divided participants by the duration of their veganism and their supplement use using the same methods as *Selinger et al.* Respondents were therefore classified as “short-term vegans” for those who were vegan for <3 years; “medium-term vegans” for 3–7 years and “long-term vegans” for >7 years. A *Chi*²

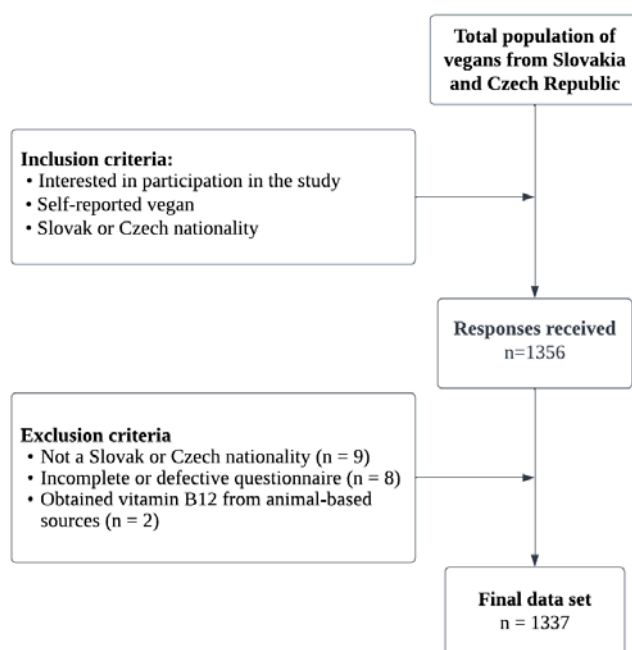


Figure 1. The inclusion and exclusion criteria and completion of participant selection

test and *Cramér's V* were used to assess the statistical significance and intercorrelation between the nominal variables, including supplementation status and duration of veganism, sex, pregnancy/breastfeeding status, nationality, education level and other factors. To assess statistical significance between nominal and metric variables, two-tailed t-test for independent samples was used. For all analyses, a p-value <0.05 was considered statistically significant.

Supplemental doses were evaluated using mean with standard deviation (SD), median, minimum, maximum and quartiles. Supplemental doses of daily and weekly supplementing participants were compared to the recommendations for the daily and weekly intake respectively in separate tables. Supplemental doses of remaining participants, who supplemented 2 – 6x times a week, were converted to the cumulative weekly intake and compared to the recommendations for weekly intake in a separate table. Cumulative weekly intake was calculated by multiplying the dose by weekly frequency. For example, supplementation of 100 µg of cobalamin three times a week would result in cumulative weekly intake of 300 µg. Basic data processing and descriptive statistics were performed using MS Excel (MS Office Professional Plus 2019). Further statistical analysis, including hypothesis testing by two tailed t-test, *Chi*² test and *Cramér's V*, were performed using DATAtab online statistics calculator <https://datatab.net/>.

RESULTS

Participants were on the vegan diet on average for 4.05±3.57 years, with the median value of 3 years. Men were on the vegan diet on average longer than women, 4.7±3.64 and 3.8±3.53 years respectively. As identified by the two-tailed t-test for the independent samples (equal variances assumed), this relation was statistically significant ($p < 0.001$, 95% CI [-1.35, -0.45]).

Women comprised 76.59% (n = 1024) of all respondents and 9.75% (n = 98) of them were currently pregnant or breastfeeding. Proportion of the male vegans was increasing with the duration of the vegan diet, up to 31.88% in the group on vegan diet for over 7 years. Most vegans in the study were from the age group of 25-34-year-olds, who comprised 46.45% of all respondents. Oldest and youngest participants were 13 and 70 years old respectively. Of all participants, 53.7% had bachelor's or higher degree. Full demographic data are shown in the Table 1.

Vitamin B₁₂ supplementation

Of all 1337 participants, 55.5% were using vitamin B₁₂ supplements regularly and 32.54% irregularly. There was a statistically significant relationship between vitamin B₁₂ supplementation and the duration

of vegan diet ($\chi^2(4) = 33.09$, $p < 0.001$, *Cramér's V* = 0.11). The highest rate of vegans, who did not use vitamin B₁₂ supplements (17.45%), was identified in the group, who followed the vegan diet “short-term” (<3 years). In contrast only 8.31% of “medium-term” and 7.50% of “long-term” vegans were not supplementing at all.

Compared to women, men were more likely to use vitamin B₁₂ supplements and supplement regularly, with the exception of the pregnant or breastfeeding women, who had the highest rates of supplementation of all groups. We have identified that there was a statistically significant relationship between the vitamin B₁₂ supplementation and pregnancy or breastfeeding status ($\chi^2(2) = 18.82$, $p < 0.001$, *Cramér's V* = 0.14).

Table 1. Demographic data

Sex (n = 1337)		
Male	313	76.59%
Female	1024	23.41%
Pregnancy or breastfeeding (n = 1005) ¹		
Yes	98	9.75%
No	907	90.25%
Nationality (n = 1337)		
Czech	518	38.74%
Slovak	819	61.26%
Duration of veganism (n = 1337)		
Under 3 yrs	556	41.59%
3-7 yrs	621	46.45%
Over 7 yrs	160	11.97%
Age (n = 1337)		
0-14	3	0.22%
15-24	319	23.86%
25-34	621	46.45%
35-44	265	19.82%
45-54	99	7.40%
55-64	26	1.94%
65+	4	0.30%
Education level (n = 1337)		
Lower secondary education or lower	39	2.92%
Upper secondary education without maturity diploma	55	4.11%
Upper secondary education with maturity diploma	525	39.27%
Bachelor's degree or equivalent	256	19.15%
Master's degree	436	32.61%
Doctorate	26	1.94%

¹ 19 out of 1024 female participants have not answered this question

There was a statistically significant difference in the vitamin B₁₂ supplementation between Czech and Slovak vegans ($\chi^2(2) = 6.23$, $p=0.044$, *Cramér's V* = 0.07). Among Slovak vegans there was 5.04% higher rate of not supplementing individuals compared to Czech vegans. Supplementation rate was also higher in the group of participants with the bachelor's or higher degree, than in the group of those with high school diploma or lower education, but this result did not reach statistical significance. Supplementation rates for all groups can be found in the Table 2.

Supplemental doses

Out of 1173 respondents, who regularly or irregularly used vitamin B₁₂ supplements, 690 provided valid details on both supplementation frequency and used dose and were supplementing on at least weekly basis. The mean dose taken by these participants was 616.53±536.82 µg and the most common frequency of

supplementation was daily (40.72%). Doses used by vegans in specific frequencies are visualized in Figure 2 and the full data are available in Table 3.

Mean cobalamin intake in regularly supplementing vegans was 55.48% higher than in irregularly supplementing vegans, as shown in the Table 4. A two-tailed t-test for independent samples (equal variances not assumed) showed that this result was statistically significant, $t(340.92)=6.88$, $p=<0.001$, 95% confidence interval [931.78, 1684.26].

Cobalamin intakes were compared to the recommended daily intake by EFSA for general population [9] and cobalamin intake targets for vegans proposed by different authors [10, 11, 12] in the Tables 5, 6 and 7. Out of 690 participants who used supplements on at least a weekly basis and provided a supplemental dose 42 (6.09%) reported one or more risk factors for the cobalamin malabsorption.

Table 2. Supplementation rates among Slovak and Czech vegans

Total	Supplement regularly		Supplement irregularly		Do not supplement		p-Value
	739	55.27%	434	32.46%	164	12.27%	
Q1 How long have you been vegan? (n = 1337)							
Under 3 years	303	54.50%	153	27.52%	100	17.99%	<0.001
3-7 years	349	56.20%	220	35.43%	52	8.37%	
Over 7 years	87	54.38%	61	38.13%	12	7.50%	
Q3 What is your highest level of education? (n = 1337)							
No college	318	51.37%	216	34.89%	85	13.73%	0.026
Some college	421	58.64%	218	30.36%	79	11.00%	
Q4 Nationality (n = 1337)							
Czech	297	57.34%	172	33.20%	49	9.46%	0.044
Slovak	442	53.97%	262	31.99%	115	14.04%	
Q5 Sex (n = 1337)							
Women	553	54.00%	338	33.01%	133	12.99%	0.171
Men	186	59.42%	96	30.67%	31	9.90%	
Q6 Are you currently pregnant or breastfeeding? (n = 1005)							
Yes	73	74.49%	18	18.37%	7	7.14%	<0.001
No	467	51.49%	314	34.62%	126	13.89%	
Q16 Have you informed your doctor about your vegan diet? (n = 1337)							
Yes	362	58.01%	208	33.33%	54	8.65%	0.001
No	377	52.88%	226	31.70%	110	15.43%	
Q17 Have you ever been tested to see if you get enough vitamin B ₁₂ ? (n = 1337)							
Yes	241	57.11%	139	32.94%	42	9.95%	0.211
No	498	54.43%	295	32.24%	122	13.33%	
Q18 Have you ever been diagnosed with insufficient vitamin B ₁₂ intake? (n = 417)							
Yes	31	57.41%	20	37.04%	3	5.56%	0.389
No	207	57.02%	117	32.23%	39	10.74%	
Q19 Have you ever consulted your doctor or nutritionist about your vegan diet? (n = 1337)							
Yes	175	60.76%	86	29.86%	27	9.38%	0.72
No	564	53.77%	348	33.17%	137	13.06%	

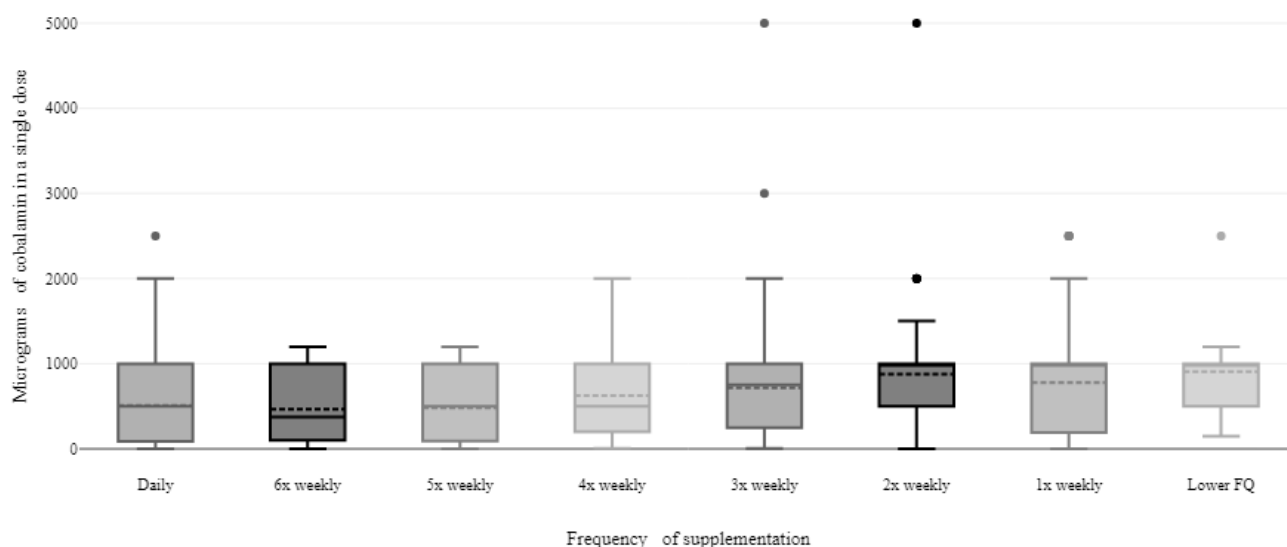


Figure 2. Supplemental doses by frequency of supplementation

Table 3. Supplementation rates and doses among Slovak and Czech vegans (in micrograms)

Frequency	N (%)	Mean dose (SD)	Median	Min	Max
Daily	281 (40.72)	509.71±428.20	500	0.50	2500
6x weekly	29 (4.20)	466.22±429.63	375	2.50	1200
5x weekly	66 (9.57)	481.24±399.75	500	2.00	1200
4x weekly	53 (7.68)	625.08±491.49	500	2.80	2000
3x weekly	117 (16.96)	716.09±635.58	750	3.00	5000
2x weekly	82 (11.88)	875.27±643.55	1000	1.80	5000
Weekly	62 (8.99)	777.64±644.80	1000	1.00	2500
Total	690 (100)	616.53±536.82	500	0.50	5000

Table 4. Cobalamin supplementation in regularly and irregularly supplementing vegans (in micrograms)

	Regularly supplementing	Irregularly supplementing
N	739	434
Mean weekly intake (SD)	2938.34±2566.60	1630.31±1949.27
Median weekly intake	2500	1000
Mean single dose (SD)	607.34±471.57	646.99±712.53
Average weekly frequency	5.27	2.93

Out of 690 participants, who included cobalamin dose and supplemented at least weekly, 537 also included information on the vitamin B₁₂ form in their supplements. The most frequently used cobalamin form was cyanocobalamin, with methyl-cobalamin being the second most popular.

Reasons for not supplementing

Out of 1337 respondents, 12.27% (n = 164) did not supplement vitamin B₁₂. To the question 12 “What are your reasons for not supplementing vitamin B₁₂?”, where it was possible to select more than one

answer, most frequently selected option was: “I have not addressed it yet” and in the second place: “I get vitamin B₁₂ from other sources”. Among the open answers under the “Other” option, respondents the most frequently stated that they were tested for vitamin B₁₂ adequacy. Detailed data are shown in Figure 3.

Respondents who have claimed that they obtain vitamin B₁₂ from other sources were able to specify these alleged sources in question 13. The most frequently cited alleged sources of vitamin B₁₂ were various types of algae, plant foods and mushrooms, which cannot be considered reliable sources of vitamin

Table 5. Cobalamin intake in daily supplementing participants (in micrograms)

Malabsorption risk	N	%	Mean daily dose (SD)	Mean weekly intake (SD)	Target	Within target	
No	265	94.31	513.84±431.13	3596.91±3017.94	4 µg [9]	257	96.98%
					10 µg [12]	245	92.45%
					50 µg [12]	214	80.75%
Yes	16	5.69	441.31±382.01	3089.19±2674.10	100 µg [11]	13	81.25%

Table 6. Cobalamin intake in weekly supplementing participants (in micrograms)

Malabsorption risk	N	%	Mean weekly dose (SD)	Target	Within target	
No	59	95.16	723.96±597.15	350 µg [10]	38	64.41%
				2000 µg [12]	3	5.08%
Yes	3	4.84	1833.33±763.76	2000 µg [11]	2	66.67%

Table 7. Cobalamin intake in two- to six-times weekly supplementing participants (in micrograms)

Malabsorption risk	N	%	Mean weekly intake (SD)	Target	Within target	
No	324	93.37	2233.97±1903.87	350 µg [10]	269	83.02%
				2000 µg [12]	187	57.72%
Yes	23	6.63	1892.78±1707.14	2000 µg [11]	11	47.83%

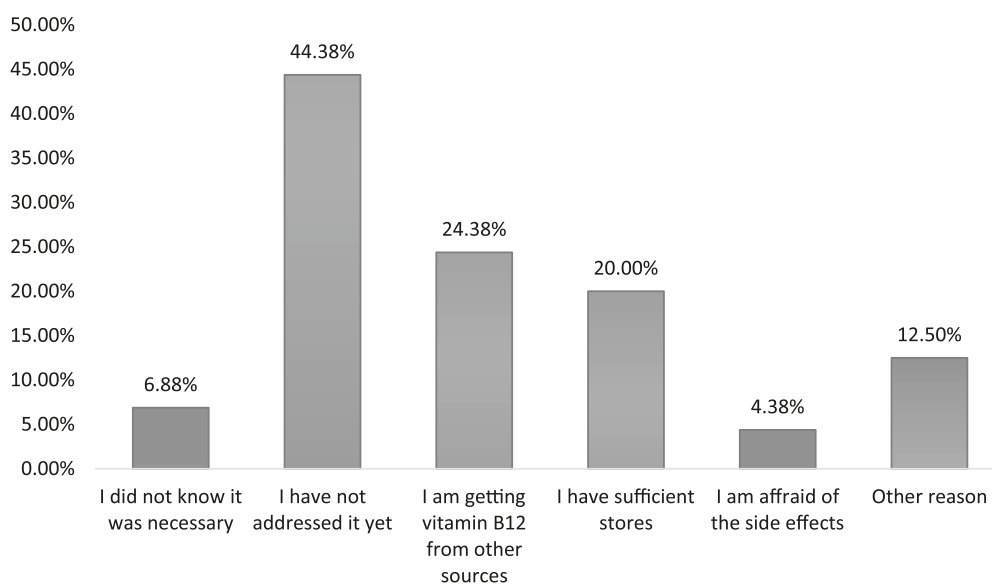
Figure 3. What are your reasons for not supplementing vitamin B₁₂?

Table 8. Cobalamin forms and averaged daily intake (in micrograms)

	N	%	Mean daily intake (±SD)	Median	Min	Max
Cyano	288	41.74	354.42±353.11	286	0.14	2143
Methyl	175	25.36	412.43±364.82	286	1	2500
Hydroxo	2	0.29	1250±1060.66	1250	500	2000
Adenosyl	2	0.29	82±8.49	82	76	88
Multiple	70	10.14	448.89±337.73	429	4	1429
Unknown	153	22.17	336.05±335.54	286	1	1400

Table 9. Claimed sources of vitamin B₁₂ among not supplementing vegans

Claimed source	No of answers
Chlorella and other seaweed	12
Various kinds of vegetables, fruit and mushrooms	11
Fortified foods	6
Unwashed vegetables, contaminated with vit. B ₁₂	4
Fermented foods	2
Vitamin B ₁₂ enriched toothpaste	1
Vitamin B ₁₂ injections	1

B₁₂. Potentially effective sources of vitamin B₁₂, such as vitamin B₁₂ fortified toothpaste and intramuscular injections, have also been mentioned, as highlighted in the Table 9 [13, 14].

DISCUSSION

First aim of our study was to determine, what proportion of Czech and Slovak vegans use vitamin B₁₂ supplements regularly, irregularly or not at all. Our research has found that most vegans used vitamin B₁₂ supplements, 55.27% (n=739) regularly and 32.46% (n= 434) irregularly. Only 12.27% (n=164) of the participating vegans did not supplement vitamin B₁₂ at all. This scale has been used in order to allow for comparison with the research by *Selinger* et al., who have presented the same question to a smaller sample of Czech vegans (n=72). In their study 11.64% vegans have not used vitamin B₁₂ supplements, but the rate of regular supplementation was higher at 61.64% [7].

With regards to the duration of the vegan diet, the highest number of not supplementing individuals was found among short-term vegans (17.99%) in comparison to medium-term vegans (8.37%) and long-term vegans (7.50%). This result is consistent with findings by *Selinger* et al. who have found that it was the short-term vegans, who had the highest prevalence of lowered cobalamin levels and supports their conclusion that it is the presence of cobalamin supplementation rather than the duration of veganism that is associated with increased risk of cobalamin deficiency [7].

There was a statistically significant difference in supplementation rate between Slovak and Czech vegans, with 14.04% of Slovak and 9.46% of Czech vegans not supplementing. In comparison, in the study of similar sample size (n = 1530) conducted in Australia, 25.3% of vegans did not use any vitamin B₁₂ supplements [15]. Other studies on cobalamin supplementation in vegans also included laboratory

testing and therefore used smaller sample size, similarly to the Czech study by *Selinger* et al. These studies were conducted in Germany (31.03% not supplementing (n = 58)) [16], Spain (29.63% not supplementing (n = 54)) [17] and Denmark (37.14% not supplementing (n = 70)) [18] and also on the Czech vegan children (10.14% not supplementing (n = 69)) [19]. These results suggest that the vitamin B₁₂ supplementation rates in Czech and Slovak vegans identified in our study are comparatively high.

Higher rate of overall supplementation as well as regular supplementation was found in male compared to female vegans in our study. However pregnant and breastfeeding women had the highest rate of supplementation of all groups, with 74.49% supplementing regularly and 18.37% irregularly. This is a positive result given since the majority of vegans in our study were women in the reproductive age (55.57% women under 35) and the need for the appropriate cobalamin supplementation in vegans is the most urgent during pregnancy and breastfeeding and in children. Only 7 out of 98 pregnant or breastfeeding vegan women (7.14%) did not supplement vitamin B₁₂ at all. This is consistent with findings by *Světnička* et al., where only 6.5% of Czech vegan women did not use cobalamin supplements during pregnancy or breastfeeding [19]. Gynecologists and pediatricians can be instrumental in ensuring that vegans are supplementing vitamin B₁₂ in these critical periods. However, as our results show, most vegans (53.33%) did not inform their doctor about being vegan.

Second aim of our study was to determine what supplemental doses vegans use. Vegans in our study used a wide variety of supplemental doses, ranging from 0.5 to 5000 µg of cobalamin in a single dose. The most popular frequency of supplementation in our study was daily (40.72%), compared to only 26.3% in Australian vegans [15]. Over half (50.29%) of our supplementing study participants used supplements two- to six-times a week and 8.99% supplemented once a week. Our results show that the irregularly supplementing vegans had 44.52% lower mean supplemental cobalamin intake compared to the regularly supplementing vegans. This was primarily caused by 44.40% lower supplementation frequency, rather than the supplemental doses, which were actually on average 6.53% higher among irregularly supplementing vegans. These results may explain the higher incidence of cobalamin deficiency among irregularly supplementing (17.90%) compared to regularly supplementing Czech vegans (5.56%) in the study by *Selinger* et al [7].

Adequate daily intake of 4 µg recommended by the EFSA for the general adult population [9] was met or exceeded by 96.98% of daily supplementing vegans without risk factors for cobalamin malabsorption. This recommendation is however based on the assumption

that the cobalamin is obtained multiple times a day in small doses from food. In such a scenario active transport is utilized multiple times a day and the overall absorption rate can exceed 40%, resulting in absorption of 1.5 µg of cobalamin [9]. However, in order to absorb the same amount from a single daily supplemental dose, higher doses of 10 or even 50 µg may be required [12]. Thresholds of 10 µg and 50 µg were achieved or exceeded by 92.45% and 80.75% of daily supplementing vegans without risk factors for cobalamin malabsorption respectively.

Various conditions and medication, as well as advanced age, may increase the risk for cobalamin malabsorption, particularly affecting the ability to utilize active transport. In such cases oral supplementation may still be effective, as 1-2% of the supplemental dose is absorbed by passive non-specific mechanisms [9]. The oral dose of 1000 µg was found to be effective in the treatment of cobalamin deficiency even in the individuals suffering from pernicious anemia [20]. However, as suggested by *Rajan et al.*, even 100 µg daily may be sufficient for the maintenance of already adequate cobalamin status in people with malabsorption [11]. This threshold was reached or exceeded by 81.25% of daily supplementing vegans with one or more risk factors for cobalamin malabsorption.

As an alternative to the daily supplementation, a weekly dose of 2000 µg has been proposed [12]. This target was however only reached by 5.08% of weekly supplementing vegans without risk factors for malabsorption. However as demonstrated by *Del Bo et al.*, even a weekly dose of 350 µg may be sufficient for the maintenance of the adequate cobalamin status in vegans without the risk factors for malabsorption [10]. Weekly target of 350 µg was reached or exceeded by 64.41% of weekly supplementing participants without the risk factors for malabsorption. It is important to stress out that the cobalamin intake thresholds for vegans are still based on limited evidence and other factors, such as cobalamin form or supplement type, may affect the individual absorption rate.

Two most popular cobalamin vitamers used by study participants were cyanocobalamin and methylcobalamin. Cyanocobalamin is the most researched vitamer of the vitamin B₁₂ and it proved to be reliable and the most stable cobalamin form [21]. Due to their lower stability, *Koeder and Perez-Cueto* [12] have suggested that the higher recommended doses may be required for vitamers other than the cyanocobalamin. Our results showed that the mean supplemental cobalamin intake among methylcobalamin users was 16.37% higher than that of the cyanocobalamin users. Currently there is however insufficient evidence to confirm whether such difference would be sufficient to compensate for lower stability.

The main strength of our research lies in the high number of participants, which allowed us to reach statistically significant results for the supplementation habits in Czech and Slovak vegans. It is at the same time a limitation, since we were not able to perform laboratory tests to evaluate actual cobalamin status of participants.

We can see several avenues future research can take to advance our understanding of vitamin B₁₂ intake and metabolism in people. Apart from vegans there are other groups which may be at risk of vitamin B₁₂ deficiency, such as lacto-ovo vegetarians or flexitarians. These groups may not abstain from animal-based foods completely, but the frequency may vary greatly and even beyond the point where the cobalamin intake from foods alone will be insufficient. Future research may therefore investigate frequency of the intake of animal-based foods and supplementation habits in these groups. Additionally, there is still a need for further research to establish optimal supplemental intake of cobalamin for vegans.

CONCLUSIONS

The first aim of our study was to identify how prevalent is the regular and irregular supplementation in Slovak and Czech vegans. We have found that most Czech and Slovak vegans used vitamin B₁₂ supplements, with 55.27% supplementing regularly, 32.46% irregularly and only 12.27% not supplementing at all. Czech vegans were 5.04% more likely to use cobalamin supplements than Slovak vegans and also the rate of the regular supplementation was higher in Czechs. The rate of nonsupplementation was significantly higher among vegans following the diet short-term (17.99%) compared to medium-term (8.37%) and long-term vegans (7.50%). These results indicate that there is still a need for education about the importance of the cobalamin supplementation, particularly focused on new vegans.

Second aim of our study was to identify supplemental cobalamin intake of Czech and Slovak vegans. We have observed statistically significant differences in the supplemental frequency and total supplemental cobalamin intake between regularly and irregularly supplementing vegans. Irregularly supplementing vegans were supplementing on average 2.93 times a week, compared to 5.27 times a week in regularly supplementing vegans, resulting in 44.52% lower mean supplemental cobalamin intake. This result supports our initial hypothesis that the reason why some of the supplementing vegans in the study by *Selinger et al.* still developed cobalamin deficiency was an inadequate supplemental cobalamin intake. Despite the cobalamin supplementation rate being higher than in other countries, our results suggest that there is still

a need to raise awareness about the importance of regular and adequate cobalamin supplementation in the Czech and particularly Slovak vegan community. This is even more urgent due to the fact that the majority of vegans in our study were women in the reproductive age and the adequate cobalamin intake is the most important in pregnant and breastfeeding vegan women and their children.

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Conflict of interest

The authors declare no conflict of interest.

Annex. Questionnaire

Q No	Condition	Question	Note	Options
1		How long have you been vegan (years)?	Please enter only the number, if you have been eating vegan for less than a year, enter 1	1 Number
2		How old are you?	Please enter a number only	2 Number
3		What is your highest level of education?		1 Secondary education or less
				2 Upper secondary education without maturity diploma
				3 Upper secondary education with maturity diploma
				4 Bachelor's degree or equivalent
				5 Master's degree
				6 Doctorate
4		Nationality		1 Slovak
				2 Czech
				3 Other
5		Sex		1 Female
				2 Male
6	Question 5 = 1	Are you currently pregnant or breastfeeding?		1 Pregnant
				2 Breastfeeding
				3 Pregnant and breastfeeding
				4 Neither
7		Do you use vitamin B ₁₂ supplements?	Tablets, drops and sprays with a defined vitamin B ₁₂ content are included, including multivitamins and B-complexes with a vitamin B ₁₂ content. Enriched foods and beverages, injectables, chewing gum, toothpaste, chlorella or other algae are not included.	1 Regularly
				2 Irregularly
				3 Not at all
8	Question 7 = 1, 2	What nutritional supplement do you use? *	Choose one or more answers (if you have more than one nutritional supplement)	1 Standalone vitamin B ₁₂ supplement
				2 Combination supplement (e.g. B-Complex, multivitamin)
9	Question 7 = 1, 2	How often do you take vitamin B ₁₂ supplements?	In case of irregular use, please estimate.	1 1x a week (weekly)
				2 2x a week
				3 3x a week
				4 4x a week
				5 5x a week
				6 6x a week
				7 7x a week (daily)
				8 Other frequency (describe)

10	Question 7 = 1, 2	What dose of vitamin B ₁₂ in micrograms (µg/mcg) are you taking?	Dose corresponds to the total amount of vit. B ₁₂ you take in one day. For example, if you take two tablets three times a week, this is the amount of vitamin B ₁₂ in two tablets. Please enter a number only. Decimal places are allowed.	1	Dose in µg:
				2	I do not know
11	Question 7 = 1, 2	What is the form of vitamin B ₁₂ in your nutritional supplement?	Select one or more answers (in case of a multiple forms)	1	Cyanocobalamin
				2	Methylcobalamin
				3	Adenosylcobalamin
				4	Hydroxocobalamin
				5	I do not know
12	Question 7 = 3	What are your reasons for not supplementing vitamin B ₁₂ ?	Select one or more answers	1	I didn't know it was necessary on a vegan diet
				2	I haven't addressed it yet
				3	I get vit. B ₁₂ from other sources
				4	I have sufficient stores
				5	I'm worried about the effects of nutritional supplements
				6	Other (describe)
13	Question 12 = 3	What other sources do you get vit. B ₁₂ from?	Please list all your sources of vitamin B ₁₂	1	text
14		Do you suffer or have suffered from any of the following diseases/problems in the past?	Pernicious anemia, celiac disease, Crohn's disease, ulcerative colitis, atrophic gastritis, pancreatic insufficiency, gastrointestinal cancer, cobalamin (vitamin B ₁₂) absorption disorder, decreased gastric acid production, gastric or small intestinal surgery. (affect the absorption of vitamin B ₁₂)	1	Yes
				2	No
15		Are you currently taking any of the following medicines?	Proton pump inhibitors (eg Pantoprazole, Omeprazole), H ₂ antagonists or other drugs to reduce stomach acid. (affect the absorption of vitamin B ₁₂)	1	Yes
				2	No
16		Have you informed your doctor about your vegan diet?		1	Yes
				2	No
17		Have you ever been tested for vitamin B ₁₂ status?	Not every blood test can detect vitamin B ₁₂ . Choose answer "yes" only if you know that test was focused specifically on the vitamin B ₁₂ .	1	Yes
				2	No
18	Question 17 = 1	Have you ever been diagnosed with vitamin B ₁₂ deficiency?		1	Yes
				2	No
19		Have you ever consulted your doctor or nutritionist about your vegan diet?		1	Yes
				2	No
20		From what sources do you get information on how to eat vegan properly?		1	Social media discussions
				2	friends and acquaintances
				3	Books
				4	Educational articles and videos
				5	Information materials for pro-vegan organizations
				6	Scientific articles and primary research
				7	Consultations with experts
				8	Other (describe)

21	Which of these sources is most important for you?	Which of these sources have you used the most to learn about vegan nutrition?	1	Social media discussions
			2	Friends and acquaintances
			3	Books
			4	Educational articles and videos
			5	Information materials for pro-vegan organizations
			6	Scientific articles and studies
			7	Consultations with experts
			8	Other (describe)
22	In what language do you get information about the vegan diet most often?		1	Slovak
			2	Czech
			3	English
			4	Other
23	How do you rate the availability of info on proper vegan nutrition in Slovak and Czech languages?		1	Sufficient
			2	Rather sufficient
			3	Rather insufficient
			4	Insufficient

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