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Dietary intake of vitamin B_6 and concentration of vitamin B_6 in blood samples of German vegans

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Abstract

Objective: The study aimed to evaluate the dietary vitamin B_6 intake and determine the vitamin B_6 concentration in blood samples of German vegans.
Design and setting: Cross-sectional study with 33 examination sites all over Germany.
Subjects: Ninety-three vegans (50 females) with a mean (± standard deviation (SD)) age of 43.7 ± 15.7 years who took no vitamin supplements.
Methods: Dietary intake was assed using a semi-quantitative food-frequency questionnaire. Erythrocyte aspartate aminotransferase activity coefficient (EAST-AC) was calculated as the ratio of stimulated (pyridoxal 5′-phosphate added) to unstimulated activity in blood samples that were provided after an overnight fast.
Results: Mean ± SD vitamin B_6 intake was 2.83 ± 0.98 mg day^{-1} and mean ± SD protein intake was 56.6 ± 21.7 g day^{-1}. Of the participants 4% showed vitamin B_6 intakes lower than daily recommended intakes for Germany, 16% showed EAST-AC >1.85, and a further 58% showed EAST-AC of 1.5–1-85. Moderate vegans were affected to a lesser extent than strict vegans. None of the established confounders was a significant predictor of EAST-AC. In logistic regression analyses the contribution of nutriments and cereals to pyridoxine intake was the only predictor of EAST-AC classified as ≤1.85 and >1.85, respectively.
Conclusions: In spite of the high total intake of vitamin B_6, an adequate concentration in blood samples could not be realised for a majority of the participants. Due to the health implications of a marginal pyridoxine status, vegans should be encouraged to include foods with a high bioavailability of pyridoxine, such as beans, lentils and bananas, in the daily diet.

Keywords
Veganism
Vitamin B_6
EAST-AC
Dietary intake

The vitamin B_6 content of foods consists of several pyridoxine derivatives (pyridoxine, pyridoxal, pyridoxamine and their phosphorylated forms)[1]. Foods of animal origin contain mainly pyridoxamine and pyridoxal[2], resulting in a bioavailability of approximately 75%[3], which approaches 100% in some foods[2]. Vitamin B_6 in foods of plant origin consists mainly of pyridoxine and the phosphorylated form – derivatives that have reduced bioavailability. Furthermore, a large proportion of the vitamin B_6 content in foods of plant origin is glucosylated, which reduces the bioavailability further[2,4]. For example, the bioavailability of pyridoxine glycosides (pyridoxine-5′-β-D-glycosides) is approximately 50–58% that of free pyridoxine applied orally[5,6]. Furthermore, pyridoxine glycosides show an antagonistic effect on the metabolism of pyridoxine[4,5]. Therefore it can be assumed that persons with a dietary regimen that consists mainly (vegetarian diet) or exclusively (vegan diet) of plant foods are at risk of inadequate blood vitamin B_6 concentrations. Some studies indicate that vegetarians have comparable vitamin B_6 status to omnivorous study populations[7,8], while other studies have found contradictory results[9]. The health implications associated with marginal pyridoxine status are increased risk for coronary diseases – independently of homocysteine concentrations[10] – and an increased risk for pancreatic cancer[11]. Furthermore, pyridoxine is important to immune function[12,13] and associations have been identified between pyridoxine and depression[14,15].

In a study on 441 Dutch elderly non-vegetarians and 32 vegetarians, the possible interrelationships between vitamin B_6 and factors in the vegetarian diet that may influence its bioavailability (i.e. dietary fibre and complexing agents) were evaluated[8]. It was concluded that dietary fibre does not have a significant impact on vitamin B_6 in elderly vegetarians, since only protein and vitamin B_6 intakes showed relationships with erythrocyte aspartate aminotransferase activity coefficient (EAST-AC) in multivariate analyses. To date it is still unknown if those conclusions are true for a vegan population as well.

Today, about 7% of the German population practises a vegetarian diet[16]. Of these, 8–10% are estimated to practise a vegan dietary regimen (i.e. about 550 000

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persons)[17]. As yet, data on the dietary intake of vitamin B_6 and its concentration in blood samples of vegans are still missing for Germany. Therefore we initiated the German Vegan Study (GVS), which aimed to investigate dietary vitamin B_6 intakes in this population. In addition, the vitamin B_6 concentration in blood samples was determined. In the present paper we report the results of further analyses conducted to evaluate whether intakes and blood concentrations of vitamin B_6 are related and whether participant characteristics, the dietary regimen *per se* or single dietary components act as predictors of blood vitamin B_6 concentration.

Materials and methods

Subject recruitment for the GVS (cross-sectional study design) was realised by advertisements placed in German magazines. As described in more detail elsewhere[18], each GVS participant was asked to complete two 9-day estimated food-frequency questionnaires (FFQs; semi-quantitative). The FFQ used was a slight modification (complemented with vegan foods; foods of animal origin were excluded with the exception of eggs, butter, milk and dairy products) of the validated FFQ used in the Giessen Raw Food Study[19]. Altogether, the GVS FFQ contained 199 vegan foods and seven non-vegan foods (i.e. eggs, milk and other dairy products). Common household measures and their equivalents in grams or millilitres were given for each food item. In some cases, portion sizes were made clear with photos or specific comments (e.g. nine strawberries each the size of a walnut weigh 150 g; an orange the size of a tennis ball equals 250 g). In addition, participants were asked for copies of recipes of home-made vegan dishes. In order to minimise seasonal differences, one FFQ was completed in autumn and the other in spring. Members of the GVS team developed software (Paradox and Access database) on the basis of the German Nutrient Data Base (BLS II.2; Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin Berlin, Germany) to calculate the concentrations of ingested nutrients. Missing nutritional information on vegan foods and dishes was obtained from the manufacturers. In the end the database provided information on 245 foods (including 19 beverages and seven non-vegan foods).

The GVS cohort was subdivided into strict and moderate vegans according to dietary intakes: strict vegans (SV; $n = 60$) consumed a pure vegan diet and moderate vegans (MV; $n = 33$) consumed a maximum of 5% of total energy from eggs and/or dairy products.

The ratio of estimated energy intake to basal metabolic rate (EI/BMR) was calculated, EI computed from the FFQ and BMR estimated using equations given by the Food and Agriculture Organization/World Health Organization/United Nations University, which account for age and body weight[20].

The body height of the GVS participants was measured with a measuring tape. Body weight was measured using a calibrated SECA scale with participants in light clothes and without shoes, and was recorded to the nearest 0.1 kg. Body mass index (BMI) was computed using the standard formula: BMI (kg m^{-2}) = body weight (kg)/body height^2 (m^2).

Blood was sampled after an overnight fast using a standard procedure. Erythrocyte aspartate aminotransferase (EAST) activity was measured photometrically with and without added pyridoxal 5′-phosphate (PLP) (Eppendorf-Photometer, Eppendorf, Germany). The EAST activity coefficient (EAST-AC) was calculated as the ratio of stimulated (PLP added) to unstimulated activity. For classification of vitamin B_6 concentration in the blood samples either as sufficient or insufficient a cut-off value of 1.85 was used, with EAST-AC values >1.85 representing an insufficient vitamin B_6 concentration[21]. Due to the cross-sectional study design (with 33 different examination sites) only one blood sample was obtained from each participant.

Ninety-three persons (who used neither vitamin supplements nor oral contraceptives, did not receive hormone replacement therapy and did not smoke) participated in all study segments and fulfilled the following study criteria: ‘strict’ or ‘moderate’ vegan nutrition for at least 1 year prior to joining the study, minimum age of 18 years, and no pregnancy or childbirth during the last 12 months. Exclusion criteria were the presence of a severe illness (such as cancer, diseases of the kidney or liver) or the intake of drugs related to chronic diseases.

Ethical considerations

The GVS was conducted in accordance with the Helsinki Declaration of 1964 as revised 1983 and 1996. Since there was no intervention, the Ethics Commission of Lower Saxony confirmed that ethical approval was not required. All subjects participated voluntarily and gave written consent prior to joining the study.

Statistical analysis

A statistical analysis program (SPSS 12.0; SPSS Inc., Chicago, IL, USA) was used to analyse the data. Results are presented as mean ± standard deviation. The independent samples *t*-test (normally distributed data) and the Mann–Whitney *U*-test (skewed data) were used to reveal significant differences between the two subgroups. Spearman correlation analysis was applied to reveal statistically significant associations between EAST-AC and estimated food and nutrient intakes. In addition, we also conducted a binary logistic regression analysis with EAST-AC (≤ 1.85 vs. >1.85) as the dependent variable and age, gender, dietary subgroup allocation, energy intake, protein intake, dietary fibre intake, vitamin B_6 intake and contribution to vitamin B_6 intake of nutriments/cereals, southern fruits, vegetables, bread and potatoes as independent variables.

All tests were two-sided; the significance level was set at 5%.

Results

Of the total cohort 53.8% were females ($n = 50$). Mean BMI, age and duration of following a vegan diet did not differ significantly between the specific dietary subgroups (Table 1). Intake of alcohol and intake of vitamin B_6 – in terms of mg day^{-1}, mg kg body weight^{-1} day^{-1} (ratio 2) and nutrient density, mg MJ^{-1} – did not differ between dietary subgroups (Table 2). The observed statistically significant difference for mg vitamin B_6 g protein^{-1} (ratio 1) was due to the higher total intake of protein in the SV cohort vegans. Table 3 shows food groups and their contribution to vitamin B_6 intake. Although only four participants (SV: $n = 3$, MV: $n = 1$) had vitamin B_6 intake below the daily recommended intake for adults in Germany (females: 1.2 mg day^{-1}, males: 1.5 mg day^{-1})[22], vitamin B_6 concentration in blood samples was classified as insufficient in 16.1% of study participants. Another 58.1% had EAST-AC between 1.5 and 1.85. When both classifications were set against each other as done in Table 4, the concordance of classifications was poor with a misclassification rate of 18.3%.

Comparing those persons with EAST-AC <1.5 with those having EAST-AC >1.85, they were comparable regarding age but the latter were vegans for a shorter time (6.6 vs. 8.0 years), ingested more vitamin B_6 from vegetables (28.3% vs. 24.2% of total pyridoxine intake), nutriments/cereals (10.6% vs. 7.4%), bread (8.6% vs. 6.9%) and potatoes (8.6% vs. 8.2%), but southern fruits contributed to a lesser extent to vitamin B_6 intake (28.3% vs. 36.9%).

In the total cohort statistically significant bivariate associations were not seen between EAST-AC and dietary intake of vitamin B_6 ($r_S = 0.007$, $P = 0.944$) and dietary fibre ($r_S = 0.105$, $P = 0.318$), respectively. The intake of dietary protein was related to EAST-AC when considered as a percentage of energy ($r_S = 0.240$, $P = 0.020$), but missed statistical significance when measured as intake in g day^{-1} ($r_S = 0.176$, $P = 0.091$). The strongest association was found for EAST-AC and the intake of nutriments/cereals ($r_S = 0.319$, $P = 0.002$). The more nutriments/cereals a person consumed, the more likely was the pyridoxine concentration in their blood sample to be classified as insufficient. EAST-AC was correlated with the proportional contribution of nutriments/cereals to total vitamin B_6 intake ($r_S = 0.252$, $P = 0.015$), confirming the previous observation, while southern fruits showed an inverse association ($r_S = -0.236$, $P = 0.023$), with persons consuming lower amounts of vitamin B_6 from southern fruits being more susceptible to high EAST-AC.

In a logistic regression analysis the influence of dietary intake of vitamin B_6 and potential confounders on EAST-AC were considered. In the GVS cohort none of the established confounders showed a significant influence on EAST-AC classification, with the exception of the contribution of nutriments/cereals to vitamin B_6 intake (odds ratio: 1.06, 95% confidence interval: 1.003–1.117). However, the coefficient of determination of the overall model was poor (Nagelkes R^2: 0.074).

Discussion

In this sample of 93 apparently healthy vegans about 16% showed EAST-AC >1.85 despite high dietary vitamin B_6 intakes. The vitamin B_6 intake in the total study population was 2.83 ± 0.98 mg day^{-1}, thus exceeding the daily recommended intake of 1.2 and 1.5 mg day^{-1} (females, males) in about 96% of all cases. This situation is comparable to the general population in Germany, where likewise only a minor proportion does not reach daily recommended intakes[23]. High total intake with a plant-only diet is documented for American vegans also[24], while mean vitamin B_6 intakes with plant-based diets (i.e. vegetarian diets) have been shown to be lower[7,9,25,26]. This is partly due to the low vitamin B_6 content of milk and milk products and the higher content of fruits, cereals and cereals products. In the GVS study population MV consumed less dietary protein than SV. At first, this seems to be illogical because MV consumed eggs and dairy products. But the ingested amounts were actually very low (eggs: 0.42 ± 1.13 g day^{-1}, milk and other dairy products: 3.95 ± 7.12 g day^{-1}, cheese and curd: 1.85 ± 4.55 g day^{-1}, butter: 1.61 ± 1.97 g day^{-1}), while SV consumed nuts and

Table 1 Characteristics of the German Vegan Study cohort and the specific dietary subgroups

	All ($n = 93$)	Strict vegans ($n = 60$)	Moderate vegans ($n = 33$)	P-value
Females/males (%)	53.8 ± 46.2	48.3 ± 51.7	63.3 ± 36.4	0.157*
Body mass index (kg m^{-2})	21.4 ± 2.33	21.6 ± 2.56	21.0 ± 1.81	0.227†
Age (years)	43.7 ± 15.7	44.4 ± 16.2	42.3 ± 15.0	0.541†
Years adhering to eating a vegan diet	6.70 ± 6.56	7.94 ± 7.78	4.45 ± 1.98	0.057‡

* Chi-square test.
† t-test for unpaired samples.
‡ Mann–Whitney U-test.

Table 2 Dietary intakes of vitamin B_6, protein and alcohol, and activity coefficient of erythrocyte aspartate aminotransferase (EAST-AC), in the German Vegan Study cohort and the specific dietary subgroups

	All ($n = 93$)	Strict vegans ($n = 60$)	Moderate vegans ($n = 33$)	P-value
Vitamin B_6 (mg day^{-1})	2.83 ± 0.98	2.88 ± 1.03	2.72 ± 0.88	0.456†
Nutrient density of vitamin B_6 (mg MJ^{-1})	0.34 ± 0.08	0.33 ± 0.08	0.35 ± 0.07	0.146†
EI/BMR	1.40 ± 0.44	1.45 ± 0.50	1.30 ± 0.30	0.142‡
Alcohol (g day^{-1})	0.42 ± 1.22	0.28 ± 0.94	0.67 ± 1.59	0.278‡
Protein (g day^{-1})	56.6 ± 21.7	60.7 ± 23.3	49.2 ± 16.4	0.013†
Protein (% of energy)	11.3 ± 2.05	11.7 ± 2.09	10.8 ± 1.82	0.048†
Ratio 1: mg vitamin B_6/g protein	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.034†
Ratio 2: mg vitamin B_6/kg body weight per day	3.30 ± 1.28	3.18 ± 1.32	3.52 ± 1.19	0.228†
EAST-AC*	1.60 ± 0.21	1.61 ± 0.22	1.58 ± 0.20	0.618‡
Proportion (%) with EAST-AC				
1.5–1.85	58.1	56.7	60.6	0.738§
> 1.85	16.1	18.3	12.1	

EI – energy intake; BMR – basal metabolic rate.
* EAST-AC was calculated as the ratio of stimulated (pyridoxine 5′-phosphate added) to unstimulated activity.
† *t*-test for unpaired samples.
‡ Mann–Whitney *U*-test.
§ Chi-square test.

seeds (SV: 39.2 ± 35.7 g day^{-1}, MV: 26.5 ± 18.5 g day^{-1}; $P = 0.098$) as well as soy products (SV: 108 ± 104 g day^{-1}, MV: 53.0 ± 80.1 g day^{-1}; $P = 0.001$) to a greater extent. In an American cohort of elderly persons fruit and vegetables were the main dietary contributors of vitamin B_6 intake (approximately 45%), while flesh foods and cereals/grains contributed each about 20%[27]. However, in the National Health and Nutrition Examination Survey II population, flesh foods, alcoholic beverages, potatoes, cereals and milk were important dietary vitamin B_6 sources[28]. Due to the absence of flesh products in the diet of vegans, the low intake of milk and other dairy products in the MV and the low intake of alcoholic beverages[18], (southern) fruits, vegetables, nutriments/cereals, bread and potatoes accounted for about 92% of dietary vitamin B_6 intake. The foods eaten by the GVS study population were mainly (or all) plant-based and therefore, with the exception of only some minor foods, were carbohydrate-rich and low-protein sources. The comparably high vitamin B_6 and the low protein intakes resulted in a ratio of vitamin B_6 to protein that was relatively high, with all of the GVS participants showing vitamin B_6/protein ≥0.3. Thus, one would expect an overall low EAST-AC in the total GVS cohort, but 58% showed EAST-AC in the range of 1.5–1.85 and a further 16% (women: 18%, men: 14%; $P = 0.597$) showed EAST-AC >1.85, indicating that dietary vitamin B_6 in the course of a vegan diet might be of reduced bioavailability. In the German general population intakes of dietary vitamin B_6 are comparable[23], but the prevalence of elevated EAST-AC is 4%[21].

EAST-AC is said to be useful as a long-term measure of vitamin B_6 status[29]. Mean EAST-AC values in other vegetarian cohorts were higher (1.72–2.10)[8,9] than in our cohort. For the elderly Dutch study population of Löwik *et al.*[8] we assume that lower intakes in combination with a higher mean age account for this difference, because resorption decreases with age. In the Taiwanese study population of Huang *et al.*[9] vegetarians had lower vitamin B_6/protein ratios (mean: 0.015), thus indicating a higher risk of inadequate intakes. Furthermore, both vegetarians and non-vegetarians had dietary vitamin B_6 intakes that were lower than in our study population.

Once again it could be shown that it is not sufficient to judge the adequacy of a diet by solely evaluating nutrient intakes and comparing them with national nutrient intake recommendations. As documented here, an overall high total intake of a specific nutrient does not automatically

Table 3 Main dietary sources of vitamin B_6 intake (% of total dietary vitamin B_6 intake)

	All ($n = 93$)	Strict vegans ($n = 60$)	Moderate vegans ($n = 33$)	P-value
Southern fruits	28.3 ± 16.8	26.3 ± 16.5	32.0 ± 16.9	0.120*
Vegetables	28.3 ± 10.8	28.0 ± 11.5	29.0 ± 9.64	0.674*
Nutriments/cereals	10.6 ± 9.35	11.3 ± 9.14	9.26 ± 9.73	0.323*
Bread	8.63 ± 6.68	9.21 ± 6.82	7.58 ± 6.38	0.260*
Potatoes	8.59 ± 6.34	9.12 ± 6.21	7.64 ± 6.35	0.284*
Fruits	7.83 ± 5.36	7.74 ± 5.80	8.01 ± 4.52	0.816*
Nuts/seeds	3.61 ± 2.99	3.86 ± 3.38	3.16 ± 2.05	0.492†
Soy (products)	2.60 ± 2.97	3.03 ± 3.03	1.81 ± 2.71	0.006†

* *t*-test for unpaired samples.
† Mann–Whitney *U*-test.

Table 4 Concordance of classifications of pyridoxine supply for 93 vegan persons: dietary intake of pyridoxine versus activity coefficient of erythrocyte aspartate aminotransferase (EAST-AC) in blood samples

	Dietary pyridoxine intake ≥ daily recommended intakes	Dietary pyridoxine intake < daily recommended intakes	Total
EAST-AC ≤ 1.85	75 (80.6)	3 (3.2)	78 (83.9)
EAST-AC > 1.85	14 (15.1)	1 (1.1)	15 (16.1)
Total	89 (95.7)	4 (4.3)	93 (100)

Values are expressed as *n* (%).
Absolute concordance: 81.7% (kappa coefficient: 0.04).

result in an adequate status for the parameter. Furthermore, a statistically significant linear association was not observed between the intake and the concentration of vitamin B_6 in blood samples. Like other studies focusing on interrelationships between dietary fibre and vitamin B_6 in the course of plant-based diets[6,8], we were not able to find an adverse effect of dietary fibre intake on vitamin B_6 status in the GVS cohort. But in logistic regression analysis EAST-AC was found to be related to the contribution of nutriment/cereals to vitamin B_6 intake. It is to be questioned whether the intake of nutriments/cereals *per se* or the intake of other factors associated with nutriment and cereal intake (such as complexing agents and dietary fibre; vegans of the GVS population consumed mainly wholemeal and full-kernel variants of foods, unpublished data from the GVS)[18] accounts for this finding. Due to the fact that confounders of vitamin B_6 metabolism such as protein and dietary fibre intakes were considered in analyses and that only minor associations were found (when at all), there is evidence to suggest that not quantified or other unknown factors influence the vitamin B_6 status in the course of a vegan diet. The association between the intake of nutriments/cereals and EAST-AC suggests something along those lines. It is known that a large proportion (about 20–30%) of vitamin B_6 in cereals is glycosylated and that glycosylated pyridoxine forms are absorbed to a lesser extent[2,4,30]. Therefore it would be desirable to quantify the content of glycosylated pyridoxines of specific foods and to consider the amount of glycosylated pyridoxine-rich foods consumed with a specific diet. In addition, special nutrient intake recommendations for the different types of diets are desirable.

The following limitations have to be considered when interpreting the results of the GVS. Due to the cross-sectional study design with 33 examination sites all over Germany and for ethical reasons, only one blood sample was taken from each participant, while the mean of two or more blood samples would have been more feasible for representing the clinical relevance and showing long-term effects. Furthermore, only EAST-AC was analysed, while we failed to analyse other biochemical indices such as plasma PLP. In addition, a separate validation for the adopted FFQ has not been conducted, but the original FFQ of the Giessen Raw Food Study was used in persons who consumed a diet consisting mainly of plant food as well. Therefore we believe the GVS FFQ to be precise and accurate. However, the strengths of the GVS are the nationwide recruitment, thus minimising regional differences in eating behaviour; the usage of two semi-quantitative FFQs, thus minimising seasonal differences in nutrient intakes; and the comparably large sample size for a vegan study population, thus allowing subgroup analyses.

In conclusion, the results of the GVS show that German vegans had vitamin B_6 intakes that lay above the national daily recommended intakes. However, 16% showed inadequate concentrations in blood samples and a further 58% showed EAST-AC of 1.5–1-85. Southern fruits were the main contributors to vitamin B_6 intake and the following associations were seen: the less southern fruits and the more nutriments/cereals contributed to pyridoxine intake, the more likely was a person to show EAST-AC >1.85. Due to the high prevalence of inadequate and borderline concentrations of pyridoxine in the blood samples, and the known associations of inadequate pyridoxine status and increased risk of coronary diseases and pancreatic cancer, vegans should be encouraged to include foods with a high bioavailability of pyridoxine such as beans, lentils and bananas in the daily diet.

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