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INFLUENCE OF DAILY DIET ON ASCORBIC ACID SUPPLY TO STUDENTS

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ABSTRACT

Background. Researchers suspect that the accepted adequate ascorbic acid plasma concentration is not being met even after dietary intake of the recommended amount of vitamin C. Current dietary intake recommendation in Poland is 60 mg per day for women and 75 mg per day for man (EAR), while in Western Europe and North America is higher and amounts to 75-90 mg per day.

Objective. The paper aimed at studying a correlation between composition of nutrients in daily diet and plasma vitamin C levels in university students.

Materials and methods. This study examined diet composition and the nutritional status of ascorbic acid in plasma of 120 university students in Szczecin, Poland. Ascorbic acid was determined in blood plasma using HPLC method. The information concerning diet composition was collected using the method of "7-days food record" prior to blood collection.

Results. Plasma ascorbic acid deficiency (<40 μmol/L) was observed in 23% of women and 28% of men. The average plasma ascorbic acid concentration was 48.65 μmol/L in women and 45.61 μmol/L in men. The average intake of vitamin C in women with observed deficiency was average 46.55 mg/day, whereas in men it was 48.56 mg/day.

Conclusions. The recommendation of dietary intake of vitamin C in Poland is low in comparison to other countries. Population-based studies are necessary to determine the actual demand for vitamin C in various population groups in Poland.

Key words: *nutrition, vitamins, dietary intake, diet, ascorbic acid, plasma,*

STRESZCZENIE

Wprowadzenie. Badacze podejrzewają, że przyjęte odpowiednie stężenie kwasu askorbinowego w osoczu nie jest spełnione nawet po spożyciu zalecanej ilości witaminy C. Aktualna norma spożycia witaminy C w Polsce wynosi 60 mg/dzień dla kobiet i 75 mg/dzień dla mężczyzn (EAR), podczas gdy w Europie Zachodniej i Ameryce Północnej jest większa i wynosi 75-90 mg dziennie.

Cel badań. Celem pracy było zbadanie korelacji między składnikami pokarmowymi całodziennej racji pokarmowej a witaminą C u studentów.

Materiał i metody. Badano skład diety oraz zawartość witaminy C w osoczu 120 studentów ze Szczecina. Stężenie kwasu askorbinowego oznaczano w surowicy krwi za pomocą HPLC. Informacje dotyczące żywienia zebrano stosując metodę 7 dniowego zapisu żywieniowego przed pobraniem krwi.

Wyniki. Niedobór kwasu askorbinowego w osoczu (< 40 μmol/L) wystąpił u 23% kobiet i 28% mężczyzn. Średnie stężenie kwasu askorbinowego w osoczu wynosiło 48.65 μmol/L u kobiet i 45,61 μmol/L u mężczyzn. U kobiet, u których stwierdzono niedobór witaminy C, jej średnie spożycie z dietą wynosiło 46,55 mg/dzień, podczas gdy u mężczyzn 48,56 mg/dzień.

Wnioski. Zalecana w Polsce norma dziennego spożycia witaminy C jest niska w porównaniu z innymi krajami. Konieczne są badania populacyjne w celu określenia rzeczywistego zapotrzebowania na witaminę C wśród różnych grup społecznych w Polsce.

Słowa kluczowe*: żywienie, witaminy, spożycie, dieta, kwas askorbinowy, osocze*

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INTRODUCTION

The discovery of ascorbic acid was associated with the occurrence of scurvy, observed as early as the middle ages, mainly in the northern regions of Europe. These areas of cool temperate zone, were deficient in fresh fruit and vegetables in winter and hence residents had a diet poor in ascorbic acid. This avitaminosis could be found among people and animals which in the course of evolution lost their ability to synthetize L-ascorbic acid in their body, due to the absence of L-gulono-γ-lactone oxidase in their liver [5].

Ascorbic acid is absorbed from the intestine in higher concentrations by passive diffusion and in lower concentrations on the basis of active transport, being transported to cells in oxidized form, as dehydroascorbic acid (DHA), with the participation of glucose [23]. It circulates freely, reaching all tissues and cells, and therefore occurs both in plasma and inside cells (especially in leukocytes and platelets). The highest concentrations of ascorbic acid have been observed in glandular tissues, and the lowest in muscle and adipose tissue.

Within cells, dehydroascorbic acid is reduced to ascorbate and stored in this form. Ascorbic acid is mainly metabolized to oxalate excreted in the urine. Ascorbic acid excretion is controlled by the so-called renal threshold, 1.5 mg/100 ml. Exceeding this threshold in the plasma leads to an increased excretion of ascorbic acid with urine in unchanged form, as ascorbic acid, and can also be excreted as dehydroascorbic acid. The absorption of ascorbic acid significantly depends on the presence of biotin and folic acid.

The reserves of ascorbic acid in the body are sufficient for 3-4 months, as the rate of catabolism is about 3% per day. Increased demand for ascorbic acid occurs in athletes, in people staying in cold or in hot weather, and during diseases. Deficiency is commonly observed in breast-fed infants, undernourished people, dialysis patients [29], during chronic diseases (tuberculosis, rheumatic disease), alcohol drinkers and smokers [20, 26]. For women, in addition to frequently occurring qualitative malnutrition, the level of supply with ascorbic acid may also be significantly affected by oral contraceptives that may affect the metabolism of ascorbic acid and cause an increased risk of cervical dysplasia [8]. Ascorbic acid excess in the diet is relatively rarely recorded, and may cause formation of kidney oxalate stones. However, the formation of oxalate stones relates to the consumption of extremely high doses of L-ascorbic acid. Some of the products may also contain ascorbic acid that especially in combination with benzoic acid can cause exposure to benzene [11]. A much greater threat is associated with ascorbic acid deficiency, because as a highly reactive antioxidant it participates in many reactions responsible for disposing of free radicals. Vitamin C supplementation is indicated in individuals exposed to free radicals (ROS), generated during pathogenic processes, among smokers [19] and athletes. The effect of vitamin C supplementation is most noticeable 2-8 h after consumption [22]. It is interesting to note that despite ascorbic acid antioxidant function, no links have been found between levels of this vitamin and the incidence of prostate cancer [1]. Currently, recommended in Poland daily intakes of vitamins C for women are 60 mg (EAR level) and 75 mg (RDA level) and for men 75 and 90 mg per day, respectively [3]. However, some the researchers express the opinion that these standards may be too low and inadequate for adults as compared to the recommended dietary intake of vitamin C in Western Europe and North America (75-90 mg per day) [9]. Moreover, taking into account that smoking increases oxidative stress and metabolic turnover of vitamin C, the requirement for smokers is increased by 35 mg/day. And the average daily intake of vitamin C in the Mediterranean countries is significantly higher and amounts to more than 140 in Greece per day [16].

The aim of this paper was to study a correlation between composition of nutrients in daily diet and plasma vitamin C levels in university students. This study was undertaken in relation to the fact that despite frequent supplementation (being the most popular in the examined population), the quantity of vitamin C absorbed from enriched foods and dietary supplements in daily food rations is rarely examined.

MATERIAL AND METHODS

Examined group

The experiments were conducted on a group of students from the Department of Food Technology and Human Nutrition at the West Pomeranian University of Technology in Szczecin. Prior to experiments the consent of the Bioethical Commission at the Pomeranian University of Medical (Declaration of Helsinki) was obtained [2]. Smoking and non-smoking students (96 women and 24 men) voluntarily took part in the research program. Disproportion between the sexes resulted from the type of the studies, where the majority of students are female. The age of the subjects ranged from 22 to 25. Nutritional status was assessed using anthropometric methods with Body Mass Index (BMI) and Waist to Hip Ratio (WHR). The interview with the participants, concerning their health status, symptoms characteristic for ascorbic acid deficiency, medical treatment, diet supplements, smoking and oral contraception, was carried out on the day of blood collection.

Dietary assessment method

The examination was performed in February. Diets were assessed by 7-days food report method. After that blood was taken from all participants, centrifuged (4˚C/10 min/2500 g/dark) and plasma was collected in -80˚C to future analysis. 7-days food reports included information on the supply of dietary supplements and foods enriched were collected. Daily diet were identified using the "Album of photographs of food products and dishes" [25]. The content of selected nutrients in daily diet such as: energy, proteins, lipids, carbohydrates, vitamins A, D, E, C, B1, B2, B6, B12, niacin, folate, and Na, K, Ca, P , Mg, Fe, Cu, Zn, Mn, fibre and cholesterol was calculated using the computer program "Dietetyk 2" (National Food and Nutrition Institute, Warsaw, Poland) taking nutrient loss into account [13].

Statistical analysis

Arithmetic mean (x), standard deviation (SD) and the significance of differences at $p \leq 0.05$ were calculated using a statistical software package Statistica version 8.0 (Statsoft, Tulsa, Oklahoma, United States).

Determination of ascorbic acid by HPLC method

Blood was collected in tubes (Sarsted) containing EDTA anticoagulant. The samples were centrifuged for 10 min at 2500 rpm at 4°C. Plasma from centrifuged blood was collected into Merck amber Eppendorf safe lock containers and stored at -80°C. All the samples were determined and stored in signed containers. 1 month after collection using the procedure described above plasma was thawed and vortexed. Ascorbic acid was determined by HPLC. After adding equal amounts of EDTA and HPO_3 solution, the samples were centrifuged for 10 min at 2°C. The final stage was the injection of the supernatant and measurement at 243 nm in Hewlett Packard liquid chromatograph. The measurement was duplicated. Quantitative determination was based on the peak area for internal standard [17]. Normal status ascorbic acid nutrition was assumed at the level above 55 µmol/L in plasma.

RESULTS

Interview and general examinations

The students were a random group. Women had a mean BMI of 21.2 and men 23.4. The average value of WHR among all women was 0.79 and men 0.93. As a result of general examination, five persons were noted as during antibiotic-therapy or shortly (up to 2 weeks) after such treatment. Oral hormonal contraception was used by an average of 34.4% of female students, while 28.1% of women and 41.6% of men were smokers. Clinical symptoms that may indicate a deficit of ascorbic acid were reported by 41.7% of women and 54.2% of men (bleeding from the gums, decrease in immune response, dry skin on knees and elbows, papules on the forearms and thighs). Because symptoms are nonspecific, we toke taken into account people who identified 3 of the 4 symptoms. In the examined population 24.6% of the students reported supplementation with vitamin C during 7 days prior to collection of blood samples. The average amount ingested ascorbic acid among women and men was respectively 66 and 60 mg/day/person. In the study group there were no people at risk of exceeding vitamin C upper tolerable level (UL). It seems that the often overlooked sources of ascorbic acid can significantly affect its status in the human body.

Table 1. Population characteristics of selected essential features of the nutritional status of vitamin C

Descriptor	Women n=96	Men n=24	Population n=120
% of students with clinical symptoms that may indicate a deficit of ascorbic acid	41.7	54.2	44.2
% of students with oral hormonal contraception used	34.4	-	-
% of students reported smoking	28.1	41.6	30.8
% of students with ascorbic acid concentration < 11 µmol/L	1.1	0.0	0.8
% of students with ascorbic acid concentration 11-28 µmol/L	5.2	12.5	6.7
% of students with ascorbic acid concentration 29-39,9 µmol/L	18.7	16.7	18.3
% of students with ascorbic acid concentration 40-55 µmol/L	50.0	45.8	49.1
% of students with ascorbic acid concentration >55 µmol/L	25.0	25.0	25.0
the average concentration of ascorbic acid in plasma [µmol/L]	48.65	45.61	48.04
the average concentration of ascorbic acid in plasma <40 µmol/L	32.2	29.6	30.0
the average concentration of ascorbic acid in plasma 40-55 µmol/L	47.63	46.97	47.5
the average concentration of ascorbic acid in plasma >55 µmol/L	65.12	64.77	64.99
The average daily intake with supplementation of vitamin C (mg). The group ascorbic acid in plasma <40 µmol/L	46.55	48.56	47.03
The average daily intake with supplementation of vitamin C (mg). The group ascorbic acid in plasma 40-55 µmol/L	46.74	45.55	46.5
The average daily intake with supplementation of vitamin C (mg). The group ascorbic acid in plasma >55 µmol/L	62.06	66.26	62.76

Provision with ascorbic acid

Ascorbic acid nutritional status in the majority of examined students was correct (94.8% women and 87.5% men) (Table 1). Biochemical evidence of marginal ascorbic acid concentration in plasma (11-28 μmol/L) was observed in 6.7% of the population. Biochemical symptom of ascorbic acid deficiency (less than 11 μmol/L) were observed in only one person. The average concentration of ascorbic acid in plasma in women amounted to 48.65 μmol/L and in men 45.61 μmol/L (Table 1). Assessment of significant differences in ascorbic acid provision between the sexes did not show better provision in any of the groups. Comparison of plasma concentrations of ascorbic acid content of the 7-day diet record have showed an average ascorbic acid intake of 47.03 mg for those at risk of ascorbic acid deficiency (concentration <40 μmol/L). People who were not at risk of ascorbic acid deficiency (concentration >55 μmol/L), consumed an average of 62.76 mg of this vitamin in daily diet (Table 1).

Daily diet (DD) with respect to concentration ascorbic acid in plasma

DD composition analysis in relation to the concentration of ascorbic acid in plasma showed that in the group, with the highest concentration of ascorbic acid in plasma (group III), both in women and men, diets were richer in nutrients (in respectively 13 and 16 tested ingredients, Table 2) than group II and group I in women. Who consumed a similar value of energy as group III, but energy was provided by disaccharides (saccharose) to a larger extent than group II. Differences in the nutrient content of DD of men were even more pronounced (Table 3). In the group of men with the lowest concentration of ascorbic acid in plasma (group I) DD was significantly richer in energy than macronutrients (protein, lipids, carbohydrates) compared with group II (ascorbic acid concentration in plasma 40-55 μmol/L). Moreover, the intake of saturated fatty acids was significantly higher and the intake of polyunsaturated fatty acids was significantly lower than group

Table 2. Energy and nutrients content in daily diet in women and the ascorbic acid status in plasma (μmol/L)

Discriminant	Plasma ascorbic acid concentration ranges		
	Group I <40 n=22	Group II 40-55 n=50	Group III >55 n=24
Energy [kcal]	1742.6^{ab} ± 324.4	1688.4^{a} ± 310.7	1885.6^{b} ± 442.3
Protein [g]	62.9 ^{a} ± 12.0	61.1 ^{a} ± 12.3	64.0^{a} ± 12.9
Lipids [g]	69.0 ^{a} ± 18.1	66.6 ^{a} ± 14.7	73.3 ^{a} ± 20.3
SUFA [g]	26.0 ^{a} ± 7.4	24.2 ^{a} ± 5.6	26.2 ^{a} ± 7.7
MUFA[g]	27.5 ^{a} ± 7.9	26.7 ^{a} ± 6.6	29.8 ^{a} ± 9.3
PUFA [g]	10.2 ^{a} ± 3.1	10.5 ^{a} ± 3.7	11.8 ^{a} ± 3.7
Cholesterol [mg]	245.5 ^{a} ± 89.9	233.5 ^{a} ± 79.6	226.2 ^{a} ± 64.1
Carbohydrates [g]	225.8^{a} ± 48.7	221.7^{a} ± 44.4	252.1^{b} ± 64.0
Saccharose [g]	57.4^{ab} ± 21.8	54.6^{a} ± 20.2	66.3^{b} ± 30.3
Lactose [g]	8.8 ^{a} ± 3.6	7.5 ^{a} ± 3.6	9.4 ^{a} ± 4.4
Dietary fibre [g]	14.2^{a} ± 3.3	15.6^{a} ± 3.7	17.8^{b} ± 4.6
Na [mg]	1697.5 ^{a} ± 400.1	1782.8 ^{a} ± 423.5	1777.8 ^{a} ± 454.5
K [mg]	2286.0^{a} ± 428.5	2332.5^{a} ± 476.3	2694.5^{b} ± 694.4
Ca [mg]	598.9 ^{a} ± 158.9	552.9 ^{a} ± 155.4	594.5 ^{a} ± 208.5
P [mg]	1028.2 ^{a} ± 184.0	1010.2 ^{a} ± 189.2	1078.0 ^{a} ± 249.5
Mg [mg]	236.6^{a} ± 48.8	230.7^{a} ± 51.6	266.3^{b} ± 67.1
Fe [mg]	8.83^{a} ± 1.58	8.79^{a} ± 2.40	10.38^{b} ± 3.70
Zn [mg]	8.23 ^{a} ± 1.54	7.99 ^{a} ± 1.57	8.96 ^{a} ± 3.69
Cu [mg]	0.93^{a} ± 0.19	0.94^{a} ± 0.22	1.23^{b} ± 0.52
Mn [mg]	3.69 ^{a} ± 0.73	3.72 ^{a} ± 1.01	4.23 ^{a} ± 1.47
Vitamin A [μg]	694.2 ^{a} ± 279.6	962.0 ^{a} ± 1180	931.9 ^{a} ± 576.6
Vitamin D [μg]	2.80 ^{a} ± 1.78	2.56 ^{a} ± 1.50	2.74 ^{a} ± 1.49
Vitamin E [mg]	7.40^{a} ± 2.79	7.56^{a} ± 2.74	9.16^{b} ± 3.55
Vitamin B1 [mg]	0.88 ^{a} ± 0.21	0.90 ^{a} ± 0.27	0.93 ^{a} ± 0.21
Vitamin B2 [mg]	1.25 ^{a} ± 0.24	1.26 ^{a} ± 0.36	1.36 ^{a} ± 0.35
Niacin [mg]	12.59^{a} ± 3.69	13.44^{ab} ± 4.59	15.63^{b} ± 9.35
Vitamin B6 [mg]	1.36^{a} ± 0.29	1.47^{a} ± 0.46	1.68^{b} ± 0.53
Folacin [μg]	142.83^{a} ± 30.40	145.25^{a} ± 38.23	178.75^{b} ± 96.82
Vitamin B12[μg]	3.30 ^{a} ± 2.11	3.37 ^{a} ± 2.83	3.60 ^{a} ± 1.98
Vitamin C [mg]	46.55^{a} ± 48.37	46.74^{a} ± 34.27	62.06^{b} ± 62.15

^{a,b}homogeneous groups according to the *Tuckey* test

Table 3 Content of nutrients in daily diet in men and the ascorbic acid status in plasma (μmol/L)

Discriminant	Plasma ascorbic acid concentration ranges		
	Group I <40 n=7	Group II 40-55 n=11	Group III >55 n=6
Energy [kcal]	2444.0[b] ± 490.7	2115.3[a] ± 404.6	2428.6[b] ± 506.1
Protein [g]	83.2[b] ± 15.7	76.0[a] ± 14.0	85.4[b] ± 14.2
Lipids [g]	100.7[b] ± 25.4	86.3[a] ± 22.4	101.1[b] ± 20.5
SUFA [g]	37.1[b] ± 12.6	31.7[a] ± 9.8	36.2[b] ± 7.8
MUFA[g]	41.0 ± 10.5	35.1[a] ± 9.8	39.7[a] ± 9.8
PUFA [g]	14.8[a] ± 2.4	13.0[a] ± 4.1	17.7[b] ± 4.3
Cholesterol [mg]	344.3[a] ± 151.7	286.5[a] ± 94.9	320.1[a] ± 76.3
Carbohydrates [g]	309.8[b] ± 68.2	275.7[a] ± 54.9	315.1[b] ± 76.2
Saccharose [g]	59.6[a] ± 10.5	61.2[a] ± 23.1	80.3[b] ± 19.1
Lactose [g]	13.0[a] ± 7.5	10.8[a] ± 6.0	11.3[a] ± 5.4
Dietary fibre [g]	20.0[a] ± 4.6	18.9[a] ± 5.4	22.6[b] ± 8.9
Na [mg]	2609.9[a] ± 754.0	2194.8[a] ± 635.1	2500.8[a] ± 364.4
K [mg]	2938.9[a] ± 686.0	2811.7[a] ± 510.2	3378.9[b] ± 1029.8
Ca [mg]	725.2[a] ± 223.4	706.0[a] ± 250.3	832.8[a] ± 238.1
P [mg]	1382.9[ab] ± 266.0	1255.6[a] ± 293.0	1473.4[b] ± 261.1
Mg [mg]	302.9[ab] ± 59.1	275.1[a] ± 73.0	337.9[b] ± 108.0
Fe [mg]	10.96[a] ± 2.19	11.91[ab] ± 2.79	12.42[b] ± 2.94
Zn [mg]	11.03[ab] ± 1.84	10.30[a] ± 2.42	11.93[b] ± 1.74
Cu [mg]	1.15[a] ± 0.19	1.12[a] ± 0.27	1.45[b] ± 0.49
Mn [mg]	4.69[a] ± 1.33	4.77[a] ± 1.89	5.61[a] ± 2.26
Vitamin A [μg]	990.38[a] ± 764.85	843.11[a] ± 381.92	1186.46[a] ± 654.42
Vitamin D [μg]	3.83[a] ± 1.68	3.07[a] ± 1.22	4.14[a] ± 2.12
Vitamin E [mg]	9.83[a] ± 1.63	8.77[a] ± 3.08	12.47[b] ± 3.13
Vitamin B1 [mg]	1.34[a] ± 0.26	1.30[a] ± 0.30	1.28[a] ± 0.52
Vitamin B2 [mg]	1.76[a] ± 0.46	1.61[a] ± 0.35	1.64[a] ± 0.32
Niacin [mg]	17.22[a] ± 5.64	16.94[a] ± 3.41	15.75[a] ± 4.33
Vitamin B6 [mg]	1.95[a] ± 0.57	1.97[a] ± 0.38	1.94[a] ± 0.58
Folacin [μg]	188.75[a] ± 65.56	172.33[a] ± 38.56	185.76[a] ± 53.44
Vitamin B12 [μg]	5.75[b] ± 3.55	3.48[a] ± 1.47	3.88[a] ± 0.63
Vitamin C [mg]	48.56[a] ± 30.79	45.55[a] ± 15.69	66.26[b] ± 10.38

[a,b]-homogeneous groups according to the *Tuckey* test

III. The intake of phosphorus, magnesium and zinc did not differ in the compared extreme groups (I and III). The intake of vitamin E and C and minerals such as potassium, iron and copper was significantly lower than group III (Table 3).

Correlation between diet and ascorbic acid contents

Table 4 show the relationship between the content of analyzed nutrients in diets and ascorbic acid contents in daily diet with reference to sexes. In the female subjects, the nutrients of the greatest importance were (in order of significance): dietary fiber, potassium, calcium, phosphor, magnesium, iron and folate (correlation significance 0.3 – 0.2). In the male group the corresponding energy, cholesterol and nutrients were: protein, lipids, MUFA, PUFA, carbohydrates, sodium, calcium, phosphorus, iron, zinc, vitamin E, folate (correlation significance 0.62 – 0.40). This suggests that the major source of ascorbic acid in DD of female students was greater consumption of fruit and vegetable in daily diet.

DISCUSSION

The most frequently reported indicator of ascorbic acid status in the human body is blood plasma concentration, considered to be correct when in the range 60 μmol/L to 180 μmol/L [18]. However, as suggested by *Lykkesfeld* [15], the optimal concentration of ascorbic acid in plasma should exceed 70 μmol/L. A decline in ascorbic acid concentration below 28 μmol/L shows a marginal deficiency of ascorbic acid. Concentrations below 11 μmol/L (0.2 mg/100 ml) were classified as biochemical evidence and/or clinical symptoms of scurvy [10]. The survey carried out in the current work shows that the concentration of ascorbic acid in plasma that demonstrates a marginal deficiency should be at least 40 μmol/L (and even least 55 μmol/L). As dietary consumption of vitamin C in these subjects was only about 45 mg per day.

It is assumed that the daily intake of vitamin C for adults amounting to 60-75 mg is sufficient to maintain

Table 4 Correlation between diet and ascorbic acid content in female and male groups

Discriminant	Female	Male
Energy [kcal]	0.54	0.16
Protein [g]	0.43	0.18
Lipids [g]	0.46	-0.03
SUFA [g]	0.35	-0.01
MUFA[g]	0.45	-0.05
PUFA [g]	0.4	-0.01
Cholesterol [mg]	0.43	-0.12
Carbohydrates [g]	0.42	0.19
Saccharose [g]	0.31	0.07
Lactose [g]	-0.03	0.08
Dietary fibre [g]	0.31	0.26
Na [mg]	0.43	0.12
K [mg]	0.36	0.3
Ca [mg]	0.44	0.23
P [mg]	0.42	0.22
Mg [mg]	0.32	0.3
Fe [mg]	0.46	0.2
Zn [mg]	0.43	0.09
Cu [mg]	0.31	0.15
Mn [mg]	0.22	0.14
Vitamin A	0.26	0.02
Vitamin D	0.25	0.13
Vitamin E	0.46	0.04
Vitamin B1	0.30	0.16
Vitamin B2	0.36	0.12
Niacin	0.18	0.19
Vitamin B6	0.21	0.18
Folacin	0.62	0.28
Vitamin B12	0.07	-0.09
Vitamin C	1.0	1.0

correlation significance for female r>0.2; correlation significance for male r>0.38

an adequate level in plasma and sufficient leukocyte saturation [18]. However, when comparing the average intake of this vitamin among the Greek the level at 140 mg, among the Japanese at 115 mg [7, 10, 16, 24] it would be prudent to consider an increase in daily ascorbic acid by Poles. In nearly a quarter of the study group, even the use of ascorbic acid supplements at around 60mg/day/person did not significantly change the trend of low concentrations of ascorbic acid in plasma.

Importantly, the level of ascorbic acid in the blood is affected not only by the diet (including the treatment of products) and possible supplementation but also factors not related to diet, such as health status, physical activity, drinking alcohol and smoking tobacco. In assessing the body's supply with vitamin C in young athletes it was found that physical exercise causes a decrease of serum in serum by about 15%, while supplementation significantly improve the players' physical fitness tests [4]. Controversial results by *Chawla* [4], exist which show that fitness results depended on vitamin E concentration in plasma and not ascorbic acid.

It seems that the cause of the adequate supply of vitamin C in Poland is the insufficient consumption of fruit and vegetables, in winter. This is due to high prices or irregular eating habits and easy access to processed foods. Troubling results for the too low intake of ascorbic acid found in the younger group of Polish population. The vitamin C intake in daily diets of girls and boys aged 13-18 was deficient [6]. Studies from *Leszczynska* confirm low intake of vitamin C in students (30.0 and 35.4 mg per day) [14]. Considerable variation in the consumption of vitamin C in daily food rations using advanced statistical analysis methods showed *Wądołowska* [28].

The trend of lower intake of vitamin C in winter was observed in students from Arizona [12], with marginal intake of vitamin C observed in 11% students in autumn and in 16% in winter (below 28 μmol/L in plasma). In contrast, vitamin C status among Indians after one month stay in the Antarctic decreased from an initial level of 1.31 to 0.81 mg/dL (74,4-45,9 μmol/L), which undoubtedly shows the influence of season on the nutritional status of the human body [27].

Comparing the average concentrations of vitamin C levels among students from different places around the globe, it should be noted that among students of Arizona the average concentration was 44.7 and 41.5 μmol/L [12], higher levels of vitamin C in plasma (54 and 62 μmol/L) were observed among students from Florida [21]. The highest concentration of vitamin C (62 and 67 μmol/L) was found in Japanese students diets rich in plant food [24].

The risk of scurvy (ascorbic acid <11 μmol/L) in a study by *Johnston* et al. [12] was measured 1-2% of the population, while among Polish students in our study it was measured only one person (0.8% of population). This could be the result of either a larger supply of ascorbic acid or - seemingly more likely - differences in the methodology of plasma sample preparation. In our study samples were strictly handled, i.e. within 15 min of collection they had been centrifuged, separated, stored in a dark room and frozen in amber Eppendorf, which certainly had a positive effect on reducing the losses of ascorbic acid in the sample. This supposition is supported by the fact that concentration of ascorbic acid in Polish populations, studied by other authors, was relatively lower. It should be noted that the average concentrations of ascorbic acid in the blood of Polish students (48.6 μmol/L in women and 45.6 μmol/L in men) did not differ from results obtained in the group of students from Arizona, but were lower than students of both sexes from Florida, and students from Japan. The percentage of the population having ascorbic acid concentrations considered marginal (12-28 μmol/l) was 6.2%.

CONCLUSIONS

1. It seems that the range of marginal ascorbic acid concentrations in plasma should be increased from 12 to 40 µmol/L and even to 55 µmol/L, because the intake of vitamin C in these fields in the plasma was significantly lower.
2. In one quarter of the study group the dietary supplementation with ascorbic acid did not cause increased ascorbic acid levels in the plasma.
3. Despite low intake of vitamin C, the risk of serious deficiency among respondent students is very low.

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Conflict of interest

The authors declare no conflict of interest.

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