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Assessment of Tocotrienols Intake in Adults— A Pilot Study †

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Abstract: Vitamin E compounds are known for their antioxidant potential. Research indicates a more effective antioxidant effect of tocotrienols compared to tocopherols. The aim of this study was to develop an FFQ for evaluation of tocotrienols intake and comparison with data obtained from a 24-h dietary record in adults. The average intake of tocotrienols for subjects (202) was: 2.165 mg/day from 24-h dietary record and 2.236 mg/day for the FFQ. The highest content of the diet was β-tocotrienol, and the lowest was δ-tocotrienol The results of the tocotrienols intake obtained with both methods were similar.

Keywords: tocotrienols; food frequency; adults

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1. Introduction

Tocotrienols are natural antioxidants and together with tocopherols formed a group of compounds known as vitamin E. These compounds are existing as four homologues: α, β, γ, δ—tocopherols and α, β, γ, δ—tocotrienols, which are different in the location and the number of methyl groups in their chemical structure [1]. Tocotrienols and tocopherols are synthesized by autotrophic organisms through photosynthesis [2], and play a significant role in protection against reactive oxygen forms [3]. Vegetable oils provide the best sources of tocopherols and tocotrienols, particularly palm oil and soy oil higher amounts of tocotrienols [4]. Moreover tocotrienols occur in rice, wheat or barley and in some plants such as Achiote (*Bixa orellana* L.) or Mangosteen (*Garcinia mangostana*) [5]. Tocotrienols have broad unique biological activities and properties such as antioxidant, analgesic, anti-inflammatory, antibacterial, antipyretic, anticoagulant, anticancer, cardioprotective, hepatoprotective and neuroprotective [6]. The research results showed the effect of tocotrienols on the inhibition of hormonal changes, oxidative stress, inflammation and the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Tocotrienols have shown a greater contribution to the treatment of chronic diseases alone, than in combination, with tocopherols [7]. According to Palozza et al. [8] δ-tokotrienol has shown a greater inhibitory effect on lipid peroxidation and reactive oxygen species production, and thus a greater antioxidant activity, compared to γ- and α-tocotrienol. Taking into account the role of tocotrienols as bioactive compounds, it is important to conduct further research to develop a tool to assess their dietary content. The aim of the study was to develop a food frequency questionnaire (FFQ) for the evaluation of tocotrienols intake and comparison with data obtained from a 24-h dietary record in a group of adults.

2. Methods

This study used a cross-sectional online survey to collect data using the FFQ method and a single 24-h dietary record. This survey was open to all Poland residents aged 18–65 years from May to September 2021. Out of 217 enrolled subjects, 202 completed the

survey, mostly aged 18 to 40 (people over 40 years of age were not taken into account, n = 5; 10 responses were rejected due to unreliable information). The FFQ questionnaire consisted of 41 questions on food consumption of 15 food categories (e.g., fruits, vegetables, oils, nuts and seeds, fish, eggs, beverages, sweets and salty snacks). A sample question in the questionnaire was "How many portions of olive oil do you consume per day? (1 portion = 10 g/1 spoon)", and the answer could be for instance 1/2, 1, 4, 6. The FFQ questionnaire included questions about the frequency of consumption of vitamin E-rich foods in the three months preceding the study. The 24-h dietary record included questions about the consumption of the same products in the 24 h preceding the study. Moreover, questions about sociodemographic data, such as age, sex, height, weight, place of living, education, occupation, and financial status, physical activity, health, dietary supplements intake were added, based on KomPAN questionnaire [9]. The body mass index (BMI) of the respondents was calculated on the basis of height and body weight declared by the respondents. BMI from 18.5 kg/m^2 to 24.9 kg/m^2 was interpreted as normal weight, from 25.0 kg/m^2 to 29.9 kg/m^2 as overweight and of 30.0 kg/m^2 or more has been considered as obesity [10]. Based on the results obtained with two methods (FFQ and 24 h dietary record), the average consumption of individual isomers and the sum of tocotrienols were calculated using the USDA database. The data on consumption obtained in the study were compared with the level of Adequate Intake (AI) for vitamin E, which for the Polish population was set at 8–10 mg of α-tocopherol equivalent [11]. Statistical analysis was performed with the use of IBM SPSS Statistics. The differences between variables dependent on gender were verified by a Chi-square test for categorical data or, due to non-parametric distribution (confirmed by using Shapiro–Wilk test), for continuous data by U Mann–Whitney test. The Bland-Altman plot was used to check the agreement between the results obtained with the two methods. For all tests, the significance level $p \leq 0.05$ was considered as significant.

3. Results and Discussion

Characteristics of Participants

The study involved 73% of women and 27% of men (Table 1). Most of the respondents were aged 18–25 (77% of the total) and lived in cities with more than 100,000. The most of the respondents described their physical activity as moderate or high. Over 60% of the respondents had a normal weight. Almost 90% of the respondents had a university degree or were in the process of studying (90% of women and 85% of men), and half of the group combined work with studies (64% of the total). There were significant differences between the BMI value in men and women, which indicated a higher percentage of women with malnutrition compared to men, and among men a higher percentage of people with excess body weight than in women was found. Consumption of dietary supplements was declared by 43% of the total, while the use of supplements was significantly more often declared by women. The most frequently taken dietary supplement among the respondents was vitamin D (data not shown).

Table 1. Characteristics of participants.

Variables	Total		Women		Men		χ (p)
	n	**%**	**n**	**%**	**n**	**%**	
	202	**100**	**148**	**73**	**54**	**27**	
Age							
18–25 years	155	77	118	80	37	69	0.095
26–40 years	47	23	30	20	17	31	
Place of residence							
Town > 100,000 inhibitants	135	67	104	70	31	57	0.176
Town < 1000,000 inhibitants	37	18	23	16	14	26	

Rural	30	15	21	14	9	17	
Education level							
Higher (university)	76	38	58	39	18	33	
During studies	103	51	75	51	28	52	
Professional	2	1	0	0	2	4	0.176
Secondary	20	10	14	9	6	11	
Primary	1	0	1	1	0	0	
Occupation status							
Full hour work	39	19	25	17	14	26	
Part-time job	8	4	6	4	2	4	
Work + studies combine	96	48	73	49	23	42	0.574
Students	51	25	37	25	14	26	
Not working	8	4	7	5	1	2	
Body Mass Index							
Underweight	16	8	16	11	0	0	
Normal weight	129	64	96	65	33	61	0.012
Overweight and obesity	57	28	36	24	21	39	
Physical activity							
Low	72	36	58	39	14	26	
Moderate	96	42	75	51	21	39	<0.001
High	34	17	15	10	19	35	

The average intake of tocotrienols obtained by the FFQ method was 2.246 mg/day in women and 2.144 mg/day in men (Table 2). This is 28% of the Adequate Intake (AI) value for the whole vitamin E for women and 21% for men. Analyzing the data obtained using the 24-h dietary record (Table 2), the average intake of tocotrienols was 1.955 mg/day in women and 2.761 mg/day in men, and it differed from the results obtained with the FFQ. It was 24.4% and 27.6% of the recommended AI for complete vitamin E for women and men, respectively. Similar results were found in Japan population [12], where total tocotrienols intake was 1.9–2.1 mg per day.

Among tocotrienols, the largest amount in the diet, regardless of the method of data collection, was β-tocotrienol, which is present in whole-grain pasta, wholemeal bread, carrot juice, brown rice and crackers. The smallest amount in the diet was δ-tocotrienol, which was found in brown rice and wholemeal bread. It was found that α- and δ-tocotrienol as well as the total sum of tocotrienols intake were significantly lower in women than in men. β-tocotrienol has a potential anti-cancer effect in the treatment of lung and brain cancer [13]. Palm oil is widely used in food processing, so γ-tocotrienol is abundant in processed foods such as nachos [14,15]. This isomer is also found in brown rice and forest fruits. γ-tocotrienol is one of the isomers with a protective effect against heart disease [16], it has the strongest adipogenesis-inhibiting potential among the tocotrienol isomers [17], and also inhibits the penetration of macrophages into adipose tissue [18]. It has also been shown that the γ form of tocotrienol has a protective effect of human neuroblastoma cells, indicating its potential role in the prevention of Parkinson's disease [19].

Table 2. Intake of individual isomers and sum of tocotrienols.

	FFQ Mean (SD); mg/Day				
	α-T3	**β-T3**	**γ-T3**	**δ-T3**	**Sum of T3s**
Total	0.418 (0.377)	1.343 (1.341)	0.419 (0.41)	0.055 (0.05)	2.236 (2.157)
Women	0.418 (0.377)	1.336 (1.354)	0.436 (0.422)	0.056 (0.058)	2.246 (2.199)
Men	0.403 (0.379)	1.314 (1.318)	0.375 (0.375)	0.051 (0.063)	2.144 (2.059)
p	0.890	0.794	0.297	0.253	0.886
	24-h Mean (SD); mg/Day				
	α-T3	**β-T3**	**γ-T3**	**δ-T3**	**Sum of T3s**
Total	0.418 (0.378)	1.207 (1.205)	0.455 (0.459)	0.084 (0.089)	2.165 (2.160)
Women	0.376 (0.335)	1.098 (1.123)	0.412 (0.408)	0.068 (0.058)	1.955 (1.953)
Men	0.540 (0.461)	1.505 (1.488)	0.584 (0.585)	0.131 (0.134)	2.761 (2.516)
p	0.018	0.064	0.057	0.005	0.020

FFQ—Food Frequency Questionnaire; SD—standard deviation; T3—tocotrienol; 24-h—24-hour dietary record.

Average α-tocotrienol intake, obtained by two methods, was almost identical and amounted to approximately 0.418 mg/person/day. The research results indicated the neuroprotective and hypercholesterolemic effects of α-tocotrienol, important in the prevention of stroke [6]. The lowest intake among all isomers was found for δ-tocotrienol. This isomer plays an important role in preventing the development of pancreatic cancer [20], and also has a protective effect of dopaminergic neurons and positively influences motor activity in Alzheimer's disease [21].

The significant differences between the results obtained with the two methods occurred for total tocotrienols, δ-tocotrienol, and γ-tocotrienol only in men (Table 3). In turn, a significant difference occurred for β-tocotrienol intake in women. For δ -tocotrienol a significant differences were found for all categories.

Table 3. Comparison of the mean intake of sum of tocotrienols and individual isomers obtained with the two methods.

	FFQ	**24-h**	***p***
	Mean (SD); mg/Day		
	Sum of T3s		
Total	2.236 (2.157)	2.165 (2.160)	0.545
Women	2.246 (2.199)	1.955 (1.953)	0.126
Men	2.144 (2.059)	2.761 (2.516)	0.022
	α-T3		
Total	0.418 (0.377)	0.418 (0.378)	0.645
Women	0.418 (0.377)	0.376 (0.335)	0.956
Men	0.403 (0.379)	0.540 (0.461)	0.002
	β-T3		
Total	1.343 (1.341)	1.207 (1.205)	0.054
Women	1.336 (1.354)	1.098 (1.123)	0.024
Men	1.314 (1.318)	1.506 (1.488)	0.390
	γ-T3		
Total	0.419 (0.410)	0.455 (0.459)	0.247
Women	0.436 (0.422)	0.412 (0.408)	0.810
Men	0.375 (0.375)	0.584 (0.585)	<0.001
Total	**δ-T3**		

Women	0.055 (0.050)	0.084 (0.089)	<0.001
Men	0.056 (0.058)	0.068 (0.058)	0.006
	0.051 (0.063)	0.131 (0.134)	<0.001

FFQ—Food Frequency Questionnaire; 24-h—24-hour dietary record.

The Bland-Altman plot (Figure 1) show differences between the results of total tocotrienol consumption obtained with the two methods. The mean absolute difference of the sum of tocotrienols intake was observed to amount to −0.071. The interval from −4.908 (lower agreement limit) to 4.766 (upper agreement limit) was obtained for the limits of agreement value (LOA) after adding a ±1.96-fold standard deviation. The number of individuals observed to be beyond the LOA value was 189 out of 202, corresponding to the Bland-Altman index of 6.4%.

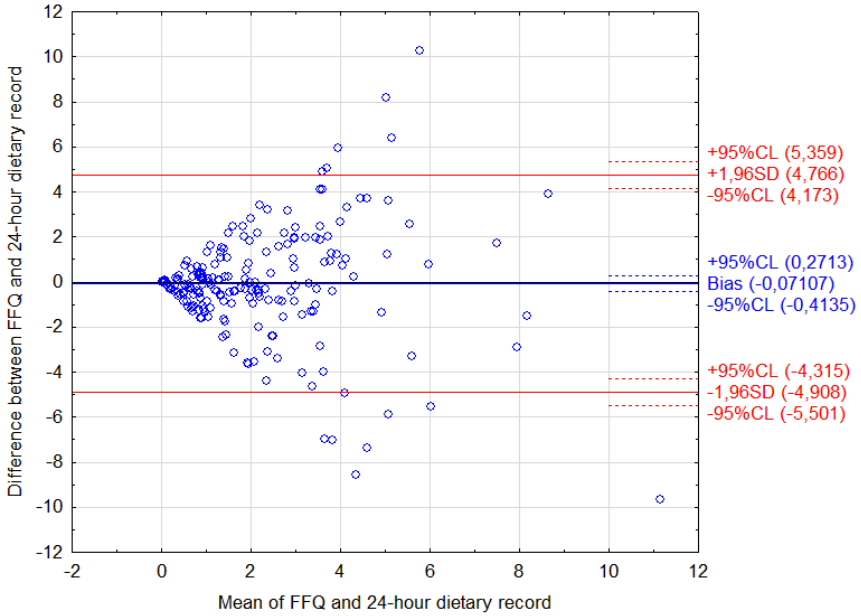

Figure 1. Bland-Altman plot comparing FFQ with 24-h dietary record for sum of tocotrienols intake (Bland-Altman index of 6.4%).

The results indicate that the developed FFQ questionnaire obtained lower total tocotrienol intake results, by an average of 0.071 mg tocotrienols than the 24-h dietary record. The FFQ questionnaire needs to be refined so that the 95% limits of agreement include the appropriate number of measurements, giving the Bland-Altman index below 5%.

4. Conclusions

The results of the tocotrienols intake obtained with both methods were similar, but the questionnaire developed in this pilot study requires further refinement in order to correctly assess the intake of these compounds.

Due to the low proportion of tocotrienols in the diet, it seems beneficial to popularize the knowledge about their influence on health and food sources.

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