ANNA HARTON[1]*, JOANNA MYSZKOWSKA-RYCIAK[1], DANUTA GAJEWSKA[1], SA'EED BAWA[1,2]
*Corresponding author
1. Warsaw University of Life Sciences, Faculty of Human Nutrition and Consumer Sciences, Department of Dietetics, Nowoursynowska Street 159C, 02-776 Warsaw, Poland
2. The University of the West Indies, St Augustine Campus, Section of Human Nutrition and Dietetics, Faculty of Food and Agriculture, The Republic of Trinidad and Tobago

Anna Harton

The estimation of the intakes of dietary flavonoids by a group of young Polish females

KEYWORDS: flavonoids intake, female, cardiovascular diseases

Abstract

There is growing evidence regarding beneficial health effects of dietary flavonoids, which have been associated particularly with the reduction in the risk for the development of cardiovascular diseases (CVDs). The aim of the study was to assess the intakes of flavonoids by a group of selected young Polish females (n=254). The mean of total daily flavonoids intake was 272.1 mg (median of 210.9 mg). The main sources of flavonoids were tea followed by vegetables and fruits. Cocoa, dark chocolate, potatoes and herbs also contributed to the consumption of flavonoids. So far, no recommendation has been established for the intake of flavonoids, but due to their protective effects on overall health, the consumption of flavonoids from diet by the general population should be encouraged.

INTRODUCTION

Flavonoids are ubiquitous plant secondary products that are best known as the characteristic red, blue, and purple anthocyanin pigments of plant tissues (1). To date, more than 5.000 different flavonoids have been identified. Flavonoids can be classified into six subgroups based on their chemical structure: anthocyanidins, flavanols or flavan-3-ols, flavonols, flavanones, flavones, isoflavones (2). Flavonoids are widely distributed in plants, and in some of them in large amounts, e.g. berry fruits are rich in anthocyanidins (blueberries 163.52 mg/100g); parsley is a good source of flavones (fresh leaves provide 227.17 mg/100g); onions contain high amounts of flavonols (27.07 mg/100g), while tea contains the largest quantities of flavan-3-ols (black, brewed 115.25 mg/100g) (3). However, depending on the structure of consumption of food products in the diet (quantity, frequency), the main sources of flavonoids may vary in countries or populations with different dietary habits.

Flavonoids are able to scavenge free radicals directly by hydrogen atom donation, and their antioxidant capacity is much stronger than that of vitamins C and E (4). Epidemiological studies have suggested that flavonoids-rich diet, especialy flavonol and flavone classes were most strongly associated with lower coronary heart disease (CHD) mortality (5). This is particularly important as CVDs remain the biggest cause of death worldwide and the percentage of premature death from CVDs ranges from 4 percent in high-income countries to 42 percent in low-income countries (6). Specially, in Poland in 2010 the standardized death rate from CVDs was almost 20 percent higher than the average in the European Union, and more than twice higher than in France, Spain or Portugal (7). However, it has been proved that CVDs are largely preventable. Controlling the main risk factors and changing to "healthy" lifestyle (balanced diet, regular physical activity) can reduce mortality rate from CVDs by 50 percent (8). A "healthy diet" should include low amount of saturated fatty acids, trans fatty acids, cholesterol, and sodium, but should be rich in dietary fiber, vitamins and flavonoids (6, 9, 10, 11).

Recently, flavonoids have been reported to exhibit other multiple biological effects, e.g. antiviral, antibacterial, anti-inflammatory, vasodilatory, anticancer, and anti-ischemic (4, 12). Studies have shown that flavonoids may be beneficial via regulating the metabolism of gut microbiota, which directly participates in host metabolism through controlling expression of host genes and degrading plant polysaccharides into short-chain fatty acids. The evidence indicates that gut microbiota may have an important role in the pathogenesis of metabolic diseases, including CVDs and type II diabetes by inducing low-grade, systemic and chronic inflammation (13).

Taking into account the vital role that flavonoids intake may play in health and disease, it is important to accurately estimate intake levels in different groups as well as determine the main sources of flavonoids in population-based studies. This could help with the formulation of dietary recommendations in the prevention of diet-related diseases, especially CVDs. It might be also interesting to compare the main sources of flavonoids in "traditional" diets in different countries.

EXPERIMENTAL SECTION

Materials and methods

The study group included 254 female volunteers who participated in the Diet-related Diseases Prevention Program conducted between September 2012 to June 2013, which was organized by the Department of Dietetics at Warsaw University of Life Sciences, Poland. The main aim of the Program was to provide nutritional education towards the prevention of diet-related diseases (especially obesity, diabetes and CVDs). All participants gave their informed consents for participating in the study. Anthropometric measurements, including height and body weight were carried out following standardized WHO procedures. Body mass index (BMI) was calculated as subjects' weight (kg) divided by the square of their height (m²). Normal weight was defined as a BMI $\geq$ 18.5 and < 25 kg/m² according to WHO classification (14). Dietary intakes of participants were assessed by three-day food record, completed during two weekdays and 1 weekend day. Participants were instructed on estimating portion sizes and how proper recording of the weights of all consumed food and edible parts of food products were used. To facilitate the record, household measurements (cups, spoons etc.) were used in addition to measures of weight of portions in grams. The diary records were reviewed by the dietitian during an individual interview with the participants. Total energy intake was calculated using Polish software Energia®, and the structure of consumed groups of food products (vegetables, fruits) was conducted. For the calculation of flavonoids from tea (black and green) the typical recipes with the average weight of tea leaves/bags for a cap of water were used. Other food sources of flavonoids (except fruits, vegetables, tea) e.g. herbs, potatoes, chocolate were classified as "other" group of food. For assessment of dietary flavonoids intake, the "U.S. Department of Agriculture Database for the Flavonoid Content of Selected Foods, Release 3" was used (3). The USDA database contains information about flavonoids content in 500 food items; isoflavones and proanthocyanidins are not included in this database, In the estimation of total dietary flavonoid legumes and wine were not included because none of the participants reported their consumption. Flavonoids content is given in mg/100g of edible part of food product. Flavonoids are divided into five following subclasses: flavonols, flavones, flavanones, flavan-3-ols and anthocyanidins. In the present study, all sources of flavonoids were calculated. Juices were recalculated for the fresh fruits and vegetables amounts, and all dishes were broken down in ingredients based on recipes. The following flavonoids were asses: flavonol, flavones, flavanones, flavan-3-ols, anthocyanidins. The results are expressed as total dietary flavonoid intake (mg/d) and the amount of flavonoids for 1000 kcal per day. Dietary flavonoids intake was estimated as aglycones Statistical analyses were performed using STATISTICA v.10Pl software (StatSoft Inc. USA)

with the nonparametric Spearman test. Differences were considered statistically significant with p-value < 0.05.

RESULTS AND DISCUSSION

The characteristics of studied group is presented in Table 1 above. The study population consisted of young, healthy, educated female, who are aware of the role of nutrition in diet-related diseases prevention, but are not representative for the Polish female population. In our study, none of the subjects were overweight or obese, thus several women had BMI below 18.5 kg/m² indicating underweight. These data are correspond with quite low energy intake (1520 kcal/d), which is lower than estimated energy requirements for this population group (1900 kcal/d with low physical activity and 2400 kcal/day for moderate physical activity. Obtained values for BMI and energy intake were lower than those seen in the general population of Poland (15), which is probably due to the young age of the examined women (median of 22 years).

Factor	Mean ± SD	Median	Range
Age (years)	22.5±0.9	22	21.0-24.9
BMI (kg/m²)	20.8±2.2	20.6	16.9-24.9
Physical activity (min./d)*	38±15.7	32	15.0-90.0
Total energy intake (kcal/d)	1520±619	1587	935-2177
Fruits intake (g/d)	193.0±140.1	165.6	0-648
Vegetables intake (g/d)	297.2±184.5	267	213-809
Tea intake (ml/d)	160.3±101.5	136	0-750

*self-reported

Table 1. Characteristics of subjects: age, BMI, energy, vegetables, fruits and tea intakes (n=254)

Besides low energy value, fruits and vegetables are important sources of vitamins, minerals, dietary fiber, and other cardioprotective components such as flavonoids. Results from many studies have shown inadequate intakes of these groups of products. It should be underlined that increasing the intake of fruits and vegetables is one of the main determinants for maintaining good health. In our study, the average fruits intake was 193 g/d, while the consumption of vegetables stood at 297 g/d. Lower consumption of fruits and vegetables by women (393 g/d) but close to recommendation in the primary prevention of coronary heart disease (11) was recorded in another Polish study (15). It is generally regarded that higher consumption of fruits and vegetables is associated with higher intake of flavonoids. In the present study, total daily flavonoids intake per day was found to be 272.1 mg (median 210.9 mg) (Table 2).

Flavonoids	Mean ± SD	Median	Range
Total dietary flavonoid			
(mg/d)	272.1±268.9	210.9	1.9-1274
(mg/1000kcal/diet)	190.8±144.1	160.9	1.3-777.2
Subclasses of flavonoids			
Flavonols (mg)	21.8±13.7	19.7	1.2-57.4
Flavones (mg)	13.0±41.3	1.0	0-299
Flavanones (mg)	11.8±19.7	1.4	0-126
Flavan-3-ols (mg)	215.1±237.5	116.3	0.3-1274
Anthocyanidins (mg)	10.5±12.5	6.2	0-58.1

Table 2. Total flavonoid content in the diets of subjects

Significantly higher intake of total flavonoids was found in a group of 1520 subjects (men and women with average age of 49.9 and 50.0 years, respectively) living in Breslau, Poland (16). However, our study included only young women, whose diets were low in energy (the average about 1520 kcal/d). Comparison of these two studies showed higher intake of fruits and vegetables and significantly lower tea consumption in our study. It should be noted that we recalculated all juices for the fresh fruits and vegetables, while in the study by Ilow et al. (16) juices were excluded for separate group. In addition, these two studies had different methodology (three-day food records vs. FFQ) and participants (young vs. middle aged, women only vs. men and women). The results from the study by Ilow et al. (16) showed that tea (no other sources, such as fruits and vegetables) provided 96 percent of all consumed bioflavonoids. Similar results and conclusions were reported in another Polish study conducted by the same authors with the same methodology (17). Data on total flavonoids intake from other countries reported lower consumption as compared to our results as well as results obtained by Ilow et al. (16, 17). The intake is stratified by gender and type of diet typical for the examined country. For example, Brazilian women consumed only 79 mg/d flavonoids per day (18) and Flemish women 166 mg flavonoids per day (19). Despite the fact that the Mediterranean diet is considered to be rich in flavonoids, data from Greek adults study showed intake of total flavonoids to be only 92 mg/d (20) and data from National Health and Nutrition Examination Survey Study indicated that the intake of these phytochemicals among American population was 190 mg/d (21). Similar amounts of total flavonoids intake were observed in Spanish women (median about 220 mg/d) (EPIC-Spain Study) (22) and in the Iowa Women`s Health Study (239 mg/d) (23). The most recent studies on a large group of 35,628 subjects showed no significant differences in total flavonoid mean intake between Mediterranean and non- Mediterranean countries (24).

Moreover, the literature shows a variation in the consumption of subclasses of flavonoids depending mainly on the type of diet. Total flavonols intake by Finns was found to be about 4 mg per day (25); the intake in our study was 21.8 mg/d, while Mediterranean diet contained 32 mg/day. The main sources of flavonols in Polish diet were vegetables – mainly onions and broccoli (Table 3). The intake of flavones in our study was 13 mg/d with parsley, celery and cabbage as the main sources. The same result (13.6 mg/d) was obtained in a study of American population (Baltimore Longitudinal Study of Aging, BLSA) in a group of 366 participants (50 percent of men, average age 68.9) (26). While in the EPIC-Spain Study (22) the consumption of flavones was only 4.3 mg per day. Flavanones intake from the Greek menu was found to be 38.45 mg/day (27), whereas

the intake in our study was 11.8 mg/day; the main sources were oranges. Flavan-3-ols accounted for 86 percent of total dietary flavonoids in our study (with the largest amounts from black tea vs. green tea). In the BLSA study the average flavan-3-ols intake was found to be 37.4 mg/day (26), while the intake in the EPIC-Spain Study was 40 mg/day, which constituted only 10.3 percent total daily flavonoids (22). While tea was the main source of flavan-3-ols in American (26, 28) and Polish diet (16, 17), red wine was the major source for Spanish population (22). Anthocyanidins intake in our study was 10.5 mg/day, in Spain – 26.3 mg/d and in the BLSA Study – 15.1 mg/d (22, 26). In general, anthocyanidins are present in fruits, and in Poland apples and bananas delivered about 40 percent of the total intake.

"Others" products such as cocoa, dark chocolate, potatoes and herbs had a relatively small share of the total amount of flavonoids in our study; however, these sources should not be omitted in the diet analysis. In our study red wine was not consumed. The main sources of total flavonoids intake in our study were tea, followed by fruits and vegetables. Compared to other Polish studies (16, 17),

	Intake (mean g/d)	Intake (median g/d)	Flavonols (mg)	Flavones (mg)	Flavanones (mg)	Flavan-3-ols (mg)	Anthocyanidins (mg)
Fruits (15% from all)							
Apple	55	53.0	2.13	0.06	0.00	6.78	2.10
Pear	40	33.0	0.00	0.00	0.00	0.06	0.00
Orange	17	15.0	0.12	0.03	7.24	0.00	0.00
Grapefruit	5	3.1	0.00	0.05	2.73	0.00	0.00
Peach	12	10.0	0.04	0.00	0.00	1.96	0.12
Lemon	3	0.0	0.05	0.06	1.49	0.00	0.00
Banana	27	26.0	0.05	0.00	0.00	1.65	2.00
Dark grapes	21	19.0	0.50	0.00	0.00	4.54	0.00
Raspberries black	1	0.0	0.00	0.00	0.00	0.00	3.24
Strawberries	12	6.5	0.69	0.00	0.03	0.51	3.02
TOTAL	193	165.6	3.59	0.19	11.48	15.50	10.47
% from subclasses			16.47	1.46	97.35	7.21	100.00
Vegetables (8.8% from all)							
Tomato	46	44.0	0.37	0.00	0.31	0.00	0.00
Carrots	62	57.6	0.30	0.07	0.00	0.00	0.00
Cabbage	50	45.0	0.62	0.44	0.00	0.00	0.00
Parsley	5	0.0	0.83	10.83	0.00	0.00	0.00
Celery	20	16.9	0.12	0.78	0.00	0.00	0.00
Onion	19	15.1	4.74	0.00	0.00	0.00	0.00
Broccoli	25	23.0	2.79	0.20	0.00	0.00	0.00
Lettuce	30	27.4	1.27	0.11	0.00	0.00	0.00
Cucumber	40	38.0	0.12	0.00	0.00	0.00	0.00
TOTAL	297	267.0	11.16	12.42	0.31	0.00	0.00
% from subclasses			51.26	95.86	2.65	0.00	0.00
Tea (70.4% from all)							
Green	32	16	1.54	0.10	0.00	37.28	0.00
Black	128	120	4.80	0.00	0.00	147.93	0.00
TOTAL	160	136	6.34	0.10	0.00	185.21	0.00
% from subclasses			29.12	0.74	0.00	86.12	0.00
Others (5.6% from all)							
Potato	140	100	0.69	0.00	0.00	0.00	0.00
Cocoa dry powder	3	0	0.00	0.00	0.00	7.84	0.00
Dark chocolate	6	5	0.00	0.00	0.00	6.52	0.00
Herbs (e.g. oregano)	1	0	0.00	0.25	0.00	0.00	0.00
TOTAL	150	105	0.69	0.25	0.00	14.35	0.00
% from subclasses			3.15	1.94	0.00	6.67	0.00
All total	800	-	21.78	12.96	11.80	215.06	10.47
Total flavonoids from all sources = 272.1 mg							

Table 3. The main sources of flavonoids subclasses in the diets of subjects

the intake of tea was much lower and the consumption of fruits and vegetables were higher, but it could also be due the methodology as we recalculated juices for the fresh, raw ingredients.

The results of our study showed correlations – the negative correlation between total energy intake and flavonols content (p=0.0041; R=-0.35), as well as flavones (p=0.0121; R=-0.31). It can be explained by the fact that the main sources of these phytochemicals were vegetables which are low in energy, thus their higher consumption is not associated with significant increase in energy intake. Precise estimation of total flavonoids intake as well as their subclasses is limited by the availability of data for all foods, lack of information about losses during different methods of preparation etc. (21, 26). Furthermore, comparison of data from different countries is difficult due to different methodology, size of groups, age of examined subjects as well as contents of flavonoids etc. (21, 22, 26, 29). The limitation of this study is nonrepresentative sample of the general population. On the other hand, the examined subjects formed very homogeneous group (sex, age, inhabitancy, level of education, and nutritional status), but representative for population of young, well educated women (30, 31). The second limitation of this study may have been underestimation of total flavonoids intake because the database does not contain proanthocyanidins and isoflavones. The intake of main dietary sources of isoflavones (legumes) was not reported by participants, but did not have influence on the final results; typical Polish diet is rather low in legumes (about 2g/d) (32).

The strength of the study was the period of food records collection: 10 months between September and June excluding holiday months. Intake of selected groups of food (e.g. fruits, vegetables) maybe higher during these months because of availability. On the other hand, nutrition during holiday period is often atypical. The other strength of this estimation is that the dietary intake of participants was assessed by three-day food record. All participants were individually instructed on estimating portion sizes and proper recording of the weight of all consumed food by qualified dietician. In our study, only edible parts of food products were used for analysis. Calculation based on only 24-h DR or FFQ may increase the risk of under- or overestimation of the intake. The last strength of our study is estimation of "other" food sources of flavonoids such as herbs, potatoes, chocolate and different types of tea (green and black) which increases precision of calculation.

In the current recommendation for "healthy diet" as well as for primary prevention of CVDs, there is no information about recommended levels of dietary flavonoids. However, there are recommendations about amounts (portions) of fruits and vegetable which should be consumed daily. A large number of studies have shown correlation between high intake of fruits and vegetables and lower risk of cardiovascular disease and/or stroke as well as other health benefits (33). Dauchet et al. (34) found that each additional serving of fruits and vegetables reduced the risk of CHD by 4 percent and risk of stroke by 5 percent. Thus raising public awareness and including nutritional education even in low risk groups can play an important role in maintaining good health and the prevention of heart disease in whole population.
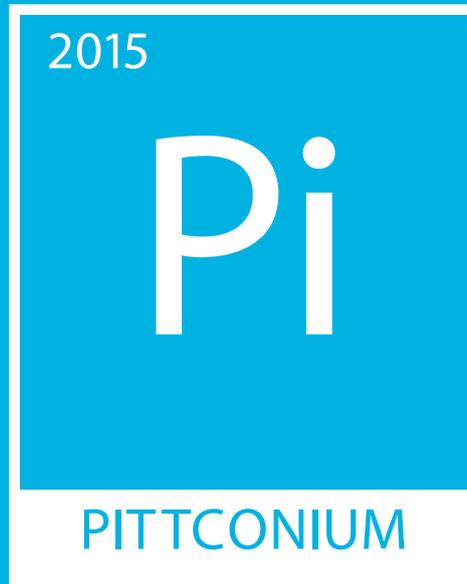
CONCLUSIONS

1. Total mean of flavonoids intake by Polish young females was similar to the intake observed in other European studies.
2. The main sources of these phytochemicals were tea, followed by vegetables and fruits; cocoa, dark chocolate, potatoes and herbs also contributed to the total intake of flavonoids.
3. A well-balanced diet in terms of the intake of vegetables, fruits and other products rich in flavonoids should be an important element of a healthy diet.

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