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Antioxidant Intakes and Food Sources in Greek Adults[1–3]

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Abstract

Antioxidants are compounds physiologically produced or provided through the diet with a potential to inhibit the oxidation of important biological molecules, such as proteins, lipids, and DNA. The contemporary Greek diet is still strongly influenced by Mediterranean dietary traditions. The traditional Mediterranean diet (MD) is a plant-based diet with apparent beneficial health properties, to which a high antioxidant content may contribute. To explore this issue in detail, a database of the content of >200 Greek foods and recipes for a wide spectrum of antioxidant compounds and indices (flavonoids, proanthocyanidins, other antioxidant microcomponents, and total antioxidant capacity) was developed. The database enabled the estimation of antioxidant intakes in Greece using the population-based European Prospective Investigation into Cancer and Nutrition study, in which >28,000 Greeks participate. The results of this work suggest that the contemporary MD in the Greek population is a rich source of a variety of antioxidants. These data can be used in studies about the relationship between antioxidant intake and chronic diseases in the Greek population. J. Nutr. doi: 10.3945/jn.110.121848.

Introduction

The term antioxidant is used to characterize compounds that can prevent, irrespectively of the mechanism used, the oxidation of molecules of biological importance, such as lipids, proteins, and DNA. The free radical theory of aging formulated >50 y ago (1) proposed that the sustained oxidation by free radicals is a main cause of aging and chronic disease. Consequently, antioxidants were viewed as health-promoting agents that may protect against accelerated aging. At about the same time period, the concept of the Mediterranean diet (MD)[4] emerged (2). Since then, studies focused on the effects of the MD on longevity have consistently indicated associations in populations of varying origins, such as Mediterraneans, Northern Europeans, Americans, and Australians (3–6). The health advantages in populations adhering to the MD are expressed through the reduction of the incidence of several chronic diseases, including cardiovascular, cancer, Parkinson's, and Alzheimer's disease (7). However, although several investigations have provided evidence on the beneficial health attributes of this diet, the mechanisms of action are not yet adequately understood.

The prevailing hypothesis, which is based mainly on observational data, focuses on to plentiful consumption of unrefined plant foods that are cooked in or supplemented with olive oil; less frequent consumption of animal products, among which fish is preferred over red meat; and moderate wine consumption, most often during meals (8).

Because many of the plant foods contain high amounts of antioxidants, a possible link between the MD and the reported health benefits may be considered (9). This association is supported by epidemiological evidence in a study in which adherence to the MD was positively associated with serum total antioxidant capacity (TAC) and negatively with oxidized LDL in some 3000 volunteers (10). A recent randomized trial on the impact of the traditional MD on high cardiovascular risk individuals also supports a relation between the MD and biochemical antioxidant markers (11). However, other trials have given diverse results (12,13). The present work aims to contribute to the field by providing information on the antioxidant content of the contemporary Greek diet, which is still strongly influenced by Mediterranean dietary traditions.

Materials and Methods

The European Prospective Investigation into Cancer and Nutrition (EPIC) study is a cohort study conducted in 10 European countries aiming to investigate the role of nutrition and lifestyle on the etiology of cancer and other chronic diseases (14). In the context of the Greek-EPIC cohort, 28,572 Greek volunteers (11,954 men, median age 51 y, and 16,618 women, median age 54 y) were recruited between 1994 and 1999, covering all Greek areas and socioeconomic strata. Written informed consent was provided by all study participants and all procedures were in accordance with the Helsinki Declaration. The study

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[3] Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.
[4] Abbreviations used: FRAP, ferric reducing ability of plasma; GAE, gallic acid equivalent; MD, Mediterranean diet; ORAC, oxygen radical absorbance capacity; TAC, total antioxidant capacity; TEAC, trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter.
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protocol was approved by the ethics committees of the International Agency for Research on Cancer and the University of Athens Medical School. Dietary assessment was performed with a validated semiquantitative questionnaire (15) including questions on the frequency of consumption of ~200 foods and recipes as well as 15 types of beverages. The questionnaire contained photographs with standard portion sizes, which facilitated the conversion of frequencies into quantities of consumption. Recipes were broken down to primary (raw) ingredients for which the content in various antioxidants as well as TAC was calculated using food composition tables (**Table 1**) (16–23). To estimate the contribution of food groups to the intake of antioxidants, primary foods were allocated into the following 14 groups: potatoes, vegetables, legumes, fruits and nuts, dairy products, cereals, meat, fish, eggs, lipids, sugar and confectionary, nonalcoholic beverages, alcoholic beverages, and condiments and sauces (24). The information on the antioxidant content of foods in combination with the consumption data provided the means to calculate median gender-specific antioxidant intakes and the contribution of individual foods and food groups to those intakes in the Greek population. Statistical procedures were performed with STATA version 10. Values in the text are medians unless noted otherwise.

TABLE 1 Sources for the EPIC-Greece antioxidant database

Class	Reference	Compounds/indexes[1]
Flavonoids	16–17	Twenty-nine individual flavonoids and related compounds
Proanthocyanidins	19	Six categories (monomers, dimers, trimers, 4–6mers, 7–10mers, and polymers)
Vitamins	20	Vitamins C and E and β-carotene
Inorganic compounds	20	Selenium
TAC indexes	18, 21–23	ORAC, TRAP, TEAC, FRAP, total phenols

[1] FRAP, ferric reducing ability of plasma; ORAC, oxygen radical absorbance capacity; TEAC, trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameter.

TABLE 2 Daily intakes of antioxidants by Greek adults participating in the EPIC study[1]

Antioxidant	Intake		
	Women	Men	All
n	16,618	11,954	28,572
Flavones, *mg*	7 (6, 9)	8 (6, 10)	7 (6, 9)
Apigenin	4 (3, 5)	5 (4, 6)	5 (4, 6)
Luteolin	3 (2, 4)	3 (2, 4)	3 (2, 4)
Flavonoles, *mg*	26 (20, 32)	32 (25, 39)	28 (22, 35)
Quercetin	17 (13, 21)	20 (16, 25)	18 (14, 23)
Kaempferol	5 (4, 8)	7 (4, 10)	6 (4, 9)
Isorhamnetin	2 (2, 3)	3 (2, 3)	2 (2, 3)
Myricetin	1 (1, 1)	1 (1, 2)	1 (1, 1)
Flavanones, *mg*	27 (15, 37)	28 (16, 42)	27 (16, 39)
Eriodictyal	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)
Hesperetin	19 (10, 26)	19 (10, 27)	19 (10, 27)
Naringenin	7 (5, 11)	8 (6, 13)	8 (5, 12)
Flavan-3-ols, *mg*	13 (8, 18)	18 (12, 28)	14 (9, 22)
Catechin	5 (3, 7)	8 (5, 13)	6 (4, 10)
Epicatechin	6 (4, 8)	7 (5, 11)	6 (4, 9)
Gallocatechin	<0.1	0.1 (0.05, 0.4)	<0.1
Epigallocatechin	0.7 (0.4, 1.4)	0.8 (0.5, 1.5)	0.7 (0.5, 1.4)
Epicatechin gallate	0.1 (0.04, 0.2)	0.1 (0.05, 0.2)	0.1 (0.05, 0.2)
Epigallocat. gallate	0.1 (0.08, 0.2)	0.1 (0.08, 0.2)	0.1 (0.08, 0.2)
Anthocyanidins, *mg*	9 (6, 13)	12 (8, 19)	10 (6, 16)
Cyanidin	4 (2, 6)	4 (2, 6)	4 (2, 6)
Delphinidin	3 (2, 4)	4 (3, 6)	4 (3, 6)
Malvidin	0.3 (0.01, 0.6)	2 (0.3, 6)	0.3 (0.01, 0.6)
Pelagronidin	<0.1	<0.1	<0.1
Peonidin	0.2 (0.07, 0.5)	0.4 (0.2, 0.9)	0.3 (0.1, 0.6)
Petunidin	<0.1	0.2 (0.03, 0.7)	<0.1
Isoflavones,[2] *mg*	<0.1	<0.1	<0.1
Proanthocyan,[3] *mg*	67 (42, 98)	89 (57, 130)	75 (47, 111)
Vitamin C, *mg*	209 (160, 270)	220 (269, 280)	214 (163, 274)
Vitamin E, *mg*	26 (18, 37)	31 (23, 45)	28 (20, 41)
β-carotene, *μg*	4532 (3362, 6143)	4828 (3629, 6440)	4660 (3470, 6269)
Selenium, *μg*	69 (54, 88)	85 (66, 110)	75 (58, 98)
TEAC, *μmol trolox equivalent*	3496 (2407, 7281)	5241 (3249, 9913)	4092 (2669, 8355)
TRAP, *μmol trolox equivalent*	2918 (1953, 9343)	4941 (2752, 12366)	3560 (2187, 10934)
FRAP, *μmol trolox equivalent*	3739 (2695, 5445)	5498 (3692, 8093)	4357 (2990, 6589)
ORAC, *μmol trolox equivalent*	9944 (7697, 12749)	12277 (9454, 16121)	10796 (8283, 14152)
Total phenols, *mg GAE*	1229 (950, 1564)	1427 (1110, 1814)	1306 (1006, 1671)

[1] Values are medians (1st, 3rd quartile).
[2] Includes genistein, daidzein, and glycitein.
[3] Includes dimers, trimers, four-sixmers, seven-tenmers, and polymers of flavan-3-ols.

TABLE 3 Contribution of individual foods to the antioxidant intake of Greek adults participating in the EPIC study[1]

Antioxidant	Women, n = 16,618	Men, n = 11,954
Flavones		
Apigenin	Parsley, Wine	Parsley, Wine
Luteolin	Peppers, Orange, Melon	Peppers, Orange, Melon
Flavonoles		
Quercetin	Onion, Okra, Zucchini	Onion, Okra, Zucchini
Kaempferol	Greens, Spinach, Onion	Greens, Spinach, Onion
Isorhamnetin	Onion, Dill, Celery	Onion, Dill, Celery
Myricetin	Tomato, Parsley, Grapes	Tomato, Wine, Parsley
Flavanones		
Eriodictyal	Lemon juice, Orange juice	Lemon juice, Orange juice
Hesperetin	Orange, Orange juice, Lemon juice	Orange, Orange juice, Lemon juice
Naringenin	Orange, Tomato, Orange juice	Orange, Tomato, Wine
Flavan-3-ols		
Catechin	Apple, Peach, Grapes	Wine, Peach, Grapes
Epicatechin	Apple, Peach, Grapes	Apple, Wine, Peach
Gallocatechin	Lentils, Grapes, Tea	Wine, Beer, Lentils
Epigallocatechin	Peach, Apple, Tea	Peach, Apple, Tea
Epic. Gallate	Grapes, Tea, Figs	Grapes, Tea, Figs
Epig. Gallate	Peach, Tea, Apple	Peach, Tea, Apple
Anthocyanidins		
Cyanidin	Cherry, Apple, Peach	Cherry, Apple, Peach
Delphinidin	Eggplant, Banana, Wine	Eggplant, Banana, Wine
Malvidin	Wine	Wine
Pelagronidin	Strawberry, Cherry, Apple	Strawberry, Cherry, Apple
Peonidin	Cherry, Wine	Wine, Cherry
Petunidin	Wine, Vinegar	Wine, Vinegar
Isoflavones	Dried beans, Chick peas, Lentils	Dried beans, Chick peas, Lentils
Proanthocyanidins	Grapes, Peach, Pear	Wine, Grapes, Peach
Vitamin C	Orange, Pepper, Tomato	Orange, Pepper, Tomato
Vitamin E	Olive oil, Olives, Tomato	Olive oil, Olives, Tomato
β-carotene	Carrots, Tomato, Melon	Carrots, Tomato, Melon
Selenium	Bread, Rice, Hard cheese	Bread, Rice, Hard cheese
TEAC	Orange, Tomato, Pepper	Orange, Wine, Tomato
TRAP	Orange, Tomato, Pepper	Orange, Wine, Tomato
FRAP	Orange, Tomato, Bread	Wine, Orange, Tomato
ORAC	Apple, Orange, Potato	Apple, Orange, Wine
Total phenols	Orange, Tomato, Potato	Orange, Tomato, Potato

[1] The most important contributors within gender to antioxidant intakes are presented in declining order from left to right.

Results

We estimated the median, gender-specific intakes of flavonoids, proanthocyanidins, antioxidant micronutrients, and TAC among the participants (**Table 2**), as well as the contributions of food groups (**Supplemental Table 1**) and the primary sources (**Table 3**).

The total median flavonoid intake was ~92 mg/d. Flavones contributed 7 mg to the total, derived mainly from vegetables such as peppers; fruits, including oranges and melons; and condiments (mainly parsley). The contribution of flavonols was ~28 mg/d, with the major flavonol sources being vegetables such as onions, greens, and tomato. Flavanone intake was ~27 mg/d and is derived mainly from citrus fruits. Flavan-3-ol intake, ~16 mg/d, was mainly accounted for by fruits such as apples, peaches, and grapes as well as by beverages, mainly wine and tea. Isoflavone intake was rather small (<1 mg/d), derived solely from pulses. Anthocyanidins contributed the remaining 10 mg/d to the total flavonoid intake. Fruits and wine were the principal sources. Proanthocyanidins were estimated as the sum of compounds with various degrees of polymerization (data on individual compounds not shown) (19). The most important sources included fruits such as grapes, peaches, and pears as well as wine. The major vitamin C sources were vegetables and fruits such as oranges, peppers, and tomatoes, whereas that of vitamin E were added lipids (mainly olive oil) and fruits (olives and tomatoes). β-Carotene was derived from fruits and vegetables, including carrots, tomatoes, and melons. Cereals and their products like bread and rice, as well as dairy products including cheese, comprised the principal selenium sources. The most prominent sources of all TAC intakes were fruit and vegetables, including oranges and tomatoes.

Discussion

Flavonoids. We have previously investigated the flavonoid content of individual Greek traditional foods and recipes on the hypothesis that the beneficial health properties of the MD could be attributed in part to the traditional foods this diet includes. In this context, ~100 traditional foods and recipes have been chemically analyzed for their flavonoid (flavones, flavonols, and flavan-3-ols) content (25). In addition, the flavonoid content of a weekly menu based on the traditional Greek diet has also been studied analytically (flavones, flavonols, and flavan-3-ols) (26), as well as via database calculations (for flavones, flavonols, flavan-3-ols, flavanones, anthocyanins, and isoflavones) (27) (**Fig. 1**). Although the present estimations seem compatible with these 2 reports, a trend toward lower flavonoid intakes is apparent in the EPIC population-based data (Fig. 1). This may reflect the changes that contemporary diet has incorporated compared with the traditional Greek diet. Nevertheless, considering the evidence of several investigations on the flavonoid intake of populations (28–32), the estimated flavonoid intake in Greece (92 mg/d) can be considered important both in terms of the amounts of flavonoids ingested as well as in the variety of the contributing food sources.

Proanthocyanidins. Proanthocyanidins are polymers of flavan-3-ols. They are considered a major flavonoid category in Western diets (33). The overall daily median intake by the Greek population is 87 mg, whereas on the basis of the USDA's Continuing Survey of Food Intakes by Individuals, the mean intake of the U.S. population (>2 y) was estimated as 58 mg/person (33). The intake in a Finnish adult population (n = 2007) was on average 83 mg/d (30) (**Fig. 2**). The most important proanthocyanidin sources in the U.S. diet are apples, chocolate, and grapes, whereas in the Finnish diet, apples, berries, tea, and chocolate prevail. In Greece, grapes, wine, and peaches provide the most prominent sources of proanthocyanidins.

Antioxidant micronutrients. The conventional approach of assessing each individual compound separately is appropriate, because besides their antioxidant activity, these compounds possess additional physiological properties. The Greek diet provides ~4660 μg/d of β-carotene, 214 mg/d of vitamin C, and 28 mg/d of vitamin E, which represents a high intake of these

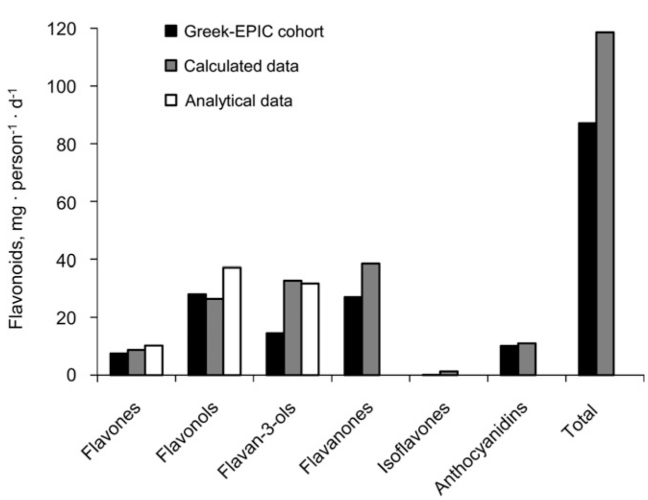

FIGURE 1 Daily flavonoid intakes from the Greek diet estimated by 3 different approaches. The Greek EPIC cohort data represent the estimated daily intakes (medians) from the present study (n = 28572); Calculated data were based on database calculations on the mean daily content of a traditional Mediterranean menu (27); Analytical data (mean daily content) were based on the chemical analysis of the traditional Mediterranean menu that the calculated data refer to (analysis not performed for flavanones, isoflavones and anthocyanidins) (26).

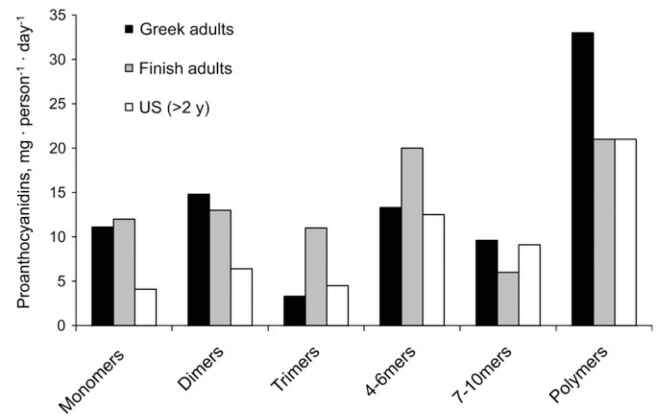

FIGURE 2 Daily proanthocyanidin intakes in a Greek (EPIC-Greece, 1994–1999), a Finnish (FINDIET 2002) (30) and a US (CSFII, 1994–1996) population (33).

antioxidant micronutrients compared with other European countries (34). The median daily intakes in the Spanish EPIC cohort were 1679 μg for β-carotene and 137 and 8.6 mg for vitamins C and E, respectively (35). The prominent vitamin C and E sources in the Greek and the Spanish diets are similar (36), whereas there are important differences between these diets and the U.S. diet with respect to vitamin E (37). The present selenium intake data are comparable to the respective data from several European countries, which range between 68 and 120 mg/d for men and 49–84 mg/d for women (38), as well as in the US, were selenium mean intake from foods was recently estimated at ~109 mg/d (37).

TAC. Although TAC assays do not quantify specific antioxidants, there are certain advantages in the use of these methods, probably the most important being the provision of a single measure of the antioxidant potential. Various methods have been developed to assess TAC, which differ in many aspects such as the chemical reaction exploited or the end points determined (39). The daily TAC intake in the Spanish EPIC cohort based on consumption data of >40,000 healthy volunteers has recently been estimated using 4 different methods (**Fig. 3**) (35). It should be noted that fruit and vegetables account for ~50–80% of the TAC intake in Greece (Supplemental Table 1). The TAC from fruit and vegetables in the U.S. diet was estimated at 5724 μmol trolox equivalents (ORAC method), with dried beans, peas, apples, and oranges being the most important contributors (40). The daily intakes of phenols from fruits and vegetables in the US and France were 450 and 278.5 mg, respectively, expressed as gallic acid equivalents (GAE), provided mainly by oranges, apples, and potatoes in the US (41) and apples and potatoes in the French diet (42). The overall median intake of total phenolic compounds in Greece is 1306 mg GAE, derived primarily from fruits and vegetables such as oranges, tomatoes, and potatoes. The Spanish diet provides ~1171 mg GAE per person per day, with the main contributor being coffee followed by wine (43). Coffee was also the major polyphenol source in the Finnish diet (30).

There is no sufficient evidence about the hypothesized beneficial role of antioxidants in health, but some beneficial effect is plausible. The traditional MD has been ascribed several health-promoting characteristics and it is likely that part of the beneficial effect may be mediated by protection against oxidation through the high quantity of antioxidants that this diet abundantly provides. In addition, we suggest that besides the

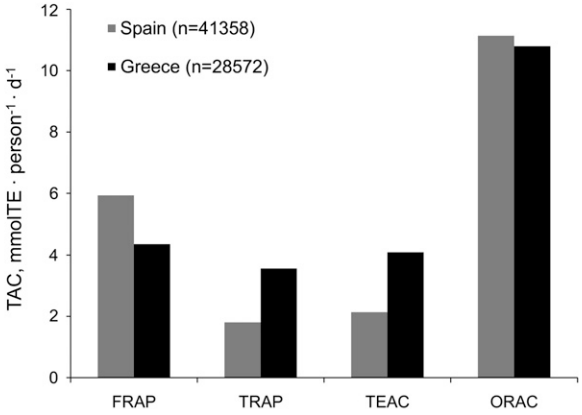

FIGURE 3 Daily median TAC intakes in the Spanish (35) and Greek EPIC cohorts as assessed by the FRAP, TRAP, TEAC, and ORAC methods.

total amount of antioxidants ingested, the variety of antioxidants in the diet may also be important. This latter hypothesis could explain the failure of some trials to show any benefits through the administration of single or a few purified antioxidant compounds. The present study indicates an advantageous antioxidant content of the Greek variant of the MD in both quantitative and qualitative terms. This seems to rely on the fact that the diets of the Mediterranean region are naturally endowed by many sources of antioxidant compounds such as fruits, vegetables, cereals, wine, and extra virgin olive oil. For instance, the dietary importance of virgin olive oil in terms of the antioxidants it contains has recently been highlighted. The daily antioxidant intake of the Greek population solely via olive oil and olives was estimated to be ~16 mg of phenolic compounds, 12 mg of α-tocopherol, 223 mg of squalene, and 2 mg of lignans (44).

The data generated in this study provide a platform to further investigate the role of antioxidants in health and disease on a population basis in Greece. A weakness of our study is that changes in the antioxidant content of foods during domestic or industrial processing have not been considered, because databases on the content of foods in antioxidants are yet far from complete in that respect. Another limitation is that the estimated intakes do not take into account the bioavailability of antioxidants, which may vary considerably. Other factors that might need to be considered in the interpretation of the results include the food coverage of the database used, the differences in the flavonoid content of foods due to varying climatological conditions, and the dietary assessment method used to document food intake. Further research is required on other antioxidants not considered in this study, such as phenolic acids, which are the predominant dietary antioxidants in other populations (30). Newly developed databases, such as the EuroFIR BASIS database (45) and the French polyphenol database (46), may assist in exploring this issue.

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V.D. was responsible for the implementation of the study. A.T. designed the study. Both authors contributed to the drafting of the manuscript and have both approved the final version.

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