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## Macro- and Micronutrients in a Traditional Greek Menu

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### Abstract

**Background/Aims:** The traditional Mediterranean diet is frequently being considered as a prototype for dietary recommendations. We have investigated a weekly menu typical of the Greek variant of the Mediterranean diet to examine the compatibility with the nutritional recommendations of the Scientific Committee for Food of the European Commission, concerning macronutrients and certain micronutrients. **Methods:** A typical weekly traditional Greek Mediterranean menu was chemically analyzed and certain food constituents, like flavonoids were theoretically estimated. **Results:** The evaluated typical menu meets all the dietary recommendations for macronutrients. The daily energy intake is derived from dietary lipids (40.3%) and carbohydrates (41.4%). The ratio of  $\alpha$ -tocopherol per gram of polyunsaturated fatty acids in the Mediterranean diet under investigation is around 0.4 mg, indicating a well-balanced diet. With respect to microcomponents, with existing recommendations of the Scientific Committee for Food of the European Commission, such as inorganic constituents, the investigated menu meets all the requirements. **Conclusion:** The diet that the Mediterranean populations developed many years ago, without any scientific input, appears to meet current dietary recommendations.

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### Introduction

The traditional Mediterranean diet is frequently being considered as a healthy diet [1–3]. Recently it was found that a diet that adheres to the principles of the traditional Mediterranean one is associated with a significant reduction in total mortality, even though no strong associations with mortality were evident for each of the individual components of this diet [3]. The health benefits of the Mediterranean diet cannot be attributed to a single component,

but are probably associated with a complex combination of favorable nutritional elements.

The composition of the Mediterranean diet and particularly the traditional Greek diet favors plant foods with antioxidant potential [4], which are considered to provide protection from coronary heart disease and cancer [5, 6]. The high phytochemical intake in conjunction with the sufficient intake of macronutrients and inorganic constituents may account for the health benefits observed in the Mediterranean basin.

We have analyzed a weekly menu typical of the dietary pattern of the Greek variant of the Mediterranean diet to examine whether it meets the nutritional recommendations developed by the Scientific Committee for Food of the European Commission.

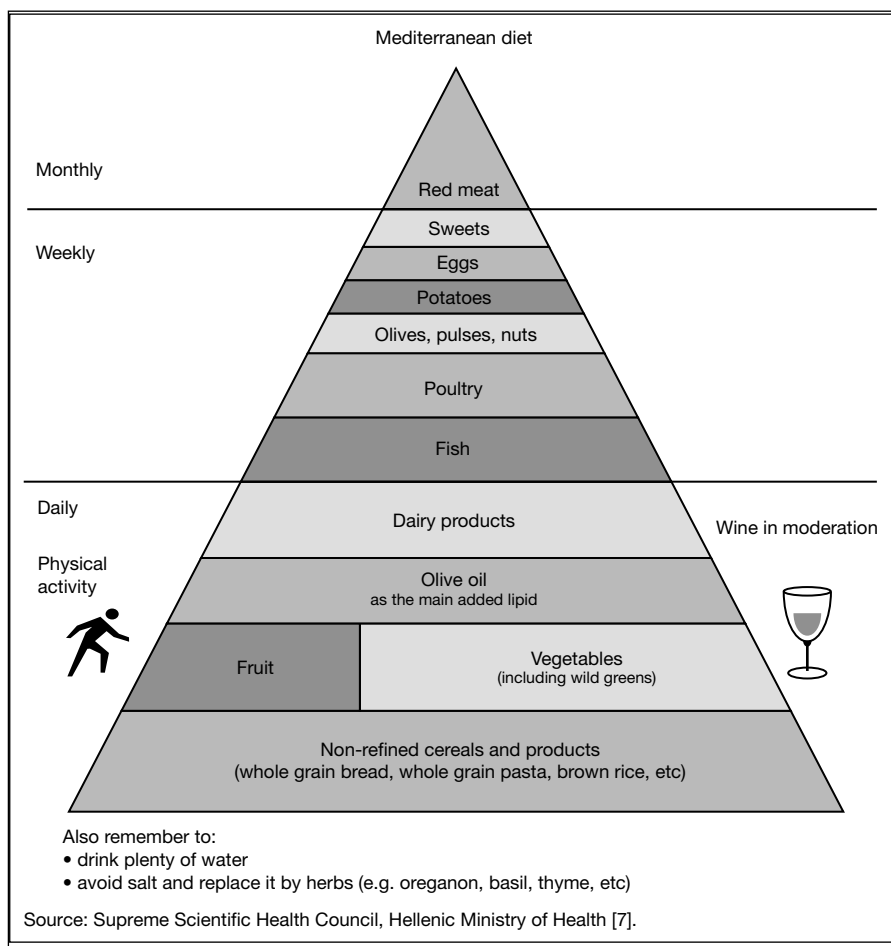
## Materials and Methods

A traditional weekly menu was developed based on the Greek dietary guidelines, which refer to the traditional Greek diet [7]. The pictorial presentation of these guidelines is presented as the traditional Mediterranean Pyramid (fig. 1). The weekly menu under investigation is given in table 1. The generated weekly menu refers to adults of both genders. The menu is compatible with the tradition, which has religious roots, of avoiding foods of animal origin every Wednesday and Friday. Portion sizes were defined according to Greek market regulations. The portion size of composite and primary foods refers to edible parts. Specification of the raw ingredients as well as detailed information on the preparation of recipes, salads and beverages are available [8].

After the preparation of each daily menu, all the weighed food portions (composite and primary foods) were homogenized forming a uniform mass, placed in a food bag and deep-frozen ( $-20^{\circ}\text{C}$ ). The homogenization procedure was performed using a household multi-mixer (Braun K600). Once all daily menus were prepared, the seven food bags (one for each day of the week), were defrosted, mixed and homogenized (Braun K600) forming the sample for analysis (weekly diet). This sample was then prepared for freeze-drying using a Martin Christ Alpha1-2, c/n 100200 freeze-dryer.

The liquid ingredients of the weekly menu (wine, coffee and tea) were not included with the solid ingredients in order to avoid dilution of the sample. They were mixed and kept separately, stored in deep freeze until distribution of the samples for analysis. Although the consumption of more than one liter of water per day is recommended, the water is not included in the liquid sample since its contribution to the nutrient content of the diet is negligible. The specific gravity of the liquid sample at room temperature was also determined.

Reference analytical methods for total diet samples do not exist. Any available method may be used provided that it is capable of providing reliable data at the required level of sensitivity. For the purpose of the current project, the following analytical methods were used for the determination of the macro- and micronutrients of the menu. The determination methods for nitrogen, fatty acids and inorganic constituents are accredited by the Hellenic Accreditation System S.A. (ESYD), while the determination methods for total lipids, dietary fiber and ash are accredited by the United Kingdom Accreditation Service.



**Fig. 1.** The traditional Mediterranean diet pyramid depicting dietary guidelines for adults in Greece.

The moisture content of the solid sample was evaluated during the freeze-drying procedure by difference, while the moisture of the liquid sample was determined by the oven method. Carbohydrates were calculated ‘by difference’ through the equation:  $Carbohydrate \% = 100 - moisture \% - lipids \% - proteins \% - ash \% - dietary\ fiber \% - ethanol \%$ , where ethanol was estimated. The values reported correspond to available carbohydrates. The energy value of the samples was calculated from the amount of protein, total lipids, available carbohydrates, dietary fiber and ethanol by the application of the general Atwater energy conversion factors 4–9–4–2–7, respectively [9].

The analytical methods performed were the following: *Nitrogen determination* – The determination of nitrogen was carried out using Kjeldahl-in house methodology based on the standard ISO/DIS 8968 –2 method [10]. Protein was calculated by using the standard nitrogen conversion factor of 6.25. *Lipid determination* – Total lipids of the samples were determined

**Table 1.** The Mediterranean menu under investigation

	Monday		Tuesday		Wednesday		Thursday		Friday		Saturday		Sunday	
	Food	Serve g	Food	Serve g	Food	Serve g	Food	Serve g	Food	Serve g	Food	Serve g	Food	Serve g
Breakfast	Herbal tea	165	Yogurt	200	Herbal tea	165	Herbal tea	165	Herbal tea	165	Herbal tea	165	Herbal tea	165
	Sugar	5	Honey	40	Sugar	5	Sugar	5	Sugar	5	Sugar	5	Sugar	5
	Feta	50			Black olives	30	Feta	50	Black olives	40	Feta	50	Feta	50
Morning Snack	Bread	60			Bread	60	Bread	60	Bread	60	Bread	60	Bread	60
	Grapes	150	Apple	160	Apple	160	Apple	160	Apple	160	Apple	160	Apple	160
Lunch	Green beans	250	Fried wet salted cod	150	Lentils with tomato	350	Chicken casserole	180	Eggplants casserole	250	Baked vegetables	250	Roast lamb	110
	Feta	50	Chicories salad	290	Green olives	40	Rice (pilaf)	150	Fish roe salad	50	Feta	50	Baked potatoes	150
	Bread	90	Bread	90	Bread	90	Bread	90	Bread	90	Bread	90	Bread	90
	Red wine	120	Red wine	120	Red wine	120	Red wine	120	Red wine	120	Red wine	120	Red wine	120
	Apple	160	Pear	160	Orange	200	Pear	160	Orange	200	Pear	160	Orange	200
					Lettuce salad	140	Mizithra	10					Lettuce salad	140
							Cabbage salad	120						



by the Soxhlet method after acid hydrolysis based on The Feeding Stuffs (Sampling and Analysis) (Amendment) Regulations 1985 SI No. 1119 and BS4401: Part 4 1970. *Fatty acid determination* – The determination of fatty acids was carried out using capillary column gas chromatography-in house methodology based on the preparation of methyl esters of fatty acids: ISO 5509:1978 (E) [11] and the analysis of methyl esters of fatty acids: Regulation of European Community 2568/91 (Appendix XA-General Case) [12]. *Sterols determination*: The determination of sterols was carried out using capillary column gas chromatography, based on the Regulation of European Community 2568/91 (Appendix V) [13]. *Tocopherols determination*: High performance liquid chromatography was used [14, 15]. *Dietary fiber determination* – Dietary fiber content was determined by the AOAC procedure (985.29) [16]. *Ash determination*: The determination of ash was performed by the oven method, based on The Feeding Stuffs (Sampling and Analysis) Regulations 1982 and BS4401: Part 1 1998. *Inorganic constituents' determination*: The microwave-assisted acid hydrolysis and analysis of elements by inductively coupled plasma–atomic emission spectroscopy was used for the determination of inorganic constituents. This method is based on the EPA method 200.7 Revision 4.4 (1994) and applies to aqueous samples, but is also considered appropriate for food samples. *Total carotenoids determination*: The determination of total carotenoids was carried out using the spectrometry method based on the prEN12136: 1997 standard method [17].

All analyses of the composite food sample were carried out in duplicate. Moreover, nitrogen and fatty acid determination were externally validated through reference material used in quality control testing conducted by the Food Analysis Performance Assessment Scheme [FAPAS-Ministry of Agriculture, Fisheries and Foods (MAFF) of the United Kingdom] and the International Olive Oil Council, respectively.

## Results

The daily intake of macro- and micronutrients of the traditional Greek menu are presented in table 2, derived from the analysis of samples, solids and liquids consumed within the week. The high intake of total lipids, in particular, monounsaturated fatty acids (MUFA) lipids is characteristic of Mediterranean diet as is the relative high intake of dietary fiber and several metals. The results of this study have been evaluated based on the relevant recommendations of the Scientific Committee for Food of the European Commission [18]. The recommendations are expressed per person per day, conceptually representing the average intake over a period of time, while the values are proposed for groups of healthy people (table 3). It is clear that the Mediterranean menu is fully compatible with recommendations of the Scientific Committee for Food of the European Commission.

The Mediterranean menu under investigation is based on an average daily energy intake; therefore, wherever recommendations were expressed separately for males and females, an averaged value was used. The energy requirement proposed by the Scientific Committee for Food of the European Commission [18] for adults (average) with desirable physical activity is 10 MJ/day. The daily

**Table 2.** Daily intake of macro- and micronutrients of the Mediterranean menu

Component	Daily intake
Protein	74.5 g
Total lipids	110.7 g
Dietary fiber	29.8 g
Carbohydrates	255.8 g
Ethanol	14 g
Energy value	2,473 kcal
Fatty acids:	
SFA	29.8 g
MUFA	63.8 g
PUFA	9.9 g
TFA	1.4 g
Total sterols	256.8 mg
Total carotenoids	65.7 mg
α-tocopherol	4.3 mg
Inorganic constituents	
K	1,774 mg
Fe	14.9 mg
Na	2,632 mg
Ca	696 mg
Mg	234 mg
Zn	10.3 mg
Cu	3.80 mg
Mn	3.51 mg

energy provided by the Mediterranean menu under investigation is 2,473 kcal/day or 10.4 MJ/day.

It is evident from table 2 that the energy of the Mediterranean menu can be attributed to the following components: proteins (12%), dietary lipids (40.3%), carbohydrates (41.4%), dietary fiber (2.4%), and ethanol (4%).

## Discussion

The traditional diets of Mediterranean countries are based mainly on fruits, vegetables, fish and seafood, legumes, cereals and olive oil. In the Mediterranean menu under investigation, energy is isothermically provided by lipids and carbohydrates. Our results are in accordance with earlier findings that indicate that total lipids correspond to 40% of total energy intake in Greece [19].

**Table 3.** Daily intake of inorganic constituents of the Mediterranean menu and European Commission daily recommendations

Inorganic constituents	Daily intake	Recommendations*
K	1,774 mg	1,600 mg Lowest threshold intake
Fe	14.9 mg	14.4 mg Average requirement
Na	2,632 mg	575–3,500 mg Acceptable range
Ca	696 mg	550 mg Average requirement
Mg	234 mg	150–500 mg Acceptable range
Zn	10.3 mg	6.5 mg Average requirement
Cu	3.8 mg	0.8 mg Average requirement
Mn	3.5 mg	1–10 mg Acceptable range

\*Source [18].

The distribution of lipid-generated daily energy of the menu is: saturated fatty acids (SFA; 10.8%), MUFA (23.2%), polyunsaturated fatty acids (PUFA; 3.6%) and trans fatty acids (TFA; 0.5%). The predominant added lipid in the Mediterranean menu is extra virgin olive oil. Consequently, the highest proportion of lipid-generated daily energy comes from monounsaturated fatty acids while the contribution from TFA is low.

Our results are compatible with the findings of the TRANSFAIR study [20], where SFA were found to provide on average between 10–19% of total energy intake, with the lowest contribution in Mediterranean countries. TFA intake ranged from 0.5–2.1% of energy intake. The TFA intake was again found lowest in Mediterranean countries. Moreover, in the Mediterranean countries more than half the intake of TFA is derived from milk and ruminant fat.

Certain PUFA (essential fatty acids) cannot be synthesized by the human body and must be supplied with the diet to avoid deficiency. It appears prudent, however, to avoid extremely high dietary intakes of PUFA because of potential untoward side effects of excessive consumption, such as lipid peroxidation, immunosuppression and bleeding. It is recommended that intakes of total PUFA should not exceed 15% of dietary energy [18]. In our study, PUFA intake corresponds to 3.6% of energy.

The PUFA requirements proposed by the Scientific Committee for Food of the European Commission [18] for adults (average) are: average requirement of 3.3 g/day and population reference intake (PRI) of 6.5 g/day. PRI is the intake that is enough for virtually all healthy people in a group. The daily PUFA intake of the traditional Greek menu under investigation is 9.9 g, which meets the lowest requirement and does not exceed the upper limit of 15% of energy.

With high dietary intakes of PUFA, it is essential to ensure that the intake of vitamin E is adequate because an increased intake of PUFA raises the need for vitamin E to prevent unwanted oxidation. Therefore a logical approach is to make dietary recommendations for vitamin E in terms of the dietary PUFA intake. There is, however, no general agreement about what the ratio in mg of  $\alpha$ -tocopherol equivalents per gram of PUFA should be, but about 0.4 seems adequate in a normal diet [18]. The ratio in mg of  $\alpha$ -tocopherol per gram of PUFA of the Mediterranean diet under investigation was exactly 0.4, indicating a well-balanced diet.

With respect to proteins, the physiological requirement of an individual is that the lowest level of dietary protein intake will balance the losses of nitrogen from the body in adults maintaining energy balance at modest levels of physical activity. The requirements proposed by the Scientific Committee for Food of the European Commission [18] for adults (average) are: average requirement 41 g/day and PRI 52 g/day. The daily protein intake of the traditional Greek menu under investigation is 74.5 g, consisting mainly of plant protein. This value meets the requirements set above. An intake value equal to or greater than the PRI indicates that every individual will almost certainly be provided for adequately. This high but not excessive protein value ensures sufficient amino acid requirements. Excessive animal protein intake may be associated with health risks, but the level of animal protein in the traditional Greek menu is far from excessive.

The Mediterranean menu under investigation meets all requirements with respect to inorganic constituents, as shown in table 3. Recommendations refer to average requirements or acceptable ranges, with the exception of potassium. For this element, the lowest threshold intake is indicated. Below this level, on the basis of current knowledge, all individuals may not be able to maintain metabolic integrity. The values proposed for iron are the intakes needed to cover the requirements based on a bioavailability of 15%. With respect to zinc intake, it is considered unwise to exceed a daily zinc intake of 30 mg in adults, while an upper limit of 10 mg/day is proposed for copper intake [18].

It is worth noting that, except for potassium which has a PRI of 3,100 mg/day, the Mediterranean menu meets the PRI of the other inorganic constituents such as calcium PRI: 700 mg/day, zinc PRI: 8 mg/day and copper PRI: 1.1 mg/day.

With respect to carotenoids there is as yet insufficient evidence to recommend the consumption of any specific amount of  $\beta$ -carotene, or carotenoids in

general, beyond what is needed to supply vitamin A. Until further findings emerge, the Scientific Committee for Food of the European Commission found it unwarranted to make any recommendation other than to encourage the consumption of vegetables and fruits. Sterols are considered to cause reductions in plasma cholesterol concentrations. Their habitual concentration in the daily diet amounts to 150–400 mg [21]. The daily intake through the Mediterranean menu under investigation is 257 mg/day.

In addition to the chemical analysis of the traditional Greek menu, a theoretical determination of the menu's flavonoid content was implemented using mainly the USDA [22] and VENUS [23] databases [24]. According to the calculations, the daily average flavonoid intake through this traditional Greek menu was 118.6 mg/day, of which flavanones contribute 32% (38.5 mg/day), catechins contribute 28% (32.7 mg/day), flavonols 22% (26.4 mg/day), anthocyanidins 9% (11 mg/day), flavones 8% (8.7 mg/day) and isoflavones contribute 1% (1.3 mg/day).

Oranges were found to be the main source of flavanones (98%), with the principal flavanone being hesperetin, the intake of which was assessed at 28.6 mg/day (74%). The daily average catechin intake was calculated at 32.7 mg/day. In the traditional Greek menu the main contributors to catechin intake were apples and red wine. The majority of the flavonol intake refers to quercetin (85%), which is considered the most widespread flavonoid, as it is present in over half the ingredients in the Mediterranean menu. The primary source of anthocyanidins was red wine, while the primary source of isoflavones was brown bread (94%). With respect to isoflavones, the calculated daily average intake is 1.3 mg/day. The majority of the flavone intake is derived from apigenin (77%). Common herbs of the Greek culinary culture such as parsley are a good source of apigenin. The major contributor to the total flavone intake is parsley (74%), followed by black olives (18%) [24]. Herbs such as parsley and dill, which are commonly used in traditional Greek dishes, although added in small quantities, significantly contribute to the overall flavonol and flavone intake because of frequent consumption. Typical Mediterranean foods such as olives and red wine also contribute substantially.

The above-presented data are merely indications of the flavonoid content of a typical plant-based Mediterranean menu since seasonal variation of diet as well as flavonoid concentration of foods could not be taken into account. Current estimates of flavonoid intakes vary considerably because food composition information with respect to flavonoids is incomplete [25]. However, in spite of the reservations involved in the theoretical determination, the indications show that the traditional Mediterranean diet has high flavonoid content. The inclusion of herbs and spices in the traditional Greek cuisine significantly contributes to the flavonoid content of the diet.

The nutritional value of the Mediterranean diet, and in particular, the traditional Greek diet, has been presented through the investigation of different typical traditional weekly menus [26], which demonstrates that the health benefits are not due to individual food items but to the traditional Mediterranean dietary pattern. Although the Mediterranean diet and lifestyle were shaped by climatic conditions, poverty and hardship, rather than by intellectual insight or wisdom, it seems as if some superior force lead the Mediterranean populations to a prudent diet by exploiting, to the utmost, the gifts that nature abundantly favored them with. The diet that the Mediterranean populations developed many years ago, without any scientific input, meets current dietary recommendations and is considered a diachronic, well-balanced, healthy diet.

### Acknowledgements

This study was supported by the International Foundation for the Promotion of Nutrition Research and Nutrition Education (ISFE). Chemical analyses were conducted by The Food Industrial Research and Technological Development Company (ETAT S.A.) Athens, The laboratory of analytical chemistry of the Mediterranean Agronomic Institute of Chania (M.A.I.CH), Crete and The Eclipse Scientific Group, Chatteris, UK.

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