Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: The PREDIMED study

A. Tresserra-Rimbau a,b,c,1, A. Medina-Remón a,b,c,1, J. Pérez-Jiménez d,1, M.A. Martínez-González c,e,1, M.I. Covas b,f,1, D. Corella b,g,1, J. Salas-Salvadó b,c,h,1, E. Gómez-Gracia c,i, J. Lapetra b,j,1, F. Arós k,1, M. Fiol b,l,1, E. Ros b,m,1, L. Serra-Majem c,n,1, X. Pinto c,o,1, M.A. Muñoz p,1, G.T. Saez c,q,1, V. Ruiz-Gutiérrez c,r,1, J. Warnberg e,s,1, R. Estruch b,c,t,1, R.M. Lamuela-Raventós a,b,c,*1

a Nutrition and Food Science Department, XaRTA, INSA, Pharmacy School, University of Barcelona (UB), Barcelona, Spain
b CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición, Madrid, Spain
c RETICS RD06/0045, Instituto de Salud Carlos III, Madrid, Spain
d Institute for Advanced Chemistry of Catalonia(IQAC-CSIC), Barcelona, Spain
e Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra, Pamplona, Spain
f Department of Cardiovascular Epidemiology Unit, Municipal Institute for Medical Research, Barcelona, Spain
1 on behalf of the PREDIMED Study Investigators.
g Department of Epidemiology, Preventive Medicine and Public Health, School of Medicine, University of Valencia, Valencia, Spain
h Human Nutrition Unit, School of Medicine, IISPV, University Rovira i Virgili, Reus, Spain
i Department of Epidemiology, School of Medicine, University of Malaga, Malaga, Spain
j Department of Family Medicine, Primary Care Division of Sevilla, San Pablo Health Center, Sevilla, Spain
k Clinical Trials Unit, Hospital Txangorritxu, Vitoria, Spain
l Institut Universitari d’Investigació en Ciències de la Salut, Palma de Mallorca, Spain
m Lipid Clinic, Endocrinology and Nutrition Service, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clinic, Barcelona, Spain
n Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
o Lipid Unit, Department of Internal Medicine, Hospital Universitari de Bellvitge, L’Hospitalet de Llobregat, FIPPEC, Barcelona, Spain
p Primary Care Division, Catalan Institute of Health, Barcelona, Spain
q Department of Biochemistry and Molecular Biology Service of Clinical Analysis, CDB, Hospital General Universitario, Universitat de Valencia, Valencia, Spain
r Lipid Clinic, Endocrinology and Nutrition Service, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clinic, Barcelona, Spain
s Nutrition and Lipids Metabolism, Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Sevilla, Spain

* Corresponding author. Nutrition and Food Science Department, XaRTA, INSA, Pharmacy School, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain. Tel.: +34 934034843; fax: +34 934035931.
E-mail address: lamuela@ub.edu (R.M. Lamuela-Raventós).

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A high consumption of polyphenols, which are bioactive compounds present mainly in plant foods and beverages, has been suggested to have beneficial effects on human health and provide protection against many chronic illnesses [1–3]. Polyphenols constitute a very heterogeneous group of compounds, with over 500 different molecules that have different properties and bioavailabilities [4]. This diversity should be considered when studying the health effects of these compounds [5] and hampers the estimation of their content in foods [6]. Polyphenols are divided into five main groups according to structure: phenolic acids, flavonoids, stilbenes, lignans and others (such as secoiridoids) [5,6].

Some studies have used the US Department of Agriculture (USDA) Flavonoid Database [7–10] to estimate flavanoid intake, with the drawback that the limited number of compounds that it contains is far from the wide diversity of polyphenols found in food [11]. In this setting, the aim of this study was to determine the major dietary sources of polyphenols in a Spanish population at high cardiovascular risk (the PREDIMED cohort, PREvención con Díeta MEDiterránea) [12] using the Phenol-Explorer database (www.phenol-explorer.eu), the most complete database currently available, which holds data on 502 polyphenols contained in 452 foods [11]. To our knowledge, this is the first study to report the intake of such a high number of polyphenols in a Spanish population using this tool. The application of this methodology will facilitate further investigation into polyphenol intake and its relation with the incidence of several diseases in the epidemiological observational studies and feeding trials such as the PREDIMED study and may be useful in establishing nutritional recommendations.

Methods

Study population, the PREDIMED cohort

Subjects were participants of the PREDIMED study, which is a large, parallel-group, multicentre, randomised, controlled 5-year feeding trial aimed at assessing the effects of the traditional Mediterranean Diet (MedDiet) in the primary prevention of cardiovascular diseases (www.predimed.org). The recruitment method and study protocol have been described in detail previously, as well as the characteristics of eligible participants and exclusion criteria [12,13]. The participants provided written informed consent and the study protocol was approved by the institutional review boards of the participating centres.
Estimation of dietary intake

Food intake among the PREDIMED cohort at baseline was estimated using a validated 137-item Food Frequency Questionnaire (FFQ) [14] and physical activity with the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire [15]. Data on other lifestyle factors, including educational level, history of illness and medication use, were collected at baseline through a 47-item questionnaire. Participants also filled in a 14-point score questionnaire on adherence to the traditional Mediterranean Diet [16]. Anthropometric and blood pressure measurements were taken. The baseline questionnaires of 7200 participants of the PREDIMED study collected from 2003 to 2009 were used to correlate food consumption with individual and total polyphenol intake.

Estimation of polyphenol intake

Data on the polyphenol content in foods were obtained from the Phenol-Explorer database (www.phenol-explorer.eu), which has been previously described [4]. The correspondence between food items in the FFQ and the Phenol-Explorer database was assessed with the following five steps: 1) all foods with no or only traces of polyphenols were excluded; 2) the yearly FFQ was converted into 24-h dietary recall interviews; 3) recipes were separated according to their ingredients; 4) the polyphenol content of each food item was searched in the Phenol-Explorer database as described by Perez-Jimenez et al., 2011 [11]; and 5) weight loss or gain during cooking was corrected using yield factors [17]. Finally, individual polyphenol intake from each food was calculated by multiplying the content of each polyphenol by the daily consumption of each food. Total polyphenol intake was calculated as the sum of all individual polyphenol intakes from all food sources reported by the FFQ.

The data used to calculate polyphenol intake correspond to normal phase high performance liquid chromatography (HPLC) for all phenolic compounds. In the case of lignans, and phenolic acids in certain foods (cereals, olives and beans), data corresponding to HPLC after hydrolysis were also collected, since these treatments are needed to release phenolic compounds that otherwise could not be analysed.

Dietary sources of polyphenols

A ratio of daily total or individual polyphenols provided by the specific food or food group over the total intake of polyphenols from all foods was used to calculate the contribution of each food or food group to the daily total intake of polyphenols.

Statistical analyses

The mean polyphenol intake was calculated for the 7200 participants who had completed the baseline FFQ, and those who had no missing values in the other questionnaires used. The Stata Statistical Package (version 10.1, Stata Corp., TX, USA) was used for the analyses. Data are presented as means (±SD) for continuous variables and frequencies, and percentages for categorical variables.

Results

Total polyphenol intake

A brief description of the PREDIMED cohort is detailed in Table 1. According to the Phenol-Explorer database, 93 of the 137 food items from the FFQ contain a total of 290 different polyphenols from 18 polyphenol subclasses.

Table 1 General characteristics of the studied PREDIMED population (n = 7200).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>67.1 ± 6.1</td>
<td>53</td>
<td>82</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>76.6 ± 11.6</td>
<td>40.0</td>
<td>130.0</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.9 ± 3.6</td>
<td>17.8</td>
<td>40.0</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>148.7 ± 19.2</td>
<td>69.0</td>
<td>234.5</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82.8 ± 10.3</td>
<td>39.0</td>
<td>145.0</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>71.0 ± 10.8</td>
<td>35.7</td>
<td>128.5</td>
</tr>
<tr>
<td>Energy intake (Kcal/d)</td>
<td>2274.9 ± 606.7</td>
<td>587.8</td>
<td>6007.6</td>
</tr>
<tr>
<td>Energy consumed due to physical activity in leisure time (METS-min/d)</td>
<td>232.6 ± 240.5</td>
<td>0.0</td>
<td>2975.1</td>
</tr>
<tr>
<td>14-point questionnaire of adherence to the traditional Mediterranean diet (score)</td>
<td>8.7 ± 1.9</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Carbohydrates intake (g/d)</td>
<td>239.2 ± 81.0</td>
<td>41.3</td>
<td>749.2</td>
</tr>
<tr>
<td>Protein intake (g/d)</td>
<td>92.5 ± 23.1</td>
<td>15.3</td>
<td>369.9</td>
</tr>
<tr>
<td>Total fat intake (g/d)</td>
<td>98.7 ± 30.4</td>
<td>16.5</td>
<td>281.5</td>
</tr>
<tr>
<td>Fibre intake (g/d)</td>
<td>25.6 ± 9.1</td>
<td>0.9</td>
<td>82.7</td>
</tr>
</tbody>
</table>

a BP: blood pressure.

b MedDiet: Mediterranean diet.
shows, in decreasing order, the mean intakes of total polyphenols, total flavonoids and phenolic acids (mg day\(^{-1}\)) from different types of food, as well as the main contributors within each type of food.

The mean total polyphenol intake was 820 ± 323 mg day\(^{-1}\), 443 ± 218 mg of which were flavonoids, 304 ± 156 mg phenolic acids and 73 mg belonged to other polyphenol groups. Fruits were the main source of polyphenols, providing almost 44% of the total polyphenol intake, more than half of total flavonoids and 23% of total phenolic acids.

The non-alcoholic beverages group provided 55% of total phenolic acids, principally coffee. Vegetables provided more than 12% of total polyphenol intake and were the third source of phenolic acids. Alcoholic beverages, cereals, olive oil, cocoa products, nuts and seeds and legumes each contributed less than 10% of the total intake of polyphenols.

Considering individual foods, coffee was the main source of total dietary polyphenols (18%), followed by two fruits: oranges (16%) and apples (12%). Olives and olive oil were the fourth source, together providing 11% of total polyphenol intake, followed by red wine, which contributed 6%.

### Intake of different classes of polyphenols

The consumption of the different classes of polyphenols was also calculated and the main food contributors determined (Table 3). Hydroxycinnamic acids were the most consumed type of polyphenol (33%), mainly provided by coffee. Flavanones were the second most consumed polyphenols, with oranges and their products being almost the single food source. Proanthocyanidins, mainly coming from red wine and apples, were the third most consumed polyphenol group, followed by flavonols, flavones and anthocyanins. Olives provided 90% of phenolic acids other than hydroxybenzoic and hydroxycinnamic acids. The remaining polyphenols were grouped into a wide class of ‘other polyphenols’, including tyrosols, alklyphenols, hydroxybenzaldehydes, furanocoumarins and hydroxycoumarins, among others, representing 8.7% of total polyphenol intake, and being mainly provided by olives and olive oil (37% and 29%, respectively).

### Intake of individual polyphenols

The mean individual polyphenol intake was also calculated. Of the 290 polyphenols, 86 were consumed in amounts >1 mg day\(^{-1}\). A list of the 35 most consumed polyphenols (intake > 4 mg day\(^{-1}\)) is given in Appendix 1 Table 1 in decreasing order, along with the polyphenol subclass to which they belong, the mean intake expressed in milligrammes per day, and the main food contributors in percentage.

These 35 major polyphenols included seven hydroxycinnamic acids, five proanthocyanidins, five flavonoids, four flavonones, three flavanones, two anthocyanins and two catechins, while seven did not belong to any of the aforementioned groups and were classified as ‘other polyphenols’.

As expected from the results described in the previous section, the polyphenols of the hydroxycinnamic acid group head the list. Notably, 7 of the 35 most consumed polyphenols belong to the ‘other polyphenols’ group and, moreover, their source was typical MedDiet foods. For example, oleuropein and its aglycone, 3,4-DHPEA-EDA (oleuropein-aglycone di-aldehyde), p-HPEA-EDA (ligstroside-aglycone di-aldehyde), 3,4-DHPEA-EA (oleuropein-aglycone di-aldehyde) and hydroxytyrosol, which together represented 39.46 mg day\(^{-1}\), came exclusively from olives.
and olive oil, except for hydroxytyrosol, since a small amount of which was also provided by red wine [18].

Polyphenols from olives and olive oil: the Mediterranean difference

Olive oil is the main source of fat in the MedDiet [19] and studies have revealed that, as well as being rich in monounsaturated fatty acids, it has a unique phenolic profile with interesting biological properties. The beneficial effects of polyphenols from olives and olive oil on plasma lipid levels and interesting biological properties. The beneficial effects of unsaturated fatty acids, it has a unique phenolic profile with studies have revealed that, as well as being rich in mono-

The health effects of polyphenols depend on the amount consumed and their bioavailability [5,22]. However, the essential step towards the understanding of the protective effects of polyphenols against chronic diseases is to estimate their consumption by FFQ or other instruments in order to identify the compounds most likely to provide the greatest protection [5,7,8]. Up to now, very few comprehensive assessments of polyphenol intake in different populations have been performed. Most of the studies on different cohorts published to date have used USDA databases that provide data only on flavonoid intake [3,7]; other studies have included other classes of polyphenols, but are based on internal laboratory data on food polyphenol content [10,23]. Another study on the overall polyphenol intake in a diet was based on spectrophotometric methods, but did not provide information on individual compounds [22]. Thus, estimates vary widely among studies making comparisons difficult. The most exhaustive data available are those obtained in the French SU.VI.MAX cohort [11], allowing comparisons with the current study, since both involved similar methodologies. Although data on some processed foods (jams, drinks, etc.) are available in the Phenol-Explorer database, general information on food polyphenol content [10,23]. Table 4 shows the contribution of olives and olive oil to the intake of different classes of polyphenols, which was more than 98% of ‘other phenolic acids’ (phenolic acids other than hydroxycinnamic and hydroxybenzoic). This table also presents individual polyphenols ingested only from olives and olive oil according to class. This does not mean that these polyphenols are found only in olives and olive oil but, rather, that other sources are scarcely or not consumed in Spain. For example, 2,4-dihydroxybenzoic acid is also found in American cranberries, isorhamnatin in peppermint, verbascoside in a herb called verbena and m-coumaric acid is also present in beers but in very small amounts (data from Phenol-Explorer database).

### Discussion

The estimated mean total intake of polyphenols in the PREMID cohort in the present study was 820 ± 323 mg day$^{-1}$, being considerably lower than the 1193 ± 510 mg day$^{-1}$ found by Pérez-Jimenez et al. in the SU.VI.MAX cohort. This could be due to the difference in phenolic acid ingestion, since flavonoid intake was similar in both cohorts (443 in the PREMID cohort and 506 mg day$^{-1}$ in the SU.VI.MAX cohort). The health effects of polyphenols depend on the amount consumed and their bioavailability [5,22]. However, the essential step towards the understanding of the protective effects of polyphenols against chronic diseases is to estimate their consumption by FFQ or other instruments in order to identify the compounds most likely to provide the greatest protection [5,7,8]. Up to now, very few comprehensive assessments of polyphenol intake in different populations have been performed. Most of the studies on different cohorts published to date have used USDA databases that provide data only on flavonoid intake [3,7]; other studies have included other classes of polyphenols, but are based on internal laboratory data on food polyphenol content [10,23]. Another study on the overall polyphenol intake in a diet was based on spectrophotometric methods, but did not provide information on individual compounds [22]. Thus, estimates vary widely among studies making comparisons difficult. The most exhaustive data available are those obtained in the French SU.VI.MAX cohort [11], allowing comparisons with the current study, since both involved similar methodologies. Although data on some processed foods (jams, drinks, etc.) are available in the Phenol-Explorer database, general information on changes in polyphenol content in cooked foods is still absent; thus, yield or cooking factors should be included when evaluating the bioavailability of polyphenols in humans [17].
Other phenolic acids

Other polyphenols

Lignans

Total polyphenols

<table>
<thead>
<tr>
<th>Polyphenol group</th>
<th>Total intake from... (mg/d)</th>
<th>% Intake derived from olives and olive oil</th>
<th>Individual polyphenols ingested only from olives and olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virgin olive oil</td>
<td>Olives</td>
<td>Full diet</td>
</tr>
<tr>
<td>Flavones</td>
<td>0.36 ± 0.33</td>
<td>1.51 ± 2.30</td>
<td>41.6 ± 26.1</td>
</tr>
<tr>
<td>Flavonols</td>
<td>0.00</td>
<td>2.67 ± 4.06</td>
<td>80.4 ± 32.7</td>
</tr>
<tr>
<td>Hydroxycinnamic acids</td>
<td>0.06 ± 0.03</td>
<td>7.62 ± 11.6</td>
<td>19.1 ± 16.8</td>
</tr>
<tr>
<td>Hydroxycinnamic acids</td>
<td>0.04 ± 0.03</td>
<td>19.0 ± 28.8</td>
<td>276 ± 146</td>
</tr>
<tr>
<td>Other phenolic acids</td>
<td>0.02 ± 0.01</td>
<td>7.43 ± 11.3</td>
<td>7.56 ± 11.3</td>
</tr>
<tr>
<td>Other polyphenols</td>
<td>20.9 ± 10.5</td>
<td>25.8 ± 39.2</td>
<td>71.2 ± 46.7</td>
</tr>
<tr>
<td>Lignans</td>
<td>0.53 ± 0.30</td>
<td>0.01 ± 0.01</td>
<td>0.08 ± 0.06</td>
</tr>
<tr>
<td>Total polyphenols</td>
<td>21.9 ± 10.9</td>
<td>68.5 ± 104.0</td>
<td>820 ± 323</td>
</tr>
</tbody>
</table>

\( ^a \) Only described in black olives.
\( ^b \) Only described in virgin olive oil.
\( ^c \) Only described in green olives.

The present work presents certain limitations. First, although the Phenol-Explorer is the most comprehensive database available nowadays, information about some foods widely consumed in Spain is still scarce because they have not been characterised or only poorly characterised (e.g., chickpeas, honey or garlic) and some polyphenolic groups are also underestimated (e.g., secoiridoids and proanthocyanidins) because a suitable quantification method is lacking. It should also be taken into account that the polyphenol content in foods can differ according to ripeness at harvest time, environmental factors, processing and storage and even plant variety [5,25,26]. Another limitation of the study is the absence of information about spices and herbs in the FFQ, which might have resulted in an underestimation of the polyphenol intake as, although consumed in low amounts, they are the richest sources of polyphenols [6]. It should also be borne in mind that the resulting estimation is only valid for the population studied (elderly men and women at high cardiovascular risk). To summarise, this study gives a complete description of the total polyphenol intake and main food contributors of dietary polyphenols in a Spanish population at high cardiovascular risk. To our knowledge, this is the first accurate estimation of polyphenol intake done in Spain. The highly detailed data obtained on dietary polyphenol intake may serve as a valuable tool to establish the future associations between the amount and type of polyphenols ingested and the risk of chronic diseases, being also useful for setting food and health policies and dietary recommendations for individuals and population groups.

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Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2012.10.008.

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