Protective effect of homovanillyl alcohol on cardiovascular disease and total mortality: virgin olive oil, wine, and catechol-methylthiation

Rafael De la Torre, Dolores Corella, Olga Castañer, Miguel A Martínez-González, Jordi Salas-Salvador, Joan Vila, Ramón Estruch, José V Sorlí, Fernando Arós, Miquel Fiol, Emili Ros, Lluís Serra-Majem, Xavier Pintó, Enrique Gómez-Gracia, José Lapetra, Miguel Ruiz-Canela, José Basora, Eva Maria Asensio, Maria Isabel Covas, Montserrat Fita

INTRODUCTION

Hydroxytyrosol is a polyphenol present in free (as a simple phenolic compound) and mainly conjugated forms (secoiridoids) in 2 key components of the traditional Mediterranean diet (TMD)\textsuperscript{12}: olive oil [particularly virgin olive oil (VOO)] and wine. Both TMD and olive oil consumption have been shown to...

be protective against cardiovascular disease (CVD) and total mortality (1–5). Hydroxytyrosol and its related phenolic compound tyrosol represent 70–80% of the total polyphenol VOO content (6). In 2011, the European Food Safety Authority released a health claim for the benefits of the daily ingestion of olive oil rich in hydroxytyrosol for preventing LDL oxidation. The panel considers that to bear the claim, 5 mg hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) in olive oil should be consumed daily within the context of a balanced diet (7).

Epidemiologic studies support that light-to-moderate alcohol consumption (10–20 g/d) may reduce the risk of CVD and all-cause mortality (8). Among other polyphenols, hydroxytyrosol and tyrosol are also present in wine. Within the framework of the PREDIMED (Prevención con Dieta Mediterránea) study (ISRCTN35739639), we reported a direct dose-dependent association between hydroxytyrosol urinary concentrations and wine or alcohol consumption in individuals at high risk of CVD (9). We recently reported (10) that alcohol, particularly red wine, can promote an endogenous hydroxytyrosol generation at moderate concentrations. Biological concentrations of hydroxytyrosol obtained after moderate red wine consumption were higher than those that, provided by VOO ingestion, had been proven to have protective effects against risk factors for CVD in human clinical trials (11, 12).

Hydroxytyrosol is absorbed from VOO in a dose-dependent manner with respect to the polyphenol content of the olive oil (13). The main biological metabolite of hydroxytyrosol is the product of the catechol-O-methyltransferase (COMT) enzyme, 3-O-methyl-hydroxytyrosol, also known as homovanillyl alcohol (HVAL) (14, 15). In experimental studies, hydroxytyrosol is one of the strongest antioxidant, antiproliferative, proapoptotic, antiplatelet, and anti-inflammatory polyphenols (16). In addition, several clinical trials have shown the benefits of hydroxytyrosol-rich olive oils on risk factors for CVD (11, 12, 17, 18). Circulating biomarkers are always subject to some degree of homeostasis, absorption, distribution, or metabolism. Metabolic influences seem to be especially relevant for the formation of hydroxytyrosol from tyrosol and for the conversion of hydroxytyrosol to HVAL (10, 14). To our knowledge, no prior studies have evaluated how biological concentrations of hydroxytyrosol and HVAL relate to CVD and total mortality. We hypothesized that urinary hydroxytyrosol and HVAL could be associated with lower fatal and nonfatal CVD events and all-cause mortality, and, in the case of HVAL concentrations, genotypes of COMT, the enzyme that catalyzes the O-methylation of various compounds such as catechol estrogens and dietary polyphenols, could be involved (15).

METHODS

Design and population

PREDIMED is a parallel-group, randomized, multicenter controlled feeding trial aimed at assessing the effects of a TMD in the primary prevention of CVD. Details of the recruitment method and study design have been described elsewhere (4, 19). Eligible participants included 7447 community-dwelling men and women from Spain aged 55–80 y free from CVD at enrollment but at high risk. The participants had either type 2 diabetes mellitus or ≥3 major risk factors: smoking, hypertension, dyslipidemia, overweight or obesity, or a family history of premature CVD. Eligible participants were randomly assigned to 1 of 3 dietary intervention groups, 2 TMD groups supplemented with extra VOO or mixed nuts or to a control (low-fat) diet. Yearly study-clinic evaluations were performed by trained personnel and included a physical examination, diagnostic testing, blood sampling, and questionnaires on health status, medical history, and lifestyle. All participants provided written informed consent, and the study protocol was approved by the institutional review boards of the participating centers. In this work, we performed observational analyses of pooled study treatment arms.

Study measures

We measured hydroxytyrosol and HVAL in a random sample of 1851 of the 7447 participants with the use of stored urine samples from the initial visit, which was considered the baseline year for this analysis. The analyses herein were conducted in these participants assuming the design of an observational cohort with a median follow-up of 4.8 y and controlling for relevant confounding factors. Hydroxytyrosol and methyl hydroxytyrosol (MOHTyr) were measured with the use of gas chromatography–mass spectrometry. Limits of detection and quantification for MOHTyr and hydroxytyrosol were 1.85 and 5.60 and 1.60 and 4.80 ng/mL, respectively. Relative SDs of low, medium, and high control urine samples for 15, 30, and 60 ng MOHTyr/mL were 8.3%, 6.4%, and 8.1%, respectively, and those for 21, 42, and 98 ng hydroxytyrosol/mL were 7.1%, 4.7%, and 2.0%, respectively.

See Supplemental Material 1 for details of cohort sampling and hydroxytyrosol and HVAL measurements. At the initial visit a 137-item validated semiquantitative food-frequency questionnaire (19) was administered to calculate energy intake and nutrients. CVD risk factors, anthropometric variables, blood pressure, and laboratory measures were evaluated with the use of standardized procedures, and alcohol use, physical activity, and adherence to the TMD were evaluated with the use of validated questionnaires (20–22). Total hydroxytyrosol (TOHTyr) was calculated as the sum of hydroxytyrosol plus HVAL.

Endpoints

The primary endpoint was a composite of myocardial infarction, stroke, and death from cardiovascular causes. The secondary endpoint was all-cause mortality. We used 4 sources of information to identify endpoints: repeated contacts with participants, contacts with family physicians, a yearly review of medical records, and consultation of the National Death Index. All medical records related to endpoints were examined by the Endpoint Adjudication Committee, whose members were blinded to the study group assignments. Only endpoints that were confirmed by the committee were included in the analyses. The criteria for adjudicating primary and secondary endpoints are detailed in Supplemental Material 1.

Genotyping of the COMT locus

Genomic DNA was extracted from buffy coat with the MagNaPure LC DNA Isolation Kit (Roche Diagnostics). The
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<td>Participants, n</td>
<td>363</td>
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<td>354</td>
</tr>
<tr>
<td>Age, y</td>
<td>66.9 ± 6.5</td>
<td>66.5 ± 6.3</td>
<td>67.0 ± 6.3</td>
<td>67.1 ± 5.8</td>
<td>66.3 ± 6.1</td>
<td>0.497</td>
</tr>
<tr>
<td>Men, %</td>
<td>40.2</td>
<td>46.7</td>
<td>43.3</td>
<td>54.7</td>
<td>63.3</td>
<td>&lt;0.001</td>
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<td>Education &gt; high school, %</td>
<td>6.2</td>
<td>8.0</td>
<td>8.4</td>
<td>7.5</td>
<td>10.3</td>
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</tr>
<tr>
<td>Current smoking, %</td>
<td>20.9</td>
<td>28.2</td>
<td>24.0</td>
<td>24.0</td>
<td>26.3</td>
<td>0.326</td>
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<tr>
<td>Diabetes mellitus, %</td>
<td>52.3</td>
<td>55.0</td>
<td>49.3</td>
<td>57.6</td>
<td>50.0</td>
<td>0.388</td>
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<td>Hypertension, %</td>
<td>80.7</td>
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<td>Dyslipidemia, %</td>
<td>72.3</td>
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<td>BMI, kg/m²</td>
<td>29.9 ± 3.9</td>
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<td>Waist, cm</td>
<td>99.5 ± 10.8</td>
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<td>98.7 ± 9.7</td>
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<td>Physical activity, MET</td>
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<td>Alcohol, g/wk</td>
<td>4.8 (0–70)</td>
<td>10.4 (0–75)</td>
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<td>56.2 (5–190)</td>
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<td>366 (206–438)</td>
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<td>Adherence to TMD³</td>
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**TABLE 1**

Baseline characteristics of urinary hydroxytyrosol, homovanillic alcohol, and total hydroxytyrosol.

1 Total hydroxytyrosol is the sum of hydroxytyrosol and O-methyl-hydroxytyrosol. P-trend across quintiles was based on linear regression for continuous variables and logistic regression for binary variables. MET, metabolic equivalent; Q, quartile; TMD, traditional Mediterranean diet.

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3 Median; IQR in parentheses (all such values).

4 Calculated by the 14-point score.

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During 13,070 person-years of follow-up, 142 cardiovascular events and 123 deaths occurred. Across quintiles and after adjusting for demographic, cardiovascular, lifestyle, and dietary factors, concentrations of HV AL were associated with a lower incidence of cardiovascular events (myocardial infarction, stroke, or cardiovascular death) (Table 2). Participants in the third or higher quintile of HV AL (≥20 mmol/L) had a 56% lower risk (P-trend < 0.001) than those in the lowest quintile. There was a significant trend for a decreasing CVD risk across quintiles for all biomarkers (P < 0.05) (Table 2). Concerning total mortality (Table 3), no differences across quintiles of biomarker concentrations were found, but a decreasing trend across quintiles was observed for MOHTyr (P = 0.017) (Tables 2 and 3). Sensitivity analyses for the association of HV AL with CVD by group of intervention showed that, despite the same trend in all groups, MOHTyr achieved the greatest significance (P < 0.001) in the group consuming the Mediterranean diet enriched with VOO (Supplemental Table 1).

In semiparametric analyses, associations of urinary hydroxytyrosol, HV AL, and TOHTyr with primary cardiovascular events were significant in a linear manner (Figure 1), with a decrease of HRs from low to high hydroxytyrosol, HV AL, and TOHTyr concentrations (P < 0.005). Associations of the biomarkers with total mortality showed that the HR decreased linearly from low to high MOHTyr concentrations (P = 0.024) only in the case of HV AL (Figure 2).

From all biomarkers, only HV AL concentrations were significantly associated with gained years of life or years free of CVD (Supplemental Figure 1) after the age of 65 y. Individuals in the highest quintile of HV AL had a mean 9.5 y (95% CI: 3.5, 20.8 y) longer life after the age of 65 y. With regard to being free of a cardiovascular event, individuals aged >65 y in the highest quintile of HV AL had a mean 6.3 additional years free of CVD (95% CI: 2.3, 12.15 y) compared with individuals with lower concentrations of HV AL (Supplemental Figure 1). Findings were similar for both sexes separately.

The COMT genotype distribution [Val/Val (G/G), Val/Met (G/A), or Met/Met (A/A)] among individuals was in Hardy-Weinberg equilibrium. Waist circumference and vegetable consumption (P < 0.05) were lower in the rs4680AG genotype (Supplemental Table 7). Individuals with the rs4680GG genotype had higher concentrations of HV AL than those with other genotypes (Table 4). The distribution of the COMT rs4680 alleles was similar among survivors and those who died and among those free of a cardiovascular event or those who had suffered one (Supplemental Table 8). No association was obtained among COMT genotypes and all-cause mortality or CVD risk. No interaction between the COMT rs4680 genotype and HV AL was found (Supplemental Table 9). Individuals with low HV AL concentrations had an ~2-fold greater risk of CVD and all-cause mortality than those with high HV AL concentrations independently of the COMT genotype, with multiplicative and additive interactions being nonsignificant (P > 0.05).
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tes were determined with the use of Cox regression analysis. CVD, cardiovascular disease; ref, reference.
P value corresponding to the interaction with the type of diet followed during the study. All models were stratified by the center.
P value corresponding to the improvement of the model when including intervention group and its interaction with quintiles.
Median; IQR in parentheses (all such values).
Adjusted for age, sex, education, current smoking, waist circumference, physical activity, diabetes, and dyslipidemia.
Further adjusted for virgin olive oil, wine, and vegetable consumption.
Sum of hydroxytyrosol and O-methyl-hydroxytyrosol.

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HVAL concentrations were associated with a 56% lower CVD risk across quintiles in individuals at high risk of CVD in this study. After the age of 65 y, our predictive model suggested that the gained years of life and the years free of CVD could be 9.5 and 6.3 y, respectively, among participants with higher urinary HV AL concentrations compared with lower ones. In addition, HV AL was associated with a lower total mortality and lower CVD risk.

Carriers of the COMT rs4680 GG genotype displayed higher HVAL concentrations. Neither an association between COMT genotypes and CVD or all-cause mortality nor an interaction between COMT genotypes and HV AL concentrations was found.
Both experimental and human studies show the benefits of hydroxytyrosol-related foods such as VOO and wine on CVD risk factors, such as: 1) decreasing heart rate, blood pressure, LDL oxidation, inflammation, thrombotic markers, and lipoprotein particle atherogenic ratios; 2) increasing HDL cholesterol and HDL lipoprotein functionality; and 3) improving endothelial function (12, 16, 27). Polyphenols from VOO have also been shown to decrease the expression of atherosclerosis-related genes (27, 28). We have recently provided a mechanistic explanation for the combined protective effect of the simultaneous consumption of the 2 key components of the Mediterranean diet: VOO and wine (10). On the one hand, VOO provides tyrosol and 
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tyrosol occurs (10). Thus, a synergetic effect of wine and VOO on increasing the human hydroxytyrosol pool in vivo is likely to occur. HVAL in vivo concentrations, however, are not only dependent on the hydroxytyrosol concentrations but also the individual metabolic capacity for promoting COMT-regulated hydroxytyrosol methylation. A substitution of Val (G) by Met (A) at codon 158 of the COMT gene affects the activity of the COMT enzyme. Individuals with the rs4680 GG genotype have a 3- to 4-fold higher activity than those with other geno-
tes were determined with the use of Cox regression analysis. CVD, cardiovascular disease; ref, reference.
P value corresponding to the interaction with the type of diet followed during the study. All models were stratified by the center.
P value corresponding to the improvement of the model when including intervention group and its interaction with quintiles.
Median; IQR in parentheses (all such values).
Adjusted for age, sex, education, current smoking, waist circumference, physical activity, diabetes, and dyslipidemia.
Further adjusted for virgin olive oil, wine, and vegetable consumption.
Sum of hydroxytyrosol and O-methyl-hydroxytyrosol.
FIGURE 1 Multivariate-adjusted relation of hydroxytyrosol, homovanillyl alcohol, and total hydroxytyrosol (hydroxytyrosol + homovanillyl alcohol) with primary cardiovascular event. Associations were evaluated with the use of restricted cubic splines. The solid lines represent the central risk estimate, and the dotted lines represent the 95% CIs adjusted for age, sex, center, education, current smoking, waist circumference, physical activity, diabetes, and dyslipidemia and virgin olive oil, wine, and vegetable consumption.

FIGURE 2 Multivariate-adjusted relation of hydroxytyrosol, homovanillyl alcohol, and total hydroxytyrosol (hydroxytyrosol + homovanillyl alcohol) with all-cause mortality. Associations were evaluated with the use of restricted cubic splines. The solid lines represent the central risk estimate, and the dotted lines represent the 95% CIs adjusted for age, sex, center, education, current smoking, waist circumference, physical activity, diabetes, and dyslipidemia and virgin olive oil, wine, and vegetable consumption.
The protective antioxidant activity of HVAL in experimental studies has been said to be greater than (29), similar to (30), and lower than (31) that of hydroxytyrosol according to the experimental model used. However, chemically, HVAL is a compound that is far more stable in biological fluids than hydroxytyrosol (32). This stability allows HVAL to exist for longer than hydroxytyrosol in biological fluids and intracellular spaces; thus, the former can exert benefits for longer. Further studies on the effect of HVAL on pathologic mechanisms, such as inflammation, endothelial function, and thrombosis, are warranted.

Contradictory data exist on the influence of COMT genotypes on CVD risk. The rs4680GG genotype has been associated with a high risk of hypertension (33) and CVD (34), whereas the rs4860AA genotype has been shown to be protective against myocardial infarction in hypertensive patients (35). In contrast, the low COMT activity of the rs4860AA genotype has been shown to be an independent risk factor for acute coronary events in Finnish men (36). In our study, however, we did not find this association. Differences between populations could explain the differences in the results obtained herein. In agreement with others (37), in this study we did not observe any association between COMT genotypes and total mortality. Taking into account the absence of a strong association between the COMT polymorphism and HVAL concentrations, as well as the high pleiotropy of the COMT enzyme, this polymorphism cannot be used as a proxy for Mendelian randomization (38). Therefore, the absence of associations between the COMT genotypes and CVD or total mortality cannot be interpreted as not causal.

Our study has strengths and limitations. All variables in the multicenter study were collected through well-established common protocols (39). The associations among biomarkers and CVD or all-cause mortality were adjusted by possible confounders. The biomarkers in this study were measured at baseline, however, and changes over time could influence the results and in some cases lead to misclassifications. In addition, the sample size could not allow enough power to detect small differences, particularly in the case of genetic data. In addition, this was an observational study and thus cannot demonstrate causality. Cardiovascular events and total mortality were adjudicated with the use of medical records that were examined by an endpoint adjudication committee. However, some misclassifications could occur. The fact that our participants were at a high risk of CVD limits the generalizability of the results to other populations.

In summary, we report herein for the first time to our knowledge an independent association between high urinary HVAL concentrations and a lower risk of CVD and total mortality in elderly individuals at a high risk of CVD. From our results, VOO and wine consumption and a high metabolic capacity of COMT-mediated methylation are key factors for achieving high HVAL concentrations. The association that stems from our results reinforces the benefits of consuming 2 key components of the Mediterranean diet.

The authors’ responsibilities were as follows—RDlT, DC, MIC, and M Fišťo: conceived and designed the study; RDlT, DC, OC, and M Fišťo: acquired, analyzed, and interpreted the data; JV: conducted the statistical analysis; and all authors: drafted the manuscript, critically revised the manuscript for important intellectual content, and read and approved the final manuscript. ER and JS-S are consultants for the California Walnut Commission and International Nut Council, respectively. None of the other authors reported a conflict of interest related to the study.

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