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The Effect of Polyphenols in Olive Oil on Heart Disease Risk Factors

A Randomized Trial

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Background: Virgin olive oils are richer in phenolic content than refined olive oil. Small, randomized, crossover, controlled trials on the antioxidant effect of phenolic compounds from real-life daily doses of olive oil in humans have yielded conflicting results. Little information is available on the effect of the phenolic compounds of olive oil on plasma lipid levels. No international study with a large sample size has been done.

Objective: To evaluate whether the phenolic content of olive oil further benefits plasma lipid levels and lipid oxidative damage compared with monounsaturated acid content.

Design: Randomized, crossover, controlled trial.

Setting: 6 research centers from 5 European countries.

Participants: 200 healthy male volunteers.

Measurements: Glucose levels, plasma lipid levels, oxidative damage to lipid levels, and endogenous and exogenous antioxidants at baseline and before and after each intervention.

Intervention: In a crossover study, participants were randomly assigned to 3 sequences of daily administration of 25 mL of 3 olive oils. Olive oils had low (2.7 mg/kg of olive oil), medium (164 mg/kg), or high (366 mg/kg) phenolic content but were otherwise similar. Intervention periods were 3 weeks preceded by 2-week washout periods.

Results: A linear increase in high-density lipoprotein (HDL) cholesterol levels was observed for low-, medium-, and high-polyphenol olive oil: mean change, 0.025 mmol/L (95% CI, 0.003 to 0.05 mmol/L), 0.032 mmol/L (CI, 0.005 to 0.05 mmol/L), and 0.045 mmol/L (CI, 0.02 to 0.06 mmol/L), respectively. Total cholesterol–HDL cholesterol ratio decreased linearly with the phenolic content of the olive oil. Triglyceride levels decreased by an average of 0.05 mmol/L for all olive oils. Oxidative stress markers decreased linearly with increasing phenolic content. Mean changes for oxidized low-density lipoprotein levels were 1.21 U/L (CI, −0.8 to 3.6 U/L), −1.48 U/L (−3.6 to 0.6 U/L), and −3.21 U/L (−5.1 to −0.8 U/L) for the low-, medium-, and high-polyphenol olive oil, respectively.

Limitations: The olive oil may have interacted with other dietary components, participants' dietary intake was self-reported, and the intervention periods were short.

Conclusions: Olive oil is more than a monounsaturated fat. Its phenolic content can also provide benefits for plasma lipid levels and oxidative damage.

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Polyphenol intake has been associated with low cancer and coronary heart disease (CHD) mortality rates (1). Antioxidant and anti-inflammatory properties and improvements in endothelial dysfunction and the lipid profile have been reported for dietary polyphenols (2). Studies have recently suggested that Mediterranean health benefits may be due to a synergistic combination of phytochemicals and fatty acids (3). Olive oil, rich in oleic acid (a monounsaturated fatty acid), is the main fat of the Mediterranean diet (4). To date, most of the protective effect of olive oil within the Mediterranean diet has been attributed to its high monounsaturated fatty acid content (5). However, if the effect of olive oil can be attributed solely to its monounsaturated fatty acid content, any type of olive oil, rapeseed or canola oil, or monounsaturated fatty acid–enriched fat would provide similar health benefits.

Whether the beneficial effects of olive oil on the cardiovascular system are exclusively due to oleic acid remains to be elucidated. The minor components, particularly the phenolic compounds, in olive oil may contribute to the health benefits derived from the Mediterranean diet. Among olive oils usually present on the market, virgin olive oils produced by direct-press or centrifugation methods have higher phenolic content (150 to 350 mg/kg of olive oil) (6). In experimental studies, phenolic compounds in olive oil showed strong antioxidant properties (7, 8). Oxidized low-density lipoprotein (LDL) is currently thought to be more damaging to the arterial wall than native LDL cholesterol (9). Results of randomized, crossover, controlled clinical trials on the antioxidant effect of polyphenols from real-life daily doses of olive oil in humans are, however, conflicting (10). Growing evidence suggests that dietary phenols (11–15) and plant-based diets (16) can modulate lipid and lipoprotein metabolism.

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Context

Olive oil, the main fat in the Mediterranean diet, contains polyphenols, which have antioxidant properties and may affect serum lipid levels.

Contribution

The authors studied virgin olive oil (high in polyphenols), refined olive oil (low in polyphenols), and a mixture of the 2 oils in equal parts. Two hundred healthy young men consumed 25 mL of an olive oil daily for 3 weeks followed by the other olive oils in a randomly assigned sequence. Olive oils with greater polyphenol content increased high-density lipoprotein (HDL) cholesterol levels and decreased serum markers of oxidation.

Cautions

The increase in HDL cholesterol level was small.

Implications

Virgin olive oil might have greater health benefits than refined olive oil.

—The Editors

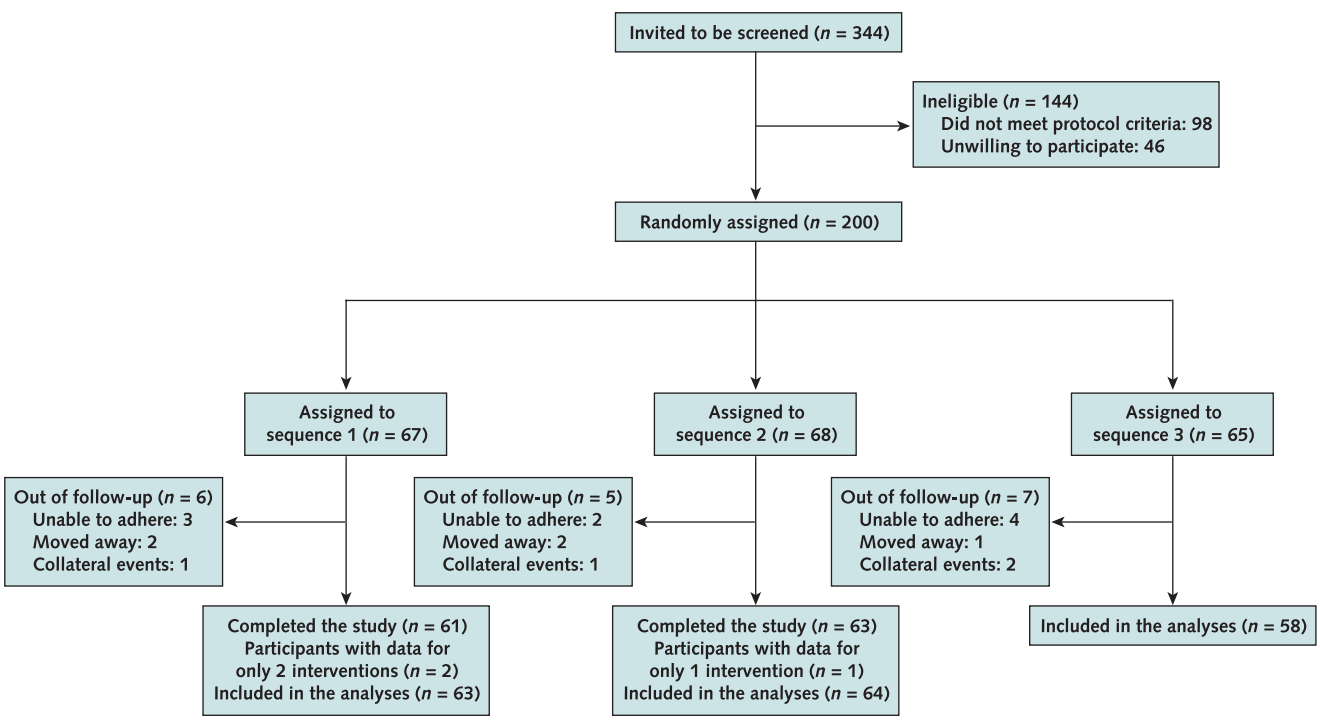
ropean Populations (EUROLIVE) Study is a multicenter, randomized, crossover, clinical intervention trial that aims to assess the effect of sustained daily doses of olive oil, as a function of its phenolic content, on the oxidative damage to lipid and LDL cholesterol levels and the lipid profile as cardiovascular risk factors.

METHODS

Participants

We recruited healthy men, 20 to 60 years of age, from 6 European cities through newspaper and university advertisements. Of the 344 persons who agreed to be screened, 200 persons were eligible (32 men from Barcelona, Spain; 33 men from Copenhagen, Denmark; 30 men from Kuopio, Finland; 31 men from Bologna, Italy; 40 men from Postdam, Germany; and 34 men from Berlin, Germany) and were enrolled from September 2002 through June 2003 (**Figure 1**). Participants were eligible for study inclusion if they provided written informed consent, were willing to adhere to the protocol, and were in good health. We preselected volunteers when clinical record, physical examination, and blood pressure were strictly normal and the candidate was a nonsmoker. Next, we performed a complete blood count, biochemical laboratory analyses, and urinary dipstick tests to measure levels of serum glucose, total cholesterol, creatinine, alanine aminotransferase, and triglycerides. We included candidates with values within the reference range. Exclusion criteria were smoking; use of antioxidant supplements, aspirin, or drugs with established

Figure 1. **Study flow diagram.**



Sequence of olive oil administration: 1) high-, medium-, and low-polyphenol olive oil; 2) medium-, low-, and high-polyphenol olive oil; and 3) low-, high-, and medium-polyphenol olive oil.

Table 1. **Baseline Characteristics***

Variable	Sequence 1 (*n* = 67)	Sequence 2 (*n* = 68)	Sequence 3 (*n* = 65)
Age, *y*	33.4 (11.2)	34.3 (11.0)	31.9 (10.7)
BMI, kg/m^2	23.7 (2.8)	23.8 (2.4)	24.0 (3.2)
Physical activity, *kcal/d*†	248 (132–413)	220 (103–408)	226 (135–399)
Systolic blood pressure, *mm Hg*	125 (14.7)	125 (11.1)	123 (12.8)
Diastolic blood pressure, *mm Hg*	77 (7.6)	78 (8.2)	76 (8.5)
Glucose level			
mmol/L	4.72 (0.54)	4.73 (0.58)	4.78 (0.60)
mg/dL	85 (9.7)	85 (10.5)	86 (10.9)
Total cholesterol level			
mmol/L	4.79 (0.96)	4.82 (1.1)	4.84 (1.1)
mg/dL	185 (37)	186 (42)	187 (42)
LDL cholesterol level			
mmol/L	3.06 (0.91)	3.11 (0.93)	2.92 (0.98)
mg/dL	118 (35)	120 (36)	113 (38)
HDL cholesterol level			
mmol/L	1.22 (0.29)	1.24 (0.29)	1.22 (0.28)
mg/dL	47.1 (11.1)	47.9 (11.3)	47.0 (11.0)
Triglyceride level†			
mmol/L	0.99 (0.75–1.36)	0.87 (0.65–1.23)	0.93 (0.71–1.29)
mg/dL	87 (66–120)	77 (57–108)	82 (63–114)
Oxidative biomarkers			
Oxidized LDL, *U/L*	51 (25)	50 (22)	47 (20)
Hydroxy fatty acids, *nmol/L*†	1241 (1023–1528)	1218 (1053–1430)	1194 (1048–1361)
$F_{2\alpha}$-isoprostanes, *μg/L*	29.2 (6.3)	29.0 (6.4)	29.7 (7.3)
Conjugated dienes, *μmol/mol of cholesterol*	2.61 (1.25)	2.59 (1.05)	2.90 (1.29)
Glutathione balance			
Reduced glutathione, *μmol/L*	4.72 (0.58)	4.50 (0.58)	4.61 (0.73)
Oxidized glutathione, *μmol/L*	1.24 (0.12)	1.27 (0.12)	1.24 (0.12)
Reduced–oxidized glutathione ratio	3.85 (0.62)	3.57 (0.57)	3.74 (0.71)
Endogenous antioxidant enzymes			
Superoxide dismutase, *U/L*	142 (20)	143 (23)	141 (19)
Glutathione peroxidase, *U/L*	717 (176)	681 (132)	691 (178)
Glutathione reductase, *U/L*	65 (18)	63 (16)	63 (15)
Paraoxonase, *U/L*	156 (112)	150 (96)	193 (143)
Exogenous antioxidants			
Ascorbic acid, *μmol/L*	61 (26)	60 (22)	61 (23)
α-Tocopherol, *μmol/L*	25 (5.5)	25 (6.3)	24 (7.2)
β-Carotene, *μmol/L*	0.45 (0.38)	0.39 (0.27)	0.34 (0.24)
Lycopene, *μmol/L*	0.46 (0.22)	0.42 (0.37)	0.43 (0.21)

* Values are expressed as means (SD) unless otherwise indicated. Sequence 1 = high-, medium-, and low-polyphenol olive oil; sequence 2 = medium-, low-, and high-polyphenol olive oil; sequence 3 = low-, high-, and medium-polyphenol olive oil. BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
† Median (25th–75th percentile).

antioxidant properties; hyperlipidemia; obesity; diabetes; hypertension; intestinal disease; or any other disease or condition that would impair adherence. We excluded women to avoid the possible interference of estrogens, which are considered to be potential antioxidants (17). All participants provided written informed consent, and the local institutional ethics committees approved the protocol.

Design and Study Procedure

The trial was a randomized, crossover, controlled study. We randomly assigned participants consecutively to 1 of 3 sequences of olive oil administration. Participants received a daily dose of 25 mL (22 g) of 3 olive oils with high (366 mg/kg), medium (164 mg/kg), and low (2.7 mg/kg) polyphenol content (**Figure 1**) in replacement of other raw fats. Sequences were high-, medium-, and low-polyphenol olive oil (sequence 1); medium-, low-, and high-polyphenol olive oil (sequence 2); and low-, high-, and medium-polyphenol olive oil (sequence 3). In the coordinating center, we prepared random allocation to each sequence, taken from a Latin square, for each center by blocks of 42 participants (14 persons in each sequence), using specific software that was developed at the Municipal Institute for Medical Research, Barcelona, Spain (Aleator, Municipal Institute for Medical Research). The random allocation was faxed to the participating centers upon request for each individual included in the study. Treatment containers were assigned a code number that was concealed from participants and investigators, and the coordinating center disclosed the code number only after completion of statistical analyses. Olive oils were specially prepared for the trial. We selected a virgin olive oil with high natural phenolic content (366 mg/kg) and measured its fatty acid and vitamin E composition. We tested refined olive oil harvested from the same cultivar and soil to find an olive

Table 2. **Daily Energy Consumption and Selected Nutrient Intake after Olive Oil Interventions***

Variable	Olive Oil Intervention		
	Low-Polyphenol Olive Oil (*n* = 182)	Medium-Polyphenol Olive Oil (*n* = 184)	High-Polyphenol Olive Oil (*n* = 183)
Energy, *kcal*	2269 (2081–2457)	2273 (2082–2464)	2302 (2114–2489)
Carbohydrate, %†	50.2 (47.2–53.2)	50.2 (47.2–53.3)	50.3 (47.3–53.3)
Protein, %†	14.7 (13.6–15.9)	15.1 (13.9–16.2)	14.9 (13.7–16.1)
Total fat, %†	34.6 (31.9–37.3)	33.6 (31.1–36.1)	33.5 (30.1–36.0)
Saturated fat, %†	12.1 (10.8–13.3)	12.0 (10.8–13.2)	12.1 (10.9–13.3)
Monounsaturated fat, %†	14.3 (13.1–15.4)	14.1 (13.0–15.2)	14.1 (12.9–15.2)
Polyunsaturated fat, %†	4.2 (3.6–4.7)	4.1 (3.6–4.7)	4.1 (3.5–4.6)
Vitamin C, *mg*	102 (77–126)	103 (78–127)	113 (87–139)
Vitamin E, *mg*	8.8 (7.8–9.8)	8.3 (7.3–9.2)	8.2 (7.2–9.2)
β-Carotene, *mg*	2.4 (1.5–3.4)	2.5 (1.5–3.5)	2.2 (1.3–3.2)
Alcohol, *g*‡	6.7 (4–12)	7.4 (4–13)	8.5 (4–15)

* Values are adjusted means (95% CI) estimated from a linear mixed model with terms for period, treatment, and center as fixed effects; participant as a random effect; and baseline values and age as covariates. From data of the 3-day dietary record obtained after each intervention period.
† Expressed as percentage of total energy intake.
‡ Log-transformed; data from alcohol consumers (77% of the study sample).

oil with similar quantities of fatty acid and a similar micronutrient profile. Vitamin E was adjusted to values similar to those of the selected virgin olive oil. Because phenolic compounds are lost in the refinement process, the refined olive oil had a low phenolic content (2.7 mg/kg). By mixing virgin and refined olive oil, we obtained an olive oil with an intermediate phenolic content (164 mg/kg). Olive oils did not differ in fat and micronutrient composition (that is, vitamin E, triterpenes, and sitosterols), with the exception of phenolic content. Three-week interventions were preceded by 2-week washout periods, in which we requested that participants avoid olive and olive oil consumption. We chose the 2-week washout period to reach the equilibrium in the plasma lipid profile because longer intervention periods with fat-rich diets did not modify the lipid concentrations (18). Daily doses of 25 mL of olive oil were blindly prepared in containers delivered to the participants at the beginning of each intervention period. We instructed participants to return the 21 containers at the end of each intervention period so that the daily amount of unconsumed olive oil could be registered.

Dietary Adherence

We measured tyrosol and hydroxytyrosol, the 2 major phenolic compounds in olive oil as simple forms or conjugates (7), by gas chromatography and mass spectrometry in 24-hour urine before and after each intervention period as biomarkers of adherence to the type of olive oil ingested. We asked participants to keep a 3-day dietary record at baseline and after each intervention period. We requested that participants in all centers avoid a high intake of foods that contain antioxidants (that is, vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer). A nutritionist also personally advised participants to replace all types of habitually consumed raw fats with the olive oils (for example, spread the assigned olive oil on bread instead of butter, put the assigned olive oil on boiled vegetables instead of margarine, and use the assigned olive oil on salads instead of other vegetable oils or standard salad dressings).

Data Collection

Main outcome measures were changes in biomarkers of oxidative damage to lipids. Secondary outcomes were changes in lipid levels and in biomarkers of the antioxidant status of the participants. We assessed outcome measures at the beginning of the study (baseline) and before (preintervention) and after (postintervention) each olive oil intervention period. We collected blood samples at fasting state together with 24-hour urine and recorded anthropometric variables. We measured blood pressure with a mercury sphygmomanometer after at least a 10-minute rest in the seated position. We recorded physical activity at baseline and at the end of the study and assessed it by using the Minnesota Leisure Time Physical Activity Questionnaire (19).

We measured 1) glucose and lipid profile, including serum glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels determined by enzymatic methods (20–23) and LDL cholesterol levels calculated by the Friedewald formula; 2) oxidative damage to lipids, including plasma-circulating oxidized LDL measured by enzyme immunoassay, plasma total $F_{2\alpha}$-isoprostanes determined by using high-performance liquid chromatography and stable isotope-dilution and mass spectrometry, plasma C18 hydroxy fatty acids measured by gas chromatography and mass spectrometry, and serum LDL cholesterol uninduced conjugated dienes measured by spectrophotometry and adjusted for the cholesterol concentration in LDL cholesterol levels; 3) antioxidant status from endogenous origin, including whole-blood superoxide dismutase activity measured by the rate of inhibition of

2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, serum glutathione peroxidase and glutathione reductase activities measured through glutathione oxidation–reduction, serum paraoxonase activity measured by its capacity to hydrolyze paraoxon, and reduced and oxidized glutathione content of cells determined by high-performance liquid chromatography; and 4) antioxidant status from exogenous origin, including plasma ascorbic acid, β-carotene, vitamin E, and lycopene measured by high-performance liquid chromatography. We performed analyses in duplicate.

Sample Size and Power Analysis

A total sample of 180 participants (30 participants per center) allowed at least 80% power to detect a statistically significant difference among the olive oil groups of 10 units in the LDL oxidative measurement, assuming a drop-out rate of 15% and type I error of 0.005 (2-sided). We retained an additional 20 participants who met the inclusion criteria after the screening procedure to ensure statistical power if the differences among the treatment groups were lower than expected.

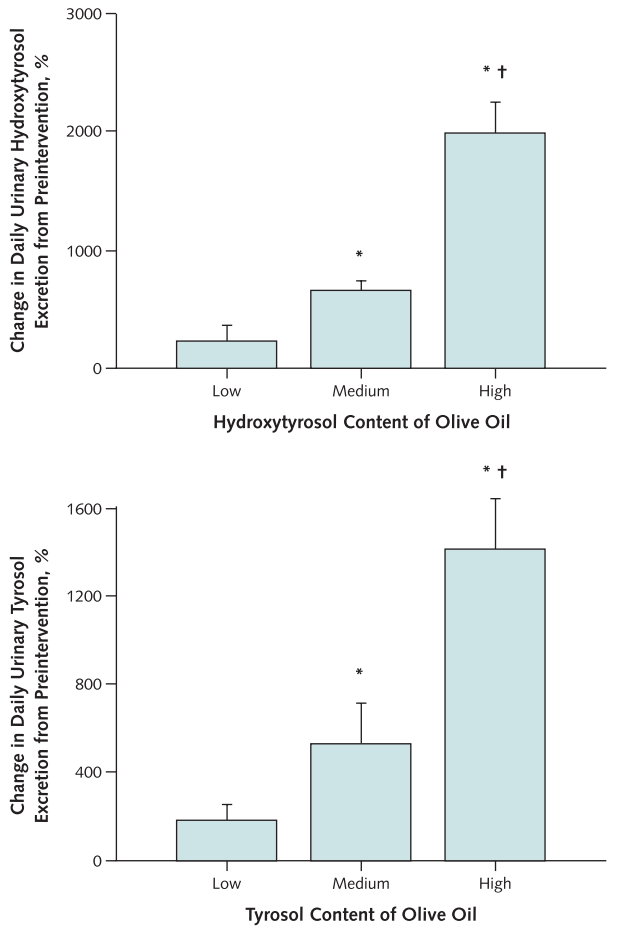
Statistical Analyses

We assessed the normality of variables by looking at normal probability plots. Triglycerides and hydroxy fatty acids were log-transformed to achieve normality. We used the 1-factor analysis of variance (ANOVA) or the Kruskal–Wallis test, as appropriate, to determine differences in baseline characteristics. We used the Student *t*-test to determine differences in baseline characteristics between participants who did and participants who did not complete the study. We checked the possible carryover effect by testing a period-by-treatment interaction term in the general linear mixed models. Because the period-by-treatment interaction term was not statistically significant in any model, we did not include period-by-treatment terms in the final models used to test the nutrient intake among the 3 olive oil interventions and the effect of the interventions on lipid profile, biomarkers of oxidative damage, and biomarkers of antioxidant status. These models used the postintervention values as the dependent variables and included independent variables of period, treatment, and center as fixed effects; participant number as random effect; and age and baseline values as covariates (24). We performed all analyses on an intention-to-treat basis. Statistical significance was defined as a *P* value less than 0.050 for a 2-sided test. We performed analyses by using SAS software, release 9.1 (SAS Institute Inc., Cary, North Carolina).

Role of the Funding Source

The EUROLIVE project was financially supported by the Commission of the European Communities Quality of Life and Management of Living Resources program (QLK1-2001-00281). The funding source had no role in the collection, analysis, or interpretation of data. The authors had full access to all data and final responsibility for the decision to submit the manuscript for publication.

Figure 2. **Changes from preintervention periods (mean percentage and upper 95% CI limit) in urinary tyrosol and hydroxytyrosol excretion as the function of the phenolic content of the olive oil administered.**



$P < 0.001$ for linear trend. *$P < 0.05$ vs. low-polyphenol olive oil. †$P < 0.05$ vs. medium-polyphenol olive oil.

RESULTS

Participant Characteristics

We observed no differences in general baseline characteristics (**Table 1**) or in the energy, macronutrient, or main antioxidant or pro-oxidant intake among the olive oil groups. We also observed no changes in physical activity from baseline to the end of the study. Comparisons of baseline characteristics among centers showed statistically significantly lower values of 1) serum glucose levels in Berlin (mean glucose level, 4.44 mmol/L [80 mg/dL]) than in Copenhagen (mean glucose level, 5.2 mmol/L [93 mg/dL]) and Postdam (mean glucose level, 4.9 mmol/L [89 mg/dL]); 2) total fat consumption in Bologna (72 g/d) than in Postdam

Table 3. **Changes in Outcome Measurements after Olive Oil Interventions***

Variable	Olive Oil Intervention						*P* Value†
	Low-Polyphenol Olive Oil (*n* = 182)		Medium-Polyphenol Olive Oil (*n* = 184)		High-Polyphenol Olive Oil (*n* = 183)		
	Postintervention	Change from Preintervention	Postintervention	Change from Preintervention	Postintervention	Change from Preintervention	
Total cholesterol level							0.36
mmol/L	4.72	0.007 (−0.08 to 0.06)	4.69	−0.011 (−0.1 to 0.07)	4.74	0.015 (−0.04 to 0.08)	
mg/dL	182	0.29 (−3.1 to 3.3)	181	−0.42 (−3.3 to 2.8)	183	0.58 (−1.7 to 3.3)	
LDL cholesterol level							0.74
mmol/L	2.97	0.015 (−0.06 to 0.09)	2.93	−0.019 (−0.1 to 0.05)	2.98	−0.010 (−0.08 to 0.06)	
mg/dL	115	0.61 (−2.3 to 3.4)	113	−0.75 (−3.8 to 1.9)	115	−0.38 (−3.1 to 2.3)	
HDL cholesterol level							0.018
mmol/L	1.27	0.025 (0.003 to 0.05)	1.27	0.032 (0.005 to 0.05)	1.30	0.045 (0.02 to 0.06)	
mg/dL	49.2	0.98 (0.1 to 1.9)	49.5	1.22 (0.2 to 1.9)	50.3	1.74 (0.95 to 2.5)	
Total cholesterol–HDL cholesterol ratio	3.88	−0.062 (−0.1 to −0.02)	3.83	−0.09 (−0.2 to −0.02)	3.82	−0.11 (−0.2 to −0.05)	0.013
LDL–HDL cholesterol ratio	2.46	−0.04 (−0.1 to 0.2)	2.41	−0.05 (−0.1 to −0.008)	2.40	−0.08 (−0.1 to −0.02)	0.050
Triglyceride level‡							0.74
mmol/L	0.96	−0.065 (−0.1 to −0.002)	0.97	−0.039 (−0.1 to 0.001)	0.96	−0.054 (−0.1 to −0.002)	
mg/dL	85	−5.8 (−10.8 to −0.2)	86	−3.1 (−7.7 to 0.1)	85	−4.8 (−8.5 to −0.2)	
Oxidative biomarkers							
Conjugated dienes, *μmol/mol of cholesterol*	2.61	−0.35 (−0.1 to 0.02)	2.55	−0.86 (−1.4 to −0.4)	2.37	−0.77 (−1.2 to −0.4)	0.011
Hydroxy fatty acids, *nmol/L*‡	179	−31 (−81 to 9)	176	−37 (−86 to 5)	157	−41 (−83 to −1)	0.038
Oxidized LDL, *U/L*	48	1.21 (−0.8 to 3.6)	47	−1.48 (−3.6 to 0.6)	46	−3.21 (−5.1 to −0.8)	0.014
$F_{2\alpha}$-isoprostanes, *μmol/L*	28.3	0.14 (−0.6 to 0.9)	27.7	−0.29 (−1.1 to 0.6)	28.1	0.08 (−0.7 to 0.8)	0.34

* Values are adjusted means (95% CI) estimated from a linear mixed model with terms for period, treatment, and center as fixed effects; participant as a random effect; and baseline values and age as covariates. HDL = high-density lipoprotein; LDL = low-density lipoprotein.
† For linear trends across oils.
‡ Log-transformed.

(106 g/d); and 3) blood pressure in Bologna (120 mm Hg) and Berlin (116 mm Hg) than in Postdam (132 mm Hg).

Attrition and Adverse Effects

Of the 200 participants, 18 (9%) did not complete the study (**Figure 1**). The dropout rates were 8.9%, 7.4%, and 10.6% in sequences 1, 2, and 3, respectively. One participant who dropped out had 1 postintervention value and another had 2 postintervention values, and we included these 2 participants in the analyses. Individuals who discontinued the study (1 participant in Barcelona, 5 participants in Copenhagen, 8 participants in Bologna, 2 participants in Postdam, and 2 participants in Berlin) had both low baseline systolic blood pressure (mean value, 117 mm Hg vs. 125 mm Hg; $P = 0.036$) and low total fat consumption (69 g/d vs. 86 g/d; $P = 0.045$). We could not identify any adverse effects related to olive oil intake.

Dietary Intake and Adherence

Table 2 shows the daily dietary intake after each intervention period. Diet was similar in all intervention groups. Participant adherence was good, as reflected in the changes in urinary tyrosol and hydroxytyrosol excretion after olive oil interventions (**Figure 2**). Urinary phenolic compounds increased in a dose-dependent manner with the phenolic content of the olive oil ($P < 0.001$ for trend). Mean changes after low-, medium-, and high-polyphenol olive oil consumption were −21 μg/d (95% CI, −67 to 23 μg/d), 18 μg/d (CI, −64 to 100 μg/d), and 316 μg/d (CI, 262 to 371 μg/d), respectively, for tyrosol and 20 μg/d (CI, −72 to 112 μg/d), 313 μg/d (CI, 217 to 409 μg/d), and 990 μg/d (CI, 840 to 1140 μg/d), respectively, for hydroxytyrosol.

Cardiovascular Risk Factors

All interventions increased HDL cholesterol levels, decreased the total cholesterol–HDL cholesterol ratio and triglyceride levels (**Table 3**), and improved the reduced glutathione–oxidized glutathione ratio (**Appendix Table 1**, available at www.annals.org). The medium- and high-polyphenol olive oil interventions decreased the LDL cholesterol–HDL cholesterol ratio. Conjugated dienes decreased after medium- and high-polyphenol olive oils. Hydroxy fatty acids and circulating oxidized LDL decreased after the high-polyphenol olive oil intervention. High-density lipoprotein cholesterol levels increased and total cholesterol–HDL cholesterol ratio decreased linearly with the phenolic content of the olive oil. We observed a linear decrease in oxidative biomarkers (conjugated dienes, hydroxy fatty acids, and circulating oxidized LDL) in association with the phenolic content of the olive oils (**Table 3**). We observed the greatest within-group effect on increasing HDL cholesterol levels and decreasing oxidative biomarkers after high-polyphenol olive oil consumption. We did not find any statistically significant period-by-treatment interaction

Table 3—Continued

Between-Group Differences		
High- vs. Low-Polyphenol Olive Oil	High- vs. Medium-Polyphenol Olive Oil	Medium- vs. Low-Polyphenol Olive Oil
0.036 (–0.07 to 0.12)	0.021 (–0.10 to 0.09)	0.024 (–0.11 to 0.09)
1.42 (–2.9 to 4.8)	0.83 (–3.9 to 3.7)	– 0.96 (–4.3 to 3.4)
0.014 (–0.07 to 0.12)	–0.041 (–0.09 to 0.07)	–0.050 (–0.13 to 0.05)
0.56 (–2.9 to 4.5)	–1.06 (–3.6 to 2.9)	–1.96 (–4.9 to 2.0)
0.029 (0.005 to 0.06)	0.025 (0.001 to 0.05)	0.006 (–0.01 to 0.02)
1.12 (0.2 to 2.2)	0.98 (0.06 to 1.9)	0.27 (–0.6 to 0.9)
–0.06 (–0.1 to –0.01)	–0.01 (–0.08 to 0.06)	–0.05 (–0.1 to 0.02)
–0.05 (–0.1 to 0.01)	–0.01 (–0.07 to 0.1)	–0.03 (–0.1 to 0.1)
0.002 (–0.07 to 0.1)	0.002 (–0.1 to 0.07)	0.004 (–0.1 to 0.08)
0.19 (–6 to 8)	–0.14 (–8 to 6)	0.36 (–8 to 7)
–0.23 (–0.4 to –0.1)	–0.16 (–0.3 to 0.09)	–0.12 (–0.4 to 0.03)
– 19 (–64 to 25)	–20 (–65 to 29)	–10 (–46 to 47)
–3.79 (–6.8 to –0.4)	–1.16 (–2.9 to 1.6)	–2.63 (–5.1 to –0.07)
–0.43 (–1.2 to 0.5)	–0.15 (–1.0 to 0.9)	–0.66 (–1.4 to 0.5)

term in any model. We observed a significant period effect ($P < 0.001$) for glutathione outcomes (mean postintervention values for the reduced–oxidized glutathione ratio were 5.0, 7.6, and 11.6 for intervention periods 1, 2, and 3, respectively). The period effect could be explained by many influences, such as a seasonal effect (24). However, the within-group increases in the glutathione outcomes were statistically significant and did not vary by olive oil intervention, showing that regardless of its phenolic content, olive oil contributes to improving the endogenous antioxidant status. We observed no differences among the centers. The changes followed a similar pattern overall (**Appendix Table 2**, available at www.annals.org).

DISCUSSION

A daily 25-mL dose of all types of olive oil, similar to the daily consumption recommended by the U.S. Food and Drug Administration (5), reduced lipid cardiovascular risk factors and improved glutathione antioxidant status (7). Daily consumption of high- and medium-polyphenol olive oil decreased oxidative damage on lipids. Consumption of olive oil with high phenolic content provided the greatest benefits by increasing HDL cholesterol levels and reducing the oxidative damage on lipids.

Low-carbohydrate, high-fat diets typically increase HDL cholesterol levels compared with high-carbohydrate, low-fat diets (25, 26). Our findings point out an independent effect of the phenolic compounds in olive oil: increasing HDL cholesterol levels. The mean increase in HDL cholesterol levels was 0.025 mmol/L (0.99 mg/dL), 0.032 mmol/L (1.22 mg/dL), and 0.045 mmol/L (1.74 mg/dL) for low-, medium-, and high-polyphenol olive oil, respectively. A 0.026-mmol/L (1-mg/dL) increase in HDL cholesterol levels has been associated with a 2% to 3% decrease in CHD risk (27). These risk decrements were based on data from cohort studies. Whether a 0.026-mmol/L (1-mg/dL) reduction in HDL cholesterol level due to olive oil and its phenolic content would lead to similar decreases in CHD risk has not been established. An increase in HDL cholesterol levels after consumption of high-polyphenol olive oil (11, 15) or other polyphenol-rich foods, such as pine bark extract (12), cocoa (13), and green tea (14), has been reported. Mechanisms by which dietary phenolic compounds increase HDL cholesterol levels are currently unclear.

Oxidative damage to lipids decreased in a linear manner with the phenolic content of the olive oil, particularly in markers that are directly associated with LDL oxidation. Oxidation of lipids present in LDL cholesterol (measured by conjugated dienes and hydroxy fatty acids) leads to a change in the lipoprotein conformation by which LDL cholesterol can better enter into the monocyte–macrophage system of the arterial wall and promote the atherosclerotic process (28). Alternatively, LDL cholesterol could also be directly oxidized through myeloperoxidase activity (29). The change in the conformation of the LDL cholesterol when oxidized is measured by circulating oxidized LDL levels in vivo, which are strong predictors of acute CHD in patients with CHD and the general population (30, 31). Although several studies report a direct relationship between oxidized LDL and CHD risk, the attributable CHD risk associated with a 1-U/L change in oxidized LDL is currently unknown. Oleate-rich LDL cholesterol is less susceptible to oxidative modification than linoleate-rich LDL cholesterol (32). Thus, mechanisms by which polyphenol-rich olive oil further reduces oxidative lipid damage would be linked to the combined effect of the phenolic and monounsaturated fatty acid content of the olive oil. The susceptibility of LDL cholesterol to oxidation depends not only on its fatty content but also on its antioxidants (for example, vitamin E and polyphenols) (33), which are protected by olive oil phenolic compounds (34). Recently, phenolic compounds bound to human LDL have been shown to increase in a dose-dependent manner with the phenolic content of the olive oil administered (35).

All olive oils improved the balance between reduced and oxidized glutathione. Reduced glutathione is a major mechanism for cellular protection against oxidative stress (36). Depletion of reduced glutathione precedes lipid oxidation and atherogenesis in vivo (37). Daily consumption of high-polyphenol olive oil did not compromise the endogenous antioxidant enzymes. In some studies, polyphe-

nol-rich food and antioxidant supplementation led to a decrease in these enzymes, presumably because of a lack of activation of their production by the decrease in free radicals (38, 39). This decrease in enzyme activity is considered to be a negative effect in situations of free radical production, such as exercise, in which the role of the antioxidant enzymes is crucial in counteracting oxidative damage (40). The absence of changes in plasma antioxidant vitamins suggests an independent effect of phenolic compounds from olive oil on oxidative damage. Changes in biomarkers were modest, which we expected with the administration of real-life doses of a single food, such as raw olive oil, that cannot be consumed in great quantities per day.

Our trial had strengths and limitations. A strength was the crossover design, which permitted the same participants to receive all olive oils and thereby minimized interferences with confounding variables. Our design, however, did not allow modeling the first- and second-order possible carryover effects. Our study had a high retention rate of enrolled participants (91%) with good adherence to the treatments. A limitation was the inability to assess potential interactions between olive oil and other diet components that might affect the generalizability of the results because of dietary differences among countries. The overall intercountry consistency of the results, however, contributes to the generalizability of the message. Measurements of dietary intake relied on self-reporting and were therefore subjective. A second limitation was in controlling whether participants fully substituted their habitually consumed raw fats with the assigned olive oil. A third limitation is the short duration of the intervention periods. Whether additional or different effects in the oxidative biomarkers would have been observed over longer periods is unknown. A longer study, however, could have impaired the adherence of the participants.

In conclusion, our study shows that olive oil is more than a monounsaturated fat. The polyphenol content of an olive oil can account for further benefits on HDL cholesterol levels and oxidative damage in addition to those from its monounsaturated fatty acid content. Our study provides evidence to recommend the use of polyphenol-rich olive oil, that is, virgin olive oil, as a source of fat to achieve additional benefits against cardiovascular risk factors.

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Appendix Table 1. **Changes in Glutathione Balance after Olive Oil Interventions***

Variable	Olive Oil Intervention						*P* Value†	Between-Group Differences		
	Low-Polyphenol Olive Oil (*n* = 182)		Medium-Polyphenol Olive Oil (*n* = 184)		High-Polyphenol Olive Oil (*n* = 183)			High- vs. Low-Polyphenol Olive Oil	High- vs. Medium-Polyphenol Olive Oil	Medium- vs. Low-Polyphenol Olive Oil
	Postintervention	Change from Preintervention	Postintervention	Change from Preintervention	Postintervention	Change from Preintervention				
Reduced glutathione, *μmol/L*	5.85	0.28 (0.2 to 0.3)	5.87	0.28 (0.2 to 0.3)	5.88	0.31 (0.3 to 0.4)	0.57	0.035 (−0.04 to 0.1)	0.012 (−0.1 to 0.1)	−0.024 (−0.1 to 0.1)
Oxidized glutathione, *μmol/L*	0.84	−0.11 (−0.14 to −0.09)	0.82	−0.14 (−0.2 to −0.1)	0.83	−0.12 (−0.15 to −0.1)	0.60	0.016 (−0.04 to 0.03)	0.010 (−0.03 to 0.09)	−0.025 (−0.04 to 0.01)
Reduced–oxidized glutathione ratio	7.91	1.79 (1.2 to 2.3)	8.02	1.94 (1.4 to 2.8)	8.16	1.59 (0.9 to 2.3)	0.58	−0.56 (−1.4 to 0.4)	−0.19 (−0.9 to 0.9)	0.42 (−0.7 to 1.1)

* Log-transformed. Values are adjusted means (95% CI) estimated from a linear mixed model with terms for period, treatment, and center as fixed effects; participant as a random effect; and baseline values and age as covariates.
† For linear trends across oils.

Appendix Table 2. **Changes after Olive Oil Interventions by Center***

Variable	Center 1 (Barcelona, Spain)	Center 2 (Copenhagen, Denmark)	Center 3 (Kuopio, Finland)	Center 4 (Bologna, Italy)	Center 5 (Postdam, Germany)	Center 6 (Berlin, Germany)
Low-polyphenol olive oil						
HDL cholesterol level						
mmol/L	0.024 (−0.03 to 0.07)	0.039 (−0.04 to 0.11)	0.028 (−0.03 to 0.08)	0.013 (−0.04 to 0.07)	0.033 (−0.03 to 0.09)	0.005 (−0.03 to 0.05)
mg/dL	0.92 (−1.1 to 2.9)	1.51 (−1.4 to 4.2)	1.08 (−1.1 to 3.2)	0.56 (−1.4 to 2.5)	1.28 (−1.1 to 3.7)	0.22 (−1.3 to 1.8)
Total cholesterol–HDL cholesterol ratio	0.05 (−0.12 to 0.19)	0.06 (−0.14 to 0.27)	−0.09 (−0.26 to 0.10)	−0.07 (−0.41 to 0.3)	−0.34 (−0.56 to 0.02)	0.10 (−0.12 to 0.3)
Oxidized LDL, *U/L*	3.22 (−3.1 to 9.4)	2.93 (−7.2 to 13)	−0.56 (−4.6 to 3.3)	2.39 (−4.6 to 8.9)	−5.38 (−12.9 to 0.7)	1.86 (−2.6 to 7.4)
Conjugated dienes, *nmol/mol of cholesterol*	−0.12 (−0.4 to 0.9)	−0.19 (−0.6 to 15)	−0.03 (−0.5 to 0.3)	0.04 (−0.2 to 0.3)	0.05 (−0.2 to 0.3)	0.26 (−0.2 to 0.5)
Hydroxy fatty acids, *nmol/L*†	11 (110 to 106)	46 (−89 to 162)	35 (−74 to 141)	−51 (−208 to 140)	−69 (−164 to 35)	−131 (−265 to 18)
Medium-polyphenol olive oil						
HDL cholesterol level						
mmol/L	0.035 (−0.002 to 0.07)	0.093 (−0.02 to 0.2)	0.014 (−0.05 to 0.07)	0.004 (−0.06 to 0.06)	0.036 (−0.01 to 0.08)	0.013 (−0.04 to 0.06)
mg/dL	1.38 (−0.08 to 2.9)	3.61 (−0.8 to 7.9)	0.56 (−1.9 to 2.6)	0.18 (−2.2 to 2.5)	1.38 (−0.6 to 3.3)	0.50 (−1.4 to 2.3)
Total cholesterol–HDL cholesterol ratio	−0.11 (−0.27 to 0.03)	−0.12 (−0.45 to 0.07)	−0.02 (−0.22 to 0.24)	0.13 (−0.16 to 0.4)	−0.25 (−0.38 to −0.05)	−0.06 (−0.2 to 0.09)
Oxidized LDL, *U/L*	−1.47 (−6.6 to 3.2)	−2.32 (−11.1 to 4.9)	−1.43 (−7.3 to 2.1)	−0.63 (−11.3 to 9.2)	−1.98 (−6.9 to 2.6)	−2.32 (−6.4 to 1.6)
Conjugated dienes, *nmol/mol of cholesterol*	0.39 (−0.7 to 1.2)	−2.3 (−3.1 to −0.9)	0.29 (−0.8 to 1.5)	−1.4 (−2.1 to −0.2)	−1.6 (−2.4 to −0.7)	−1.6 (−3.4 to −0.2)
Hydroxy fatty acids, *nmol/L*†	−5 (−136 to 125)	−12 (−120 to 150)	− 26 (−142 to 93)	−175 (−296 to −47)	19 (−81 to 115)	−73 (−194 to 33)
High-polyphenol olive oil						
HDL cholesterol level						
mmol/L	0.055 (0.009 to 0.1)	0.034 (−0.04 to 0.11)	0.047 (−0.005 to 0.1)	0.068 (0.007 to 0.13)	0.069 (0.01 to 0.12)	0.016 (−0.05 to 0.08)
mg/dL	2.13 (0.36 to 3.8)	1.32 (−1.2 to 4.6)	1.82 (−0.2 to 3.9)	2.64 (0.3 to 5.2)	2.68 (0.6 to 4.5)	0.62 (−1.8 to 3.1)
Total cholesterol–HDL cholesterol ratio	−0.10 (−0.24 to 0.04)	0.01 (−0.18 to 0.24)	−0.27 (−0.45 to −0.12)	−0.16 (−0.54 to 0.2)	−0.26 (−0.40 to −0.11)	−0.36 (−0.7 to −0.01)
Oxidized LDL, *U/L*	−2.94 (−7.1 to 0.9)	0.49 (−7.8 to 8.4)	−1.67 (−5.1 to 1.6)	−8.03 (−16.8 to −2.3)	−7.41 (−15.0 to −3.1)	−2.09 (−6.8 to 0.65)
Conjugated dienes, *nmol/mol of cholesterol*	−0.56 (−1.8 to 0.3)	−1.4 (−2.8 to −0.2)	−0.98 (−2.8 to 0.4)	−0.93 (−1.8 to 0.05)	− 0.22 (−1.1 to 0.7)	−1.3 (−2.0 to −0.2)
Hydroxy fatty acids, *nmol/L*†	−20 (−116 to 79)	−101 (−232 to 22)	−57 (−201 to 65)	−104 (−209 to −9)	−21 (−95 to 56)	−85 (−191 to −6)

* Values are adjusted means (95% CI) estimated from a linear mixed model with terms for period, treatment, and center as fixed effects; participant as a random effect; and baseline values and age as covariates. HDL = high-density lipoprotein; LDL = low-density lipoprotein.
† Log-transformed.