Olive oil polyphenols decrease LDL concentrations and LDL atherogenicity in men in a randomized controlled trial

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Footnotes to the title

i. Existence of OSM

Supplemental Table 1 and **Supplemental Figure 1** are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

ii. List of abbreviations

HPCOO: high-polyphenol content olive oil

LDL: low-density lipoprotein

LPCOO: low-polyphenol content olive oil

LPL: lipoprotein lipase

VLDL: very low-density lipoproteins

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ABSTRACT

Background. Olive oil polyphenols have shown protective effects on cardiovascular risk factors. Their consumption decreased oxidative stress biomarkers and improved some features of the lipid profile. However, their effects on low-density lipoprotein (LDL) concentrations in plasma and LDL atherogenicity have not yet been elucidated.

Objective. Our objective was to assess whether the consumption of olive oil polyphenols could decrease LDL concentrations (measured as apolipoprotein B-100 levels and the total number of LDL particles) and atherogenicity (the number of small LDL particles and LDL oxidizability) in humans.

Methods. The study was a randomized, cross-over, controlled trial with 25 healthy European males, aged 20-59, in the context of the EUROLIVE Study. Volunteers ingested 25 mL/day of low-polyphenol content olive oil (LPCOO, 366 mg/kg) or high-polyphenol content olive oil (HPCOO, 2.7 mg/kg), raw, for 3 weeks. Interventions were preceded by 2-week washout periods. Effects of olive oil polyphenols on plasma LDL concentrations and atherogenicity were determined in the sample of 25 men. Effects on the lipoprotein lipase (*LPL*) gene expression were assessed in another sample of 18 men from the EUROLIVE Study.

Results. Plasma apolipoprotein B-100 concentrations and the number of total and small LDL particles decreased (mean ± SD) 5.94±16.6%, 11.9±12.0% and 15.3±35.1%, respectively, from baseline after the HPCOO intervention. These changes differed significantly from those after the LPCOO intervention, which were increases of 6.39±16.6%, 4.73±22.0% and 13.6±36.4% from baseline, and were significant (P<0.03). LDL oxidation lag-time increased 5.0±10.3% from baseline after the HPCOO intervention, significantly only relative to pre-intervention values (P=0.038). *LPL* gene expression tended to increase 26% from baseline after the HPCOO intervention (P=0.08), and did not change after the LPCOO intervention.

Conclusions. The consumption of olive oil polyphenols decreased plasma LDL concentrations and LDL atherogenicity in healthy, young men.

Clinical Trial Registration Number: ISRCTN09220811.

Keywords: olive oil polyphenols, randomized clinical trial, low-density lipoproteins, apolipoprotein B-100, LDL particle number, small LDL particles, LDL oxidation, lipoprotein lipase, healthy individuals

INTRODUCTION

Virgin olive oil consumption protects against the development of cardiovascular diseases (1) due to its monounsaturated fatty acids (2) and polyphenols (3). To assess the beneficial properties of olive oil polyphenols on lipid profile and oxidation, the EUROLIVE Study (The Effect of Olive Oil on Oxidative Damage in European Population) was performed. This project showed that the consumption of polyphenol-rich olive oil was beneficial for the oxidative status of low-density lipoproteins (LDLs). Olive oil polyphenols dose-dependently decreased the circulating levels of oxidized LDLs, C18 hydroxy-fatty acids, and uninduced conjugated dienes (4). In previous studies, our group also observed that olive oil polyphenols induced changes in LDL composition: the consumption of olive oil polyphenols increased LDL content of oleic acid, vitamin E, and olive oil phenolic compounds (5).

However, effects of olive oil polyphenols on LDL concentrations, LDL pro-atherogenic properties, such as LDL size, and the expression of some key genes related to LDL concentrations, such as lipoprotein lipase, have not been determined *in vivo* in humans. Thus, our objective was to determine whether the consumption of a polyphenol-rich olive oil would be able to improve all these properties.

METHODS

Study participants

Our study population is a sub-sample of the EUROLIVE Study. It was a parallel, crossover, randomized controlled trial performed with 180 healthy men, aged 20-59, from six different European cities. The purpose of the study was to determine the effects of olive oil polyphenols on lipid profile and oxidative stress biomarkers. Local institutional ethics committees approved the protocol of the study, whose details have been previously described (4). In all cases, written informed consent was provided by the participants before joining the trial. The protocol of the EUROLIVE Study is registered with the International Standard Randomized Controlled Trial Number ISRCTN09220811 (www.controlled-trials.com).

We studied the effects of olive oil polyphenols on the participants' lipid profile, apolipoprotein B-100 levels, LDL particle distribution and LDL oxidizability *ex vivo* in a random sub-sample of 25 EUROLIVE volunteers of three centers (9 from Potsdam, Germany; 9 from Kuopio, Finland; and 7 from Barcelona, Spain). We assessed the polyphenols effects on the expression of the lipoprotein lipase gene (*LPL*) in another random sub-sample of 18 volunteers of the EUROLIVE Study, 8 of which are also present in the first subsample of individuals. Blood samples were taken from fasting participants before and after dietary interventions with high-polyphenol content olive oil (HPCOO, a natural virgin olive oil with 366 mg/kg of polyphenols) and low-polyphenol content olive oil (LPCOO, a refined olive oil with 2.7 mg/kg of polyphenols). Polyphenols become degraded during the refinement process and, thus, the refined olive oil presented a lower phenolic content. Composition of both olive oils was identical, except for their polyphenol content (4). Samples were stored at -80ºC until the present experiments commenced. No thaw-freeze cycles were applied to the samples before the present work.

As shown in the crossover design (**Supplemental Figure 1**), volunteers followed 3-week intervention periods in which they ingested 25 mL/day of raw olive oil, distributed among meals. Participants were taught to replace other dietary fats with olive oil. Intervention periods

were preceded by 2 weeks of washout, during which olive oil, olives, and antioxidant-rich foods were avoided. A 2-week washout period was enough to eliminate olive oil polyphenols between interventions, considering the half-life of the sum of the main olive oil phenolic compounds (8 h) (6). The washout period was also enough if the half-life of the LDL particle is considered (3 days) (7). A more detailed description of the diet of the participants has been previously published (4,8).

Study measurements

Diet adherence, physical activity and oxidative status of the volunteers

Diet adherence was determined through the 24-hour urinary excretion of tyrosol and hydroxytyrosol. These are the two main phenolic compounds in olive oil and were considered as biomarkers of compliance of the olive oil ingested. These compounds were determined by gas chromatography and mass spectrometry, as previously described (6).

Participants' diet was controlled through a 3-day dietary record. Diet control was performed at the beginning of the study and after each dietary intervention. Volunteers were asked to maintain their usual diet during the whole study.

Physical activity of the volunteers was also measured. It was calculated at the beginning and the end of the study, using a validated Minnesota Leisure Time Physical Activity Questionnaire (4).

Oxidative status of the volunteers was also assessed by means of the determination of different oxidative biomarkers (oxidized LDLs, C18 hydroxy-fatty acids, and reduced ascorbic acid and dehydroascorbic acid), as previously reported (4).

Lipid profile and apolipoprotein B-100 determination

We performed the lipid profile and apolipoprotein B-100 analyses in an ABX Pentra 400 autoanalyzer (Horiba Diagnostics). Triglycerides and total cholesterol were measured using enzymatic methods (ABX Pentra Triglycerides CP, and ABX Pentra Cholesterol CP, respectively, from Horiba Diagnostics), HDL cholesterol was determined by the Accelerator Selective Detergent method (ABX Pentra HDL Direct CP, Horiba Diagnostics), and

apolipoprotein B-100 levels were measured by immunoturbidimetry (ABX Pentra Apo B, Horiba Diagnostics). The inter-assay coefficients of variation of the previous determinations are the following: 1.48% for triglycerides, 1.54% for total cholesterol, 3.34% for HDL cholesterol and 1.95% for apolipoprotein B. We also calculated LDL cholesterol concentrations by the Friedewald formula, whenever triglycerides were below 300 mg/dL.

LDL particle analyses

We determined LDL cholesterol concentrations (directly measured) and the number of total LDL particles, total very low-density lipoprotein (VLDL) particles, small LDL particles, large LDL particles, small VLDL particles and medium+large VLDL particles by nuclear magnetic resonance spectroscopy. Values were calculated from the measured amplitudes of the nuclear magnetic resonance signals of the lipid methyl groups in the samples (9). These analyses were performed in a Vantera Clinical Analyzer (LipoScience Inc.) and could only be performed in the available samples of 20 of the volunteers. The technique presents an inter-assay coefficient of variation of 5.30% for the determination of total LDL particle number, for the range of low values (typical in healthy individuals), under the most unfavorable conditions.

Analyses of LDL resistance against oxidation

Before the analyses, LDLs were isolated from K_2-EDTA plasma of the volunteers by density gradient ultracentrifugation (10) and stored at -80ºC in 2.5% sucrose until the experiments.
In the isolated LDL samples, we determined LDL resistance against oxidation by measuring the accumulation of Cu^{2+}-induced conjugated dienes in the lipoprotein. First, we dialyzed the isolated LDLs with PBS to discard possible contaminants. We then incubated the dialyzed LDLs (at a final concentration of 10 mg/dL of LDL cholesterol) in the presence of $CuSO_4$ to induce the oxidation reaction (at a final concentration of 5 µM $CuSO_4$), at 37ºC during 4 hours. During the incubation, absorbance at 234 nm was determined each 3 minutes, in an INFINITE M200 reader (Tecan Group Ltd.).

Consecutive measurements of absorbance defined the LDL oxidation kinetic curves. For each of these curves, we calculated: 1) the lag time (the time when maximal oxidation started, in minutes) and 2) the oxidation rate (the slope of the kinetic curve at peak velocity, expressed as the increase in the concentration –mM– of conjugated dienes per minute and mg of LDL cholesterol), as previously described (5). All determinations were performed in duplicates. We used an LDL pool of healthy volunteers as inter-assay control. The inter-assay coefficients of variation of the previous determinations are the following: 2.89% for lag-time, and 4.77% for oxidation rate.

LPL gene expression analyses

First, we isolated total RNA from peripheral blood mononuclear cells by means of a liquid-liquid method. We then checked RNA purity and integrity and converted RNA to complementary DNA. Afterwards, *LPL* gene expression was quantified using a real-time polymerase chain reaction in TaqMan® Low Density microfluidic cards (Applied Biosystems, Life Technologies). Four replicates of each RNA sample were used in the experiments. Data were analyzed using the Sequence Detection System software (SDS 2.1., Applied Biosystems, Life Technologies), following the manufacturer's instructions. *LPL* gene expression was finally calculated by the relative quantification method (using the $2^{-\Delta\Delta Ct}$ formula). This technique presents an inter-assay coefficient of variation of 0.98%, calculated in the control pool for the housekeeping gene (*GAPDH*).

Sample size calculation

Biochemical determinations. A sample size of 25 individuals allowed a $\geq$ 80% power to detect significant differences of 4 mg/dL of apolipoprotein B-100 levels between both olive oil interventions, considering a 2-sided type I error of 0.05. Calculations were made using previous data of our group, considering the standard deviation of apolipoprotein B-100 levels in healthy volunteers.

158 *LPL gene expression*. A sample size of 18 individuals allowed a ≥ 80% power to detect
159 significant differences of 0.5 units of $\log_2$ ratio relative quantification in the expression of a
160 reference gene (human interferon-γ –*IFNG*–) between both olive oil interventions, considering a
161 2-sided type I error of 0.05. Calculations were made using previous data of our group,
162 considering the standard deviation of *IFNG* gene expression in healthy volunteers (8).
163
164 *Statistical analyses*
165 We confirmed the normal distribution of the continuous variables by normal probability plots
166 and the Shapiro-Wilk test. To take into consideration the inter-individual variability of the
167 parameters studied, we investigated possible differences in baseline values between the two
168 interventions by a paired T-test, and we expressed the differences between baseline and post-
169 intervention as percentage changes. These percentage changes were calculated as follows: (Post-
170 intervention value – Baseline value)/Baseline value x 100.
171 We evaluated the effect of olive oil interventions compared with their baseline, and the
172 differences between treatments, in a mixed linear model. We considered the interaction between
173 treatment (LPCOO or HPCOO) and the pre-post intervention differences as the term of interest,
174 and we included the following variables as adjustment variables: study period, age and country
175 of origin of the volunteers. Moreover, taking into consideration the fact that we performed
176 repeated measurements in the study, due to the study design (cross-over), we introduced the
177 individual as a factor of random effect in the model. We checked the period-by-treatment
178 interactions to discard possible carry-over effects. We tested the relationships among variables
179 through Pearson's correlation analyses.
180 In all cases, we considered significant any *P* value below 0.05. All the previous analyses were
181 performed with R Software, version 3.0.2 (R: A language and environment for statistical
182 computing. R Foundation for Statistical Computing, Vienna, Austria) and with SPSS Software,
183 version 18.0 (IBM Corp.). Mixed models were adjusted using the lme4 package in R Software
184 (11).

RESULTS

General characteristics of the participants

Supplemental Figure 1 shows the design of the study. No significant differences in baseline values were found amongst our sub-samples and the total EUROLIVE study population (**Table 1**). Dietary patterns and energy expenditure in leisure-time physical activity did not change either. As previously reported (4), participants' compliance was correct, as the urinary excretion of tyrosol and hydroxytyrosol were 9-fold and 18-fold higher, respectively, after the HPCOO intervention, relative to baseline. These increases were significantly greater than those after the LPCOO intervention, which were 0.3-fold and 0.7-fold higher, respectively, and were significant ($P<0.001$, in both cases). Lipid profile of the volunteers (triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol calculated by the Friedewald formula) did not differ between the intervention periods (data not shown).

The consumption of olive oil polyphenols improved the oxidative status of the volunteers. We observed significant decreases in the concentrations of oxidized LDL and C18 hydroxy-fatty acids, equivalent to the ones previously reported (4). The ratio between the reduced and the oxidized forms of ascorbic acid increased significantly after the HPCOO intervention when compared with baseline values ($P=0.018$). Data for these and other variables are in the **Supplemental Table 1**.

Olive oil polyphenols decreased LDL concentrations

We directly determined LDL concentrations by three different approaches: directly-measured LDL cholesterol, apolipoprotein B-100 levels, and the number of total LDL particles. As shown in **Figure 1A**, after the HPCOO intervention, apolipoprotein B-100 levels and the number of total LDL particles decreased 5.9±16.6% and 11.9±12.0%, respectively, relative to baseline values. These parameters increased 6.4±16.6% and 4.7±22.0%, respectively, after the LPCOO intervention. Both decreases in apolipoprotein B-100 levels and the number of total LDL

particles after the HPCOO intervention were significant compared to the LPCOO intervention (P=0.004 and P=0.013, respectively).

Olive oil polyphenols decreased the number of small LDL particles

As observed in **Figure 1B**, the number of small LDL particles decreased 15.3±35.1% after the HPCOO intervention relative to baseline values. However, after the LPCOO intervention, it increased 13.6±36.4% relative to baseline. The decrease in the number of small LDL particles after the HPCOO intervention was statistically significant when compared to the LPCOO intervention (P=0.029). No significant changes in the number of large LDL particles were found after either intervention.

High adherence to the consumption of olive oil polyphenols (reflected as an increase in urinary tyrosol excretion) and improvements in the oxidative status after the HPCOO intervention (reflected as an increase in the reduced/oxidized ascorbic acid ratio) correlated with greater decreases of small LDL particle numbers (r=-0.53 and r=-0.66, P=0.042 and P=0.005, respectively).

Olive oil polyphenols increase the resistance of LDL against oxidation

As seen in **Figure 1C**, the LDL oxidation lag-time increased 5.01±10.3% after the HPCOO intervention, and 3.17±19.1% after the LPCOO intervention. After the HPCOO intervention, lag-time values were significantly higher only compared to baseline (P=0.038). Changes in lag-time did not differ between interventions.

LDL oxidation rate did not change significantly after either intervention.

Changes in the LPL gene expression

LPL gene expression tended to increase 26%, relative to baseline, after the HPCOO intervention (P=0.08) (**Figure 1D**). It did not change significantly after the LPCOO intervention. Changes in this variable did not differ between the interventions.

DISCUSSION

The present work shows that a 3-week consumption of olive oil polyphenols decreases LDL concentrations and LDL atherogenicity *in vivo*. To date, this has been one of the most considerable decreases in the number of total and small LDL particles that has been reported in humans due to dietary bioactive compounds.

LDL cholesterol concentrations are directly and strongly associated with coronary heart disease risk (12). This association justifies their determination in most epidemiological and interventional cardiovascular studies. Although the direct quantification of LDL cholesterol is possible, in several studies LDL cholesterol concentrations are calculated using indirect equations such as the Friedewald formula (an approximation based on triglycerides, total and HDL cholesterol) (13). These formulae may underestimate LDL cholesterol concentrations when they are compared to direct measurements, particularly in non-pathological ranges of triglyceride values. Thus, the direct and more precise determinations of apolipoprotein B-100 levels or the total number of LDL particles are recommended (13). Moreover, apolipoprotein B-100 levels and the total number of LDL particles are more accurate than LDL cholesterol to quantify cardiovascular risk in high-risk patients (e.g., individuals who have suffered premature coronary events or with metabolic syndrome) (14) and both are directly related with a greater incidence of cardiovascular events (15,16).

In this context, we directly assessed the effects of olive oil polyphenols on LDL concentrations. The consumption of olive oil polyphenols was significantly associated with a decrease in apolipoprotein B-100 levels and total number of LDL particles (5.9% and 11.9%, respectively). Similar decreases have been reported after other antioxidant-rich dietary approaches. Apolipoprotein B-100 levels decreased after consuming a hazelnut-enriched diet (17) and a concentrated red grape juice (18). The number of LDL particles also decreased after a long-term consumption of a high-fiber oat cereal (19).

The decrease in LDL concentrations may be explained through an improvement in the systemic oxidative status, or an increase in the gene expression of lipoprotein lipase, as observed in our

study. Three different mechanisms may be involved in this hypothesis. First, oxidative stress states are associated with increased LDL concentrations, especially due to an increased number of small LDL particles (20). An improved oxidative status due to the consumption of olive oil polyphenols may counteract increases in LDL concentrations by decreasing small LDL particles, as we reported. Second, increases in the expression of lipoprotein lipase may help the organism to decrease the levels of triglyceride-rich lipoproteins (such as LDL), since lipoprotein lipase is the main enzyme involved in the removal of triglycerides from the blood and it presents some LDL receptor activity (21). Finally, improvements in general oxidative status have been associated with a better activity of lipoprotein lipase (22,23).

LDLs are more atherogenic when they are small and dense because: 1) lipoprotein lipase does not recognize them properly; 2) they easily traverse the endothelial barrier; and 3) they are easily oxidized in the sub-endothelial space (24). They are thus associated with early atherosclerosis and high cardiovascular risk (25,26) and are directly related with a greater incidence of cardiovascular events in some studies (16). In our data, the number of small LDL particles decreased by 15.3% after the consumption of olive oil polyphenols. This decrease was greater when there was a higher adherence to the HPCOO intervention.

The decrease in the number of small LDL particles may be explained by an improvement in oxidative status. As we previously commented, a better oxidative status may result in a lower production of small LDLs, since the number of small LDL particles increases when the levels of oxidative stress are higher (20). This hypothesis concurs with the significant correlation between the decrease in the number of small LDL particles and the increase in the ratio between reduced and oxidized ascorbic acid in our data. In addition, similar effects have also been observed after other antioxidant-rich dietary interventions, such as a Mediterranean diet supplemented with nuts (27), and the consumption of a polyphenol-rich supplement made from freeze-dried strawberries (28).

LDL oxidation is considered to be a trigger for the biochemical processes that take place in the sub-endothelial space and lead to the formation of an atherosclerotic plaque (29). In particular, LDL resistance against oxidation *ex vivo* predicts artery dysfunction, even when adjusted for

other cardiovascular risk factors (30). In our study, olive oil polyphenols increased LDL resistance against oxidation. Increases in LDL antioxidant defenses after the consumption of olive oil polyphenols justify this beneficial effect (5). Our results confirm the decrease in this LDL atherogenic trait after consuming virgin olive oil (31), and after following an antioxidant-rich, vegetarian diet (32).

One of the strengths of the present study was its crossover design, which reduced interferences from confounding variables. We administered real-life doses of a food that cannot be consumed in great quantities. Thus, some of the changes observed were modest. However, the LDL-related traits that we have described in this work help to explain residual cardiovascular risk (33) and have been directly related to a greater incidence of cardiovascular diseases (15,16). Therefore, even modest decreases in these parameters may be protective against the development of cardiovascular events. A possible limitation of our work was that we performed systemic and gene expression analyses in two different sub-samples of individuals. However, both sub-groups did not present significantly different baseline characteristics and were representative of the whole EUROLIVE population. Other limitations of the study are that the amount of polyphenols equivalent to that provided by the HPCOO intervention could have proceeded from other food types, or that synergistic effects between olive oil polyphenols and other olive oil components on LDL biology have not yet been identified.

In conclusion, the consumption of olive oil polyphenols decreased LDL concentrations, directly measured as the levels of apolipoprotein B-100 and the total number of LDL particles. The consumption of olive oil polyphenols also decreased LDL atherogenicity, reflected in the lower number of small LDL particles and enhanced LDL resistance against oxidation. An improved oxidative status, and an increased gene expression of lipoprotein lipase, may contribute to explain these changes. These data support the previous evidence indicating that olive oil polyphenols can contribute highly to the control of cardiovascular risk.

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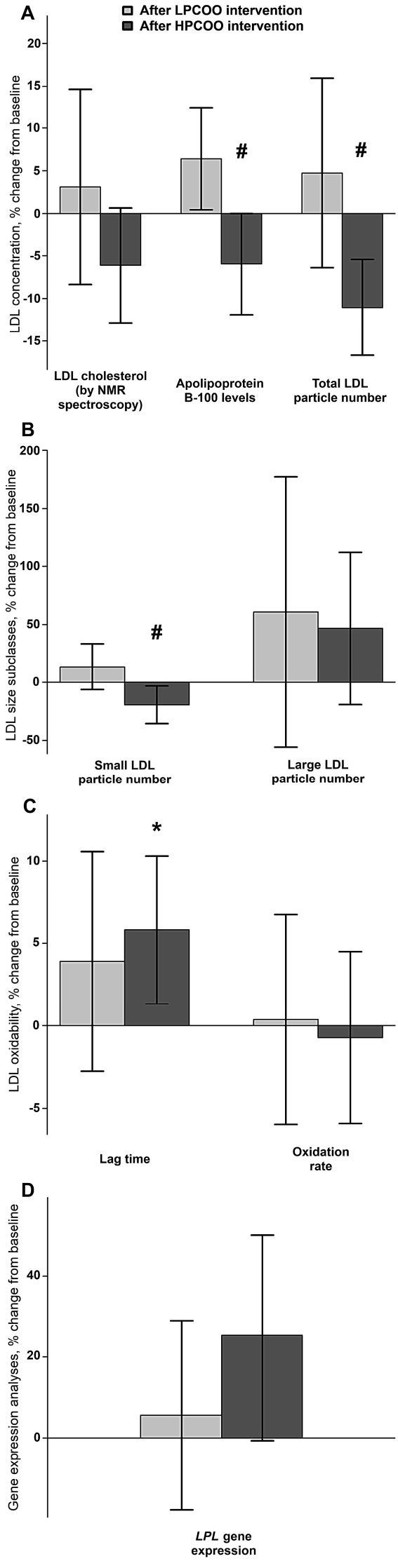
TABLES

Table 1

Baseline characteristics and plasma lipid profile of the healthy, male participants, in the two sub-samples of volunteers of the study, compared with the whole EUROLIVE Study population[1]

	Biochemical analyses subsample *n*=25	Gene expression subsample *n*=18	EUROLIVE study population *n*=180
Age, years	32.3 ± 11.2	36.9 ± 12.3	33.2 ± 11.0
Weight, kg	78.2 ± 10.9	78.1 ± 10.9	76.4 ± 10.5
Height, m	1.79 ± 0.08	1.79 ± 0.08	1.79 ± 0.07
Total cholesterol, mmol/L	4.5 ± 1.2	4.8 ± 0.9	4.5 ± 1.1
LDL cholesterol, mmol/L	2.6 ± 1.0	2.8 ± 0.9	2.5 ± 0.9
HDL cholesterol, mmol/L	1.3 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
Triglycerides, mmol/L	1.1 (0.8 to 1.4)	1.2 (0.9 to 1.5)	1.1 (0.8 to 1.5)

[1] Values are means ± SDs, except triglycerides –median (1st quartile to 3rd quartile)–.


A
After LPCOO intervention
After HPCOO intervention
LDL concentration, % change from baseline
20
15
10
5
0
-5
-10
-15
#
#
LDL cholesterol (by NMR spectroscopy)
Apolipoprotein B-100 levels
Total LDL particle number
B
LDL size subclasses, % change from baseline
200
150
100
50
0
-50
#
Small LDL particle number
Large LDL particle number
C
LDL oxidability, % change from baseline
10
5
0
-5
*
Lag time
Oxidation rate
D
Gene expression analyses, % change from baseline
40
20
0
LPL gene expression

FIGURES

Figure 1

Changes from baseline values of directly measured LDL concentrations (determined as LDL cholesterol, apolipoprotein B-100 levels and total LDL particle number) (A), LDL size distribution (B), LDL oxidizability (C), and lipoprotein lipase gene expression (D), in healthy European males, aged 20-59, after a 3-week consumption of a low-polyphenol content olive oil or a high-polyphenol content olive oil. Values are means ± SEMs of the percentage changes of the variables, relative to baseline values. n=25 (for A, B and C), n=18 (for D). *: Significant change from baseline, $P<0.05$. #: Different from LPCOO intervention, $P<0.05$. HPCOO, high-polyphenol content olive oil. LPCOO, low-polyphenol content olive oil.

ONLINE SUPPORTING MATERIAL (OSM)

SUPPLEMENTAL TABLE 1

Post-intervention values and changes (in percentage, relative to baseline values) of all the parameters of the study, in healthy European males, aged 20-59, after a 3-week consumption of a low-polyphenol content olive oil or a high-polyphenol content olive oil.[1].

Variable	LPCOO intervention		HPCOO intervention	
	Post-intervention	Change (%)	Post-intervention	Change (%)
BIOMARKERS OF COMPLIANCE				
Urinary excretion of tyrosol, µg/day	113 ± 240	34.8 ± 177	389 ± 195	885 ± 727*#
Urinary excretion of hydroxytyrosol, µg/day	125 ± 125	72.5 ± 251	1190 ± 892	1760 ± 1990*#
OXIDATIVE STRESS BIOMARKERS IN PLASMA				
Plasma oxidized LDL, U/L	42.6 ± 20.7	13.3 ± 41.1	40.4 ± 16.1	-7.48 ± 15.1#
Plasma C18 hydroxy-fatty acids, nmol/L	1.33 ± 0.55	9.36 ± 28.4	1.24 ± 0.32	-7.10 ± 25.1*#
Reduced vs. oxidized ascorbic acid ratio in plasma	1.13 ± 0.14	-1.02 ± 12.8	1.17 ± 0.17	2.49 ± 7.79*
PLASMA LDL CONCENTRATIONS				
LDL cholesterol (Friedewald formula), mg/dL	99.3 ± 44.9	5.26 ± 20.6	97.2 ± 43.6	-4.45 ± 15.4
Apolipoprotein B-100, mg/dL	76.3 ± 33.5	6.39 ± 16.6	72.5 ± 33.1	-5.94 ± 16.6*#
LDL cholesterol (NMR spectroscopy), mg/dL	87.2 ± 36.3	3.11 ± 22.6	86.4 ± 38.8	-8.36 ± 13.5
NMR LIPOPROTEIN ANALYSES IN PLASMA				
Total LDL particle number, nmol/L	892 ± 385	4.73 ± 22.0	831 ± 423	-11.9 ± 12.0*#
Small LDL particle number, nmol/L	341 ± 173	13.6 ± 36.4	283 ± 155	-15.3 ± 35.1*#
Large LDL particle number, nmol/L	217 ± 177	65.3 ± 199	234 ± 169	41.6 ± 146
Total VLDL particle number, nmol/L	48.9 ± 21.0	4.16 ± 51.0	47.6 ± 17.8	3.95 ± 49.7
Small VLDL particle number, nmol/L	32.9 ± 15.1	26.0 ± 78.3	32.4 ± 13.3	25.8 ± 108
Medium+large VLDL particle number, nmol/L	17.0 ± 11.2	-18.7 ± 43.9	15.7 ± 6.34	-5.95 ± 38.9
LDL OXIDIZABILITY ANALYSES (IN ISOLATED LDLs)				
Lag-time, minutes	50.4 ± 8.27	3.17 ± 19.1	50.4 ± 8.27	5.01 ± 10.3*
Oxidation rate, $mmol \cdot L^{-1} \cdot min^{-1} \cdot mg$ LDL-$cholesterol^{-1}$	2.78 ± 1.40	0.02 ± 17.6	2.62 ± 1.38	-0.21 ± 13.4
LDL-RELATED GENE EXPRESSION ANALYSES				
Lipoprotein lipase gene expression (log_2 ratio relative quantification)	4.87 ± 5.82	5.60 ± 49.3	6.32 ± 8.52	25.5 ± 51.4

1. Values are mean ± SDs. *n*=25 (except for LDL-related gene expression analyses, *n*=18). *: Significant change from baseline (*P*<0.05). #: Different from LPCOO intervention (*P*<0.05). HPCOO, high-polyphenol content olive oil. LPCOO, low-polyphenol content olive oil. NMR, nuclear magnetic resonance.

ONLINE SUPPORTING MATERIAL (OSM)

SUPPLEMENTAL FIGURE 1

Intervention study design. Randomized, controlled, cross-over trial in healthy European males, aged 20-59, to determine the effects of 3 weeks of consumption of high-polyphenol content olive oil or low-polyphenol content olive oil.