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Olive oil polyphenols enhance the expression of cholesterol efflux related genes *in vivo* in humans. A randomized controlled trial

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Abstract

Both oleic acid and polyphenols have been shown to increase high-density lipoprotein (HDL) cholesterol and to protect HDL from oxidation, a phenomenon associated with a low cholesterol efflux from cells. Our goal was to determine whether polyphenols from olive oil could exert an *in vivo* nutrigenomic effect on genes related to cholesterol efflux in humans. In a randomized, controlled, cross-over trial, 13 pre/hypertensive patients were assigned 30 ml of two similar olive oils with high (961 mg/kg) and moderate (289 mg/kg) polyphenol content. We found an increase in ATP binding cassette transporter-A1, scavenger receptor class B type 1, peroxisome proliferator-activated receptor (PPAR)BP, PPARα, PPARγ, PPARδ and CD36 gene expression in white blood cells at postprandial after high polyphenol olive oil when compared with moderate polyphenol olive oil intervention ($P<.017$), with COX-1 reaching borderline significance ($P=.024$). Linear regression analyses showed that changes in gene expression were related to a decrease in oxidized low-density lipoproteins and with an increase in oxygen radical absorbance capacity and olive oil polyphenols ($P<.05$). Our results indicate a significant role of olive oil polyphenols in the up-regulation of genes involved in the cholesterol efflux from cells to HDL *in vivo* in humans. These results are in agreement with previous ones concerning the fact that benefits associated with polyphenol-rich olive oil consumption on cardiovascular risk could be mediated through an *in vivo* nutrigenomic effect in humans.

Keywords: ATP binding cassette transporter-A1 (ABCA1); Olive oil polyphenols, gene expression; Peroxisome proliferator-activated receptor (PPAR); Scavenger receptor class B member-1(SR-B1); CD36 molecule (thrombospondin receptor) (CD36)

1. Introduction

Data from human studies show that olive oil polyphenols are protective against risk factors for coronary heart disease (CHD) [1], particularly in individuals submitted to an oxidative stress situation (i.e., hypertensive, CHD patients) [2,3]. A crucial event for the development of the atherosclerosis plaque is the accumulation of cholesterol in macrophages that leads to the formation of foam cells. In response to a lipid loading, macrophages activate a compensatory pathway for cholesterol efflux from cells to the high-density lipoprotein (HDL): the reverse cholesterol transport (RCT), in which accumulated cholesterol is removed from macrophages in the subintima of the vessel wall and collected by HDL and ApoA-I [4,5]. Several mechanisms are involved including passive diffusion and protein transmembrane transporters such as sterol 27-hydroxylase, the ATP-binding membrane cassette system or the scavenger receptor class B type 1 (SR-B1) [4,5]. Results of the European EUROLIVE study, performed in 200 healthy individuals, showed that olive oil consumption promotes an increase in plasma HDL cholesterol and a decrease in low-density lipoprotein (LDL) oxidative damage in a direct relationship with the polyphenol content of the olive oil administered [6].

We have previously reported that oxidation of HDL reduces the HDL functionality by impairing cholesterol efflux from macrophages [7] and that oleic acid consumption reduces HDL oxidation *in vivo* in humans [8]. In experimental studies, polyphenols from red wine have been shown to protect HDL and LDL from oxidation [9]. Polyphenols, however, can exert protective effects not only through the scavenging of free radicals but also by modulating signal transduction, cell signaling, gene expression and cellular communications in various pathways [10]. From our data and others, olive oil polyphenols modulate, towards a protective mode, the expression of inflammation-related genes [11–13], a common target of dietary intervention

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Table 1
Characteristics of the olive oils administered

	Type of olive oil	
	MPC	HPC
Quality parameters		
Free acidity (% of oleic acid)	0.19	0.26
Peroxide value (mEq O_2/kg)	16.76	6.10
Fatty acids (% of total)		
Monounsaturated	72	72
Polyunsaturated	11	11
Saturated	17	17
Total polyphenols* (mg/kg of olive oil)	288.9	961.2
Free hydroxytyrosol	0.37	6.64
Free tyrosol	1.03	8.7
Secoroid derivatives	123	680
Vanillic acid	0.37	3.94
p-Coumaric acid	0.08	0.84
Vanillin	0.16	1.44
Pinoresinol	115.8	173.1
Luteolin	1.44	6.28
Apigenin	0.27	0.80

studies [14]. Concerning cholesterol efflux related genes, hydrocinnamic acid derivatives have proven to be potent dual peroxisome proliferator-activated receptor (PPAR) α/γ agonists [15]. Ingestion of a phenolic-rich beverage, such as coffee, has been shown to enhance the cholesterol efflux from human macrophages to HDL, while increasing the messenger ribonucleic acid (mRNA) and protein levels of ATP binding cassette transporter G1 (ABCG1) and SR-B1 [16]. These effects were also observed to be mediated by caffeic and ferulic acid in experimental models [16]. Therefore, we assessed the *in vivo* human transcriptome response related with cholesterol efflux after sustained consumption of similar olive oils, but with differences in their phenolic content, in a pre/hypertensive population.

2. Methods and materials

2.1. Olive oil preparation and characteristics

A virgin olive oil with a high phenolic content (HPC) was prepared, as previously described [17], by the addition of a phenolic-rich extract [oleuropein complex or secoiridoids (89.4%); hydroxytyrosol, tyrosol or phenil alcohols (3.5%); and flavonoids (6.0%)], obtained from the olive cake, to a natural virgin olive oil with a moderate content of phenolic compound (MPC). Briefly, olive cake phenolic extract (7 mg/ml oil) and 0.3% (p/v) of lecithin (Emulpur; Cargill, Barcelona, Spain) were dissolved in ethanol–water (50:50, v/v) and added to the MPC using a Polytron (Kinematica, Littau, Switzerland) until full homogenization. The MPC was also submitted to the process of lecithin addition to ensure a similarity with HPC. Total polyphenol content of the olive oils was 289 and 961 mg/kg oil for MPC and HPC, respectively, measured by ultraperformance liquid chromatography coupled to a tandem mass detector as previously described [18]. Fatty acid type content of olive oils was measured by gas chromatography. Table 1 shows the composition of the olive oils used in the study.

2.2. Study design

2.2.1. Participants

Between January and July 2009, 22 participants, aged 20 to 75, were recruited through a volunteer center database. Participants were community dwelling with prehypertension [systolic blood pressure (SBP) ≥120 mmHg to 139 mmHg and/or diastolic blood pressure (DBP) ≥80 mmHg to 89 mmHg] or stage 1 hypertension (SBP ≥140 mmHg to 159 mmHg and/or DBP ≥90 mmHg to 99 mmHg) without antihypertensive treatment. We selected this type of patients because they are submitted to a higher oxidative stress than healthy individuals [19]. Due to this, pre- and hypertensive individuals could be more susceptible to receiving benefits from polyphenol-rich olive oil. Exclusion criteria included the following: LDL cholesterol >4.9 mmol/L, triglycerides (TG) >3.97 mmol/L or current hypolipemic treatment; diabetes mellitus; any chronic disease and body mass index (BMI) >30 kg/m^2. Participants provided written informed consent prior to enrollment in the trial. After a screening visit, eligibility or exclusion was assessed by the attending physician based on a review of the clinical records. The study was approved by the Clinical Research Ethical Committee of the Hospital Universitari Sant Joan de Reus, Spain. Protocols were according to the Helsinki Declaration. This trial is registered with the International Standard Randomized Controlled Trial Number (identifier: ISRCTN03450153).

2.2.2. Randomization and intervention

The trial was randomized, controlled, double blind and crossover (Fig. 1). The randomization scheme was generated by using a Web site (http://www.randomization.com). Participants received 30 ml of one of the two types of olive oil, MPC or HPC, with bread (80 g). Washout periods were 2 weeks in which participants were instructed to follow a stabilization diet with 10% of saturated fatty acids during the week before the postprandial test. The day before the intervention, participants followed a polyphenol-free diet, avoiding olive oil, olives, fresh fruit or juices, vegetables, legumes, soya, chocolate, coffee, tea, wine and beer. Compliance was assessed using a 3-day dietary record (2 working days and a holiday or weekend one) before the intervention days. Trained dieticians explained to participants how to complete these questionnaires. Participants were instructed to avoid intense physical activity 3 days prior to the intervention day. Physical activity was evaluated by the Minnesota Leisure Time Physical Activity Questionnaire validated for its use in Spanish men and women [20,21]. Venous blood was collected at baseline of each intervention period (0 h) and at several periods after olive oil administration. Serum and plasma were obtained by centrifugation of blood at 1500g at 4°C for 20 min and stored at −80°C in the central laboratory's biobank. Whole blood was collected at baseline and at 5 h after olive oil intake in PAXgene tubes for gene expression analyses and stored at −80°C after 2 h at room temperature.

2.3. Systemic biomarkers

Anthropometric data were obtained by standardized methods. After 15 min of resting, blood pressure was measured in triplicate (1-min intervals) using an automatic sphygmomanometer (OMRON HEM-907; Peroxfarma, Barcelona, Spain).

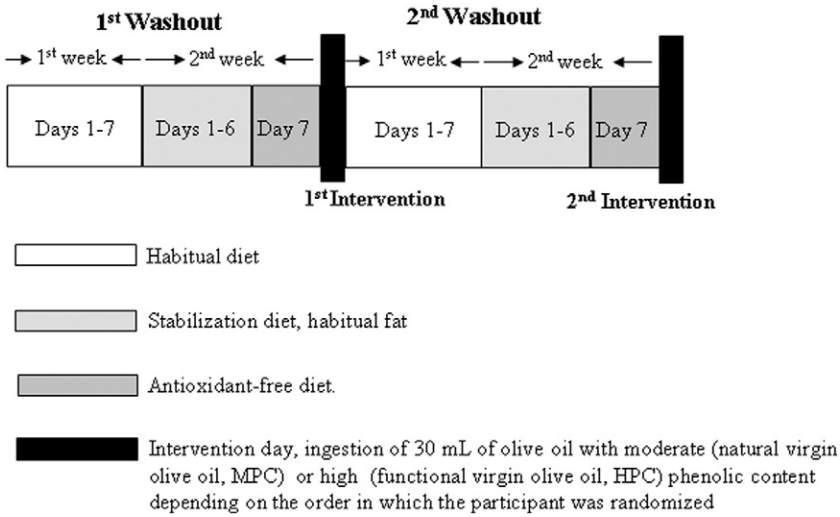

Fig. 1. Design of the crossover study.

Table 2
Baseline characteristics of the participants

Gender (male/female)	7/6
Age, years	51.15±16.67
BMI, kg/m^2	25.74±1.89
Waist circumference, cm	87.81±6.11
Systolic blood pressure, mmHg	136.46±19.76
Diastolic blood pressure, mmHg	81.77±10.19
Glucose, mmol/L	5.54±0.48
Total cholesterol, mmol/L	5.09±0.79
TG, mmol/L	0.86 (0.72 to 1.23)
HDL-C, mmol/L	1.70±0.40
LDL-C, mmol/L	2.88±0.68
Oxidized LDL, U/L	74.5±17.9
ORAC, μmol TE/ml	0.59±0.26
FRAP, μmol TE/ml	51.6±6.4
F_2-isoprostanes, pg/ml	82.3±60.4

Results are expressed as mean±S.D. or median (25th to 75th percentile) (n=13).

Serum total and HDL cholesterol, TG and glucose measurements were performed using standardized enzymatic methods in an autoanalyzer (Beckman Coulter-Synchron, Galway, Ireland). Low-density lipoprotein cholesterol (LDL-C) was calculated by means of the Friedewald formula whenever TG were <300 mg/dl. Plasma circulating oxidized LDL (Mercodia AB, Uppsala, Sweden) was measured by immunoassay. Olive oil polyphenols and their biological metabolites were measured in plasma, as markers of compliance, as previously described [18]. Ferric reducing ability of plasma (FRAP) was determined by 2,4,6-tripyridyl-s-triazine chelation at 593 nm in a UV–VIS spectrophotometer (Lambda 25, Perkin Elmer, Beaconsfield, UK). Plasma oxygen radical absorbance capacity (ORAC) was measured by peroxyl radical generation by 2,2′-azobis(2-amidinopropane) dihydrochloride using fluorescein as a fluorescence probe in a Fluoroskan Ascent fluorescence plate reader (Labsystems, Helsinki, Finland). F_2-isoprostanes were measured by enzyme-linked immunosorbent assay (Cayman Chemical, Ann Arbor, MI, USA).

2.4. Gene expression analyses

Total RNA was obtained from white blood cells (WBC) by using the PAXgene extraction kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland). For mRNA expression analyses, isolation of total RNA (tRNA) was performed by a liquid–liquid method. RNA purity and integrity were assessed by Agilent (Agilent Technologies, Santa Clara, CA, USA). After cDNA conversion, gene expression was measured by real-time polymerase chain reaction with TaqMan® Low Density Microfluidic cards in triplicate and analyzed by the Sequence Detection System 2.1 software according to the manufacturer's instructions (Applied Biosystems-Life Technologies Corporation, Carlsbad, CA, USA). Changes in gene expression were calculated using the relative quantification method (RQ) and applying the $2^{-\Delta\Delta Ct}$ formula. The selection of candidate genes was performed on the basis of their relationship with cholesterol efflux.

2.5. Sample size and power analyses

A total sample size of 16 participants allows for a more than 80% power to detect a significant difference between olive oil groups of 1 U of RQ in the gene expression of *ABCA1* measurement with consideration of a two-sided type I error of 0.05. This sample size takes into account a 20% dropout rate. Calculations were made from our previous data concerning the standard deviation of ABCA1 gene expression in healthy volunteers [13].

2.6. Statistical methods

Normality of continuous variables was assessed by normal probability plots. Nonnormally distributed variables were log transformed, and values were expressed as antilogarithm. Pearson correlation analyses were used to evaluate relationships among variables. A general lineal model was performed to assess the effect of each intervention compared to its baseline and to assess the effect between interventions for systemic and gene expression variables. No interactions with age and gender were observed. Due to this, models were performed without covariates. Multiple regression analyses were performed, adjusted by gender and age, in order to explore the relationship among systemic and gene expression variables. A value of P<.05 was considered significant. For gene comparison adjustment, a P<.017 was considered significant due to the fact that we explored three gene families (cholesterol transporters, PPAR nuclear receptor factors and prostaglandins). Statistical analyses were performed by SPSS 13.0 software.

3. Results

3.1. Participants and baseline characteristics

From the 22 participants recruited, 16 were eligible. Three participants dropped out before starting the study, two due to an incompatible work timetable and one for problems with blood collection. Finally, 13 participants (7 men and 6 women) entered the study. We could not identify any adverse effects related to olive oil intake. Participants' baseline characteristics are shown in Table 2. No changes were observed in dietary patterns throughout the study. No changes in daily energy expenditure in leisure-time physical activity were observed from the beginning to the end of the study.

3.2. Systemic biomarkers

Changes in cardiovascular risk biomarkers from baseline to 5 h postprandial are shown in Table 3. As expected, a postprandial hypertriglyceridemia occurred after olive oil ingestions. A significant decrease in oxidized LDL was observed after both interventions. Plasma concentration of hydroxytyrosol sulphate and that of hydroxytyrosol acetate sulphate, the main biological metabolite of hydroxytyrosol, increased in a dose-dependent manner with the polyphenol content of the olive oil (P<.05), reaching a peak at 120 min after ingestion of the oils. Areas under the curve from 0 to 5 h (AUC_{0-5h}) for hydroxytyrosol sulphate were (mean±standard deviation, μmol × min/L) 46±37 and 139± 33, and those for hydroxytyrosol acetate sulphate were 335±165 and 727± 352, for MPC and HPC, respectively (P<.005). No changes were observed in blood pressure, weight or waist circumference throughout the study.

3.3. Gene expression

The expressions of ABCA1, SR-B1, PPARBP, PPARa, PPARγ, PPARδ and CD36 genes increased significantly after the HPC intervention

Table 3
Changes in cardiovascular risk systemic biomarkers at 5 h postprandial after olive oil ingestion

	Olive oil interventions			
	MPC		HPC	
	Postintervention	Change	Postintervention	Change
Glucose, mmol/L	5.08±0.35	−0.362±0.344 *	5.07±0.31	−0.305±0.464 *
TG, mmol/L	1.23(0.75 to 1.53)	0.22(0.03 to 0.30) *	1.24 (0.99 to 1.77)	0.40(0.16 to 0.69) *
Cholesterol, mmol/L				
Total	5.09±0.84	0.029±0.218	4.99±0.85	0.031±0.192
HDL	1.71±0.39	0.004±0.094	1.65±0.36	−0.014±0.071
LDL	2.82±0.70	−0.023±0.078	2.70±0.70	0.026±0.175
Oxidized LDL, U/L	63.3±14.1	−5.98±8.52 *	65.4±16.4	−5.18±5.37 *
ORAC, μmol TE/ml	50.1±6.1	−1.34±7.38	50.1±7.02	1.91±8.95
FRAP, μmol TE/ml	0.57±0.25	−0.010±0.107	0.59±0.24	−0.013±0.099
F_2-isoprostanes, pg/ml	60.6±38.3	−15.6±42.0	64.9±38.0	−20.1±40.2

Values expressed as mean (S.D.) or median (25th to 75th percentile) (n=13).
* P<.05 versus its corresponding baseline.

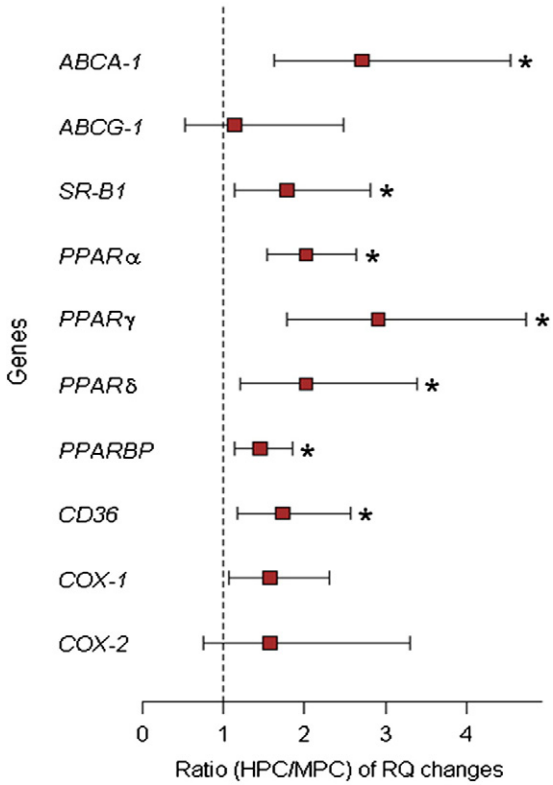

Fig. 2. Geometric mean (95% confidence interval) of the ratio (HPC olive oil/MPC olive oil) of RQ changes in gene expression after interventions. Dot axis displays the significance between olive oils at $P<.05$ level. *$P<.017$ significance after HPC versus MPC intervention.

when compared to the MCP one ($P<.017$), with changes in COX-1 reaching a borderline significance ($P=.024$). Fig. 2 shows the ratio of changes between the two interventions considering the dot axis at $P<.05$ to be of significance.

Analyses of gene expression changes after HPC showed cross-linked correlations among genes related with the cholesterol efflux cascade. The increase in the expression of ABCA1 directly correlated with that of PPARα. Changes in the expression of the PPARs gene family directly correlated among them, and those of PPARα with the increase in its coactivator, the PPARBP. The increase in SR-B1 expression directly correlated with that of CD36, COX-1 and COX-2, whose changes were also directly correlated ($P<.01$).

Multiple regression analyses showed that, after HPC intervention, values of ABCA1, PPARα and PPARγ gene expression increased with the decrease in oxidized LDL ($P<.05$). For each decrease in 1 U/L of oxidized LDL, there was a decrease of 18.2%, 5.2% and 13.8% in the gene expression of ABCA1, PPARα and PPARγ, respectively. Values of ORAC postprandial were directly related with the increase in PPARBP and PPARs gene expression ($P<.025$). For each increase of 1 μmol/L of ORAC, there was an increase of 9.6%, 7.2%, 18.8% and 41.3% in the gene expression of PPARBP, PPARα, PPARδ and PPARδ, respectively. A direct relationship was observed between the concentrations of hydroxytyrosol acetate sulphate at postprandial and changes in ABCA1 expression ($P=.036$). For each increase in 1 μmol/L of hydroxytyrosol acetate sulphate at 5 h postprandial, there was an increase of 43.2% in ABCA1 expression. These associations were not found after MPC ingestion.

4. Discussion

These outcomes showed that a randomized, crossover, controlled intervention with HPC olive oil increased the gene expression of ABCA1 and SR-B1, the main transmembrane transporters of cholesterol efflux, in WBC of pre- and stage 1 hypertensive individuals in comparison with MPC olive oil. Other related genes involved in the cholesterol efflux such as PPARα, PPARγ, their activator PPARBP, PPARδ/β, CD36 and COX-1 showed an increase in their expression when comparing HPC versus MPC intervention. To our knowledge, this is the first time changes in cholesterol efflux related genes linked to the polyphenol content of the olive oil have been reported *in vivo* in humans after a randomized trial. Our data also provide further first-level evidence that atheroprotective molecular mechanisms can be promoted by a polyphenol-rich olive oil intervention.

HDL-mediated cholesterol efflux is the natural rate-limiting step of reverse cholesterol transport [4,5]. Two main transmembrane transport systems are responsible for the removal of cholesterol from cells to the HDL lipoprotein: the ABC complex and the SR-B1 complex. ABCA1 and ABCG1 are responsible for cholesterol efflux. ABCA1 transports intracellular free unesterified cholesterol and phospholipids to the extracellular Apo-A1 in nascent HDL. ABCA1 mediates the efflux of cellular cholesterol to lipid poor apolipoproteins, but not to full HDL particles, a task performed by ABCG1. HDL presents or accepts cholesterol while anchored to plasma membranes via its receptor SR-B1 [4,5]. The density gradient of cholesterol between HDL and the cell surface determines whether HDL gives or accepts cholesterol. In perithelial cells, the main direction is the cholesterol efflux from cells [4]. In experimental models, high-fat–high-cholesterol diets decreased SR-BI expression [22], the posttranslational down-regulation of SR-BI occurring via the PDZ domain PDZK1 [23]. Genetic variants at the PDZK1-interacting domain of SR-BI interact with diet to influence the risk of metabolic syndrome in obese individuals with high polyunsaturated fatty acid and carbohydrate intakes [24]. The up-regulation of ABCA1 observed after HPC versus MPC intervention could be related to the unsaturated fatty acids/polyphenols ratio present in the olive oils tested. In fact, a repression of ABCA1 expression in macrophages by unsaturated fatty acids, including oleic acid [25], has been reported in experimental studies. The transcriptional repression of ABCA1 induced by unsaturated fatty acids has been shown to be abrogated by histone deacetylase (HDAC) inhibitors, which promote an increase in ABCA1 expression [25]. Polyphenols, such as those of green tea or quercetin, are HDAC inhibitors [26,27], a mechanism proposed as anticarcinogenic. Thus, we can hypothesize that, in HPC treatment, polyphenols present in the olive oil were able to counteract the repression induced by the oleic acid and other unsaturated fatty acids present in olive oil. This fact promoted an enhancement of ABCA1 gene expression linked to the polyphenol content of the olive oil. The direct relationship observed between plasma levels of hydroxytyrosol acetate sulphate and ABCA1 expression after HPC intervention would support this hypothesis. In a previous work with healthy volunteers [13], we did not detect significant differences in ABCA1 expression associated with a Mediterranean diet or virgin olive oil consumption. The fact that the virgin olive oil provided to participants had similar characteristics to those of the MPC reinforces the results obtained in the present study.

Many nuclear receptor factors are involved in regulating the expression of RCT-related genes. In this paper, we have focused on the PPAR family, comprising three homeotypic isomerides, PPARα, PPARγ and PPARδ, because fatty acids and also polyphenols are natural PPARs ligands [4,28]. PPARBP, also referred to as MED1, is a coactivator for PPARγ and PPARα [29]. The results obtained in this study are in agreement with our previous ones in which we observed an increase in both the expression of PPARBP and that of the lipoic acid synthase at 6 h after polyphenol-rich olive oil ingestion [30]. Lipoic acid is a powerful antioxidant that can activate PPARα and PPARγ [31]. Ligand-activated PPARs decrease inflammatory responses [32]. PPARγ and PPAR α ligands have been shown to induce a decrease in CD40, monocyte chemotactic protein MCP1 and vascular

endothelial growth factor (VEGF) secretion and to inhibit interferon γ (IFNγ) and intracellular cell adhesion molecule-1 (ICAM-1) expression in cultured cells [33]. In animal models, PPARδ agonists inhibit inflammatory gene expression including those of IFNγ, MCP1 and ICAM-1[33]. In agreement with this, and with the increase in the PPARs expression observed in our study, we have recently reported a down-regulation of CD40-ligand expression and its downstream and related products, such as VEGF, ICAM-1, IFNγ and MCP1, *in vivo* in humans linked to the polyphenol content of the olive oil [12]. A PPARδ agonist has been shown to increase HDL cholesterol *in vivo* in humans [34]. Thus, the fact that polyphenols from olive oil can increase PPARδ expression could explain the higher increase in HDL cholesterol (HDL-C) levels observed after polyphenol-rich olive oil sustained consumption in human studies [6]. PPARα, PPARγ and PPARδ ligands have been shown to stimulate cholesterol efflux in cultured macrophages by inducing the expression of ABCA1 [32,33]. PPARα has also been reported to up-regulate the expression of SR-B1 [4]. The olive oil polyphenol effect on ABCA1 and SR-B1 up-regulation observed in our study could be mediated through the enhancement of PPARs expression. In this sense, incubation of mouse peritoneal macrophages and macrophage-derived foam cells with anthocyanines led to dose-dependent induction in cholesterol efflux and in PPARγ and ABCA1 mRNA expression [35]. Resveratrol has been shown to up-regulate PPARα, PPARγ and PPARδ expression in macrophages [36]. Both quercetin and resveratrol attenuated the suppression of PPARγ mediated by tumor necrosis factor α in human adipocytes [37]. In some experimental studies, but not all [38], hydroxytyrosol, the main phenolic compound from olive oil, has been shown to be able to up-regulate the gene expression of PPARα and PPARγ [39]. In agreement with that referred to above, we observed an increase in PPARs, PPARBP, ABCA1 and SR-B1 expression after HPC olive oil ingestion.

CD36 is a scavenger receptor that promotes uptake of oxidized LDL. PPARγ promotes lipid uptake by up-regulating CD36 expression [32]. We observed an increase in CD36 expression after HPC versus MPC olive oil. This fact is in agreement with our previous results in which a single oral load of polyphenol-rich olive oil, besides an increase in the PPARγ co-activator, the PPARBP, promoted an increase in CD36 expression [30]. Oxidized LDL has been shown to increase monocyte CD36 expression [40]. This activation in turn promotes that of PPARγ via a MAPK-dependent COX-2 pathway [41]. In our setting, oxidized LDL was decreased after both treatments. This fact, together with a lack of COX-2 activation, supports the idea of an oxidized-LDL-independent PPARγ activation. Oxidized HDL decreases CD36 expression via PPARγ [40]. Whether a higher decrease in HDL oxidation after HPC versus MPC could enhance the PPARγ expression leading to an increase in that of CD36 is unknown. The direct relationship between the increase in PPARγ expression with an increase in the antioxidant capacity (ORAC) and the decrease in oxidized LDL would support this hypothesis. An increase in CD36 has been associated with atherosclerosis in experimental models [32]. However, recent data show that CD36 deficiency is associated with enhanced atherosclerotic cardiovascular diseases in humans [42].

Although ABC-dependent RCT is the primary mechanism of cholesterol removal, the 27-hydroxylase pathway provides an alternative to the apoA-I-dependent process [5]. At gene expression level, 27-hydroxycholesterol up-regulates ABCA1 expression via LXR receptor [43]. Selective COX-2 inhibition reduced the mRNA expression of cholesterol 27-hydroxylase and ABCA1, whereas selective COX-1 inhibition down-regulated the expression of 27-hydroxylase mRNA [44]. This interference with RCT has been proposed as an explanation for the fact that COX-2 inhibition elevates cardiovascular risk [45]. In experimental studies, olive oil polyphenols differed from an inhibition of COX-1 and COX-2 activity [46] to a noneffect [47] and also to a repression of the anoxia-induced COX-2 expression [48]. A PPARα and PPARγ control of COX1 and COX-2 expression in macrophages and platelet cultures has been reported, whereas PPARδ appears to be a target of COX-1[49]. Thus, one explanation for the increase in COX-1 expression observed in our study could be via the increase in PPAR expression.

In summary, an up-regulation of the expression of cholesterol efflux related genes — ABCA1, SR-B1, PPARBP, PPARα, PPARγ, PPARδ, CD-36 and COX-1 — in human white blood cells occurs after a polyphenol-rich olive oil ingestion versus a moderate one. Our results point out a significant role of olive oil polyphenols in the up-regulation of genes involved in an enhancement of the cholesterol efflux from cells. Changes in the gene expression were related to a decrease in oxidized LDL and an increase in ORAC and antioxidant polyphenols. Olive oil, a recognized healthy food, cannot be consumed in large quantities. Thus, the enrichment of olive oil with its phenolic compounds is a way of increasing its healthy properties whilst consuming the same amount of fat. Our results are in agreement with previous ones concerning the fact that benefits associated with polyphenol-rich olive oil consumption on cardiovascular risk could be mediated through a nutrigenomic effect. Long-term randomized intervention trials examining the effects of high-polyphenol olive oil diets on HDL functionality are warranted.

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