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Cardiovascular Benefits of Phenol-Enriched Virgin Olive Oils: New Insights from the Virgin Olive Oil and HDL Functionality (VOHF) Study

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Scope: The main findings of the "Virgin Olive Oil and HDL Functionality" (VOHF) study and other related studies on the effect of phenol-enriched virgin olive oil (VOO) supplementation on cardiovascular disease are integrated in the present work.

Methods and results: VOHF assessed whether VOOs, enriched with their own phenolic compounds (FVOO) or with those from thyme (FVOOT), improve quantity and functionality of HDL. In this randomized, double-blind, crossover, and controlled trial, 33 hypercholesterolemic subjects received a control VOO (80 mg kg^{-1}), FVOO (500 mg kg^{-1}), and FVOOT (500 mg kg^{-1}; 1:1) for 3 weeks. Both functional VOOs promoted cardioprotective changes, modulating HDL proteome, increasing fat-soluble antioxidants, improving HDL subclasses distribution, reducing the lipoprotein insulin resistance index, increasing endogenous antioxidant enzymes, protecting DNA from oxidation, ameliorating endothelial function, and increasing fecal microbial metabolic activity. Additional cardioprotective benefits were observed according to phenol source and content in the phenol-enriched VOOs. These insights support the beneficial effects of OO and PC from different sources.

Conclusion: Novel therapeutic strategies should increase HDL-cholesterol levels and enhance HDL functionality. The tailoring of phenol-enriched VOOs is an interesting and useful strategy for enhancing the functional quality of HDL, and thus, it can be used as a complementary tool for the management of hypercholesterolemic individuals.

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1. Introduction

A large body of knowledge provides evidence of the benefits of virgin olive oil (VOO) consumption, mainly attributed to the phenolic compounds (PC), on chronic diseases including cardiovascular diseases (CVD).[1] Monounsaturated fatty acids (MUFA) are the major components of VOO of which oleic acid represents 55–83% of the total lipid composition. The minor components constitute 1–2% of the VOO composition and are classified into two fractions: a) the unsaponifiable fraction and b) the hydrophilic fraction, that includes the PC.[2]

In randomized clinical trials, VOO consumption has been shown to promote benefits on secondary endpoints related to CVD such as lipid profile, insulin sensitivity, oxidation, inflammation, endothelial function, thrombotic factors, and blood pressure.[1,3] In cohort studies, VOO consumption has been inversely associated with coronary heart disease (CHD) mortality in the European Prospective Investigation into Cancer and Nutrition study[4–6] and with stroke risk in women in the Three-City Study.[7] The *Prevención con Dieta Mediterránea* (PREDIMED) study demonstrated that VOO consumption decreases the incidence of major CVD outcomes and CVD mortality within the framework of the Mediterranean diet in people at high CVD risk.[8]

In November 2004, the Food and Drug Administration of the United States permitted a claim concerning the benefit on the risk of CHD of eating about two tablespoons of olive oil (OO) daily, due to its MUFA content.[9] However, recent evidence indicates that VOO minor components exert a major contribution to the benefits of its consumption including antiatherogenic, hypoglycemic, anti-inflammatory, antitumor, antiviral, and immunomodulatory activities.[10] In the Effect of Olive Oil on Oxidative Damage on European Population (EUROLIVE) study, phenol-rich OO intake increased high-density lipoprotein cholesterol (HDL-c) levels, decreased oxidized low-density lipoprotein (LDL; oxLDL), and increased HDL cholesterol efflux capacity (CEC) from macrophages,[11,12] among others, according to the PC content of the OO administered. Supporting these data, a functional VOO enriched with its own PC has also been shown to increase the expression of CEC-related genes.[13] In November 2011, the European Food Safety Authority released a claim stating that OO PC contribute to the protection of blood lipids from oxidative stress and that this health claim may only be used for OO containing at least 5 mg of hydroxytyrosol (HT) and its derivatives per 20 g of OO.[14] However, PC concentration in most VOO available on the market is too low to allow the daily consumption of 5 mg of HT and its derivatives within the context of a balanced diet. For this reason, a good approach to ensure the optimal intake of PC in the context of a balanced diet is the enrichment of VOO with PC, that allows increasing VOO health-promoting properties, while consuming the same amount of fat.[15,16] However, high concentrations of OO PC result in a bitter and pungent taste due to the presence of secoiridoids (HT and its derivatives), which could lead to rejection by consumers.[17] Moreover, high doses of a single type of antioxidant could even promote lipid peroxidation and therefore increase atherosclerotic areas in animal models. In contrast, the combination of different antioxidants is effective in reducing atherosclerosis in human trials.[18,19] Herbs are traditionally added to OO in Mediterranean cuisine to enhance its aroma and taste. Besides affecting oil's organoleptic characteristics, the enrichment of a VOO with PC from aromatic herbs has an impact on the nutritional value for the flavored oils.[20,21] Thyme (*Thymus zygis*) could enhance the benefits of a phenol-enriched VOO because it is one of the richest sources of flavonoids and phenolic acids.[17]

On this basis, VOO, enriched VOOs, and its bioactive components (mainly PC) have been largely investigated in in vitro/in vivo animal models and in human intervention studies to examine their cardioprotective effects. Most of the studies are based on assessing HDL-c and the results are controversial. However, the antiatherogenic effect of HDL does not reside in the HDL quantity (i.e., HDL-c) but in HDL biological activities (such as CEC, antioxidant, anti-inflammatory, vasodilatory, and antiapoptotic capacities). HDL functionality has been found to be a better atheroprotection marker than circulating HDL-c levels.[22] Thus, therapies should focus their efforts on increasing not only HDL quantity but also HDL functionality. In this regard, the "Virgin Olive Oil and HDL Functionality: a model for tailoring functional food" (VOHF) project was designed to assess whether functional VOOs enriched with their own PC or with them plus additional complementary PC from thyme could act as nutraceuticals concerning the in vivo quantity (HDL-c levels) and quality (functionality) of the HDL particle in hypercholesterolemic subjects.

In the present work, the authors aimed to succinctly integrate the main published results of the VOHF study. Moreover, the authors also aimed to survey the scientific evidence of the effects of OO PC on human CVD risk biomarkers, other than that from the VOHF project. These data would allow us to uncover the new insights in CVD and HDL functionality obtained from the VOHF study.

2. Experimental Section

2.1. Methodology of the Review

The studies were eligible for inclusion if they examined the sustained effects of OO PC on human CVD risk biomarkers.

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Studies that met the following criteria were included: 1) original articles, 2) sustained randomized controlled trials (RCTs) conducted in healthy humans, in subjects with CVD or with a defined CVD-related outcome, 3) crossover design, 4) clinical trials where the amount of PC ingested or the PC concentration of the VOO consumed is specified, 5) articles published from 1997 up to October 2017, and 6) articles written in English. Relevant articles were identified by searching PubMed database (http://www.ncbi.nlm.nih.gov/pubmed). The key search terms used were as follows: "olive oil," "virgin olive oil," "functional virgin olive oil," "olive oil phenols," "olive oil phenolic compounds," "olive oil polyphenols," AND "oxidation," "oxidative stress," "endothelial function," "inflammation," "thrombosis," "blood pressure," "hypertension," "lipid profile," "LDL," "HDL," "cholesterol," "triglycerides." References from retrieved articles were manually searched to identify additional eligible studies. Search for eligible studies began in June 2017 and ended in November 2017. Those publications related to the VOHF project were excluded from the revision process.

Two authors independently reviewed the literature and identified relevant studies for possible inclusion. The title and abstract of each paper were initially screened and, from there on, the full texts were obtained for selecting the studies to be included in the review. A data extraction sheet was developed and the information from the included studies were extracted and tabulated. The information was extracted as follows: authors, publication year, number of participants, study design, intervention, key outcomes, and main observed effects.

2.2. Methodology of the VOHF Study

The VOHF project comprised two randomized, controlled, double-blind, crossover trials: an acute-intake study and a sustained-intake study. The methodology of the VOHF study is comprehensively detailed in the supporting information file. The design and the flowchart of the VOHF study are detailed in Figures S1 and S2, Supporting Information, respectively. The phenolic intake and the fat-soluble micronutrients and fatty acids composition of the OOs used in the sustained-intake VOHF study are detailed in Tables S1 and S2, Supporting Information, respectively.

3. Results and Discussion

3.1. Results of the Review

The initial screening provided a total of 1267 citations. After removing duplicates, 484 articles were retained. Of these, 412 were excluded for not meeting the predetermined eligibility criteria after reviewing title and abstracts. Further inspection of the full texts of the remaining 72 articles revealed that 49 studies did not meet the eligibility criteria. Thus, a total of 23 studies were included in this review (see flow diagram in Figure S3, Supporting Information).

Most of the included studies evaluated plasma lipid profile, glucose, blood pressure, oxidative balance, inflammation, and endothelial function. Few of the included studies also evaluated other CVD biomarkers related to HDL functionality such as lipoprotein composition, CEC, HDL monolayer fluidity, enzymes related to HDL metabolism, changes in gene expression, and proteomic biomarkers. However, the information obtained in this review for these more specific biomarkers was scarce, emphasizing the need of the development of an RCT assessing the effects of OO PC on HDL functionality, such as the case of the VOHF study. The results of all the included studies and also the main characteristics of each RCT are summarized in **Table 1**. The main results of the most commonly studied biomarkers in the articles investigated are shortened below.

Fifteen RCTs investigated HDL-c and LDL-cholesterol (LDL-c) levels thus being the most studied biomarkers related to lipid profile. Regarding HDL-c levels, no clear conclusion can be obtained because most authors found no observable impact on HDL-c levels,[12,23–30] while others observed increases[3,31–33] or decreases[34,35] in HDL-c levels. Results from the included studies indicated that OO PC have no effect on LDL-c levels since 14 of the studies[3,12,23–33,35] reported no changes after an OO PC intervention. Likewise, blood glucose[27,31,32,34] and blood pressure[29,33–35] did not show changes in four of the six RCTs that evaluated the effect of OO PC on these CVD risk biomarkers. The oxidative balance was determined by different biomarkers, plasma oxLDL being the most frequently used in the RCTs included. Out of the 12 studies that evaluated oxLDL, nine[3,12,23,25,31,36–39] reported significant reductions according to the phenolic content indicating a clear beneficial effect of OO PC on this biomarker. Concerning inflammation, no clear conclusion can be obtained as out of the five RCTs that evaluated c-reactive protein (CRP) concentrations, three[27,34,39] of them observed a decrement while two[29,35] of them found no significant changes. Endothelial function was measured by ischemic reactive hyperaemia (IRH)[39] and reactive hyperemia index (RHI)[29] in two different articles and a significant improvement was observed in both of them indicating a possible beneficial effect of OO PC although more studies would be necessary.

3.2. Results from the VOHF Study

3.2.1. Characteristics of the Study Participants

In the acute-intake study, 13 participants were recruited and 12 were eligible and completed the study. In the sustained-intake study, 62 participants were recruited and 33 were eligible and enrolled in the study. Baseline characteristics of participants are described in Table S3, Supporting Information.[40–42]

3.2.2. Phenolic Compounds Intake Biomarkers

To verify dietary adherence, the phenolic biological metabolites were determined by Ultra Performance Liquid Chromatography coupled with electrospray ionization tandem mass spectrometry in plasma and in 24 h-urine before and after each VOO intervention. Two HT phase-II metabolites, HT sulfate, and HT acetate sulfate were identified as compliance biomarkers for FVOO whereas thymol sulfate and hydroxyphenylpropionic acid sulfate appeared to be the best compliance biomarkers for thyme

Table 1. Randomized, controlled sustained studies on the effect of VOO phenols on CVD risk biomarkers ($n = 23$).

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
Ramirez-Tortosa, et al., 1999	24 Men with peripheral vascular disease	Randomized, crossover	Free OO per day of: - VOO (800 mg of phenols per kg) - ROO (60 mg of phenols per kg) 3 months of intervention. 3 months of wash-out periods consuming their usual diets.	**Plasma lipid profile**	
				Triglycerides [mmol L^{-1}]	↑ According to phenolic content (dose-dependent).
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL triglycerides [% total lipoprotein lipids]	↑ According to phenolic content (dose-dependent).
				HDL triglycerides [% total lipoprotein lipids]	No changes versus baseline nor interventions.
				VLDL triglycerides [% total lipoprotein lipids]	No changes versus baseline nor interventions.
				LDL free cholesterol [% total lipoprotein lipids]	↑ According to phenolic content (dose-dependent).
				HDL free cholesterol [% total lipoprotein lipids]	↑ According to phenolic content (dose-dependent).
				VLDL free cholesterol [% total lipoprotein lipids]	↑ According to phenolic content (dose-dependent).
				LDL total cholesterol [% total lipoprotein lipids]	↓ According to phenolic content (dose-dependent).
				HDL total cholesterol [% total lipoprotein lipids]	No changes versus baseline nor interventions.
				VLDL total cholesterol [% total lipoprotein lipids]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				Plasma oxLDL [nmol TBARS mg^{-1} LDL protein per μmol Cu^{2+} L^{-1}]	↓ According to phenolic content (dose-dependent).
				Macrophage uptake of plasma oxLDL [%]	↓ According to phenolic content (dose-dependent).
Vissers, et al., 2001	46 Healthy subjects	Randomized, crossover	69 g per day of: - high phenol VOO (308 mg of phenols per kg) - low phenol VOO (43 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with no consume of olives, OO, and OO products.	**Plasma lipid profile**	
				Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				LDL oxidizability (lag time [min])	No changes versus baseline nor interventions.
				HDL oxidizability (lag time [min])	No changes versus baseline nor interventions.
				Plasma MDA [μmol L^{-1}]	No changes versus baseline nor interventions.
				Plasma lipid hydroperoxides [μmol L^{-1}]	No changes versus baseline nor interventions.
				Plasma protein carbonyls [nmol mg^{-1} protein]	No changes versus baseline nor interventions.
				Plasma ferric reducing ability of plasma [mmol L^{-1}]	No changes versus baseline nor interventions.

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
Moschandreas, et al., 2002	25 Normolipemic smokers	Randomized, crossover	70g OO per day of: - VOO (308 mg of phenols per kg) - OO (43 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with no consume of olives and OO products.	**Oxidative balance biomarkers** Total plasma oxidizability (lag time [min])	No changes versus baseline nor interventions.
				FRAP [mmol L^{-1}]	No changes versus baseline nor interventions.
				MDA [μmol L^{-1}]	No changes versus baseline nor interventions.
				Lipid hydroperoxides [μmol L^{-1}]	No changes versus baseline nor interventions.
				Protein carbonyls [nmol mg^{-1} protein]	No changes versus baseline nor interventions.
Marrugat, et al., 2004	30 Healthy men	Randomized, crossover	25 mL per day of: - VOO (150 mg of phenols per kg) - OO (68 mg of phenols per kg) - ROO (0 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Plasma lipid profile and glucose** Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	↑ According to phenolic content (dose-dependent).
				Glucose [mmol L^{-1}]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers Plasma oxLDL [U L^{-1}]	↓ According to phenolic content (dose-dependent).
				LDL oxidizability (lag time [min])	↑ According to phenolic content (dose-dependent).
				OLAB [U L^{-1}]	No changes versus baseline nor interventions.
Weinbrenner, et al., 2004	12 Healthy men	Randomized, crossover	25 mL per day of: - high phenol OO (486 mg of phenols per kg) - moderate (133 mg of phenols per kg) - low phenolic content (10 mg of phenols per kg) 4 days of intervention. 10 days of wash-out period (three phases—Days 1–3: habitual diet; Days 4–7: controlled diet avoiding excess of phenolic compounds; Days 8–10: low phenolic diet).	**Plasma lipid profile and glucose** Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}; % change from baseline]	↑ According to phenolic content (dose-dependent).
				Glucose [mmol L^{-1}]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers Plasma oxLDL [U L^{-1}; % change from baseline]	↓ According to phenolic content (dose-dependent).
				Plasma 8-oxo-dG in mitDNA [% change from baseline]	↓ According to phenolic content (dose-dependent).
				Plasma 8-oxo-dG in urine [nmol $mmol^{-1}$ creatinine; % change from baseline]	↓ According to phenolic content (dose-dependent).
				Urinary MDA [nmol $mmol^{-1}$ creatinine; % change from baseline]	↓ According to phenolic content (dose-dependent).
				Plasma GSH-Px activity [U L^{-1}; % change from baseline]	↑ According to phenolic content (dose-dependent).

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
Fitó, et al., 2005	40 Men with stable CHD	Randomized, crossover	50 mL per day of: - VOO (161 mg of phenols per kg) - ROO (14.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Plasma lipid profile and blood pressure**	
				Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				Lipoprotein (a) [g L^{-1}]	No changes versus baseline nor interventions.
				Systolic blood pressure [mm Hg]	↓ According to phenolic content (dose-dependent).
				Diastolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				oxLDL [μmol L^{-1}]	↓ According to phenolic content (dose-dependent).
				OLAB [U L^{-1}]	No changes versus baseline nor interventions.
				Lipoperoxides [μmol L^{-1}]	↓ According to phenolic content (dose-dependent).
				GSH-Px [U L^{-1}]	↑ According to phenolic content (dose-dependent).
				Total antioxidant status [mmol L^{-1}]	No changes versus baseline nor interventions.
Visioli, et al., 2005	22 Mildly dyslipidemic subjects	Randomized, crossover	40 mL per day of: - EVOO (166 mg L^{-1}) - ROO (2 mg L^{-1}) 7 weeks of intervention. 4 weeks of wash-out period with ROO.	**Plasma lipid profile**	
				Triglycerides [mg dL^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mg dL^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				Serum TXB_2 [μg mL^{-1}]	↓ According to phenolic content (dose-dependent).
				Antioxidant capacity of plasma [μmol Cu^{++} reduced]	↑ According to phenolic content (dose-dependent).
				Urinary 8-iso-$PGF_{2\alpha}$ [pg]	No changes versus baseline nor interventions.
Covas, et al., 2006	200 Healthy men	Randomized, crossover (EUROLIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - OO (164 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Plasma lipid profile**	
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	↑ According to phenolic content (dose-dependent).
				Total cholesterol/HDL cholesterol ratio	↓ According to phenolic content (dose-dependent).
				Oxidative balance biomarkers	
				Conjugated dienes [μmol mol^{-1} of cholesterol]	↓ According to phenolic content (dose-dependent).

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
				Hydroxy fatty acids [mmol L^{-1}]	↓ According to phenolic content (dose-dependent).
				oxLDL [U L^{-1}]	↓ According to phenolic content (dose-dependent).
				F2α-isoprostanes [μmol L^{-1}]	No changes versus baseline nor interventions.
				GSH:GSSG ratio	↑ Regardless of phenolic content (non dose-dependent).
Hillestrom, et al., 2006	28 Healthy men	Randomized, crossover (EURO-LIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - OO (164 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Oxidative balance biomarkers** Urinary etheno-DNA adducts [pmol per 24 h]	No changes versus baseline nor interventions.
Salvini, et al., 2006	10 Post-menopausal women	Randomized, crossover	50 g per day of: - high phenol EVOO (592 mg of phenols per kg) - low phenol EVOO (147 mg of phenols per kg) 8 weeks of intervention period. 8 weeks of wash-out period between treatments with their habitual fats and oils.	**Oxidative balance biomarkers** Oxidized DNA bases [% DNA in comet tail]	↓ According to phenolic content (dose-dependent).
				Basal DNA breaks [% DNA in comet tail]	No changes versus baseline nor interventions.
				Plasma antioxidant capacity [mmol L^{-1}]	No changes versus baseline nor interventions.
Gimeno, et al., 2007	30 Healthy men	Randomized, crossover	25 mL per day with: - VOO (825 μmol of CAE per kg) - OO (370 μmol of CAE per kg) - ROO (0 μmol of CAE per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Oxidative balance biomarkers** Plasma oxLDL [U L^{-1}]	↓ According to phenolic content (dose-dependent).
				LDL oxidizability (lag time [min])	↑ According to phenolic content (dose-dependent).
Machowetz, et al., 2007	182 Healthy men	Randomized, crossover (EURO-LIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - OO (164 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Oxidative balance biomarkers** Urinary 8-oxo-guanine [nmol per 24 h urine]	No changes versus baseline nor interventions.
				Urinary 8-oxo-guanosine [nmol per 24 h urine]	No changes versus baseline nor interventions.
				Urinary 8-oxo-deoxyguanosine [nmol per 24 h urine]	↓ Regardless of phenolic content (non dose-dependent).
Fitó, et al., 2008	28 Subjects with stable CHD	Randomized, crossover	50 mL per day of: - VOO (161 mg of phenols per kg) - ROO (14.67 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods using ROO.	**Plasma lipid profile and glucose** Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				Glucose [mmol L^{-1}]	No changes versus baseline nor interventions.
				Inflammation biomarkers CRP [mg dL^{-1}]	↓ According to phenolic content (dose-dependent).
				IL-6 [pg mL^{-1}]	↓ According to phenolic content (dose-dependent).
				sICAM-1 [ng mL^{-1}]	No changes versus baseline nor interventions.

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
				sVCAM-1 [ng mL^{-1}]	No changes versus baseline nor interventions.
Machowetz, et al., 2008	38 Healthy men	Randomized, crossover (EUROLIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - OO (164 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Plasma lipid profile**	
				Triglycerides [mmol L^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				Total cholesterol [mmol L^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				Inflammation biomarkers	
				Serum resistin [ng mL^{-1}]	No changes versus baseline nor interventions.
de la Torre-Carbot, et al., 2010	182 Healthy men	Randomized, crossover (EUROLIVE study)	25 mL per day of: - VOO (629 mg L^{-1}) - ROO (0 mg L^{-1}) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Lipoprotein composition**	
				LDL cholesterol [mg mg^{-1} Apo B]	No changes versus baseline nor interventions.
				LDL triglycerides [mg mg^{-1} Apo B]	No changes versus baseline nor interventions.
				Apo B [g L^{-1}]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				Plasma oxLDL [U L^{-1}]	↓ According to phenolic content (dose-dependent).
				Serum conjugated dienes [μmol L^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				Plasma hydroxy fatty acids [μmol L^{-1})]	↓ According to phenolic content (dose-dependent).
Konstantinidou, et al., 2010	90 Healthy subjects	Randomized, parallel	Intervention with: - Mediterranean diet + VOO (328 mg of phenols per kg) - Mediterranean diet + ROO (55 mg of phenols per kg) - Control habitual diet. 12 weeks of intervention. No wash-out period.	**Plasma lipid profile, glucose and blood pressure**	
				Triglycerides [mg dL^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mg dL^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				LDL-c [mg dL^{-1}]	↓ According to phenolic content (dose-dependent).
				HDL-c [mg dL^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				Glucose [mg dL^{-1}]	No changes versus baseline nor interventions.
				Systolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Diastolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				Plasma oxLDL [U L^{-1}]	No changes versus baseline nor interventions.
				Isoprostanes [pg per mmol of urine creatine]	↓ Regardless of phenolic content (non dose-dependent).
				8-oxo-dG [nmol per mmol of urine creatine]	↓ Regardless of phenolic content (non dose-dependent).
				Inflammation biomarkers	
				CRP [mg dL^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				IFN-γ [pg mL^{-1}]	↓ Regardless of phenolic content (non dose-dependent).

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
				MCP-1 [pg mL^{-1}]	No changes versus baseline nor interventions.
				s-P-selectin [ng mL^{-1}]	↓ Regardless of phenolic content (non dose-dependent).
				Expression changes in genes	↓ Of proatherogenic gene according to phenolic content and in the context of a Mediterranean diet.
Castañer, et al., 2011	200 Healthy men	Randomized, crossover (EUROLIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - OO (164 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Oxidative balance biomarkers** OLAB [mU L^{-1}]	↑ According to phenolic content (dose-dependent).
Moreno-Luna, et al., 2012	24 Women with mild hypertension	Randomized, crossover	60 mL per day of: - VOO rich in phenolic compounds (564 mg of phenols per kg) - polyphenol-free OO 2 months of intervention. 4 weeks of wash-out period with sunflower or corn oil.	**Blood pressure** Systolic blood pressure [mm Hg]	↓ According to phenolic content (dose-dependent).
				Diastolic blood pressure [mm Hg]	↓ According to phenolic content (dose-dependent).
				Oxidative balance biomarkers Plasma oxLDL [μg L^{-1}]	↓ According to phenolic content (dose-dependent).
				Inflammation biomarkers CRP [mg L^{-1}]	↓ According to phenolic content (dose-dependent).
				Endothelial function NOx [μmol L^{-1}]	↑ According to phenolic content (dose-dependent).
				ADMA [μmol L^{-1}]	↓ According to phenolic content (dose-dependent).
				IRH [PU]	↑ According to phenolic content (dose-dependent).
Widmer, et al., 2013	82 Subjects with early atherosclerosis	Randomized, parallel	30 mL per day of: - OO enriched with EGCG (600 mg of phenols per kg) - OO (340 mg of phenols per kg) 4 months of intervention. No wash-out period.	**Plasma lipid profile and blood pressure** Triglycerides [mg dL^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mg dL^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				Systolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Diastolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers Plasma oxLDL [mg dL^{-1}]	No changes versus baseline nor interventions.
				Plasma 8-isoprostanes [ng mL^{-1}]	↑ Regardless of phenolic content (non dose-dependent).
				Inflammation biomarkers sICAM-1 [ng mL^{-1}]	↓ Regardless of phenolic content (non dose-dependent).

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
				hsCRP [mg L^{-1}]	No changes versus baseline nor interventions.
				Il-6 [pg mL^{-1}]	No changes versus baseline nor interventions.
				sVCAM-1 [ng mL^{-1}]	No changes versus baseline nor interventions.
				Endothelial function	
				RHI [AU]	↑ Regardless of phenolic content (non dose-dependent).
Hernáez, et al., 2014	47 Healthy men	Randomized, crossover (EUROLIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Plasma lipid profile**	
				Triglycerides [mg dL^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mg dL^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				Lipoprotein profile	
				Large HDLs particles	↑ According to phenolic content (dose-dependent).
				Small HDLs particles	↓ Regardless of phenolic content (non dose-dependent).
				HDL CEC	↑ According to phenolic content (dose-dependent).
				HDL monolayer fluidity	↑ Regardless of phenolic content (non dose-dependent).
				Enzymes related to HDL metabolism	
				CETP activity [U L^{-1}]	No changes versus baseline nor interventions.
				LCAT activity [U L^{-1}]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				Plasma oxLDL [U L^{-1}]	↓ According to phenolic content (dose-dependent).
Hernáez, et al., 2015	25 Healthy men	Randomized, crossover (EUROLIVE study)	25 mL per day of: - VOO (366 mg of phenols per kg) - ROO (2.7 mg of phenols per kg) 3 weeks of intervention. 2 weeks of wash-out periods with ROO.	**Plasma lipid profile**	
				Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				Apo B concentrations [% change from baseline]	↓ According to phenolic content (dose-dependent).
				Lipoprotein profile	
				Number of total LDL particles [% change from baseline]	↓ According to phenolic content (dose-dependent).
				Number of small LDL particles [% change from baseline]	↓ According to phenolic content (dose-dependent).
				Oxidative balance biomarkers	
				LDL oxidizability (lag time [min])	↑ Regardless of phenolic content (non dose-dependent).

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
Silva, et al., 2015	69 Healthy subjects	Randomized, parallel	Single intake of 20 mL of: - high phenol OO (286 mg of CAE per kg) - low phenol OO (18 mg of CAE per kg) 6 weeks of intervention. No wash-out period.	**Plasma lipid profile, glucose and blood pressure**	
				Triglycerides [mmol L^{-1}]	No changes versus baseline nor interventions.
				Total cholesterol [mmol L^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mmol L^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mmol L^{-1}]	↑ Regardless of phenolic content (non dose-dependent).
				Glucose [mmol L^{-1}]	↑ Regardless of phenolic content (non dose-dependent).
				Systolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Diastolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Oxidative balance biomarkers	
				Plasma oxLDL [μg L^{-1}]	No changes versus baseline nor interventions.
				FRAP [mmol L^{-1}]	No changes versus baseline nor interventions.
				Proteomic biomarkers	
				Urinary proteomic biomarkers of coronary artery disease	↑ Proteomic CAD score regardless of phenolic content (non dose-dependent).
Santangelo, et al., 2016	11 Overweight and DMT2 without insulin therapy subjects	Randomized, crossover	25 mL per day of: - EVOO (577 mg of phenols per kg) - ROO (no phenols) 4 weeks of intervention. The 4 week period of ROO consumption was considered as wash-out period.	**Lipid profile, glucose and blood pressure**	
				Triglycerides [mg dL^{-1}]	No changes versus baseline nor interventions.
				Total Cholesterol [mg dL^{-1}]	No changes versus baseline nor interventions.
				LDL-c [mg dL^{-1}]	No changes versus baseline nor interventions.
				HDL-c [mg dL^{-1}]	↓ According to phenolic content (dose-dependent).
				Glucose [mg dL^{-1}]	↓ According to phenolic content (dose-dependent).
				Systolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Diastolic blood pressure [mm Hg]	No changes versus baseline nor interventions.
				Inflammation biomarkers	
				hsCRP [mg dL^{-1}]	No changes versus baseline nor interventions.
				AST [UI L^{-1}]	↓ According to phenolic content (dose-dependent).
				ALT [UI L^{-1}]	↓ According to phenolic content (dose-dependent).
				IL-6 [pg mL^{-1}]	No changes versus baseline nor interventions.
				TNF-α [pg mL^{-1}]	No changes versus baseline nor interventions.
				Adiponectin [pg mL^{-1}]	No changes versus baseline nor interventions.

(*Continued*)

Table 1. Continued.

Authors, year	Subjects	Type of study	Intervention	Outcome/biomarkers	Effects
				Visfatin [ng mL^{-1}]	↓ According to phenolic content (dose-dependent).
				Apelin [ng mL^{-1}]	No changes versus baseline nor interventions.

ADMA, asymmetric dimethylarginine; ALT, alanine aminotransferase; Apo, Apolipoprotein; AST, aspartate aminotransferease; AU, arbitrary Units; CAE, caffeic acid equivalents; CAD, coronary artery disease; CEC, HDL cholesterol efflux capacity; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; DMT2, diabetes mellitus type 2; EUROLIVE, effect of olive oils on oxidative damage in European populations; EVOO, extra virgin olive oil; F2α-isoprostanes, FRAP, ferric reducing ability of plasma; GPx, glutathione-peroxidase; GSH,GSSG ratio, reduced–oxidized glutathione ratio; GSH-Px, glutathione peroxidase; HDL-c, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IRH, ischemic reactive hyperemia; LCAT, lecithin–cholesterol acyltransferase; LDL-c, low-density lipoprotein cholesterol; MDA, malondialdehyde; NOx, nitrites/nitrates; OLAB, oxidized LDL serum antibodies; OO, olive oil; oxLDL, oxidized LDL; PGF2α, prostaglandin F2α; PU, perfusion units; RHI, reactive hyperemia index; ROO, refined olive oil; sICAM-1, soluble intercellular adhesion molecule 1; SOD, superoxide dismutase; sVCAM-1, soluble vascular adhesion molecule 1; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor α; TXB2, thromboxane B2; VOO, virgin olive oil.

PC of FVOOT.[43] In previous studies, attempts to monitor OO consumption with biomarkers of intervention compliance have been focused on analyzing the total HT in urine and plasma.[11,44] The VOHF project is the first one to determine PC metabolites in these biological fluids after the intake of OO. Moreover, this is also the first time that PC metabolites were detected in erythrocytes after each VOO intervention. HT sulfate was the only phenolic metabolite derived from OO PC detected in erythrocytes, whereas hydroxyphenylpropionic acid sulfate and thymol sulfate were detected in erythrocytes as thyme phenolic metabolites similar to plasma and urine. Hydroxyphenylpropionic acid sulfate appeared to be an erythrocyte biomarker for thyme PC, as it was only detected after FVOOT intervention.[45] In this sense, the VOHF study enabled robust quantitative and qualitative compliance biomarkers after the ingestion of phenol-enriched VOO to be determined and provided a thorough analysis of the true phenolic exposure after a sustained consumption that could be further related to expected biological effects.

3.2.3. Sustained Effects on HDL Composition and Function

3.2.3.1. HDL Lipid Composition. An increase in phospholipids/free cholesterol and esterified cholesterol/free cholesterol ratios in HDL was observed after FVOOT intake.[42] These changes could promote CEC, as the PREDIMED study previously reported.[46] In the PREDIMED study has recently shown that a Mediterranean diet enriched with VOO increases the phospholipids/free cholesterol ratio content in the HDL monolayer. These changes resulted in the enhancement of CEC, among other functions.[46] Accordingly, in the VOHF study, we have described that a decrease in free cholesterol and an increase in triglycerides are major determinants of monolayer fluidity and therefore of CEC.[47]

3.2.3.2. Enzymes Related to HDL Metabolism. FVOOT intake increased lecithin–cholesterol acyltransferase (LCAT) activity versus VOO intake. Although being no significant, an increase in LCAT (FVOO vs VOO) and cholesteryl ester transfer protein (CETP) activities (FVOO and FVOOT vs VOO) was also observed, which in turn may contribute to CEC and HDL maturation enhancement.[42]

Once activated by Apolipoprotein (Apo)A-I, LCAT esterifies the free cholesterol effluxed from cells and located on the HDL surface. Because of its hydrophobicity, cholesterol esterified by LCAT is partitioned into the hydrophobic core of HDL generating a free cholesterol gradient in the HDL monolayer and enhancing HDL maturation from small (s-HDL) to large HDL (l-HDL) particles. These l-HDL particles are further remodeled by the CETP enzyme, which transfers esterified cholesterol to triglyceride-rich lipoproteins and delivers triglycerides to HDL in return, contributing to HDL maturation. Triglycerides-rich and esterified cholesterol-poor HDL is delipidated and converted into s-HDL and lipid-free ApoA-I, which are eventually reintegrated in the reverse cholesterol transport pathway. The free cholesterol gradient resulting from LCAT activation and the conversion phenomena resulting from CETP activity contribute to reverse cholesterol transport and HDL maturation,[22] in agreement with our results obtained in the VOHF study[48,49] and detailed in Sections 3.2.3.4 and 3.2.3.8. Supporting these data, other authors have reported that HDL maturation is compromised when CETP and LCAT activation is inhibited.[50,51]

3.2.3.3. HDL Proteome. To our knowledge, the VOHF study is the first one assessing the effects of PCs on the HDL protein cargo. The three VOOs consumed in the VOHF study produced changes in the expression of HDL protein cargo. One hundred and twenty-seven proteins were identified, 15 of them being commonly modified after the three VOOs intake. These 15 proteins were associated with a broad range of HDL cardioprotective functions. The common upregulated proteins were related to cholesterol homeostasis, blood coagulation, and protection against oxidation. The common downregulated proteins were implicated in acute-phase response, lipid transport, immune response, and proteolysis. The 15 common proteins were mainly involved in the liver X receptor/retinoid X receptor activation, acute-phase response, and atherosclerosis signaling pathways, which could be related to the capacity of OO PC to regulate gene transcription. These results demonstrate that VOOs consumption exerts a cardioprotective impact on the HDL proteome that could enhance HDL functionality.[52]

3.2.3.4. HDL Lipoprotein Profile. The intake of phenol-enriched VOOs modified HDL subclass distribution toward

larger and more mature HDL particles. FVOO and FVOOT increased HDL size and l-HDL number, while FVOO also decreased s-HDL number.[42,49] These changes are translated into changes in HDL function because each HDL subclass exhibits differences in functionality irrespective of its cholesterol content. For instance, s-HDL are more efficient in promoting CEC and inhibiting inflammation than l-HDL.[22,53,54] However, in most studies, s-HDL particles are more strongly associated with increased CHD risk than l-HDL particles,[55,56] and high levels of s-HDL and/or low levels of l-HDL particles are often present in CHD, ischemic stroke, and type 2 diabetes mellitus (T2DM).[57–59] Moreover, in a cohort of asymptomatic older adults, CEC was inversely associated with s-HDL particle levels and was directly associated with l-HDL, medium HDL, and HDL size.[60] In concordance with this evidence, in the VOHF study, we have found that CEC was directly related to HDL size, with s-HDL being inversely related to CEC.[47] This paradoxical evidence on the presence of high levels of s-HDL in CVD, together with s-HDL being more functional than l-HDL, may be explained by the hypothesis that increased s-HDL particles in the serum may indicate an aberration in the maturation of s-HDL particles, therefore increasing the risk of CVD.[60,61] According to this evidence, an enhancement of HDL maturation was observed in the VOHF study, surely due to the increase in LCAT activity reported after FVOOT intake.[42] Similarly, the EUROLIVE and the PREDIMED studies demonstrated that the intake of phenol-rich VOOs induced the formation of larger HDL particles, and increased CEC, LCAT, and CETP activities.[12,62]

Both phenol-enriched VOOs also decreased s-HDL/l-HDL and HDL-c/HDL particle number (HDL-P) atherogenic ratios.[42,49] The latter ratio is considered a new potential measure of HDL function and it is directly related to atherosclerosis progression in CVD-free individuals.[63–65] This ratio indicates the enrichment of the HDL particle in cholesterol and reflects the presence of cholesterol-overloaded HDL-P. These particles appear to exert a negative impact on the cardioprotective function of HDL by impairing CEC, HDL clearance, and HDL anti-inflammatory and antioxidant properties.[63,66–68] Thus, the decrease in the HDL-c/HDL-P ratio after FVOO and FVOOT intakes observed in the VOHF study is indicative of the decrease in cholesterol-overloaded HDL particles and therefore the enhancement of HDL function.

The enhancement of HDL maturation observed in the VOHF study can be explained, in part, by the increase in LCAT activity reported after the consumption of FVOOT.[42] Similarly, the EUROLIVE and the PREDIMED studies demonstrated that the intake of phenol-rich VOOs induced the formation of larger HDL particles. These changes in HDL size and distribution were accompanied by increases in CEC, LCAT, and CETP activities, antioxidant and anti-inflammatory properties, and vasodilatory capacity.[12,62]

3.2.3.5. Endogenous HDL Antioxidant Compounds. The concentration of the endogenous antioxidants present in HDL particle improved after phenol-enriched VOOs sustained intake. Both FVOO and FVOOT increased lipophilic antioxidants (retinol, ubiquinol, α-tocopherol, and carotenoids, such as lutein and β-cryptoxanthin), whereas FVOO also increased hydrophilic antioxidants (phenolic metabolites such as thymol sulfate, caffeic acid sulfate, and hydroxyphenylpropionic acid sulfate) in HDL.[48]

The coexistence of these lipophilic and hydrophilic antioxidants linked to HDL may confer additional benefits by protecting lipids and proteins from oxidative damage via different antioxidant mechanisms, since the antioxidant system is a complex network of interacting molecules.[69,70] In response to oxidative stress, antioxidant molecules are oxidized and converted into harmful free radicals that need to be converted back to their reduced form by complementary antioxidants. The benefits of antioxidant complementarity are supported by the fact that supplementing high-risk individuals with a high dose of a single type of antioxidant promotes, rather than reduces, lipid peroxidation, whereas the combination of different antioxidants has been shown to be effective in reducing atherosclerosis in human trials.[18]

It is worth highlighting that α-tocopherol is one of the main antioxidants in human plasma, and it is present in the circulation anchored to HDL and LDL. α-Tocopherol is the main initial chain-breaking antioxidant during lipid peroxidation and, subsequently, the resultant α-tocopherol is recycled back to its biologically active reduced form by Coenzyme Q (CoQ) and some active phenolic acids, such as rosmarinic and caffeic acids.[71–74] In the VOHF study, the FVOOT intervention increased α-tocopherol, ubiquinol (the reduced form of CoQ), caffeic acid sulfate, and hydroxyphenyl propionic acid sulfate, while FVOO only increased ubiquinol but not α-tocopherol, caffeic acid sulfate, and hydroxyphenyl propionic acid sulfate. These data suggest better α-tocopherol regeneration, leading to enhanced protection against oxidation after FVOOT intake, which is in agreement with the previous results on DNA protection against oxidation following FVOOT.[45] Thus, the FVOOT intervention may be better at improving HDL antioxidant activity and may consequently preserve HDL protein structures. To date, there are no data regarding the effects of OO PC on the CoQ system. However, some authors have evaluated MUFA- and PUFA-rich diets on CoQ. In this sense, MUFA-rich dietary fats have been shown to increase CoQ levels exerting its beneficial effects on oxidative stress, as recently reviewed by Varela-López et al.[74] Some of these CoQ properties are involved in age-related diseases such as atherosclerosis, diabetes, CHD, and neurodegenerative diseases.[75,76] Moreover, when PUFA-rich diets were supplemented with CoQ, beneficial effects similar to those observed after a MUFA-rich diet were observed decreasing lipid peroxidation and increasing antioxidant capacity among others.[75–77]

The antioxidant content of HDL observed following the consumption of both phenol-enriched VOOs is translated into improvements in HDL physicochemical characteristics and therefore HDL functionality. In this sense, the EUROLIVE study revealed that the intake of phenol-rich VOOs resulted in PC binding to HDL, contributing to the enhancement of HDL functionality, CEC in particular, according to the phenolic content of the consumed VOO.[12]

3.2.3.6. Enzymes Related to HDL Antioxidant Activity. Platelet-activating factor acetylhydrolase (PAF-AH), glutathione selenoperoxidase-3 (GSPx-3), and paraoxonase (PON) family are the main antioxidant enzymes present in HDL.[78] Although no changes were observed in either PAF-AH or GSPx-3 activities,[48] phenol-enriched VOOs modulate the PON enzyme family based on PC content and source. In this sense, the intake of VOO

and FVOO decreased PON1 protein levels and increased PON3 protein levels and PON1-associated activities (lactonase and paraoxonase). Conversely, the intake of FVOOT induced the opposite results and increased PON1 aryl-esterase activity.[41,42]

High PON1 protein levels and low associated activities are characteristic of CVD[79–82] and other diseases characterized by dysfunctional HDL particles and increased CVD risk such as T2DM,[83] inflammatory diseases,[84,85] cancer, and several hepatic and renal diseases.[51,86] Furthermore, it is worth mentioning that PON1 positively correlates with the improvement of HDL antioxidant properties to such an extent that PON1 activities have been proposed as new biomarkers of HDL function and CVD risk.[81,87] Therefore, the modulation of PON family observed after VOO and FVOO intake can be perceived as beneficial, as they might be indicative of a proper oxidative balance and HDL function enhancement. Similarly, the PREDIMED study reported that a Mediterranean diet enriched with VOO increased PON1 activity and other related HDL functions.[46] The contrary effects in PON family observed after FVOOT may be due to the combination of OO PC with thyme PC intake, rather than the sole presence of thyme PC since mechanistic studies revealed that single-type PC modulated PON1 synthesis, while no effects were observed when multiple types of PC were combined.[41]

The increase in PON3 protein levels observed after VOO and FVOO intakes may play a cardioprotective role since PON3 protein depletion from HDL is associated with subclinical atherosclerosis and chronic liver disease.[88] These results partially agree with the proteomic study carried out in the HDL from the VOHF participants, in which an increase in PON3 protein was reported after all VOOs intake.[52]

Several mechanisms may explain the modulation of PON status observed in the VOHF study: 1) hepatic PON synthesis modulation observed after single-type PC intake;[41] 2) the increase of antioxidant presence in HDL observed in the VOHF study,[48] which confers better antioxidant protection to ApoA-I, stabilizing PON1 binding to HDL and enhancing PON1 activation; and 3) the HDL maturation enhancement observed after phenol-enriched VOOs,[49] which has been described as being a key factor in modulating the PON system.[50,51]

3.2.3.7. HDL Monolayer Fluidity. No significant changes were observed in HDL monolayer fluidity after any VOO intervention.[48] However, when all VOO interventions were tested together, the fluidity of the HDL monolayer was one of the main determinants for CEC enhancement.[47] The EUROLIVE study showed that phenol-rich VOO intake increases HDL monolayer fluidity in healthy subjects and that this increase was accompanied by an increase in CEC.[12] The PREDIMED study recently reported that a Mediterranean diet enriched with VOO increases the phospholipid content in the HDL monolayer, resulting in the enhancement of CEC, although HDL monolayer fluidity was not assessed in this study.[46] The importance of HDL monolayer fluidity lies in the fact that it reflects the functional behavior of HDL to such an extent that fluidity has been considered as an intermediate marker of HDL functionality. In particular, the more fluid the HDL monolayer is, the greater the cholesterol efflux rate from lipid-laden macrophages to HDL.[89] Moreover, lipid peroxidation is known to rigidify HDL monolayer fluidity, resulting in less CEC in in vitro–ex vivo experiments.[90,91] In concordance with this evidence, we have reported that increases in fluidity and decreases in HDL oxidative status are major determinants for CEC.[47]

3.2.3.8. HDL Cholesterol Efflux Capacity. FVOOT intake increased CEC versus FVOO and tended to increase versus its baseline. Moreover, when all VOO interventions were tested together, CEC also increased versus the baseline. This increase may be due to the increase in HDL maturation observed in the VOHF study, as we have demonstrated that CEC is directly related to HDL size, with s-HDL being inversely related to CEC.[47–49]

The increase in the antioxidant content of the HDL particle observed after phenol-enriched VOOs intake may enhance CEC. That is, antioxidants present in HDL provide a proper oxidative balance, inhibiting lipid peroxidation and in turn increasing HDL monolayer fluidity as a result.[47,48] Concordantly, the EUROLIVE study showed that phenol-rich VOOs intake increased HDL monolayer fluidity in healthy subjects and that this increase was accompanied by an increase in CEC.[12] The differences observed between these two studies may arise from the different characteristics of the studied populations, since the EUROLIVE study was carried out in healthy volunteers, while the VOHF study was undertaken in hypercholesterolemic subjects. Moreover, in the VOHF study, a phenol-enriched VOO (80 mg of TPC per kg oil) was used as a control, while a refined OO was employed in the EUROLIVE study. The increase in the antioxidant content of the HDL particle may also protect proteins from oxidative damage. That is the case of proteins involved in both CEC and HDL maturation, such as LCAT and ApoA-I, which become dysfunctional when oxidized. Functional ApoA-I stabilizes ATP-binding cassette transporter A1 (ABCA1) and activates LCAT, promoting CEC.[22,92] Although no changes in ApoA-I sera concentrations were observed in the VOHF study after any VOO intake,[42] the higher content of antioxidants in HDL observed in the VOHF study may confer better antioxidant protection to ApoA-I, and this fact could be partly responsible for the observed enhancement of CEC. Consistent with this evidence, we have revealed a close inter-relationship between HDL oxidative status, ApoA-I, and HDL monolayer fluidity, which are the three main factors that appear to be related to CEC in the VOHF study.[47]

3.2.4. Sustained Effects on other CVD-Related Parameters

3.2.4.1. Lipid Profile. No changes were observed in EDTA-plasma TG, TC, LDL-c, HDL-c, ApoA-I, or ApoB-100.[42] An exception was the 8.5% decrease in LDL-c levels as estimated by nuclear magnetic resonance (NMR) after FVOO consumption,[49] which is in agreement with that reported at postprandial state after extra-VOO consumption.[93]

3.2.4.2. LDL, VLDL, and IDL Lipoprotein Profile. FVOO decreased LDL particle number (LDL-P), ApoB-containing particles number, small LDL, medium VLDL, LDL size, VLDL size, and the LDL-P/HDL-P ratio, while FVOOT only decreased medium VLDL.[49] All these parameters are commonly associated with the risk of CHD and dyslipidemia in individuals with T2DM.[94–97] The results observed after FVOO are in agreement with the decrease in LDL-P observed in the EUROLIVE study after the

intake of VOOs with similar phenolic content to the FVOO (366 vs 500 mg of TPC per kg oil, respectively).[12] Moreover, the PREDIMED study has recently demonstrated that a Mediterranean diet enriched with VOO increases the estimated LDL size decreasing; therefore, the LDL atherogenicity in high CVD risk individuals.[98]

Of all the LDL and VLDL particle biomarkers tested, the LDL-P/HDL-P ratio shows the strongest independent association with CHD, as the higher the LDL-P/HDL-P ratio is, the higher the CHD risk observed, leading to significant net reclassification improvements in the American Heart Association/American College of Cardiology CHD risk scores.[99]

3.2.4.3. Glucose and Insulin Resistance. No changes were observed in glucose levels after any intervention[49] as previously reported by other authors after the intake of VOO with lower or similar phenolic content.[27,31,32,34] The VOHF project is the first study assessing the effect of VOO intake on the lipoprotein insulin resistance (LP-IR) index, a measure of insulin resistance derived from lipoprotein NMR measurements.[100] In this sense, both FVOO and FVOOT decreased LP-IR,[49] which is directly related to the Homeostatic Model Assessment index and inversely to the glucose disposal rate, the gold standard for assessing insulin sensitivity. This index has been proposed as a simple method for assessing the risk to develop a prediabetic or diabetic state.[100]

3.2.4.4. Oxidative Stress. The intake of phenol-enriched VOOs used in the VOHF study ameliorates the oxidative balance. In particular, both FVOO and FVOOT increased the endogenous antioxidant enzymes, superoxide dismutase, glutathione peroxidase, and catalase[45] which constitute the first line of antioxidant defense. These changes can be perceived as beneficial, as they might be indicative of a proper oxidative balance as detailed in Section 3.2.3.6. Both functional VOOs also increased DNA protection from oxidation by decreasing 8-hydroxy-2′-deoxyguanosine (8OHdG).[45] Since these effects resulted to be greater after FVOOT than after FVOO intake, FVOOT may be better at enhancing a proper oxidative balance.

Furthermore, FVOOT also ameliorated the antioxidant status of LDL by decreasing oxLDL in a subsample of 12 individuals[101] and enhanced HDL antioxidant function by increasing PON1-associated arylesterase activity.[42] The optimal balance in PC from OO and from thyme seems to act synergistically, decreasing the oxidative stress by trapping free and peroxy radicals and chelating metal ions. In addition, catabolism of PC by bifidobacteria increases the presence of colonic metabolites in feces with significant antioxidant action, especially for the protection of lipoproteins,[101] as detailed in Section 3.2.4.8. Mechanistic studies carried out in rats revealed that the consumption of thyme PC inhibits the NF-κB activation[45] reducing, therefore, ROS production and oxidative damage to lipids and DNA.[102] However, FVOO also exerts an antioxidant role by additionally decreasing PON1 protein levels and increasing PON3 protein levels and PON1-associated specific activities.[41] These changes can be perceived as beneficial, as they might be indicative of a proper oxidative balance as detailed in Section 3.2.3.6.

3.2.4.5. Inflammation. In the VOHF study, no changes in plasminogen activator inhibitor-1 (PAI-1) concentrations were observed after the consumption of phenol-enriched VOO,[40] although other acute-phase response proteins (α-1-acid glycoprotein 1, α-2-antiplasmin, α-2-HS-glycoprotein, and haptoglobin) were found to be downregulated in HDL after all VOO interventions.[52] These acute-phase proteins are increased during inflammation and could be used as inflammatory biomarkers related to CVD.[103] Similarly, the consumption of phenol-rich VOO has also been related to favorable modulation of inflammation.[104] Therefore, all VOOs tested in the VOHF study may hold anti-inflammatory effects. However, other inflammatory biomarkers should be measured in the future to obtain a clear conclusion. In this sense, some authors have reported that the intake of phenol-rich VOOs decreases C-reactive protein (CRP), interleukin-6 (IL-6), soluble vascular cell adhesion molecule (sVCAM), soluble intercellular adhesion molecule (sICAM), soluble p-selectin, and interferon gamma (IFN-γ) according to or irrespective of phenolic content of the oil ingested.[27,29,34,39]

3.2.4.6. Fat-Soluble Vitamins. After FVOO intervention, a significant increase in α- and γ-tocopherol plasma levels was observed. After FVOOT intervention, a significant increase in retinol, β-carotene, β-cryptoxanthin, lutein, and α-tocopherol plasma levels was observed. Moreover, plasma concentrations of retinol, β-cryptoxanthin, lutein, and α-tocopherol were significantly higher in both functional VOOs compared to the control VOO.[40] Similarly, both functional VOOs increased the fat-soluble vitamin content in HDL, as previously described in Section 3.2.3.5. Our results are in concordance with the increase in plasma concentrations of β-carotene after an intervention with a Mediterranean diet rich in VOO compared to another diet with the same percentage of dietary fat and β-carotene concentration.[105] It is noteworthy that the three VOOs tested in the VOHF study had the same composition and concentration of fat-soluble vitamins and fatty acids so the significant increases observed in plasmatic levels were associated with the phenolic supplementation.

3.2.4.7. Endothelial Function. Both phenol-enriched VOOs ameliorated endothelial function by increasing IRH,[40] as previously described in hypertensive women after the consumption of VOO with a high PC content.[39] Previous studies have begun to uncover the potential mechanisms by which OO PC may induce endothelial improvements. The endothelial function enhancement has been described to be mediated via the modulation of NOx metabolites and endothelin-1.[106] The results of the VOHF study do not confirm this mechanism since no effects were observed in endothelin-1 nor NOx levels after the intake of phenol-enriched VOO.[40] Other mechanisms, such as the reduction in oxidative stress, are also involved in the improvement of endothelial function.[106] In this sense, a proper oxidative balance has been observed in the VOHF study, since both phenol-enriched VOOs exerted beneficial changes in antioxidant HDL proteins[41,52] and in the content of antioxidants in HDL[48] and in plasma.[40] Accordingly, a positive post-intervention correlation was observed for IRH values and plasma concentrations of β-cryptoxanthin, lutein, and α-tocopherol, supporting a relation between the improvements in endothelial function and the increased levels of plasmatic fat-soluble vitamins.[40] Our results are in concordance

with those of Marin et al.,[105] who observed an increment in plasma concentrations of β-carotene after an intervention with a Mediterranean diet rich in VOO. In this study, they also described positive correlations between β-carotene and circulating endothelial progenitor cells, which favor the regenerative capacity of the endothelium. Similarly, Karppi et al.[107] suggested that high plasma concentrations of β-cryptoxanthin, lycopene, and α-carotene may be associated with decreased intima-media thickness of the carotid artery wall.

In the VOHF study, a positive relationship was observed between IRH values and plasma concentrations of HDL-c,[40] which was also described in hypercholesterolemic patients after the acute consumption of a VOO with a high PC content.[106] Although HDL-c concentrations did not increase in the VOHF study, an increase in HDL functionality was observed, which can explain the improvement in endothelial function by HDL ability to inhibit monocyte adhesion.[108] However, additional studies are needed to confirm this association.

3.2.4.8. Gut Microbiota. Within the context of the VOHF study, it was investigated whether the changes generated on blood lipid profile after phenol-enriched VOOs could be related to changes in gut microbiota populations and activities in a subsample of 12 subjects. Quantitative significant changes in gut microbiota were only observed after FVOOT intake, by increasing *Bifidobacterium* group numbers.[101] Since recent studies in animals and humans have shown improvements in blood lipid profile with the ingestion of bifidobacteria and lactobacilli mixtures,[109] the increase in *Bifidobacterium* could be responsible at least in part for the decrease in oxLDL levels observed with the ingestion of FVOOT.[101] In the VOHF study, it was investigated whether not absorbed PC could serve as energy source for microbiota, therefore generating PC metabolites with antioxidant activities during the colonic transit. Results showed an increase in the protocatechuic acid (PCA) in the feces of volunteers after FVOOT intake. Additionally, the FVOO diet supplementation resulted in an increase in coprostanone (metabolite resulted from bacterial cholesterol degradation), free HT, and dihydroxyphenylacetic acids in feces.[101]

It has been demonstrated that bioactivity of some microbial metabolites from undigested PC is physiologically more relevant on CVD risk than the native form present in the diet.[110,111] In the VOHF study, the increase of fecal PCA after FVOOT intake could be due to the microbial transformation of PCA precursors provided by the thyme extract and absent in VOO.[112] We also found an increase in free HT after FVOO and FVOOT, which could be due to two factors, or the superposition of both, the microbial transformation of OO secoiridoids (HT precursors) and demonstrates a high stability of HT under the physiological conditions of the gut.[113] Although the increase in HT with FVOOT did not reach statistical significance, it could be behind, in combination with the increase in PCA, the decrease in LDL oxidation observed after the FVOOT intervention.

It was concluded that the cardioprotective effects observed after FVOOT could be mediated in part by the increases in populations of bifidobacteria together with increases in PC microbial metabolites with antioxidant activities. The specific growth stimulation of bifidobacteria in human gut suggests for the first time a potential prebiotic activity of FVOOT.[101]

The VOHF study also evaluated the influence of phenol-enriched VOO on human intestinal immune function, because abnormal microbiota has been described in many inflammatory and autoimmune diseases. FVOO increased the proportion of IgA-coated bacteria, which suggests a stimulation of the mucosal immunity at intestinal level.[114] This result confirms the hypothesis that a dietary strategy based on PC as prebiotics could be available for modulating either the composition or metabolic/immunological activity of the human gut microbiota.

3.3. New Insights Obtained from the VOHF Study

In general, phenol-rich VOOs are effective in reducing oxidative damage in a wide range of populations (Table 1). The improvement in the oxidative balance is accomplished through changes not only in lipids, mainly oxLDL levels in plasma,[3,12,23,25,26,31,39] but also in DNA.[32,34] These changes are observed according to the phenolic content of the OO tested. In the VOHF study, an improvement in the oxidative balance was observed after the intake of phenol-enriched VOOs, increasing the antioxidant content of HDL particle, upregulating proteins related to protection against oxidation, and modulating the PON antioxidant enzymes family.

As described in Table 1, controversial results on the effects of OO and its PC on HDL-c levels have been reported, since HDL-c concentrations increase or decrease in some studies, while in other studies no changes were observed. In the VOHF study, no changes in HDL-c levels were reported, although significant changes were observed in HDL functionality. Before the VOHF study, the EUROLIVE study was the only study that assessed HDL functionality in response to OO PC intake. The EUROLIVE study showed an enhancement of CEC after OO PC intakes.[12,30] Similarly, the VOHF study reported an increase in CEC together with an enhancement of HDL antioxidant, vasodilatory, and anti-inflammatory capacities after VOO consumption (**Figure 1**). To the best of our knowledge, this is the first time that HDL proteome and PON enzymes family modulation have been assessed after a VOO intervention. Results derived from these analyses allow us to explain mechanisms involved in HDL functionality enhancement.

To date, only EUROLIVE and VOHF studies have assessed the effects of OO PC on lipoprotein profile (Table 1). While both studies demonstrated that OO PC increased l-HDL and decreased s-HDL particles, the VOHF study showed for the first time that OO PC consumption also increases total HDL-P and HDL mean size, and decreases several HDL-related atherogenic ratios.[42,49]

Furthermore, few authors have evaluated the endothelial function in vivo through reactive hyperaemia assessment.[29,39] The increase in IRH values observed in the VOHF study is of great relevance, since it verifies previous scientific evidence and properly reflects the in vivo endothelial function status.

It is important to note that the VOHF study is the first one suggesting a potential prebiotic activity of functional VOOs because specific growth stimulation of bifidobacteria in the human gut is observed following FVOOT intake.[101] Another novel aspect of the VOHF study is that the parental matrix used as a control condition and for the elaboration of both functional VOOs contains indeed PC. Even with the presence of PC in the control

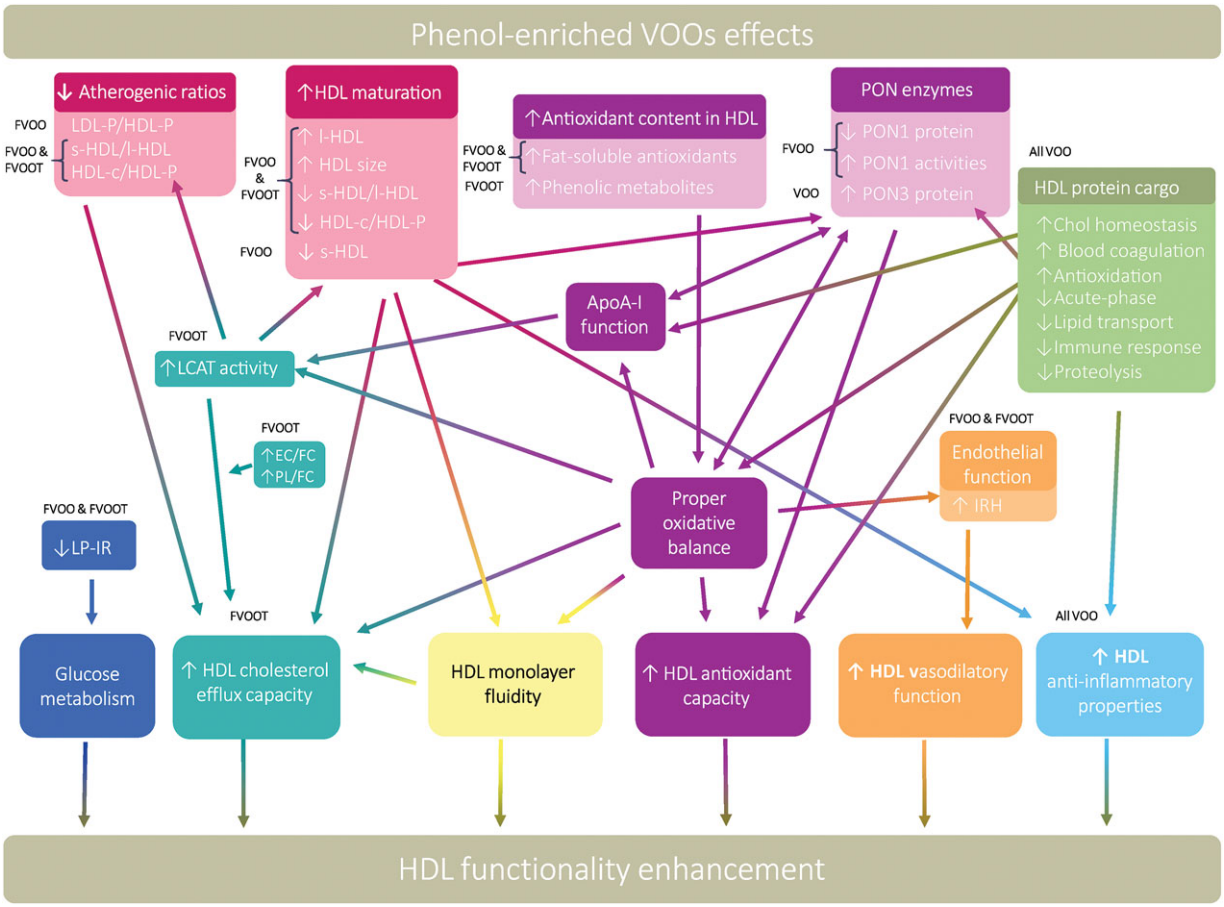

Figure 1. Scheme integrating the results and the relationships of the phenol-enriched VOOs effect on the HDL functionality in the VOHF study. Color boxes denote the different effects on HDL of phenol-enriched VOOs tested in the VOHF study: red, lipoprotein profile; blue, glucose metabolism; mint, CEC; yellow, HDL monolayer fluidity; purple, HDL antioxidant capacity; orange, HDL vasodilatory function; green, protein cargo; light blue, HDL anti-inflammatory properties. ApoA-I, apolipoprotein A-I; EC, esterified cholesterol; FC, free cholesterol; FVOO, functional VOO enriched with its own phenolic compounds; FVOOT, functional VOO enriched with its own phenolic compounds plus additional complementary ones from thyme; HDL, high-density lipoprotein; HDL-c, HDL cholesterol; HDL-P, HDL particle number; IRH, ischemic reactive hyperaemia; l-HDL, large HDL particles; LCAT, lecithin–cholesterol acyltransferase; LDL-P, low-density lipoprotein particle number; LP-IR, lipoprotein insulin resistance index; PL, phospholipids; PON, paraoxonase; s-HDL, small HDL particles; TC, total cholesterol; and VOO, virgin olive oil.

VOO (80 mg of TPC per kg oil), beneficial effects were observed after phenol-enriched VOO versus control VOO. One of the most relevant contributions of the VOHF study is the data obtained by blending OO PC with complementary ones from thyme. Although flavonoid effects on the cardiovascular system have been broadly assessed, this blending allows us to reveal, for the first time, the synergic effects of both types of PC.

3.4. Mechanisms of Action: the MEFOPC Project

In order to reveal the molecular target of the VOO PC, the "Metabolic Fate of Olive Oil Phenolic Compounds in Humans: Nutrigenomic Effects (MEFOPC) Project," a sequel project from the VOHF study, was performed. The main results obtained from this study are comprehensively detailed in supporting information file and summarized in **Figure 2**.

4. Limitations and Strengths

A potential concern in the VOHF study is the method used to isolate HDL to measure CEC because it does not accurately represent the contribution of pre-β HDL. This limitation could account for the fact that CEC after FVOOT reached significance versus FVOO, but not versus control VOO. An alternative would have been the use of ApoB-depleted plasma, but cholesterol acceptors other than HDL subfractions are also present and may interfere with CEC assessment. Another limitation is the inability to assess potential synergies and interactions in HDL parameters from PC and other VOO constituents. Nevertheless, the controlled diet followed throughout the trial should have limited the scope of these interactions.

One of the strengths of the VOHF study is its crossover, randomized, and controlled design, which enables collection of the first level of scientific evidence. In addition, the three VOOs employed in this study had the same parental matrix, fact

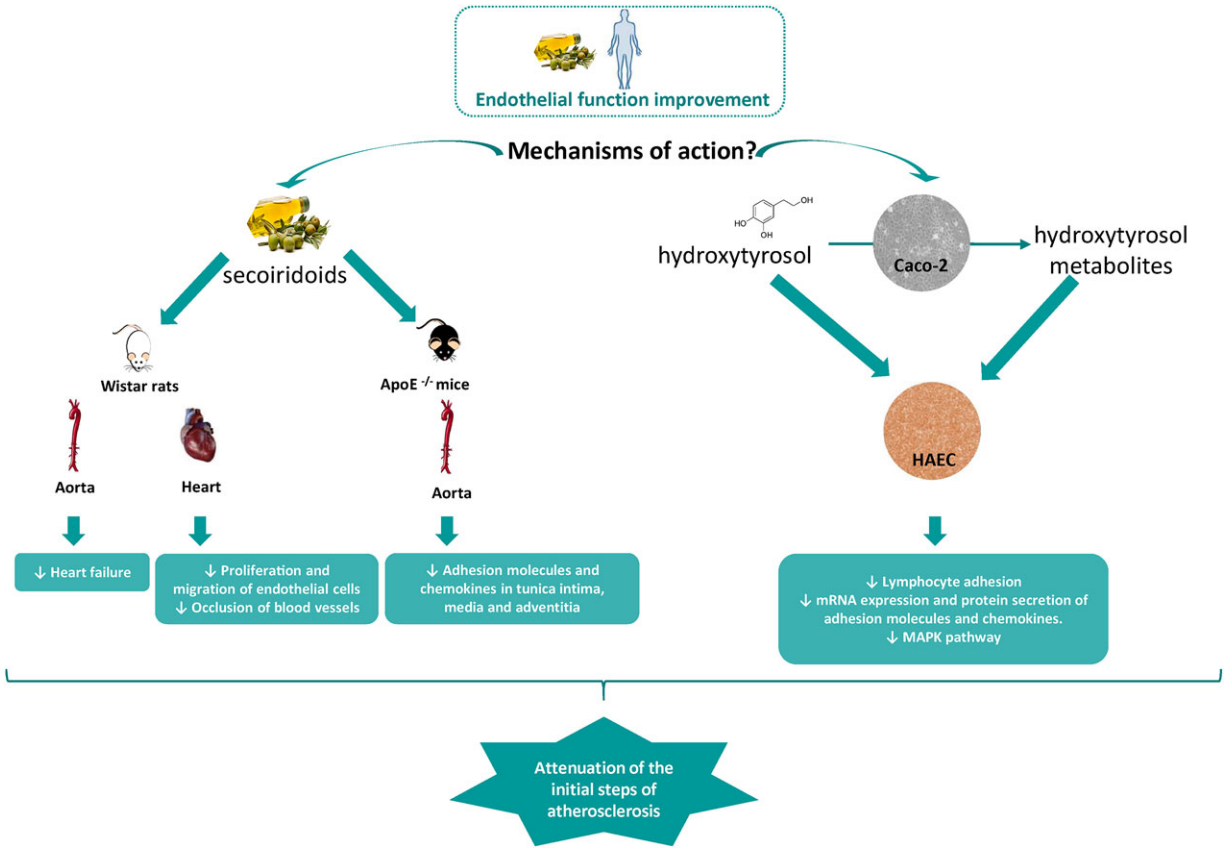

Figure 2. Scheme integrating the main results of the MEFOPC project. Caco-2, human cancer colon cell line; HAEC, human aortic endothelial cells, MAPK, mitogen-activated protein kinase.

that enables isolation of PC effects without the interference of additional nutrients.

5. Global Conclusions

The major results derived from the VOHF study confirms that phenol-enriched VOOs, enriched with their own PC or with them plus additional complementary ones from thyme, can act as nutraceuticals in regard to the in vivo HDL quantity (HDL-c levels), HDL quality (functionality), and other CVD-related parameters. These insights obtained from the VOHF study provide new evidence supporting the beneficial effects of OO and PC from different sources.

The changes observed are performed according to phenol source and content in the tested phenol-enriched VOO, since different effects have been observed after FVOO and FVOOT consumption, as detailed in **Table 2** and integrated in Figure 1. In particular, data obtained provide first level of evidence that both FVOO and FVOOT intake modulate HDL protein cargo toward a cardioprotective mode, improve HDL subclass profile and their associated atherogenic ratios, increase fat-soluble antioxidants in HDL and plasma, decrease the LP-IR index, protect DNA from oxidation, increase the endogenous antioxidant enzymes, ameliorate the endothelial function, and increase the fecal microbial metabolic activity in a protective way. Moreover, only FVOO intake exerts a beneficial impact on the PON enzyme family, improves LDL and VLDL subclass profile and their associated atherogenic ratios, and increases the fecal microbial immunological activity in a protective way. Furthermore, only FVOOT intake increases several lipid ratios and LCAT activity, increases PC metabolite content in HDL, increases CEC, decreases oxLDL, and increases fecal *Bifidobacterium* populations and PC bacterial metabolites in a protective way.

These phenol-enriched VOOs would allow the management of not only hypercholesterolemic subjects but also diabetic individuals since these VOOs modulate parameters commonly associated with T2DM, such as LP-IR and the LDL and VLDL subclasses profile.

Taking all these findings into consideration, novel therapeutic strategies should focus their efforts on not only increasing HDL-c levels but also enhancing HDL functionality through dietary, nutraceutical, or pharmaceutical interventions. The enrichment of VOOs with PC is a way of increasing the healthy properties of VOO without increasing the individual's caloric intake. Therefore, the tailoring of functional VOOs is an interesting and useful strategy for enhancing the functional quality of HDL and other cardiovascular risk factors, and thus, it could be a complementary tool for the management of cardiovascular risk individuals.

Table 2. Summary of the results obtained from the sustained-intake VOHF study.

Parameter		VOO intake	FVOO intake	FVOOT intake	Ref.
EFFECTS ON HDL COMPOSITION AND FUNCTION					
HDL lipid composition	**HDL fatty acids**	No changes	No changes	No changes	[42,52]
	PL/FC and EC/FC ratios	No changes	No changes	↑ PL/FC ratio (vs VOO and FVOO) ↑ EC/FC ratio (vs VOO and FVOO)	
Enzymes related to HDL metabolism	**LCAT activity**	No changes	(↑) LCAT activity (vs VOO)	↑LCAT activity (vs VOO)	[42]
	CETP activity	No changes	(↑) CETP activity (vs VOO)	(↑) CETP activity (vs VOO)	
HDL proteome			↑ Cardioprotective HDL protein cargo (vs pre-intervention)		[52]
HDL lipoprotein profile	**HDL particle number**	↓HDL-P (vs pre-intervention) ↓l-HDL(vs pre-intervention, VOO, and FVOOT) ↓m-HDL (vs pre-intervention) ↑s-HDL (vs pre-intervention, FVOO)	↓HDL-P (vs pre-intervention, and FVOOT) ↑l-HDL (vs VOO) ↓s-HDL (vs VOO, and FVOOT)	↑HDL-P (vs FVOO) ↑l-HDL (vs VOO) ↓m-HDL(vs pre-intervention) ↑s-HDL (vs pre-intervention, FVOO)	[49]
	HDL size	↓HDL mean size (vs pre-intervention, FVOO, and FVOOT)	↑HDL mean size (vs pre-intervention, VOO, and FVOOT)	↑HDL mean size (vs VOO) ↓HDL mean size (vs FVOO)	
	HDL-related atherogenic ratios	No changes	↓s-HDL/l-HDL ratio (vs VOO, and FVOOT) ↓HDL-c/HDL-P ratio (vs VOO) ↓ LDL-P/HDL-P ratio (vs VOO, and FVOOT)	↓s-HDL/l-HDL ratio (vs VOO) ↓HDL-c/HDL-P ratio (vs VOO)	
Endogenous HDL antioxidant compounds	**Lipophilic antioxidants**	No changes	↑Lutein (vs pre-intervention and VOO) ↑β-Criptoxanthin (vs pre-intervention and VOO) ↑Retinol (vs pre-intervention, VOO, and FVOOT) ↑Ubiquinol (vs pre-intervention and VOO)	↑Lutein (vs pre-intervention) ↑β-Criptoxanthin (vs pre-intervention and VOO) ↑α-tocopherol (vs pre-intervention) ↑Ubiquinol (vs pre-intervention)	[42]
	Hydrophilic antioxidants (phenolic metabolites)	No changes	No changes	↑ Thymol sulphate (vs pre-intervention, VOO, and FVOO) ↑ Caffeic acid sulphate (vs pre-intervention, VOO, and FVOO) ↑ Hydroxyphenylpropionic acid sulfate (vs pre-intervention, VOO, and FVOO)	
Enzymes related to HDL antioxidant activity	**PAF-AH activity**	No changes	No changes	No changes	[42]
	GSPx-3 activity	No changes	No changes	No changes	
	PON family	↓PON1 protein (vs pre-intervention, and FVOOT) ↑PON1 lactonase activity (vs pre-intervention, and FVOOT) ↑PON1 paraoxonase activity (vs pre-intervention, and FVOOT) ↑PON3 protein (vs pre-intervention, FVOO, and FVOOT)	↓PON1 protein (vs pre-intervention, and FVOOT) ↑PON1 lactonase activity (vs pre-intervention, and FVOOT) ↑PON1 paraoxonase activity (vs pre-intervention, and FVOOT)	↑PON1 protein (vs pre-intervention, VOO, and FVOO) ↑PON1 aryl-esterase activity (vs pre-intervention)	[41,42]
HDL monolayer fluidity		No changes	No changes	No changes	[48]
HDL cholesterol efflux capacity	**Interventions tested separately**	No changes	No changes	↑CEC (vs FVOO) (↑) CEC (vs pre-intervention)	[48]
	Interventions tested together	↑ CEC increased (vs pre-intervention)			[47]

(*Continued*)

Table 2. Continued.

Parameter		VOO intake	FVOO intake	FVOOT intake	Ref.
EFFECTS ON OTHER CVD-RELATED PARAMETERS					
Lipid profile	**TG, TC, LDL-c, HDL-c, ApoA-I, ApoB-100**	No changes	↓LDL-c measured by NMR (vs pre-intervention)	No changes	[42,49]
LDL lipoprotein profile	**LDL particle number**	No changes	↓LDL-P (vs pre-intervention, VOO, FVOOT) ↓IDL-P (vs pre-intervention, VOO, FVOOT) ↓s-LDL (vs FVOOT)	↑s-LDL (vs pre-intervention)	[49]
	LDL size	No changes	↑ LDL mean size (vs pre-intervention, VOO, FVOOT)	No changes	
VLDL lipoprotein profile	**VLDL particle number**	No changes	↓m-VLDL particle number (vs pre-intervention, VOO, and FVOOT) ↑s-VLDL particle number (vs pre-intervention)	↓m-VLDL particle number (vs pre-intervention, and FVOOT)	[49]
	VLDL size	No changes	↓VLDL mean size (vs pre-intervention, VOO, and FVOOT)	No changes	
ApoB-containing lipoproteins	**Particle number**	No changes	↓ ApoB-containing lipoproteins number (vs pre-intervention, VOO, and FVOOT)	No changes	[49]
Glucose and insulin resistance	**-**	No changes	↓ LP-IR ratio (vs VOO)	↓ LP-IR ratio (vs VOO)	[49]
Oxidative stress	**oxLDL (n = 12)**	No changes	No changes	↓ oxLDL (vs pre-intervention)	[45]
	SOD activity in erythrocytes	No changes	↑ SOD activity (vs pre-intervention, and VOO)	↑ SOD activity (vs pre-intervention, VOO, and FVOO)	[45]
	GSH-Px activity in erythrocytes	No changes	↑ GSH-Px activity (vs VOO)	↑ GSH-Px activity (vs VOO and FVOO)	
	CAT activity in erythrocytes	No changes	↑ CAT activity (vs VOO)	↑ CAT activity (vs VOO)	
	8OHdG	No changes	↓ 8OHdG (vs pre-intervention, and VOO)	↓ 8OHdG (vs pre-intervention, VOO and FVOO)	
	MetSO	↑ MetSO (vs pre-intervention)	↑ MetSO (vs pre-intervention)	↑ MetSO (vs pre-intervention)	
	8-iso PGF2α	No changes	No changes	No changes	
Inflammation	**PAI-1**	No changes	No changes	No changes	[40]
	Acute-phase response proteins	↓ α-1-Acid glycoprotein, α-2-antiplasmin, α-2-HS-glycoprotein, haptoglobin (vs pre-intervention)			[52]
Fat-soluble vitamins	**Fat-soluble vitamins**	No changes	↑ α- and γ-Tocopherol (vs pre-intervention) ↑ retinol, β-cryptoxanthin, lutein, and α-tocopherol (vs VOO)	↑ retinol, β-carotene, β-cryptoxanthin, lutein, and α-tocopherol (vs pre-intervention) ↑ retinol, β-cryptoxanthin, lutein, and α-tocopherol (vs VOO)	[40]
Endothelial function	**IRH values**	No changes	↑ IRH values (vs VOO)	↑ IRH values (vs VOO)	[40]
	NOx	No changes	No changes	No changes	
	Endothelin-1	No changes	No changes	No changes	
Gut microbiota	**Bifidobacterium group**	No changes	No changes	↑Bifidobacterium number	[101]
	Metabolic activity	No changes	↑ Coprostane (vs FVOOT) ↑ HT (vs pre-intervention) ↑ Dihydroxyphenylacetic acid (vs VOO)	↑ PCA metabolite (vs VOO) (↑) HT (vs Pre-intervention)	[112]
	Immunological activity	No changes	↑ IgA-coated bacteria (vs pre- intervention	No changes	[114]

8-iso PGF2α, 8-iso prostaglandin F2α; 8OHdG, 8-hydroxy-2′-deoxyguanosine; Apo, Apolipoprotein; CAT, catalase; CEC, HDL cholesterol efflux capacity; CETP, cholesteryl ester transfer protein; EC, esterified cholesterol; FC, free cholesterol; FVOO, functional VOO enriched with its own phenolic compounds; FVOOT, functional VOO enriched with its own phenolic compounds plus additional complementary ones from thyme; GSH-Px, glutathione peroxidase; GSPx-3, glutathione selenoperoxidase-3; HDL, high-density lipoprotein; HDL-P, total HDL particle number; HT, hydroxytyrosol; IDL-P, total IDL particle number; IgA, immunoglobulin A; IRH, ischemic reactive hyperemia; l-HDL, large HDL particle number; l-LDL, large LDL particle number; LCAT, lecithin–cholesterol acyltransferase; LDL, low-density lipoprotein; LDL-c, LDL cholesterol; LDL-P, total LDL particle number; LP-IR, lipoprotein insulin resistance index; m-HDL, medium HDL particle number; m-VLDL, medium VLDL particle number; MetSO, methionine sulfoxide; NOx, nitrites/nitrates; oxLDL, oxidized LDL; PAF-AH, platelet-activating factor acetylhydrolase; PAI-1, plasminogen activator inhibitor-1; PCA, protocatechuic acid; PL, phospholipids; PON, paraoxonase; s-HDL, small HDL particle number; s-VLDL, small VLDL particle number; SOD, superoxide dismutase; TC, total cholesterol; TG, triglycerides; VLDL, very-low-density lipoprotein; VOO, virgin olive oil. (↑) and (↓) denote borderline significances.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

cardiovascular, HDL functionality, phenolic compounds, polyphenols, virgin olive oil

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