Olive oil phenolic compounds and high-density lipoprotein function

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ABSTRACT

Purpose of review

The functional capacities of high-density lipoproteins (HDLs) reflect the physiological role of the particle better than the quantity of HDL cholesterol. Due to its phenolic compounds, the consumption of virgin olive oil has emerged as a promising therapy to promote these capacities. This review highlights the human studies that explain these benefits and explores some possible mechanisms.

Recent findings

The consumption of olive oil phenolic compounds increased the ability of HDLs to pick up cholesterol excess in peripheral cells (the cholesterol efflux capacity). Olive oil phenolic compounds have also been shown to improve HDL antioxidant capacities and some anti-inflammatory traits. These changes respond to an improvement of HDL oxidative status and composition.

Summary

Novel strategies to increase HDL functional capacities are in demand from clinicians. The attainment of a fully-functional HDL through dietary or lifestyle changes is a priority in cardiovascular research. Within this context, the consumption of virgin olive oil, due to its phenolic compounds, may be a relevant protective approach. Further studies in large-scale, randomized controlled trials are, however, required to confirm these effects in HDL functionality.

KEYWORDS

HDL function, olive oil phenolic compounds, cholesterol efflux capacity, HDL antioxidant capacity

45 **ABBREVIATIONS**

46 ApoA-I: apolipoprotein A-I

47 CEC: cholesterol efflux capacity

48 CVD: cardiovascular disease

49 FVOO: functional virgin olive oil, enriched with olive oil phenolic compounds

50 FVOOT: functional virgin olive oil, enriched with olive oil phenolic compounds +

51 phenols from thyme

52 HDL: high-density lipoprotein

53 HDL-C: high-density lipoprotein cholesterol

54 LCAT: lecithin-cholesterol acyltransferase

55 LDL: low-density lipoprotein

56 MD: Mediterranean Diet

57 PAF-AH: platelet-activating factor acetylhydrolase

58 PON-1: paraoxoase-1

59 PPAR: peroxisome proliferator-activated receptor

60 VOO: virgin olive oil

INTRODUCTION

High-density lipoprotein (HDL) cholesterol (HDL-C) levels are inversely and independently associated with cardiovascular disease (CVD) [1]. Several interventions, based on pharmacological and natural products, have been able to increase HDL-C concentrations although high levels have not always been associated with low cardiovascular risk [2]. Moreover, pharmacologically raised HDL-C levels have not always led to a decrease in cardiovascular risk and some studies have even reported an increased mortality risk [3]. The physiological role of HDL seems, therefore, to be better reflected by its function than HDL-C quantity.

HDLs play a central role in reverse cholesterol transport. They remove excess cholesterol from peripheral cells (the cholesterol efflux capacity, CEC) and transport it to the liver for further metabolism and excretion [4]. CEC has been shown to predict coronary event incidence [5] and be inversely related to the development of early atherosclerosis [6]. HDLs present other atheroprotective capacities: they counteract the oxidation of low-density lipoproteins (LDLs), present anti-inflammatory functions, and help preserve endothelium integrity [7].

Olive oil, the main source of fat in the Mediterranean Diet (MD), is considered to play a major role in its protective effects on CVD [8]. In observational studies adherence to the MD decreased the development of chronic CVD [9] whilst intervention studies demonstrated the beneficial effect of this diet on CVD primary and secondary prevention [10,11]. Our group reported that the consumption of virgin olive oil (VOO) increased HDL-C and decreased *in vivo* lipid oxidative damage. This effect was dose-dependently associated with the olive oil phenolic compound content [12]. Taking into account the rise in HDL-C quantity, an increase in the functional capacities of HDL due to the consumption of VOO could be expected. The whole matrix of phenolics in olive

oil is complex and diverse (**Table 1**) [13] and would be responsible for these potentially beneficial effects.

Our aim is to review the reported benefits of the consumption of phenolic compounds in VOO on the functionality of HDLs in humans. Further discussion of the possible related mechanisms is also provided.

HDL CHOLESTEROL EFFLUX CAPACITY

The consumption of phenolic compounds has increased CEC in some *in vivo* studies [14]. With respect to olive oil phenolic compounds, our group provided first-level evidence for the first time in humans of an increase in CEC after the consumption of a real-life dose of VOO (366 mg/kg) [15**]. To explain this change, we observed, on the one hand, an increase in the biological metabolites of olive oil phenolic compounds bound to the HDLs (hydroxytyrosol sulfate, and homovanillic acid sulfate and glucuronate). These compounds could exert a local antioxidant protection in HDLs [16,17,18*] that may prevent oxidative modifications of the apolipoprotein A-I (ApoA-I), the main HDL protein involved in CEC [4], and of other HDL proteins. Such protection would also avoid oxidative modifications of HDL lipids, making the lipoprotein more fluid and thus more functional [19]. Improvements in CEC and HDL fluidity have also been described in a non-controlled trial with VOO in healthy volunteers [20]. On the other hand, we found a decrease in the relative content of triglycerides in the HDL core. A triglyceride-poor HDL core is related to a more stable conformation of ApoA-I in the lipoprotein [21] which could lead to better HDL capacity to perform CEC.

Functional VOOs are also a promising therapy to improve CEC. Our group recently developed a randomized controlled trial in hypercholesterolemic individuals to test the

effects on the HDL characteristics related to CEC of two functional VOOs: one enriched with olive oil phenolic compounds (500 mg/kg; FVOO) and another with phenolic compounds from olive (250 mg/kg) plus additional complementary phenolic compounds from thyme (250 mg/kg) (total: 500 mg/kg; FVOOT), compared with a standard VOO (80 mg/kg) [22*]. The functional oils increased the quantity and bioavailability of the phenolic compounds in the real food matrix without raising the final fat intake [23]. Three main effects were observed. First, an increase in the lecithin-cholesterol acyltransferase (LCAT) mass in plasma after the FVOOT intervention versus the control VOO was reported. LCAT mediates the esterification of free cholesterol and its migration into the particle core. This process leads to the conversion of the immature HDLs into small particles and, finally, into larger ones [4]. The increase in LCAT mass could, therefore, be linked to a decrease in the relative content of free cholesterol and an increase in the relative content of phospholipids in the HDL surface, both observed after the FVOOT intervention versus the control VOO [22*]. Such changes may result in more fluid HDLs, indirectly associated with a greater CEC [19]. Second, in a pooling sample proteomic approach, we observed an increase in the ApoA-I content in HDL after the consumption of VOO and both functional VOOs [24*]. ApoA-I-rich HDLs may be more efficient in exerting CEC since ApoA-I is the main HDL protein involved in the process. Finally, in the same proteomic approach, we also found an increase in the content of affamin in HDL after the FVOOT intervention [24*]. Affamin is a transporter of α -tocopherol and, as a result, we would expect an increased α -tocopherol content in HDL which could complement the local antioxidant protection of the phenolic compounds.

CEC can also be modulated by the direct effect of olive oil phenolic compounds on CEC-related gene expression. In a postprandial study with pre-/hypertensive

individuals, a real-life dose of phenolic compound-enriched olive oil (961 mg/kg) enhanced the gene expression of transmembrane cholesterol transporters (ABCA1, SR-B1), and some transcription factors related to the peroxisome proliferator-activated receptors (PPAR α , PPAR γ , PPAR δ and PPARBP), when compared with a control VOO (289 mg/kg) [25]. The improvement in ABCA1 and SR-B1 expression could be mediated through the up-regulation of the PPAR-dependent pathway [26]. We additionally reported an augmentation in the PPAR-related gene expression in a previous postprandial study with an acute dose of 50mL of VOO [27]. With respect to long-term studies, similar increases in the expression of some transmembrane cholesterol transporters (ABCA1, ABCG1) in macrophages were described in a non-randomized trial with VOO in healthy volunteers [20], although no significant changes in ABCA1 expression were reported after the consumption of a VOO-rich MD in healthy individuals [28].

HDL ANTIOXIDANT ACTIVITY

HDLs protect LDLs against oxidative modifications; a relevant property since oxidized LDLs are a key trigger for the onset of atherosclerotic plaque [29]. The consumption of VOO has been dose-dependently associated *in vivo* with a decrease in oxidized LDLs [12], part of this protection could take place through an induction of the HDL antioxidant capacities. After the consumption of a VOO-rich diet an increase in HDL antioxidant activity was observed in apoE-deficient mice [29], a finding that could be expected to occur in humans. The main agents involved in HDL antioxidant capacity are ApoA-I and paraoxonase-1 (PON1). Nevertheless, other proteins, such as the LCAT and the platelet-activating factor acetylhydrolase (PAF-AH), and the indirect protection exerted by HDL antioxidant content, may also collaborate [4].

As previously commented, the consumption of olive oil phenolic compounds may enhance the ApoA-I functionality in HDL particles, preventing oxidative modifications of the protein and leading to a more stable conformation of ApoA-I in HDLs [15**]. These characteristic might also improve HDL capacity to pick up oxidized lipids [4]. In parallel, olive oil phenolic compounds could enhance the function of some HDL-related antioxidant enzymes, such as PON1. PON1 is present in the circulation mainly linked to HDLs and is one of the principal agents involved in the hydrolysis of oxidized lipids in plasma [30]. PON1 antioxidant activity was enhanced in healthy humans after a 3-week VOO-rich intervention [17] and also significantly upgraded after the consumption of the previously described FVOOT [22*]. These improvements in PON1 function are in line with others found after supplementation with VOO in several murine models [31–33]. Three possible mechanisms may explain this increment in PON1 function. First, olive oil phenolics could increase the biosynthesis of PON1, since the consumption of VOO induced a rise in the PON1 plasma concentrations of healthy subjects [18*]. The capacity of these phenolic compounds to stimulate the *Nrf2*-dependent gene expression could indirectly enhance PON1 production [33]. Second, HDLs may become rich in PON1, in a similar way to their becoming abundant in PON3 (a PON1 genotypic isoform), as observed after the consumption of VOO and the previously described FVOOT [24*]. Finally, the consumption of VOO increased the HDL content of olive oil phenolic compound metabolites which may protect PON1 structure and function as reported for ApoA-I [15**,17,18*].

With respect to other enzymes related to HDL antioxidant capacity, we have previously commented on an increment of LCAT mass after the consumption of the FVOOT when compared to a control VOO [22*]. In the case of the PAF-AH, no significant changes in PAF-AH quantity or activity in HDLs due to VOO have as yet been reported.

HDL ANTI-INFLAMMATORY AND VASOPROTECTIVE EFFECTS

In addition to their CEC and antioxidant properties, HDLs are also considered to be relevant anti-inflammatory and vasoprotective agents. Endothelial dysfunction and the inflammatory responses of macrophages and endothelial cells, key factors for the perpetuation of atherosclerosis [29], all seem to be counteracted by HDLs [7]. VOO consumption has been shown to be highly protective for vascular response and endothelial integrity, as observed in a number of VOO-rich interventions in humans [34–36]. HDLs could act as transporters of several derivatives of olive oil phenolic compounds to the endothelial cells where they may prevent oxidative damage in cell mitochondria and preserve the production of nitric oxide, as described *in vitro* [35,37]. Regarding HDL anti-inflammatory capacity, the consumption of VOO increased the ability of HDLs to block the secretion of intracellular adhesion molecule-1 and the adhesion of monocytes to endothelial cells in healthy humans [18*]. Such an enhancement could be due to two hypothetical mechanisms. First, the improvement in HDL antioxidant function caused by olive oil phenolic compounds may partially explain these benefits. HDL antioxidant and anti-inflammatory functions are intimately related [7] and PON1 is one of the principal agents involved [7,30]. In agreement with this, the anti-inflammatory properties of PON1 activity increased significantly after the consumption of VOO in healthy subjects [18*]. Second, the consumption of VOO may enhance HDL functionality by decreasing the levels of acute-phase proteins in the lipoprotein. Inflammatory states increase the content of acute-phase proteins in HDLs, transforming the lipoproteins into pro-inflammatory, dysfunctional particles [4]. The consumption of different VOOs [24*] and a VOO-rich MD [38] has been reported to

decrease the content of acute-phase proteins in HDLs and may, therefore, promote a less pro-inflammatory state of the particles.

OTHER HDL-RELATED PROPERTIES

In addition to the functional properties of HDLs there are other novel characteristics related to their quantity and quality. The number of HDL particles in circulation, determined by NMR-spectroscopy, has shown to be a promising biomarker of HDL concentration, for instance in the prediction of CVD incidence [39]. A short-term consumption of VOO in healthy individuals induced a non-significant increasing trend in HDL particle number [15**]. This protective effect was significantly confirmed after a long-term consumption of an MD supplemented with nuts [40]. A rise in systemic ApoA-I levels, such as those reported after some VOO-based dietary interventions in humans [41,42], may justify these changes. Mechanistically, a regular intake of VOO may enhance the intestinal expression of *ApoA1* gene, as observed in a rat model [43]. Olive oil phenolic compounds may also decrease ApoA-I degradation, as described in individuals whose HDL-C concentrations increased after a short-term consumption of an MD [44].

High CVD risk patients usually present a profile characterized by low levels of large HDLs, high levels of small HDLs, and variable values of lipid-poor/lipid-free HDLs (the pre- β fraction, the most effective one for CEC) [45]. The consumption of VOO has shown to increase large HDLs and decrease small ones [15**]. The change towards greater HDL size has been confirmed after the consumption of an FVOOT [22*], a VOO-rich MD [40,46], and in a rat model after supplementation with VOO [47]. There is however some controversy with respect to this issue. Some authors consider that the pre- β HDL and lipid-free ApoA-I are the more functional particles [48]. In contrast, a

number of *in vitro* studies indicate that small HDLs have similar effects to the large ones [49]. Furthermore, increased levels of small HDLs in plasma may indicate an aberration in HDL maturation and decreased reverse cholesterol transport [4]. Large HDLs also bind better to the ABCG1 and SR-B1 cholesterol transporters, promoting cholesterol efflux via these receptors [4]. Moreover, some HDL physicochemical modifications (such as the ones in inflammatory states) can transform the lipoprotein into a small, dysfunctional particle [4]. As a result, the interpretation of HDL size without taking into account the overall biochemical context is controversial. Finally, some VOO-based interventions, particularly the FVOOT, have increased the content of other active proteins in HDLs, such as that of apolipoprotein A-IV [24*]. This effect has also been observed in *apoE*-deficient mice after the consumption of a VOO-rich diet [31]. A rise in HDL apolipoprotein A-IV content may be cardioprotective since a decreased apolipoprotein A-IV content in HDLs appears in patients with stable or acute coronary syndrome [50].

CONCLUSIONS

HDL functions reflect the physiological role of the lipoprotein better than HDL-C quantity. As indicated in **Figure 1**, the intake of olive oil phenolic compounds resulted in an improvement in CEC, HDL antioxidant defenses, HDL size distribution, and other characteristics related to HDL quality. Olive oil phenolic compounds bound to HDLs, or surrounding the lipoprotein, improve their oxidative/inflammatory status which may justify an increase in HDL functionality. Modifications in HDL composition due to the consumption of VOO might also explain these changes. However, large-scale, randomized controlled trials with VOO-rich dietary interventions are required to

259 definitively confirm the protective role of olive oil phenolic compounds in HDL
260 biological functions.

Key points

- HDL function reflects the physiological role of the lipoprotein better than HDL cholesterol
- Lifestyle changes that may increase HDL functional capacities *in vivo* are in high demand from clinicians
- The phenolic compounds in virgin olive oil are able to improve HDL cholesterol efflux capacity
- Olive oil phenolic compounds also increase HDL antioxidant capacities
- Large-scale, long-term randomized controlled trials with virgin olive-rich dietary interventions are required to confirm the protective effects of olive oil phenolic compounds on HDL function

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288 **Conflicts of interest**

289 None.

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FIGURE TITLES

Figure 1.

Heading: Olive oil phenolic compounds and HDL function: hypothetical mechanisms.

Legend: The consumption of olive oil phenolic compounds enhances HDL cholesterol efflux capacity, HDL antioxidant capacities and some HDL anti-inflammatory properties. Olive oil phenolic compounds increase cholesterol efflux capacity by improving HDL size distribution, increasing the gene expression of cholesterol transporters, and enhancing ApoA-I function and HDL fluidity (in both cases due to a better HDL oxidative/inflammatory status and changes in HDL composition). HDL antioxidant capacity is incremented after the consumption of virgin olive oil through the preservation of ApoA-I and PON1 function, and an increase in LCAT levels. Finally, HDL anti-inflammatory properties are also augmented, mainly because of the increase in HDL antioxidant function.

Continuous lines reflect well-established relations between variables, and discontinuous lines hypothetical associations.

Olive oil phenolic compounds and HDL function: hypothetical mechanisms