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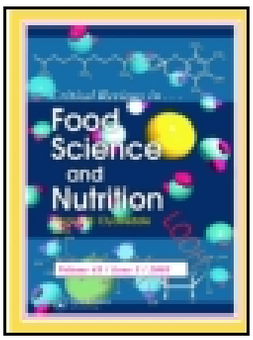

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The effect of high-polyphenol extra virgin olive oil on cardiovascular risk factors: a systematic review and meta-analysis

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Abstract

The polyphenol fraction of extra-virgin olive oil may be partly responsible for its cardioprotective effects. The aim of this systematic review and meta-analysis was to evaluate the effect of high versus low polyphenol olive oil on cardiovascular disease (CVD) risk factors in clinical trials. In accordance with PRISMA guidelines, CINAHL, PubMed, Embase and Cochrane databases were systematically searched for relevant studies. Randomized controlled trials that investigated markers of CVD risk (e.g. outcomes related to cholesterol, inflammation, oxidative stress) were included. Risk of bias was assessed using the Jadad scale. A meta-analysis was conducted using clinical trial data with available CVD risk outcomes. Twenty-six studies were included. Compared to low polyphenol olive oil, high polyphenol olive oil significantly improved measures of malondialdehyde (MD: -0.07µmol/L [95%CI: -0.12, -0.02µmol/L]; I^2: 88%; p=0.004), oxidized LDL (SMD: -0.44 [95%CI: -0.78, -0.10µmol/L]; I^2: 41%; P=0.01), total cholesterol (MD 4.5mg/dL [95%CI: -6.54, -2.39mg/dL]; p<0.0001) and HDL cholesterol (MD 2.37mg/dL [95%CI: 0.41, 5.04mg/dL]; p=0.02). Subgroup analyses and individual studies reported additional improvements in inflammatory markers and blood pressure. Most studies were rated as having low-to-moderate risk of bias. High polyphenol oils confer some CVD-risk reduction benefits; however, further studies with longer duration and in non-Mediterranean populations are required.

Keywords: olive oil; polyphenol; review; cardiovascular; oxidative stress; Mediterranean diet

Introduction

Numerous epidemiological studies and landmark clinical trials suggest that the traditional Mediterranean diet is cardioprotective (de Lorgeril et al. 1999, Estruch et al. 2006, Itsiopoulos et al. 2011, Itsiopoulos et al. 2011). There are many components of this dietary pattern that provide cardioprotective effects and mediate health benefits including red wine, high vegetable and fish intake, and the high consumption of extra virgin olive oil (EVOO). Clinical and animal studies demonstrate that EVOO can improve cardiovascular disease (CVD) outcomes including blood pressure, inflammation, and cholesterol levels (Perona et al. 2004, Beauchamp et al. 2005, Farras et al. 2015).

EVOO is high in monounsaturated fatty acids (MUFAs) which may mediate the prevention and management of CVD and associated risk factors through various mechanisms including the favorable modulation of cholesterol levels and improvement of insulin sensitivity (Schwingshackl and Hoffmann 2012). In addition to the high MUFA content, the polyphenol content of EVOO may also be cardioprotective (Covas, Konstantinidou and Fito 2009). Studies that have directly compared olive

oil with other high-MUFA oils, including flaxseed and sunflower oil, have shown superior outcomes in low-density lipoprotein (LDL) oxidation, lipoprotein concentration, and LDL particle size with provision of olive oil (Aguilera et al. 2004, Harper, Edwards and Jacobson 2006). A systematic review and meta-analysis demonstrated that compared with seed oils, olive oil significantly improved total, high-density lipoprotein (HDL) (Ghobadi et al. 2018). Emerging preclinical and observational evidence suggests that dietary polyphenol intake may reduce inflammation and is associated with improved all-cause mortality (Tresserra-Rimbau et al. 2014, Joseph, Edirisinghe and Burton-Freeman 2016). EVOO, compared to other dietary fats,(Perez-Jimenez et al. 2010) contains a unique composition of polyphenols. In particular, EVOO contains a high concentration of the polyphenols hydroxytyrosol and oleuropein, which in preclinical studies, have demonstrated cardioprotective properties including the favorable modulation of pathways related to inflammation, oxidative stress, homocysteine, cholesterol levels and cell adhesion (Parkinson and Cicerale 2016, Peyrol, Riva and Amiot 2017).

To determine the relative contribution of olive oil polyphenols to the known beneficial properties of the fatty acid profile present in olive oil, numerous trials have investigated the effect of high polyphenol olive oil (HPOO) versus low polyphenol olive oil (LPOO). The aim of this systematic review and meta-analysis was to examine the evidence for modulation of cardiovascular risk factors in existing clinical trials that have compared the effect of HPOO versus LPOO. We examined whether polyphenols, specifically, elicited superior health outcomes and if the evidence supports recommendations for the preferential use of EVOO over refined olive oil.

Methods

Literature search

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al. 2009) and as registered on PROSPERO (42017070060), relevant studies were retrieved from PubMed, Embase, The Cochrane Library, and CINAHL for articles published since journal inception up to June 2017. Search terms related to polyphenols (e.g. polyphenol, phenol, phytochemical) and olive oil were used.

Studies were required to meet each of the following eligibility criteria to be included in this review: used a randomized or non-randomized, parallel or cross-over trial study design; investigated olive oil as a stand-alone intervention; conducted in adult participants (healthy or otherwise); compared higher polyphenol olive oil to an olive oil with a lower polyphenol content; and included markers of CVD (including lipids, hemodynamic, and inflammatory measures) and/or oxidative stress outcomes.

Data extraction

Screening of the title and abstracts for individual studies was conducted in duplicate by three authors (GLT, AJR or ACL) with disagreements resolved by consensus or fourth reviewer (WM). Articles deemed eligible for full-text review were assessed for eligibility independently by two authors (GLT, ACL) and agreement reached via group consensus (ESG, HM, GLT, WM). The following parameters were extracted from included studies: author/date, study design, sample size, total study period, population characteristics (including age, gender, and co-morbidities), intervention

characteristics (including polyphenol content and duration of exposure), length of follow up and cardiovascular outcomes including lipids, hemodynamic, inflammatory measures, weight measures, endothelial function, and/or oxidative stress outcomes.

If two manuscripts reported on the same outcomes using the same or a sub-sample of a participant cohort, data were only extracted for the manuscript that included the largest sample size. If the larger study reported outcomes with insufficient detail to be included in meta-analyses, outcomes from the smaller study were extracted and both were reported qualitatively. Data for study arms that did not meet the eligibility criteria of this review were not extracted.

Assessment of study risk of bias

Risk of bias was assessed independently by three authors (ESG, AF, ACT) using the Jadad scale (Jadad et al. 1996). The Jadad scale is a five-item scale that assesses risk of bias due to randomization, blinding, and follow up. Studies can receive a score between zero and five, with lower scores indicating a higher risk of bias. Conflicting scores were resolved collaboratively and if disagreements persisted, a fourth author (WM) made the final judgment. If two or more manuscripts reported on the same cohort (or sub-cohort), details regarding blinding and randomization were extracted from all manuscripts to assess bias.

Data analysis

For qualitative analysis, difference in end intervention measures between groups and change between groups were reported, depending on the analysis reported for individual studies. Data were considered statistically significant if the reported p-value was <0.05.

When outcomes of included studies were sufficiently reported, data were pooled using Review Manager (Version 5.3, The Cochrane Collaboration 2014). Only outcomes relating to HPOO and LPOO were considered for comparison. To calculate the overall treatment effect, the difference between the outcomes at follow up of the intervention and comparison groups were considered. Continuous outcome variables were calculated using the inverse variance test as mean differences (MD) for studies which used the same measurement, or standardized mean differences (SMD) for studies which used different measures for the same construct; where SMD effect sizes of <0.4 were considered small, 0.4–0.7 moderate, and >0.7 large (Higgins, Julian and Green 2011). However, where biochemistry variables were reported via different units (e.g. mmol/L versus mg/dL); the measures were converted to the same unit and a MD was calculated. No categorical variables were pooled.

To assist clinical interpretation, SMD effect sizes were transformed into the scale of one the clinical measures and presented as a product of the total baseline standard deviation of a measure (Higgins, Julian and Green 2011). Due to the complex nature of interpreting a single variable upon nutrition-related health measures, a random effects model was used for all meta-analyses. An I^2 statistic of >50% was considered substantially heterogeneous. Sensitivity analysis was applied with pooled effect sizes with substantial heterogeneity and/or a non-significant trend towards an effect. For outcomes related to lipid profile and hemodynamics, subgroup analyses were undertaken for healthy patients versus those with hyperlipidemia or hypertension, respectively. Meta-analyses with significant results are presented as a figure within the manuscript and meta-analyses with non-significant results are included as supplementary material.

Results

Study selection

The literature search identified 4241 citations after the removal of duplicates (Figure 1). Forty articles were retrieved for full text screening and after a further 14 studies were excluded, 26 articles were included for this review and meta-analysis.

Study Characteristics

The majority of the included manuscripts (15/26) reported on outcomes from two separate cohorts: the Effect of Olive Oil on Oxidative Damage in European Populations study (abbreviated as EUROLIVE; 8/26 studies), and the Virgin Olive Oil and HDL Functionality study (VOHF; 6/26 studies). The EUROLIVE study was a multi-center randomized, double-blind, controlled, cross-over trial in 200 healthy males. Three of the 8 EUROLIVE studies reported on the full cohort while 5 studies reported on a subset. The VOHF study was a double-blind, randomized, controlled, crossover clinical trial of 33 hyper-cholesterolemic adults. Four of the 6 VOHF studies reported on the full cohort, while 2 studies reported on a subset. Perona et al. 2011 reported new outcomes using predominately the same cohort that was reported on in the study by Marrugat et al. 2004. Likewise, the paper by Fito et al.2008 reported outcomes using a sub-set of patients from Fito et al. 2005. The remaining 8 studies reported on separate cohorts (see Table 1).

Overall, the sample size of the included studies was relatively small; most studies included 10 to 49 participants, with the exception of the EUROLIVE cohort, which included 200 participants. Twelve studies recruited healthy adult participants while the remaining studies recruited specific populations (such as smokers (Moschandreas et al. 2002) and post-menopausal women (Salvini et al. 2006)) or participants with dyslipidemia, high blood pressure, fibromyalgia, and peripheral vascular disease (Ramirez-Tortosa et al. 1999, Fito et al. 2005, Visioli et al. 2005, Fito et al. 2008, Moreno-Luna et al. 2012, Rus et al. 2016).

Studies included participants recruited from either a combination of European countries (Spain, Denmark, Finland, Italy, Germany; 8/26 studies) or the following individual countries: Spain (13/26 studies), Italy (2/26 studies), Netherlands (1/26 study), Greece (1/26 study), and Jordan (1/26 study).

Trial intervention duration ranged from 3 weeks to 3 months. A cross-over study design that incorporated two 3-week intervention periods and one 2-week washout period was the most common study design with 21 of 26 studies (EUROLIVE, 8/21 studies; VOHF, 6/21 studies) using this design.

Interventions

There was a wide range in the polyphenol content of both the HPOO (150mg-800mg polyphenols per kg of oil) and LPOO (0-132mg polyphenols per kg of oil) interventions. The LPOO intervention in the VOHF cohort was a virgin olive oil, and the high polyphenol groups were the same oil infused with additional polyphenols. Al-Rewashdeh et al. 2010, as well as 5 studies from the EUROLIVE cohort included an additional intervention phase comprising olive oil with moderate amounts of polyphenols (366-368mg/kg of oil (Al-Rewashdeh 2010)); however, only the LPOO (2.7-132mg/kg) and HPOO (366-753mg/kg) arms were considered in this review.

The most commonly prescribed volume of olive oil was 25ml per day (n = 16), and ranged from 25ml-75ml per day. Additional dietary instructions varied, with most (22/26 studies) requesting participants restrict either high polyphenol, high antioxidant, or high vitamin E foods during the study intervention period.

Study Results

Oxidative stress

Twenty studies reported on measures of oxidative stress (see Table 1). These outcomes included: malondialdehyde and thiobarbituric acid reactive substances (TBARS), measures of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) oxidation, lipid oxidation, glutathione peroxidase, total antioxidant capacity and antioxidant status, isoprostane excretion, protein carbonyl, 8-hydroxy-2'-deoxyguanosine, superoxide dismutase, catalase, ferric reducing ability of plasma, measures of oxidative DNA damage, paraoxonase-3 (PON-3) protein, lactonase activity, paraoxonase activity, hydroxy fatty acids, and conjugated dienes.

Meta-analysis of studies with sufficient data demonstrated that HPOO significantly improved malondialdehyde (MD: -0.07µmol/L [95%CI: -0.12, -0.02µmol/L]; I^2: 88%; p=0.004; Figure 2) and oxidized LDL (SMD: -0.44 [95%CI: -0.78, -0.10µmol/L]; I^2: 41%; p=0.01; Figure 3) compared to LPOO. Sensitivity analysis did not improve the substantial heterogeneity in malondialdehyde. Pooling of data did not reveal a significant difference in total antioxidant capacity (SMD: 0.30 [95%CI: -0.26, 0.86]; I^2: 67%; p=0.29) (Fito et al. 2005, Salvini et al. 2006, Rus et al. 2016). A sensitivity analysis that removed the study by Rus et al. 2016 (the only group of participants with fibromyalgia) from analysis improved heterogeneity (I^2: 0%); however, there was still no significant effect (MD: -0.00 [95%CI: -0.05, 0.04]; I^2: 0%, p=0.86) (Fito et al. 2005, Salvini et al. 2006). There was also no significant effect in glutathione peroxidase (SMD: -0.04 [95%CI-0.69, 0.61]; I^2: 75%; p=0.91), and the heterogeneity was not improved upon sensitivity analysis.

For results that could not be entered into a meta-analysis, compared to LPOO, HPOO significantly improved conjugated dienes (p=0.011), (Covas et al. 2006) glutathione peroxidase (p=0.033) (Fito et al. 2005), protein carbonyl (p=0.023), (Rus et al. 2016) antioxidant status (p<0.0001) (Visioli et al. 2005), measures of oxidative DNA damage (p=0.019) and PON-3 protein (p<0.05) (Fernandez-Castillejo et al. 2017), lactonase activity (p<0.05), (Fernandez-Castillejo et al. 2017) paraoxonase

activity (p<0.05), (Fernandez-Castillejo et al. 2017) hydroxy fatty acids (p=0.038) (Covas et al. 2006). No other significant results were reported.

Inflammation

Five studies investigated the effect of HPOO on inflammatory markers compared to LPOO; (Fito et al. 2008, Machowetz et al. 2008, Castaner et al. 2012, Moreno-Luna et al. 2012, Martin-Pelaez et al. 2016) however, none were pooled because of heterogeneous measures reported or insufficient outcome and variance data. Three studies measured C-reactive protein (CRP) (Fito et al. 2008, Moreno-Luna et al. 2012, Martin-Pelaez et al. 2016) while interleukin-6 (IL-6), (Fito et al. 2008) soluble intercellular adhesion molecule-1 (sICAM-1),(Fito et al. 2008) soluble vascular adhesion molecule-1 (sVCAM-1), (Fito et al. 2008) monocyte chemotactic protein-1 (MCP-1), (Castaner et al. 2012) fecal tumor necrosis factor (TNF-α), (Martin-Pelaez et al. 2016) fecal calprotectin, (Martin-Pelaez et al. 2016) and resistin (Machowetz et al. 2008) were each measured in one study. Two studies reported a decrease in CRP after HPOO supplementation (p=0.024 (Fito et al. 2008) and p<0.001 (Moreno-Luna et al. 2012)) while one study reported an increase in CRP in the HPOO group (Martin-Pelaez et al. 2016). IL-6 was reduced in one study (p<0.002) (Fito et al. 2008). In one study, resistin was improved in the LPOO group only (Fito et al. 2008). MCP-1 also improved in one study (p=0.022) (Castaner et al. 2012). No significant differences were reported for all other measures.

Blood pressure

Five studies reported measures of blood pressure; however, participants were predominantly normotensive, excepting Moreno-Luna et al. 2012, in which all 48 female participants had mild hypertension. Meta-analysis indicated that HPOO had no effect on systolic blood pressure compared to LPOO (MD: -2.03mmHg [95%CI: -6.57-2.50]; I^2=79%; p=0.38). There was a non-significant trend towards decreased diastolic blood pressure in the HPOO group (MD: -2.70mmHg [95%CI: -5.71-0.31]; I^2=78%); p=0.08 [n=1 study was removed, as comparator was not true LPOO to improve

sensitivity (Martin-Pelaez et al. 2016)]); however, the effect size was small and a significant unexplained heterogeneity remained.

Lipid profiles

Twelve studies reported on measures of cholesterol levels and/or function (Ramirez-Tortosa et al. 1999, Vissers et al. 2001, Marrugat et al. 2004, Fito et al. 2005, Visioli et al. 2005, Al-Rewashdeh 2010, Perona et al. 2011, Hernaez et al. 2014, Farras et al. 2015, Hernáez et al. 2015, Fernandez-Castillejo et al. 2016, Martin-Pelaez et al. 2017). These included total, LDL and HDL cholesterol; triglycerides; apolipoprotein B-100 (ApoB), A1 (ApoA1), and A2 (ApoA2); LDL and HDL particle size; HDL cholesterol efflux capacity; HDL fluidity, and cholesterol esters.

Meta-analysis of studies with sufficient data demonstrated that HPOO significantly improved total cholesterol by 4.47mg/dL (95%CI: -6.54, -2.39mg/dL; p<0.0001, Figure 4). In a subgroup analysis, there was no significant difference in total cholesterol between healthy and CVD subgroups (p=0.94). Compared with LPOO, HPOO improved HDL cholesterol by 2.37mg/dL ((95%CI: 0.41, 5.04mg/dL; p=0.02); Figure 5). The substantial heterogeneity in HDL is somewhat explained by subgroup analysis, where participants with CVD had significantly different outcomes than healthy participants (p=0.09). Healthy participants still maintained substantial heterogeneity (I^2=79%) but HPOO groups had significantly lower HDL cholesterol compared to LPOO (by 3.95mg/dL [95%CI: 0.89-7.01; p=0.01]; Figure 5). Conversely, the samples with CVD had no heterogeneity (I^2=0%) and HPOO had no significant effect on HDL cholesterol in this sub-sample (MD: 0.14 [95%CI: -2.93-3.22] p=0.93).

HPOO also had a non-significant trend to lower LDL cholesterol by 3.73mg/dL (95%CI: -7.60, -0.15mg/dL; I^2: 70%; p=0.06; Figure 6) compared to LPOO; however, subgroup analysis found a significant difference between healthy versus CVD samples (p=0.01). Similar to the HDL analysis, the LDL-cholesterol in the healthy samples maintained high heterogeneity (I^2=71%) but was significantly lower by 5.31mg/dL (95%CI: -9.83- -0.79; p=0.02; Figure 6) in the HPOO groups compared to the

LPOO groups. However, the samples with CVD showed no heterogeneity (I^2=0%) and no effect on LDL cholesterol following intervention with HPOO (MD: 1.12mg/dL [95%CI: -1.30-3.53]; p=0.37). HPOO had no effect on plasma triglycerides compared to LPOO in a mixed sample of healthy and hypercholesterolemia adults (MD 0.34mg/dL (95%CI: -3.24, 3.92mg/dL; I^2: 33%; p=0.85). There were also no significant difference between healthy versus CVD subgroups.

For results that could not be entered into a meta-analysis, HPOO significantly improved ApoB (*p*<0.001, (Fernandez-Castillejo et al. 2016) p<0.05, (Perona et al. 2011) and p<0.03 (Hernáez et al. 2015)), measures of LDL and/or particle size (p<0.05 (Hernáez et al. 2015) and p<0.05 (Fernandez-Castillejo et al. 2016)), HDL cholesterol efflux capacity (p=0.042 (Hernaez et al. 2014)) and LDL cholesterol esters (p<0.05 (Ramirez-Tortosa et al. 1999)).

Other measures

Six studies reported weight or BMI outcomes, with no significant difference between interventions (Ramirez-Tortosa et al. 1999, Vissers et al. 2001, Moschandreas et al. 2002, Machowetz et al. 2008, Martin-Pelaez et al. 2016, Rus et al. 2016). Moreno-Luna et al. 2012 reported that HPOO improved measures of endothelial function (asymmetric dimethylarginine, hyperemic area after ischemia, and total plasma nitrites/nitrates) in a hypertensive cohort. Of the four studies that reported on blood glucose, (Marrugat et al. 2004, Fito et al. 2005, Visioli et al. 2005) one study reported an increase in blood glucose after HPOO consumption compared to LPOO (p=0.015) (Martin-Pelaez et al. 2016). In a proteomic analysis, HPOO up-regulated proteins related to cholesterol homeostasis, antioxidant pathways, and blood coagulation. In contrast, HPOO down-regulated proteins implicated in acute-phase inflammatory response, lipid transport, and immune response (Pedret et al. 2015). Oxidized

LDL autoantibodies (p=0.023) and pro-atherogenic gene expression (p<0.05) were also demonstrated to improve in two separate studies (Castaner et al. 2011, Castaner et al. 2012).

Adverse events

Adverse events were monitored in the VOHF and EUROLIVE study cohorts and two of the twelve individual studies. No adverse events were reported during their trial periods.

Risk of Bias

Using the Jadad Scale, most studies (15/26) received a score between 4 and 5 (out of 5), indicating a low risk of bias (Supplementary Material 2). The most common reason for receiving a lower score was due to inadequate reporting regarding withdrawals and/or dropouts and method of blinding.

Discussion

The results of this review indicate that olive oil polyphenols may provide cardioprotective benefits that are independent of the high MUFA content of olive oil. Specifically, the results of this meta-analysis suggest that high polyphenol olive oil can improve outcomes related to cholesterol (total and HDL cholesterol) and oxidative stress (malondialdehyde and oxidized LDL). Furthermore, for measures that were unable to be included in a meta-analysis, individual studies have generally reported improvements in inflammation, additional measures of oxidative stress, and endothelial function.

A recent systematic review and meta-analysis indicated that olive oil is superior compared to other plant oils in improving HDL cholesterol but not total and LDL cholesterol and triglycerides (Ghobadi et al. 2018). Furthermore, although the effect of polyphenol content was not examined in this

review, sensitivity analyses that examined the effect of virgin olive oil compared to refined olive oil reported mixed outcomes. This study builds on these findings by reporting similar improvements that are attributed to polyphenols.

Sensitivity analyses demonstrated that CVD risk factors such as HDL and LDL cholesterol significantly improved in healthy participants, while no effect was present in participants with existing CVD risk factors. A possible explanation for these results is that participants with CVD risk factors are likely to be undergoing lipid-lowering pharmacotherapy although this was not reported or controlled for in studies. A possible explanation for these results is that participants with CVD risk factors are likely to be undergoing lipid-lowering pharmacotherapy, which would make it difficult achieve additional reductions in CVD risk factors through dietary interventions, particularly within the short intervention periods (≤12 weeks) reported in these trials. Furthermore, the small effect sizes (e.g. HDL and LDL cholesterol) and non-significant differences (e.g. blood pressure) identified in the pooled analysis may be explained by there being little likelihood of large reductions in clinical outcomes for healthy participants with lipid profiles and blood pressure within reference range. Further research in participants with chronic diseases that are either not managed by pharmacotherapy or where the study interventions are for longer durations may report larger effect sizes. Furthermore, a small subset of studies assessed the functionality of cholesterol and reported improvements in measures such as HDL cholesterol efflux capacity. As emerging evidence suggests that traditional measures of HDL cholesterol may not be a reliable marker of cardiovascular health, (Rohatgi et al. 2014, Sacks et al. 2017) further research on functional outcomes of HDL cholesterol, rather than particle count, may be a more clinically relevant marker to evaluate the cardioprotective effects of polyphenols.

As discussed in our previous review, (Marx et al. 2017) clinical trials involving polyphenol interventions should implement measures to control for background polyphenol intake, as this may influence study results. Most studies in our review provided dietary advice to control for this, although there was no discussion regarding adherence to this advice. The common use of a cross-over trial design in the included studies may also provide some control for these factors. Adherence to the prescribed olive oil dosage was also not reported, posing an additional limitation to these trials. In addition, although LPOO and HPOO were directly compared in this review, there was considerable variability in the concentration of polyphenols and volume of olive oil prescribed for both groups. Therefore, total absolute daily dose varied considerably. There are also numerous considerations that need to be acknowledged regarding polyphenol concentration. Polyphenol concentrations within olive food products differ based on a variety factors including olive variety, soil, climate, maturation at harvest, and processing (Tripoli et al. 2005). Furthermore, there may be a difference in the class of polyphenols within naturally occurring high polyphenol EVOO compared to olive oil that has been fortified with polyphenols. Globally, the regulatory frameworks for labelling polyphenol concentration in foods and olive oil are lacking. With additional evidence to support the proposed benefits of polyphenols in EVOO, it will become increasingly important that labelling becomes more transparent to highlight the potential benefits to consumers. All of the reviewed studies, in a commonly shared strength of study design, measured and declared polyphenol concentration. This will assist in providing future recommendations on the concentration and volume of olive oil consumption required to achieve clinical benefit.

There is evidence to suggest that the ways in which polyphenols are consumed influence total polyphenol bioavailability and absorption. For example, exposure to prolonged heat may deplete the total polyphenol content (Brenes et al. 2002). None of the studies included in this review reported any information related to cooking and consumption methods used by participants. Further data regarding the consumption of olive oil during a trial may be worthwhile investigating, to ascertain the potential interactions between interventions and cooking methods. This will also inform the

translatability of these interventions into practical applications for prevention and management of CVD.

While the existing research provides promising evidence for the unique benefits of olive oil polyphenols, additional research is warranted. Most studies were relatively short in duration with most intervention phases lasting on average, 3 weeks. Additional studies that evaluate the long-term effects of high polyphenol olive oil are required to demonstrate sustainability of health benefits. Furthermore, while all studies included a control group, it is possible that due to the nature of the intervention (i.e. distinct taste and color difference between high and low polyphenol oils), blinding may not have been completely effective. This is an inherent problem in many dietary intervention studies and future studies should implement measures to assess the adequacy of blinding measures such as participant interview at the end of study.

Finally, most of the research reported herein has come from two major European cohorts (i.e. EUROLIVE and VOHF cohorts) and so additional research is required to replicate these findings. As stated in a previous review, (Hohmann et al. 2015) most studies were conducted in Mediterranean populations, predominantly throughout Spain, Italy, Germany, Berlin, Denmark and Finland. Additional studies with diverse populations and ethnicities are required to confirm the effect of high polyphenol olive oil. This may include investigation in of the feasibility and sustainability of regular EVOO consumption in non-Mediterranean populations that are not accustomed to a high consumption of olive oil and to determine if there are genetic differences that may predispose individuals to the cardiovascular benefits associated with polyphenol consumption.

Conclusion

In summary, the results of our systematic review and meta-analysis suggest that olive oil polyphenols provide unique cardioprotective properties, particularly for cholesterol and oxidative stress-related outcomes. Despite the identified beneficial properties reported in the existing studies, a large proportion of included studies were derived from only two cohorts. Studies were also conducted within a primarily Mediterranean population. Further research is needed to confirm these results in adequately powered, non-Mediterranean cohorts. Longer durations are also required to determine sustainability of health outcomes.

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Figure 1. PRISMA Flow Diagram

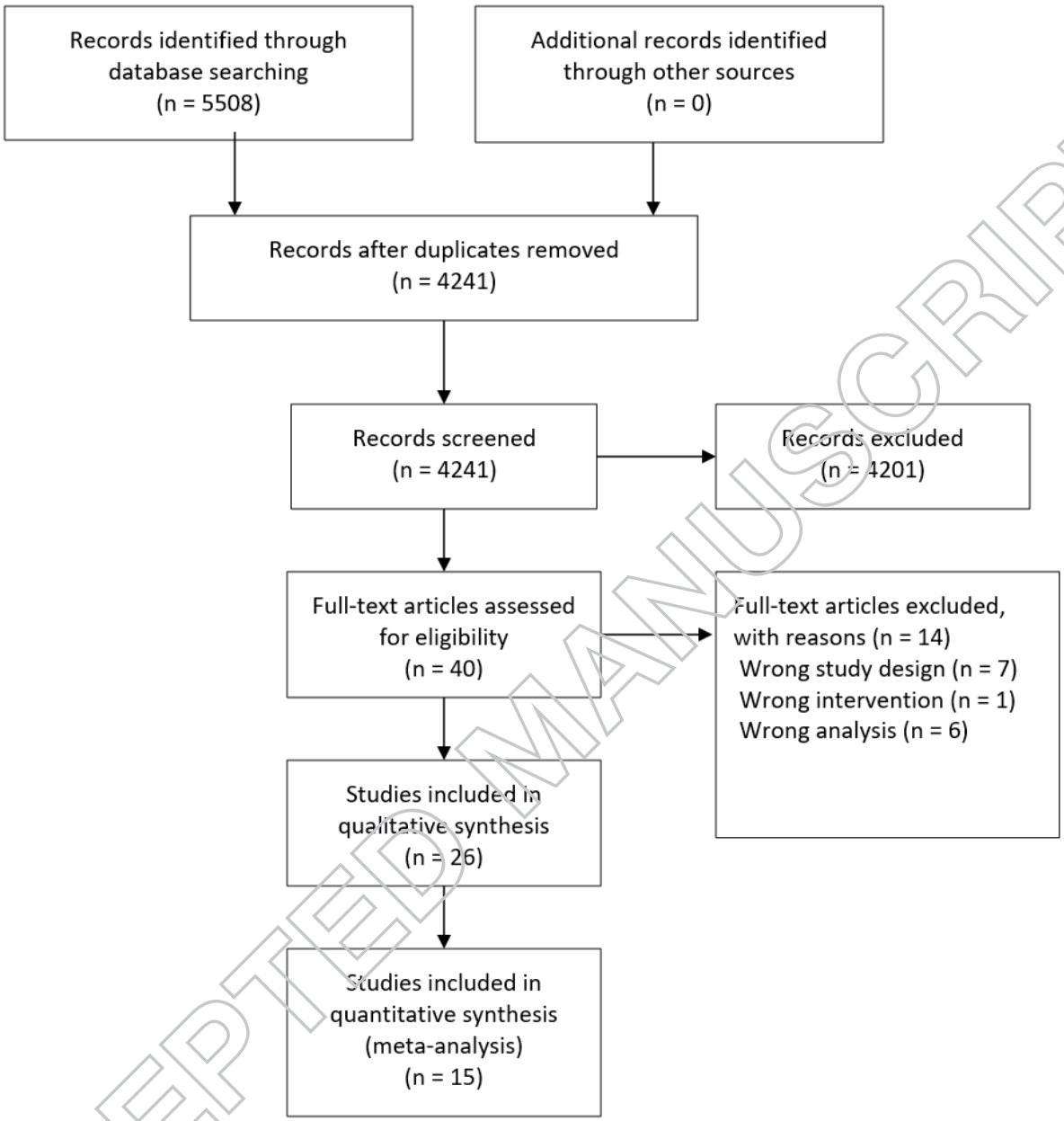

Figure 2. Meta-analysis on the effect of HPOO on plasma malondialdehyde compared to LPOO.

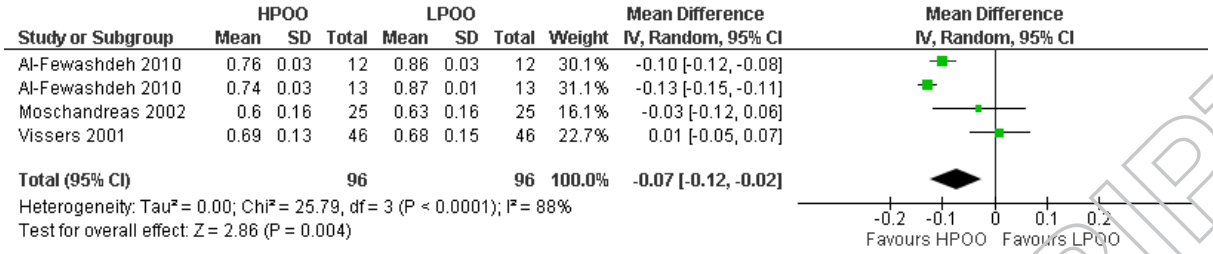

Figure 3. Meta-analysis on the effect of HPOO on oxidized LDL compared to LPOO

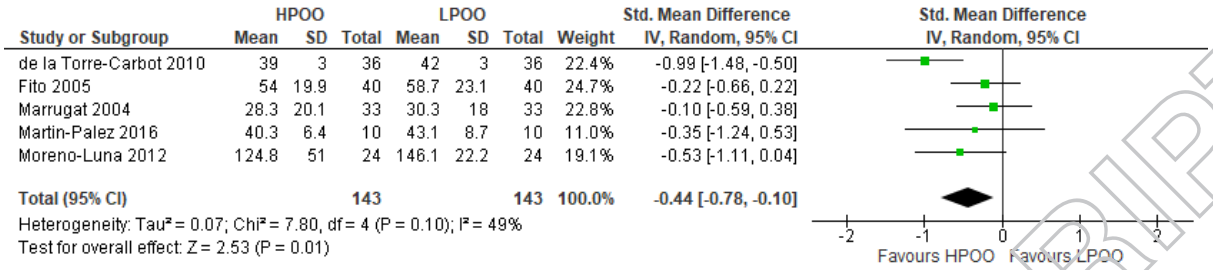

Figure 4. Meta-analysis on the effect of HPOO on total cholesterol compared to LPOO.

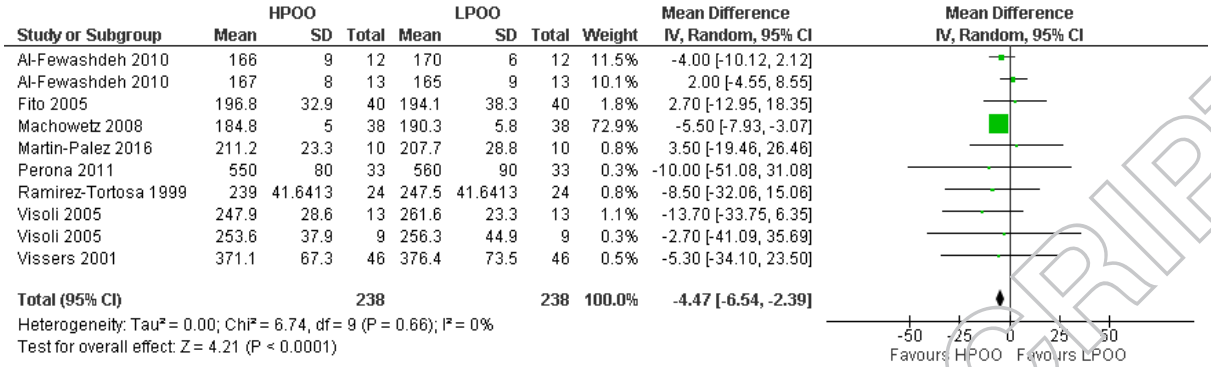

Figure 5. Meta-analysis on the effect of HPOO on LDL cholesterol compared to LPOO.

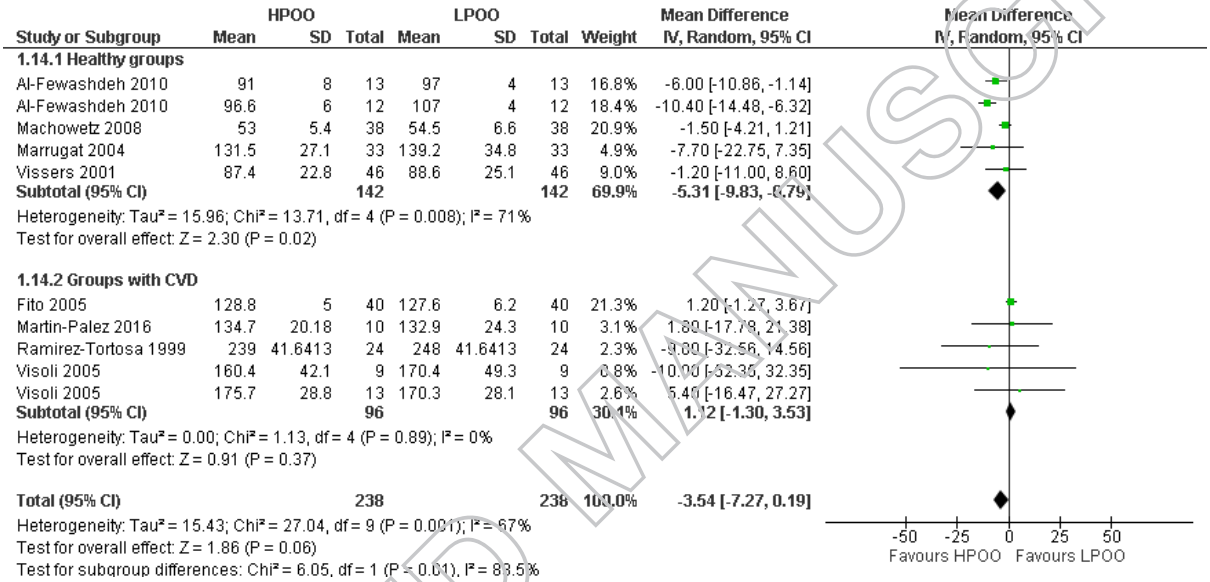

Figure 6. Meta-analysis on the effect of HPOO on HDL cholesterol compared to LPOO.

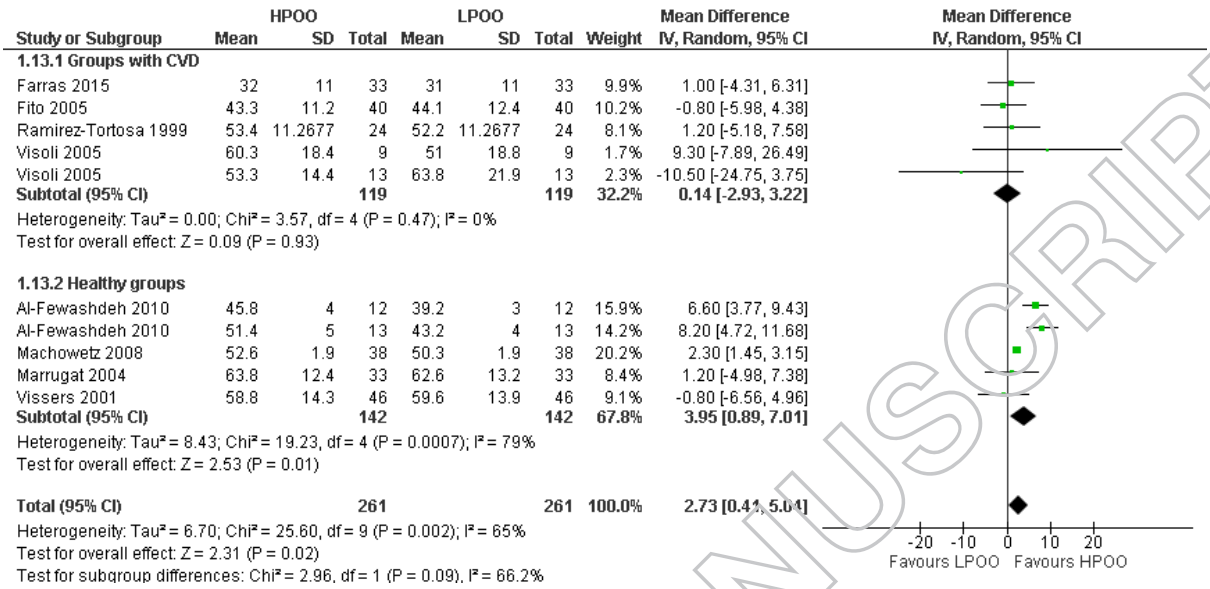

Table 1. Summary Table of Included Studies (n=26)

Author, year, country, study period	**Study Design**	**Population, Attrition rate**	**Olive oil arms**	**Duration and structure**	**Results, *differences between high polyphenol compared to low polyphenol olive oils**[a]**
Independent studies					
Ramirez-Tortosa et al. 1999, Spain. Study period: not reported	Randomized Controlled, Cross-over Trial	n=24 free-living men with peripheral vascular disease, without diabetes, hypothyroidism, obesity, cardiac	Dose: Not specified Arms: 1. HPOO; 300mg/kg polyphenols 2. LPOO; 60mg/kg	3-month interventions, 3-month wash-out period between interventions (usual diets)	Difference in end intervention measures between groups *Classic CVD markers* ↔Weight/BMI ↔HDL-C ↔LDL-C ↑ Triglycerides Lipoprotein composition of: Triglycerides (↔VLDL,↑ LDL, ↔HDL) Phospholipids (↔VLDL,↔ LDL, ↔HDL)

		episodes Age (mean±std): 70±2 years Attrition: not reported	polyphenols Method: Instruction to replace usual saturated fat intake (butter, margarine, lard and visible fat on meat) with the olive oil. Recommended to increase		Total-C (↔VLDL, ↑LDL, ↔HDL) Cholesterol Esters (↔VLDL, ↓LDL, ↔HDL) Free cholesterol (↑VLDL, ↑LDL, ↓HDL) *Oxidative Stress / Antioxidant Status* ↓ Copper- mediated LDL oxidation ↓ Macrophage uptake of oxidized LDL

			fruits, legumes and vegetables to ensure adequate intake of fibre and antioxidant vitamins. Restrict eating out to 1/week. Advised to walk at least 1 km/day		

			and stop smoking.		
Vissers et al. 2001, Netherlands. Study period: not reported	Randomized Controlled, Cross-over Trial Blinding of participants to olive oil sequence	n=49 healthy adults (32 women, 17 men), Age (range): 18-58 years, Attrition: n=6 withdrew	Dose: based on energy needs, mean 69g/day Arms: 1. HPOO; 308mg/kg polyphenols 2. LPOO; 43mg/kg polyphenols Method: daily	3-week interventions, 2-week wash-out periods before each intervention (diets without olives, olive oil and olive oil products)	Difference in end intervention measures between groups *Classic CVD markers* ↔Weight ↔Total-C ↔HDL-C ↔LDL-C ↔Triglycerides *Oxidative Stress / Antioxidant Status* LDL oxidizability (↓lag time, ↔max rate) HDL oxidizability (↔lag time, ↔max rate) ↔Malondialdehyde ↔Lipid hydroperoxides ↔Protein carbonyls

			olive oil in provided foods (40% in mayonnaise, 30% in sauces and 30% in cookies and raisin rolls). Half was consumed at lunch in presence of		

			researchers and remainder at home. Usual diet maintained, except followed instructions for low vitamin E.		
Moschandreas et al. 2002, Greece. Study period: not reported	Randomized, single-blind,	n=25 Adult smokers (11 men, 14 females)	Dose: 70 g/day Arms: 1. HPOO;	3-week intervention, 2-week	Difference in change between groups *Classic CVD markers* ↔Weight

	crossover trial, Participants were blinded to the type of oil they received	Age (mean±std): 30±9 years Attrition: n=3 dropout	308mg/kg polyphenols 2. LPOO; 43mg/kg polyphenols Method: Oil was subdivided over two meals and participants instructed to pour it over	washout periods before each intervention (diet without olives or olive oil products)	*Oxidative Stress / Antioxidant Status* Total plasma resistance to oxidation (↔lag time, ↔max rate) ↔Protein carbonyl ↔Malondialdehyde ↔Lipid hydroperoxides ↔Ferric reducing ability of plasma

			the food consumed. Participants requested to maintain their usual food and fluid intake and not consume olives and other oil-containing		

			products		
Marrugat et al. 2004, Same cohort as Perona et al. 2011, Spain. Study period: not reported	Placebo-controlled, double-blind, randomized, crossover trial	n=33 healthy men Age (mean±std): HPOO-MPOO-LPOO: 55±21 years MPOO-LPOO-HPOO: 61±19 years LPOO-HPOO-MPOO: 57±19 years Attrition: 3 withdrawals	Dose: 25 mL/day Arms: 1. HPOO: 150mg/kg of phenols 2. MPOO: 68mg/kg of phenols 3. LPOO: Undetected polyphenols Method: Participa	3-week intervention, 2-week washout periods before each intervention (LPOO used for raw and cooking purposes)	Difference in change between baseline and treatment values (change between groups not reported) *Classic CVD markers* ↔Total-C ↑HDL-C^{HPOO} ↔LDL-C ↔Triglycerides ↔Glucose *Oxidative Stress / Antioxidant Status* ↓Oxidized LDL^{HPOO} Resistance of LDL to oxidation (↑lag time^{HPOO,MPOO}, ↔rate, ↔max amount of dienes, ↔antibodies against oxidized LDL Percentage of change (baseline to end of intervention) between groups ↓Oxidized LDL^{a,c} Resistance of LDL to oxidation (↑lag time)^{a,b}

			nts instructed to consume Treatment oil raw, was, distributed over 3 meals of the day. Other cooking fats were replaced by LPOO and participants requeste		

			d to avoid a high intake of foods listed as containing phenolic compounds		
Fito et al. 2005, Spain. Study period: not reported	Placebo controlled, crossover, double-blind randomized	n=40 men with stable CHD Age (mean±std): 67±9 years Attrition: n=3 dropped out, n=3	Dose: 50mL/day Arms: 1. HPOO; 161mg/kg polyphenols	3-week intervention period, 2-week washout periods before each interventi	Difference in change between groups *Classic CVD markers* ↔Total-C ↔LDL-C ↔HDL-C ↔Triglycerides ↔Lipoprotein (a) ↔Glucose

	trial	excluded due to lack of compliance	2. LPOO; 14.7mg/kg polyphenols Method: administered raw over 3 meals, other cooking fats replaced with the LPOO during both interventions	on (LPOO as source of crude fat)	↓SBP ↔DBP *Oxidative Stress / Antioxidant Status* ↓Oxidized LDL-C ↔ Antibodies against oxidized ↓Lipoperoxides ↑Glutathione peroxidase ↔Total antioxidant status

Visioli et al. 2005, Italy. Study period: not reported	Randomized, single-blind, crossover trial. Laboratory personnel were blinded to treatments	n=22 mildly dyslipidaemic adults (12 men, 10 females) Age (range): 18 to 65 years Attrition: not reported	Dose: 40 mL/ day Arms: 1. HPOO; total hydroxytyrosol content 166 mg/L 2. LPOO; total hydroxytyrosol content 2 mg/L Method: Raw olive oil was subdivided	7-week intervention, 3-week washout period prior to commencement, 4-week washout period between interventions (40 mL/day of LPOO)	Difference in change between groups *Classic CVD markers* ↔Total-C ↔HDL-C ↔LDL-C ↔Triglycerides ↔ BMI ↔ Mean blood pressure ↔ Glucose *Oxidative Stress / Antioxidant Status* ↑Antioxidant capacity ↓Thromboxane B_2 (TXB_2) ↔Isoprostane excretion (8-iso-PGF2α)

			between lunch and dinner and participants instructed to consume with pasta or vegetables. Other polyphenol-rich foods in the diet were controlle		

			d for		
Salvini et al. 2006, Italy. Study period: September–November 2002 to January – March 2003	Randomized, double-blind, crossover trial	n=10 healthy postmenopausal women Age (range): 47 to 67 years Attrition: n=2 dropout	Dose: 50 g/day Arms: 1. HPOO: 592 mg/kg polyphenols 2. LPOO: 147 mg/kg polyphenols Method: Participants instructed to substitut	8-week intervention, 8-week washout period (habitual fats and oils)	Difference in change between groups *Oxidative Stress / Antioxidant Status* Oxidative DNA damage (↓oxidized DNA bases, ↔basal DNA breaks) ↔Total Antioxidant Status ↔DNA breakage induced by H_2O_2 (*in vitro*)

			e all fats and oils with the study oil and to consume at least 50 g daily in raw form in addition to the oil necessar y for cooking. Apart from the fat substituti on,		

			participants instructed to stay on their habitual diet		
Fito et al. 2008, Subset of Fito et al. 2005, Spain. Study period: not reported	Placebo controlled, crossover, double-blind randomised trial	n=28 men with stable CHD Age (mean±std): 68±7 years Attrition: not reported	Dose: 50mL/day Arms: 1. HPOO; 161mg/kg polyphenols 2. LPOO; 14.7mg/kg polyphen	3-week intervention period, 2-week washout periods before each intervention (LPOO as source of crude fat)	Difference in change between groups *Inflammatory markers* ↓CRP ↓IL-6 ↔sICAM-1 ↔sVCAM-1

			ols Method: administered raw over 3 meals, other cooking fats replaced with the LPOO during both interventions		
Al-Rewashdeh, 2010, Jordan. Study period: October 2008 to March 2009	Controlled, Cross-over	n=25 healthy adults (12 men, 13 women)	Dose: Not prescribed,	4-week interventions, 4-week	Difference in change between groups *Classic CVD markers* ↑HDL-C

	Trial	Age(range): 37 to 50 years (men), 33 to 44 years (women) Attrition: not reported	consumed about 70g per day Arms: 1. HPOO; 753mg/kg polyphenols 2. MPOO; 368mg/kg polyphenols 3. LPOO; 132mg/kg polyphen	wash out periods before each intervention (habitual diet with use of usual fats hydrogenated, refined oil and blend of seed oils)	↓LDL-C[abc] ↓Total /HDL-C[abc] ↓LDL /HDL-C[abc] ↔Triglycerides ↔Phospholipids ↔Total-C ↔Free cholesterol ↔Cholesterol Ester ↓SBP[ab] (men only) ↓DBP[ab] *Oxidative Stress / Antioxidant Status* ↓Malondialdehyde[abc]

			ols Method: Habitual diets plus intervention to replace usual fat intake in cooking, salad dressing, and on bread		
Perona et al. 2011. Same cohort as Marrugat et al. 2004, Spain. Study period: not reported	Placebo-controlled, double-blind,	n=33 healthy men Age(range): 23 to 91 years Attrition: 3	Dose. 25 mL/day 1. HPOO: 825 mmol caffeic	3-week intervention, 2-week washout periods	Difference in change between groups *Classic CVD markers* Serum lipid concentrations ↔Total-C ↔Triglycerides

	random ized, crossov er trial	withdrawals	acid equivale nts/kg 2. MPOO: 370 mmol caffeic acid equivale nts/kg 3. LPOO: 0 mmol caffeic acid equivale nts/kg Method: Participa nts	before each interventi on (LPOO used for raw and cooking purposes)	↓VLDL-cholesteryl esters[c] ↓VLDL-Triglycerides[a,c] ↓VLDL-C[a,c] ↓VLDL-Phospholipids[a,c] ↓VLDL-Apolioprotein B[a,b] ↑VLDL Triglyceride/Apolipprotein B ratio[a,b]

			instructed to consume treatment oil raw, distributed over 3 meals of the day. Other cooking fats were replaced by LPOO and participants requested to avoid a		

			high intake of foods listed as containing phenolic compounds		
Moreno-Luna et al. 2012, Spain. Study period: not reported	Randomized, single-blind, crossover trial	n=24 women with high-normal BP or stage 1 essential hypertension Age (Range): 24 to 27 years Attrition: n=10	Dose: 60 mL/day 1. HPOO: 564mg/kg 2. LPOO: 0mg/kg Method: Mediterranean-style diet	2-month intervention, 4-month washout period prior to commencement, 4 week washout	Difference in change between baseline and treatment values (change between groups not reported) *Classic CVD markers* ↓SBP^{HPOO} ↓DBP^{HPOO} *Oxidative Stress / Antioxidant Status* ↓Oxidized LDL^{HPOO} *Inflammatory markers*

		dropout	in addition to the treatment oil were prescribed. Participants instructed to avoid foods classified as highly rich in polyphenols	period between interventions (provided a set menu plan [Mediterranean-style diet] containing the same calories as their habitual diets and sunflower or corn oil	↓hs-CRP[HPOO] *Additional outcomes* Endothelial function measures (↓Asymmetric dimethylarginine[HPOO] ↑Hyperemic area after ischemia[HPOO] ↑Total plasma nitrites/ nitrates[HPOO])

				was permitted)	
Rus et al. 2017, Spain. Study period: not reported	Randomized, controlled, double-blind, parallel trial	n=23 women with fibromyalgia Age (mean±std): HPOO; 54±6 years, LPOO; 48±8 years Attrition: not reported	Dose: 50 mL/day Arms: 1. HPOO (n=11); polyphenol content not reported 2. LPOO (n=12); polyphenol content not reported	3-week intervention, 2-week washout period prior to commencement (50 mL/day LPOO)	Difference in change between groups *Classic CVD markers* ↔BMI ↔SBP ↔DBP ↔Cardiac frequency(bpm) *Oxidative status* ↓Thiobarbituric acid reactive substances (TBARS) ↓Protein carbonyl content ↔8-hydroxy-2'-deoxyguanosine *Antioxidant status* ↔Total antioxidant capacity ↔Superoxide dismutase (SOD) ↔Glutathione peroxidase (GPx) ↔Catalase

			Method: Treatment olive oil was consumed raw but LPOO was used for cooking. Intake of antioxidants was normalized and participants recommended to avoid an		↔Antioxidant compounds (copper, zinc, ceruloplasmin, iron, ferritin, transferrin, uric acid, albumin, bilirubin)

			excess of calories and/or lipids		
VOHF Cohort					
Farras et al. 2015, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical trial	n=33 hypercholesterolemic adults (19 men, 14 women) Age (range): 35 to 80 years Attrition: n=3 discontinued trial	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3.	3-week intervention period, 2-week washout periods before each intervention ("common" olive oil)	Difference in end intervention measures between groups (controlled for baseline values) *Classic CVD markers* ↔ HDL composition (total-C, triglycerides, Apo-A1, Apo-AII, free cholesterol, esterified-cholesterol, phospholipids, free cholesterol/total-C, esterified cholesterol/total-C, phospholipids/free cholesterol, esterified cholesterol/free cholesterol)

			HPOO+thyme (data not reported) Method: all raw oils replaced with olive oil, consumed with meals. Participants advised to limit consumption polyphen		

			ol-rich food.		
Pedret et al. 2015, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical trial	n=33 hypercholesterolemic adults (19 men, 14 women), Age (range): 35 to 80 years Attrition: n=3 discontinued trial	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not	3-week intervention period, 2-week washout periods before each intervention ("common" olive oil)	*Additional outcomes* All interventions upregulated proteins related to cholesterol homeostasis, protection against oxidation and blood coagulation, while down-regulating proteins related to in acute-phase response, lipid transport, and immune response. HPOO had a stronger effect on the following proteins: PON-3 and PPBP which were up-regulated.

			reported) Method: all raw oils replaced with olive oil, consumed with meals. Participants advised to limit consumption polyphenol-rich food.		
Fernandez-Castillejo et al. 2016, Spain.	Double-	n=33	Dose: 25	3-week	Difference in change between groups

Study period: April 2012 to September 2012	blind, randomized, controlled, crossover clinical trial	hypercholesterolemic adults (19 men, 14 women) Age (range): 35 to 80 years Attrition: n=3 discontinued trial	mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw	intervention period, 2-week washout periods before each intervention ("common" olive oil)	*Classic CVD markers* ↓LDL-C ↔ApoB100 NMR LDL particle concentration (↓total, ↓IDL, ↔large, ↔small) ↔HDL-C ↔ApoA1 NMR HDL particle concentration (↓total, ↑large, ↔medium, ↓small) and ↑size ↔Triglycerides ↔VLDL Triglycerides NMR VLDL particle concentration (↔total, ↔large, ↓medium, ↔small) and ↓size ↓ApoB100 containing lipoproteins ↓LDL particles /HDL particles ↓HDL-C/HDL particles ↓small HDL/ large HDL ↓Lipoprotein insulin resistance index

			oils replaced with olive oil, consumed with meals. Participants advised to limit consumption polyphenol rich food.		
Martin-Pelaez et al. 2016, Spain. Study period: April 2012 to September 2012	Double-blind, randomized,	n=10 hypercholesterolemic adults (5	Dose: 25 mL/day Arms: 1. HPOO;	3-week intervention period, 2-week	Difference in change between groups *Classic CVD markers* ↔Weight/BMI

	controlled, crossover clinical trial	men, 5 women) Age (range): 35 to 80 years Attrition: not reported	enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw oils replaced with	washout periods before each intervention ("common" olive oil)	↔Waist circumference ↑Glucose ↔SBP ↔DBP *Oxidative status* ↔ Oxidized LDL-C *Inflammatory markers* ↑CRP ↔Fecal TNF-α ↔Fecal calprotectin *Additional markers* ↑Total fecal bacteria ↔Ratio Firmicutes/Bacteroidetes ↔Fecal IgA coated bacteria ↔Fecal IgA

			olive oil, consumed with meals. Participants advised to limit consumption polyphenol-rich food.		
Fernandez-Castillejo et al. 2017, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossov	n=33 hypercholesterolemic adults (19 men. 14 women) Age (range):	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/k	3-week intervention period, 2-week washout periods before	Difference in change between groups *Oxidative status* ↑ PON-3 protein ↔PON-1 protein Lactonase activity (↓ raw, ↔ specific) Paraoxonase activity (less ↑ raw, ↔ specific)

	er clinical trial	35 to 80 years Attrition: not reported	g polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw oils replaced with olive oil, consumed with	each intervention ("common" olive oil)	

			meals. Participants advised to limit consumption polyphenol-rich food.		
Martin-Pelaez et al. 2017, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical trial	n=12 hypercholesterolemic adults (7 men, 5 women) Age (range): 46 to 67 years Attrition: not	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols,	3-week intervention period, 2-week washout periods before each intervention	Difference in change between groups *Classic CVD markers* ↔Total-C *Oxidative status* ↔ Oxidized LDL-C *Additional markers* ↔ Bacterial Enumerations

		reported	2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw oils replaced with olive oil, consumed with meals. Advised to limit	("common" olive oil)	↔ Short chain fatty acids ↔ Neutral sterols ↔Bile acids

			consumption polyphenol-rich food.		
EUROLIVE Cohort					
Covas et al. 2006. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover, controlled trial	n=200 healthy men Age (range): 20 to 60 years Attrition: n=18 dropout	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. MPOO; 164 mg/kg polyphenols	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil consumption)	Difference in change between groups *Oxidative status* ↓Conjugated dienes[b,c] ↓Hydroxy fatty acids[c] ↓Oxidized LDL-C[c] ↔$F_{2\alpha}$-isoprostanes

			3. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants asked to avoid high intake of high-antioxidant foods (e.g.		

			vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Machowetz et al. 2007. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover, controlled trial	n=200 healthy men Age(range): 20 to 60 years Attrition: n=18 dropout	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. MPOO;	3-week interventions, 2-week washout periods before each intervention (avoid	Difference in change between groups *Oxidative status* ↔Markers of DNA /RNA oxidative damage (urinary excretion rates of guanine, guanosine, and deoxyguanosine and their corresponding oxidation products)

			164 mg/kg polyphenols 3. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid	olive and olive oil consumption)	

			high intake of high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Machowetz et al. 2008. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June	Single centre, double-blind,	n=38 healthy men Age(mean±std): 36±2	Dose: 25 mL Arms: 1. LPOO;	3-week interventions, 2-week	Difference in change between groups *Classic CVD markers* ↔BMI

2003	randomized, crossover, controlled trial	years Attrition: not reported	2.7 mg/kg polyphenols 2. MPOO; 164 mg/kg polyphenols 3. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervent	washout periods before each intervention (avoid olive and olive oil consumption)	*Inflammatory markers* ↓resistin[LPOO]

			ion oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine,		

			and beer).		
de la Torre-Carbot et al. 2010. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicenter, double-blind, randomized, crossover, controlled trial	n=36 nonsmoking males Age (range): 20 to 60 years Attrition: not reported	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervent	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil consumption)	Difference in change between baseline and treatment values (change between groups not reported) *Oxidative status* ↓plasma oxLDL

			ion oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine,		

			and beer).		
Castaner et al. 2011. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover, controlled trial	n=200 healthy men Age(range): 20 to 60 years Attrition: n=18 dropout	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. MPOO; 164 mg/kg polyphenols 3. HPOO, 366 mg/kg polyphen	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil consumption)	Difference changes between each arm of the study (dose dependent increase related to polyphenol content of olive oil): *Oxidative status* ↑ OLAB

			ols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables,		

			legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Castaner et al. 2012. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover, controlled trial	n=18 healthy men Age(mean±std): 38±12 Attrition: not reported	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. HPOO, 366 mg/kg polyphen	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil	Difference in change between groups *Inflammatory markers* ↓MCP1 Difference changes between baseline and treatment values: *Additional markers* ↓Atherosclerosis-related gene expression (CD40L, IL23A, IL7R, IL8RA, and OLR1 genes)

			ols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables,	consumption)	

			legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Hernaez et al. 2014. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover, controlled trial	n=47 healthy men Age (mean±std): 30±9 years Attrition: not reported	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. HPOO, 366 mg/kg polyphen	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil	Difference in change between groups *Classic CVD markers* ↔Phospholipids ↔Apolipoprotein A1 and A2 ↑ HDL cholesterol efflux capacity ↑large HDL_2 particles ↔HDL particle count ↔Triglycerides in HDL core ↔HDL fluidity

			ols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables,	consumption)	

			legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Hernaez et al. 2015. 3 Cities (Potsdam, Germany; Kupio Finland, Barcelona, Spain)	Multicentre, double-blind, randomized, crossover, controlled trial	n=25 Healthy men (lipid-related outcomes) Age (mean±std): 32±11 years n=18 Healthy men (gene expression outcomes) Age	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. HPOO, 366 mg/kg polyphen	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil	Difference in change between groups *Classic CVD markers* ↓Apolipoprotein B-100 ↓Total LDL particles ↓Small LDL particles ↔Large LDL particles ↔Lipoprotein Lipase gene expression *Oxidative status* ↔LDL oxidation lag time ↔LDL oxidation rate

		(mean±std): 37±12 years Attrition: not reported	ols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables,	consumption)	

			legumes, fruits, tea, coffee, chocolate, wine, and beer).		

Author, year, country, study period	**Study Design**	**Population, Attrition rate**	**Olive oil arms**	**Duration and structure**	**Results, *differences between high polyphenol compared to low polyphenol olive oils**[6]**
Independent studies					
Ramirez-Tortosa et al. 1999, Spain. Study period: not reported	Randomized Controlled, Cross-	n=24 free-living men with peripheral vascular	Dose: Not specified Arms: 1. HPOO;	3-month interventions, 3-month wash-out	Difference in end intervention measures between groups *Classic CVD markers* ↔Weight/BMI ↔HDL-C

	over Trial	disease, without diabetes, hypothyroidism, obesity, cardiac episodes Age (mean±std): 70±2 years Attrition: not reported	800mg/kg polyphenols 2. LPOO; 60mg/kg polyphenols Method: Instruction to replace usual saturated fat intake (butter, margarine, lard and visible fat	period between interventions (usual diets)	↔LDL-C ↑ Triglycerides Lipoprotein composition of: Triglycerides (↔VLDL,↑ LDL, ↔HDL) Phospholipids (↔VLDL,↔ LDL, ↔HDL) Total-C (↔VLDL, ↑LDL, ↔HDL) Cholesterol Esters (↔VLDL, ↓LDL, ↔HDL) Free cholesterol (↑VLDL, ↑LDL, ↓HDL) *Oxidative Stress / Antioxidant Status* ↓ Copper- mediated LDL oxidation ↓ Macrophage uptake of oxidized LDL

			on meat) with the olive oil. Recommended to increase fruits, legumes and vegetables to ensure adequate intake of fibre and antioxidant vitamins. Restrict eating		

			out to 1/week. Advised to walk at least 1 km/day and stop smoking.		
Vissers et al. 2001, Netherlands. Study period: not reported	Randomized Controlled, Cross-over Trial Blinding of participants to olive oil	n=49 healthy adults (32 women, 17 men), Age (range): 18-58 years, Attrition: n=6 withdrew	Dose: based on energy needs, mean 69g/day Arms: 1. HPOO; 308mg/kg polyphenols	3-week interventions, 2-week wash-out periods before each intervention (diets without olives,	Difference in end intervention measures between groups *Classic CVD markers* ↔Weight ↔Total-C ↔HDL-C ↔LDL-C ↔Triglycerides *Oxidative Stress / Antioxidant Status* LDL oxidizability (↓lag time, ↔max rate)

	sequence		2. LPOO; 43mg/kg polyphenols Method: daily olive oil in provided foods (40% in mayonnaise, 30% in sauces and 30% in cookies and raisin rolls).	olive oil and olive oil products)	HDL oxidizability (↔lag time, ↔max rate) ↔Malondialdehyde ↔Lipid hydroperoxides ↔Protein carbonyls

			Half was consumed at lunch in presence of researchers and remainder at home. Usual diet maintained, except followed instructions for low		

			vitamin E.		
Moschandreas et al. 2002, Greece. Study period: not reported	Randomized, single-blind, crossover trial, Participants were blinded to the type of oil they received	n=25 Adult smokers (11 men, 14 females) Age (mean±std): 30±9 years Attrition: n=3 dropout	Dose: 70 g/day Arms: 1. HPOO; 308mg/kg polyphenols 2. LPOO; 43mg/kg polyphenols Method: Oil was subdivided over two meals	3-week intervention, 2-week washout periods before each intervention (diet without olives or olive oil products)	Difference in change between groups *Classic CVD markers* ↔Weight *Oxidative Stress / Antioxidant Status* Total plasma resistance to oxidation (↔lag time, ↔max rate) ↔Protein carbonyl ↔Malondialdehyde ↔Lipid hydroperoxides ↔Ferric reducing ability of plasma

			and participants instructed to pour it over the food consumed. Participants requested to maintain their usual food and fluid intake and not		

			consume olives and other oil-containing products		
Marrugat et al. 2004, Same cohort as Perona et al. 2011, Spain. Study period: not reported	Placebo-controlled, double-blind, randomized, crossover trial	n=30 healthy men Age (mean±std): HPOO-MPOO-LPOO: 55±21 years MPOO-LPOO-HPOO: 61±19 years LPOO-HPOO-MPOO:	Dose: 25 mL/day Arms: 1. HPOO: 150mg/kg of phenols 2. MPOO: 68mg/kg of phenols 3. LPOO:	3-week intervention, 2-week washout periods before each intervention (LPOO used for raw and cooking	Difference in change between baseline and treatment values (change between groups not reported) *Classic CVD markers* ↔Total-C ↑HDL-C^{HPOO} ↔LDL-C ↔Triglycerides ↔Glucose *Oxidative Stress / Antioxidant Status* ↓Oxidized LDL^{HPOO} Resistance of LDL to oxidation (↑lag time^{HPOO,MPOO}, ↔rate, ↔max amount of dienes,

		57±19 years Attrition: 3 withdrawals	Undetected polyphenols Method: Participants instructed to consume Treatment oil raw, was distributed over 3 meals of the day. Other cooking fats were	purposes)	↔antibodies against oxidized LDL Percentage of change (baseline to end of intervention) between groups ↓Oxidized LDL[a,c] Resistance of LDL to oxidation (↑lag time)[a,b]

			replaced by LPOO and participants requested to avoid a high intake of foods listed as containing phenolic compounds		
Fito et al. 2005, Spain. Study period: not reported	Placebo controlled,	n=40 men with stable CHD	Dose: 50mL/day	3-week intervention period,	Difference in change between groups *Classic CVD markers*

	crossover, double-blind randomized trial	Age (mean±std): 67±9 years Attrition: n=3 dropped out, n=3 excluded due to lack of compliance	Arms: 1. HPOO; 161mg/kg polyphenols 2. LPOO; 14.7mg/kg polyphenols Method: administered raw over 3 meals, other cooking fats replaced	2-week washout periods before each intervention (LPOO as source of crude fat)	↔Total-C ↔LDL-C ↔HDL-C ↔Triglycerides ↔Lipoprotein (a) ↔Glucose ↓SBP ↔DBP *Oxidative Stress / Antioxidant Status* ↓Oxidized LDL-C ↔ Antibodies against oxidized ↓Lipoperoxides ↑Glutathione peroxidase ↔Total antioxidant status

			with the LPOO during both interventions		
Visioli et al. 2005, Italy. Study period: not reported	Randomized, single-blind, crossover trial. Laboratory personnel were blinded to treatm	n=22 mildly dyslipidaemic adults (12 men, 10 females) Age (range): 18 to 65 years Attrition: not reported	Dose: 40 mL/ day Arms: 1. HPOO; total hydroxytyrosol content 166 mg/L 2. LPOO; total hydroxytyrosol content 2	7-week intervention, 3-week washout period prior to commencement, 4-week washout period between interventi	Difference in change between groups *Classic CVD markers* ↔Total-C ↔HDL-C ↔LDL-C ↔Triglycerides ↔ BMI ↔ Mean blood pressure ↔ Glucose *Oxidative Stress / Antioxidant Status* ↑Antioxidant capacity ↓Thromboxane B_2 (TXB_2)

	ents		mg/L Method: Raw olive oil was subdivided between lunch and dinner and participants instructed to consume with pasta or vegetables. Other	ons (40 mL/day of LPOO)	↔Isoprostane excretion (8-iso-PGF2α)

			polyphenol-rich foods in the diet were controlled for		
Salvini et al. 2006, Italy. Study period: September–November 2002 to January – March 2003	Randomized, double-blind, crossover trial	n=10 healthy postmenopausal women Age (range): 47 to 67 years Attrition: n=2 dropout	Dose: 50 g/day Arms: 1. HPOO: 592 mg/kg polyphenols 2. LPOO: 147 mg/kg polyphenols	8-week intervention, 8-week washout period (habitual fats and oils)	Difference in change between groups *Oxidative Stress / Antioxidant Status* Oxidative DNA damage (↓oxidized DNA bases, ↔basal DNA breaks) ↔Total Antioxidant Status ↔DNA breakage induced by H_2O_2 (*in vitro*)

			Method: Participants instructed to substitute all fats and oils with the study oil and to consume at least 50 g daily in raw form in addition to the oil necessary for		

			cooking. Apart from the fat substitution, participants instructed to stay on their habitual diet		
Fito et al. 2008, Subset of Fito et al. 2005, Spain. Study period: not reported	Placebo controlled, crossover, double-blind	n=28 men with stable CHD Age (mean±std): 68±7 years Attrition: not	Dose: 50mL/day Arms: 1. HPOO; 161mg/kg	3-week intervention period, 2-week washout periods before	Difference in change between groups *Inflammatory markers* ↓CRP ↓IL-6 ↔sICAM-1 ↔sVCAM-1

	random ised trial	reported	polyphen ols 2. LPOO; 14.7mg/k g polyphen ols Method: administ ered raw over 3 meals, other cooking fats replaced with the LPOO during both	each interventi on (LPOO as source of crude fat)	

			interventions		
Al-Rewashdeh, 2010, Jordan. Study period: October 2008 to March 2009	Controlled, Cross-over Trial	n=25 healthy adults (12 men, 13 women) Age(range): 37 to 50 years (men), 33 to 44 years (women) Attrition: not reported	Dose: Not prescribed, consumed about 70g per day Arms: 1. HPOO; 753mg/kg polyphenols 2. MPOO; 368mg/kg	4-week interventions, 4-week wash out periods before each intervention (habitual diet with use of usual fats hydrogenated, refined oil and blend	Difference in change between groups *Classic CVD markers* ↑HDL-C ↓LDL-C[abc] ↓Total /HDL-C[abc] ↓LDL /HDL-C[abc] ↔Triglycerides ↔Phospholipids ↔Total-C ↔Free cholesterol ↔Cholesterol Ester ↓SBP[ab] (men only) ↓DBP[ab] *Oxidative Stress / Antioxidant Status* ↓Malondialdehyde[abc]

			polyphenols 3. LPOO; 132mg/kg polyphenols Method: Habitual diets plus intervention to replace usual fat intake in cooking, salad dressing, and on bread	of seed oils)	

Perona et al. 2011. Same cohort as Marrugat et al. 2004, Spain. Study period: not reported	Placebo-controlled, double-blind, randomized, crossover trial	n=33 healthy men Age(range): 23 to 91 years Attrition: 3 withdrawals	Dose: 25 mL/day 1. HPOO: 825 mmol caffeic acid equivalents/kg 2. MPOO: 370 mmol caffeic acid equivalents/kg 3. LPOO: 0 mmol caffeic	3-week intervention, 2-week washout periods before each intervention (LPOO used for raw and cooking purposes)	Difference in change between groups *Classic CVD markers* Serum lipid concentrations ↔Total-C ↔Triglycerides ↓VLDL-cholesteryl esters[c] ↓VLDL-Triglycerides[a,c] ↓VLDL-C[a,c] ↓VLDL-Phospholipids[a,c] ↓VLDL-Apolioprotein B[a,b] ↑VLDL Triglyceride/Apoliprotein B ratio[a,b]

			acid equivalents/kg Method: Participants instructed to consume treatment oil raw, distributed over 3 meals of the day. Other cooking fats were replaced by LPOO		

			and participants requested to avoid a high intake of foods listed as containing phenolic compounds		
Moreno-Luna et al. 2012, Spain. Study period: not reported	Randomized, single-blind, crossov	n=24 women with high-normal BP or stage 1 essential	Dose: 60 mL/day 1. HPOO: 564mg/kg	2-month intervention, 4-month washout	Difference in change between baseline and treatment values (change between groups not reported) *Classic CVD markers* ↓SBP^{HPOO}

	er trial	hypertension Age (Range): 24 to 27 years Attrition: n=10 dropout	2. LPOO: 0mg/kg Method: Mediterranean-style diet in addition to the treatment oil were prescribed. Participants instructed to avoid foods classified	period prior to commencement, 4 week washout period between interventions (provided a set menu plan [Mediterranean-style diet] containing the same	↓DBP[HPOO] *Oxidative Stress / Antioxidant Status* ↓Oxidized LDL[HPOO] *Inflammatory markers* ↓hs-CRP[HPOO] *Additional outcomes* Endothelial function measures (↓Asymmetric dimethylarginine[HPOO] ↑Hyperemic area after ischemia[HPOO] ↑Total plasma nitrites/ nitrates[HPOO])

			as highly rich in polyphenols	calories as their habitual diets and sunflower or corn oil was permitted)	
Rus et al. 2017, Spain. Study period: not reported	Randomized, controlled, double-blind, parallel trial	n=23 women with fibromyalgia Age (mean±std): HPOO; 54±6 years, LPOO; 48±8 years Attrition: not reported	Dose: 50 mL/day Arms: 1. HPOO (n=11); polyphenol content not reported 2. LPOO	3-week intervention, 2-week washout period prior to commencement (50 mL/day LPOO)	Difference in change between groups *Classic CVD markers* ↔BMI ↔SBP ↔DBP ↔Cardiac frequency(bpm) *Oxidative status* ↓Thiobarbituric acid reactive substances (TBARS) ↓Protein carbonyl content

			(n=12); polyphenol content not reported Method: Treatment olive oil was consumed raw but LPOO was used for cooking. Intake of antioxidants was normaliz		↔8-hydroxy-2'-deoxyguanosine *Antioxidant status* ↔Total antioxidant capacity ↔Superoxide dismutase (SOD) ↔Glutathione peroxidase (GPx) ↔Catalase ↔Antioxidant compounds (copper, zinc, ceruloplasmin, iron, ferritin, transferrin, uric acid, albumin, bilirubin)

			ed and participants recommended to avoid an excess of calories and/or lipids		
VOHF Cohort					
Farras et al. 2015, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical	n=33 hypercholesterolemic adults (19 men, 14 women) Age (range): 35 to 80 years	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphen	3-week intervention period, 2-week washout periods before each interventi	Difference in end intervention measures between groups (controlled for baseline values) *Classic CVD markers* ↔ HDL composition (total-C, triglycerides, Apo-A1, Apo-AII, free cholesterol, esterified-cholesterol, phospholipids, free cholesterol/total-C, esterified cholesterol/total-C, phospholipids/free cholesterol, esterified cholesterol/free cholesterol)

	trial	Attrition: n=3 discontinued trial	ols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw oils replaced with olive oil, consumed with meals. Participa	on ("common" olive oil)	

			nts advised to limit consumption polyphenol-rich food.		
Pedret et al. 2015, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical trial	n=33 hypercholesterolemic adults (19 men, 14 women), Age (range): 35 to 80 years Attrition: n=3 discontinued	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg	3-week intervention period, 2-week washout periods before each intervention ("common" olive	*Additional outcomes* All interventions upregulated proteins related to cholesterol homeostasis, protection against oxidation and blood coagulation, while down-regulating proteins related to in acute-phase response, lipid transport, and immune response. HPOO had a stronger effect on the following proteins: PON-3 and PPBP which were up-regulated.

		trial	polyphenols, 3. HPOO+thyme (data not reported) Method: all raw oils replaced with olive oil, consumed with meals. Participants advised to limit	oil)	

			consumption polyphenol-rich food.		
Fernandez-Castillejo et al. 2016, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical trial	n=33 hypercholesterolemic adults (19 men, 14 women) Age (range): 35 to 80 years Attrition: n=3 discontinued trial	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3.	3-week intervention period, 2-week washout periods before each intervention ("common" olive oil)	Difference in change between groups *Classic CVD markers* ↓LDL-C ↔ApoB100 NMR LDL particle concentration (↓total, ↓IDL, ↔large, ↔small) ↔HDL-C ↔ApoA1 NMR HDL particle concentration (↓total, ↑large, ↔medium, ↓small) and ↑size ↔Triglycerides ↔VLDL Triglycerides NMR VLDL particle concentration (↔total, ↔large, ↓medium, ↔small) and ↓size

			HPOO+thyme (data not reported) Method: all raw oils replaced with olive oil, consumed with meals. Participants advised to limit consumption polyphen		↓ApoB100 containing lipoproteins ↓LDL particles /HDL particles ↓HDL-C/HDL particles ↓small HDL/ large HDL ↓Lipoprotein insulin resistance index

			ol rich food.		
Martin-Pelaez et al. 2016, Spain. Study period: April 2012 to September 2012	Double-blind, randomized, controlled, crossover clinical trial	n=10 hypercholesterolemic adults (5 men, 5 women) Age (range): 35 to 80 years Attrition: not reported	Dose: 25 mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not	3-week intervention period, 2-week washout periods before each intervention ("common" olive oil)	Difference in change between groups *Classic CVD markers* ↔Weight/BMI ↔Waist circumference ↑Glucose ↔SBP ↔DBP *Oxidative status* ↔ Oxidized LDL-C *Inflammatory markers* ↑CRP ↔Fecal TNF-α ↔Fecal calprotectin *Additional markers*

			reported) Method: all raw oils replaced with olive oil, consumed with meals. Participants advised to limit consumption polyphenol-rich food.		↑Total fecal bacteria ↔Ratio Firmicutes/Bacteroidetes ↔Fecal IgA coated bacteria ↔Fecal IgA
Fernandez-Castillejo et al. 2017, Spain.	Double-	n=33	Dose: 25	3-week	Difference in change between groups

Study period: April 2012 to September 2012	blind, randomized, controlled, crossover clinical trial	hypercholesterolemic adults (19 men, 14 women) Age (range): 35 to 80 years Attrition: not reported	mL/day Arms: 1. HPOO; enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw	intervention period, 2-week washout periods before each intervention ("common" olive oil)	*Oxidative status* ↑ PON-3 protein ↔PON-1 protein Lactonase activity (↓ raw, ↔ specific) Paraoxonase activity (less ↑ raw, ↔ specific)

			oils replaced with olive oil, consumed with meals. Participants advised to limit consumption polyphenol-rich food.		
Martin-Pelaez et al. 2017, Spain. Study period: April 2012 to September 2012	Double-blind, randomized,	n=12 hypercholesterolemic adults (7	Dose: 25 mL/day Arms: 1. HPOO;	3-week intervention period, 2-week	Difference in change between groups *Classic CVD markers* ↔Total-C

	controlled, crossover clinical trial	men, 5 women) Age (range): 46 to 67 years Attrition: not reported	enriched with 500mg/kg polyphenols, 2. LPOO; 80 mg/kg polyphenols, 3. HPOO+thyme (data not reported) Method: all raw oils replaced with	washout periods before each intervention ("common" olive oil)	*Oxidative status* ↔ Oxidized LDL-C *Additional markers* ↔ Bacterial Enumerations ↔ Short chain fatty acids ↔ Neutral sterols ↔Bile acids

			olive oil, consumed with meals. Advised to limit consumption polyphenol-rich food.		
EUROLIVE Cohort					
Covas et al. 2006. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover,	n=200 healthy men Age (range): 20 to 60 years Attrition: n=18 dropout	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols	3-week interventions, 2-week washout periods before each	Difference in change between groups *Oxidative status* ↓Conjugated dienes[b,c] ↓Hydroxy fatty acids[c] ↓Oxidized LDL-C[c] ↔$F_{2\alpha}$-isoprostanes

	controlled trial		2. MPOO; 164 mg/kg polyphenols 3. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants asked to avoid	intervention (avoid olive and olive oil consumption)	

			high intake of high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Machowetz et al. 2007. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June	Multicentre, double-blind,	n=200 healthy men Age(range): 20 to 60	Dose: 25 mL Arms: 1. LPOO;	3-week interventions, 2-week	Difference in change between groups *Oxidative status* ↔Markers of DNA /RNA oxidative damage (urinary excretion rates of guanine, guanosine, and

2003	randomized, crossover, controlled trial	years Attrition: n=18 dropout	2.7 mg/kg polyphenols 2. MPOO; 164 mg/kg polyphenols 3. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervent	washout periods before each intervention (avoid olive and olive oil consumption)	deoxyguanosine and their corresponding oxidation products)

			ion oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine,		

			and beer).		
Machowetz et al. 2008. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Single centre, double-blind, randomized, crossover, controlled trial	n=38 healthy men Age(mean±std): 36±2 years Attrition: not reported	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. MPOO; 164 mg/kg polyphenols 3. HPOO, 366 mg/kg polyphen	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil consumption)	Difference in change between groups *Classic CVD markers* ↔BMI *Inflammatory markers* ↓resistin^{LPOO}

			ols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables,		

			legumes, fruits, tea, coffee, chocolate, wine, and beer).		
de la Torre-Carbot et al. 2010. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicenter, double-blind, randomized, crossover, controlled trial	n=36 nonsmoking males Age (range): 20 to 60 years Attrition: not reported	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. HPOO, 366 mg/kg polyphen	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil	Difference in change between baseline and treatment values (change between groups not reported) *Oxidative status* ↓plasma oxLDL

			ols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of high-antioxidant foods (e.g. vegetables,	consumption)	

			legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Castaner et al. 2011. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized, crossover, controlled trial	n=200 healthy men Age(range): 20 to 60 years Attrition: n=18 dropout	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg polyphenols 2. MPOO; 164 mg/kg	3-week interventions, 2-week washout periods before each intervention (avoid olive and olive oil	Difference changes between each arm of the study (dose dependent increase related to polyphenol content of olive oil): *Oxidative status* ↑ OLAB

			polyphenols 3. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of	consumption)	

			high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Castaner et al. 2012. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized,	n=18 healthy men Age(mean±std): 38±12 Attrition: not reported	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg	3-week interventions, 2-week washout periods	Difference in change between groups *Inflammatory markers* ↓MCP1 Difference changes between baseline and treatment values:

	crossover, controlled trial		polyphenols 2. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of	before each intervention (avoid olive and olive oil consumption)	*Additional markers* ↓Atherosclerosis-related gene expression (CD40L, IL23A, IL7R, IL8RA, and OLR1 genes)

			high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Hernaez et al. 2014. 5 European Countries (Spain, Denmark, Finland, Italy, Germany) Study period: September 2002 to June 2003	Multicentre, double-blind, randomized,	n=47 healthy men Age (mean±std): 30±9 years Attrition: not	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg	3-week interventions, 2-week washout periods	Difference in change between groups *Classic CVD markers* ↔Phospholipids ↔Apolipoprotein A1 and A2

	crossover, controlled trial	reported	polyphenols 2. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of	before each intervention (avoid olive and olive oil consumption)	↑ HDL cholesterol efflux capacity ↑large HDL_2 particles ↔HDL particle count ↔Triglycerides in HDL core ↔HDL fluidity

			high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		
Hernaez et al. 2015. 3 Cities (Potsdam, Germany; Kupio Finland, Barcelona, Spain)	Multicentre, double-blind, randomized,	n=25 Healthy men (lipid-related outcomes) Age (mean±std):	Dose: 25 mL Arms: 1. LPOO; 2.7 mg/kg	3-week interventions, 2-week washout periods	Difference in change between groups *Classic CVD markers* ↓Apolipoprotein B-100 ↓Total LDL particles ↓Small LDL particles

	crossover, controlled trial	32±11 years n=18 Healthy men (gene expression outcomes) Age (mean±std): 37±12 years Attrition: not reported	polyphenols 2. HPOO, 366 mg/kg polyphenols Method: Replace all raw fats with intervention oil. Participants were also asked to avoid high intake of	before each intervention (avoid olive and olive oil consumption)	↔Large LDL particles ↔Lipoprotein Lipase gene expression *Oxidative status* ↔LDL oxidation lag time ↔LDL oxidation rate

			high-antioxidant foods (e.g. vegetables, legumes, fruits, tea, coffee, chocolate, wine, and beer).		

*Results represented by ↓ = significantly decreased more or lower ↑ = significantly increased more or higher or ↔ = no significant difference in change or measures. Where there are more than 2 groups, which groups had the significant differences is indicated by: [a]between HPOO and LPOO, [b]between MPOO and LPOO, and [c]between HPOO and MPOO.

[β] Outcomes for studies that used subsamples of a larger cohort were not extracted if another paper included a larger sample.

Abbreviations: BMI, Body Mass Index; BP, Blood Pressure; CD40L, CD40 Ligand; CHD, Coronary Heart Disease; CRP, C-reactive Protein; CVD, Cardiovascular Disease; HDL, High Density Lipoprotein; HPOO, High polyphenol Olive Oil; IL23A, Interleukin-23 alpha; IL7R, Interleukin-7 receptor; IL8RA, Interleukin 8 receptor alpha; IgA, Immunoglobulin A; LPOO, Low Polyphenol Olive Oil; LDL, Low Density Lipoprotein; MCP1, Monocyte chemotactic protein 1; MPOO, Medium Polyphenol Olive Oil; NMR, Nuclear magnetic resonance; OLAB, oxidized low density lipoprotein autoantibodies; oxLDL, Oxidized Low Density Lipoprotein; OLR1, Oxidized low-density lipoprotein receptor 1; sICAM-1, PPBP, platelet basic protein; Soluble Intercellular Adhesion Molecule-1; sVCAM-1, Soluble Vascular Adhesion Molecule-1; Total-C, Total cholesterol; TNF-α, Tumour Necrosis Factor Alpha