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Effects of virgin olive oil phenolic compounds on health: solid evidence or just another fiasco?

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SUMMARY: Current research suggests that virgin olive oil (VOO) phenolics are potent preventive and therapeutic agents against metabolic diseases associated with inflammation and oxidative stress. Evidence-based medicine requires these effects be proved in randomized controlled trials (RCT), which are then assessed in meta-analyses, to ensure that the alleged health benefits really proceed in humans. The available evidence is limited to the ability of VOO phenolic compounds to protect lipoproteins from oxidation and to reduce systolic pressure in hypertensive individuals. No RCT assessing the effects of VOO phenolics on diabetes and neurodegenerative diseases have been performed, and those focused on osteoarthritis and cancer provided very scarce information. Therefore, RCT in extensive and diverse population groups, with different disorders and phenolic doses adjusted to usual VOO consumptions are necessary to achieve high quality scientific evidence before nutritional recommendations can be given to the general public.

KEYWORDS*: Evidence; Health; Hydroxytyrosol; Oleocanthal; Phenolic Compounds; Virgin Olive Oil*

RESUMEN: ***Efectos de los compuestos fenólicos del aceite de oliva virgen en la salud: ¿evidencia sólida o simplemente otro fiasco?*** Las investigaciones actuales indican que los compuestos fenólicos del aceite de oliva virgen (AOV) son potentes agentes preventivos y terapéuticos contra las enfermedades metabólicas asociadas con la inflamación y el estrés oxidativo. La medicina basada en la evidencia requiere que estos efectos se prueben en ensayos aleatorizados controlados (RCT), que son después evaluados en meta-análisis, para garantizar que los supuestos beneficios para la salud realmente se registran en humanos. La evidencia disponible se limita a la capacidad de los compuestos fenólicos del AOV para proteger las lipoproteínas de la oxidación y reducir la presión sistólica en individuos hipertensos. No se han realizado RCT que evalúen el efecto de estos compuestos sobre la diabetes y las enfermedades neurodegenerativas, y los que se centraron en la osteoartritis y el cáncer han proporcionado información muy escasa. Por lo tanto, nuevos RCT, en grupos de población extensos y diversos, con diferentes patologías y con dosis de fenoles ajustadas a los consumos habituales de VOO, deben desarrollarse, para lograr evidencia científica de alta calidad antes de que se puedan dar recomendaciones nutricionales al público en general.

PALABRAS CLAVE*: Aceite de Oliva Virgen; Compuestos Fenólicos; Evidencia; Hidroxitirosol; Oleocantal; Salud*

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1. INTRODUCTION

In 2014, Francesco Visioli published an editorial response to a review by Tang *et al*., (2014) on the effects on cardiovascular health of resveratrol, a natural phenolic compound found in red wine (Visioli, 2014). Resveratrol was alleged to interact with multiple targets in a variety of cardiovascular disease models to exert protective effects or induce a reduction in cardiovascular risks. However, Visioli's article was entitled "The resveratrol fiasco" and concluded that after more than 20 years of well-funded research, resveratrol had no proven human activity. He claimed that there was a lack of clinical trials and the majority of available evidence came from cell culture and animal models. He also suggested that the resveratrol fiasco is not the only one of that kind in pharma-nutrition research. Considering that the association of virgin olive oil (VOO) phenolic compounds and health was initiated in the late 90s (some of the early studies were carried out by Visioli himself (Visioli *et al*., 1998)) and that the bulk of experimental data were obtained from *in vitro* and animal models, are VOO phenolics another example of scientific fiasco? In order to answer this question, the present review summarizes the current knowledge on the effects of VOO phenolic compounds on human health, focusing on the data obtained from randomized clinical trials (RCT) and their meta-analyses and systematic reviews, which provide the highest level of scientific evidence.

2. VOO PHENOLIC COMPOUNDS

There is ample evidence indicating that VOO consumption provides benefits in key processes associated with the development of a number of diseases and pathophysiological conditions. These include atherosclerosis, diabetes mellitus, obesity, metabolic syndrome, cancer, arthritis and neurodegenerative diseases (Covas *et al*., 2015). Despite its high oleic acid content, VOO is more than just a monounsaturated fatty acid-rich fat. VOO contains minor compounds with potent pharmacological activity, which are classified into two large groups: those that form part of the unsaponifiable fraction and those with a phenolic nature. The former are lipophilic and may be extracted with organic solvents after saponification of the oil, while the latter are water soluble.

More than 30 phenolic compounds that can play a role in the health promoting qualities of VOO have been identified, among which there is considerable variation regarding their concentration (0.02 to 600 mg/kg) (Servili *et al.,* 2009). This variability depends on the type of phenolic compound, but also on many other factors such as the olive tree variety, geographical origin, cultivation techniques, ripening stage at the time of harvest, processing and storage. Among VOO phenolics, secoiridoids are present in the greatest amount, but the most interesting ones from the point of view of health are probably oleuropein aglycone and its metabolite hydroxytyrosol. However, the presence of oleocanthal should not be ignored, as this compound has received a great deal of attention since it was suggested that it might have anti-inflammatory activity (Beauchamp *et al.*, 2005).

Several experimental studies in *in vitro* systems and animal models have shown that the possible benefits of VOO phenolics are associated with their anti-inflammatory, antioxidant and vasodilatory activity, which in theory makes them key preventive or therapeutic agents for metabolic diseases related to oxidative stress and inflammation (Covas *et al*., 2006). However, most of these investigations used supraphysiological doses of phenolics (> 10 μM) (Catalán *et al.*, 2015) and, therefore, it is difficult to translate their results into physiological relevance for humans. VOO phenolics are bioavailable in humans, i.e. they are susceptible to being absorbed and to exert a bioactive effect on the organism (de la Torre, 2008). It has been proposed that in humans hydroxytyrosol is dose-dependently absorbed and excreted in urine after the intake from VOO (Rubió *et al*., 2012; Oliveras-López *et al.*, 2014). However, a more recent study in Sprague-Dawley rats suggested that different dosages of hydroxytyrosol do not provide a linear, dose-dependent plasma concentration or excretion in urine (Domínguez-Perles *et al*., 2017). Nevertheless, it has been estimated that the amount of phenolics ingested from VOO consumption does not exceed 9 mg/day in Mediterranean countries (Parkinson and Cicerale., 2016).

Since 2011, the European Food Safety Authority (EFSA) accepts a claim about the benefits of daily intake of VOO rich in phenolic compounds over the oxidation of low density lipoproteins (LDL), maintenance of normal (fasting) blood levels of triglycerides, HDL-cholesterol and blood glucose

(EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011). The acceptance of these claims by EFSA authorizes their inclusion on the labels of olive oil bottles. However, this health claim is focused on the protection provided by hydroxytyrosol and there are currently no accepted claims for the other benefits that have been attributed to VOO or its phenolic compounds.

Although a large number of *in vitro* and experimental animal studies are available, according to Evidence-Based Medicine (Woolf *et al*., 1990), the healthy properties of a food and/or its components must be proved in RCT, in order to ensure that the alleged health benefits really stand out. The highest degree of scientific evidence is obtained through meta-analyses and systematic reviews of these RCT. However, at the moment the number and variety of RCT carried out using VOO phenolic compounds is very limited and there is only one meta-analysis and less than five systematic reviews assessing the healthy effects of VOO phenolic compounds. With regard to RCT, there are currently about 50 published articles that can be classified as such in which the effect of VOO phenolic compounds on health has been evaluated. A high number of these studies were published by the group of Covas and their collaborators and the rest by three or four research teams, the absolute majority of which were Spanish or Italian.

3. VOO PHENOLIC COMPOUNDS AND CARDIOVASCULAR DISEASE

The role of VOO phenolics as cardiovascular protectors has been a matter of research for a long time. However, as very recently displayed by Visioli *et al*., (2020), most investigations used isolated compounds in pharmacological *in vitro* approaches, rather than more nutritional human trials. Still, enough data from human studies assessing surrogate markers of cardiovascular disease have been generated (Bogani *et al*., 2007; Visioli *et al*., 2005; Covas *et al*., 2006) to allow for the completion of a meta-analysis of RCT (Hohmann *et al.,* 2015) and two systematic reviews (Bahramsoltani *et al*., 2019; Schwingshackl *et al*., 2019).

Indeed, this meta-analysis was aimed at assessing the effects of high phenolic olive oil on risk factors for cardiovascular disease. It was published in 2015 and included the results of 8 human RCT (Hohmann *et al.,* 2015). The sample size of these trials was quite variable, ranging from 24 participants in the study published by Moreno-Luna *et al*., (2012), to 200 individuals in the one carried out by Covas *et al*., (2006). In addition, there was variability in the health status of the participants. In five of these studies, the subjects were healthy and in the remaining three ones, the recruited individuals already had a cardiovascular event. The doses used in these tests ranged from 0 to 19.5 mg of total phenolics. Furthermore, in all these trials what was compared was the intake of a high phenolic VOO with a refined olive oil. Therefore, only the total phenolic content was considered and the concentrations in different phenolic compounds were not distinguished.

The intake of high phenolic VOO only seemed to produce a modest but significant reduction in oxidized LDL-cholesterol ($Z=1.98$, $p=0.05$) and blood pressure ($Z=4.04$, $p<0.001$). For the rest of parameters related to cardiovascular disease (malondialdehyde, LDL-cholesterol, HDL-cholesterol, total cholesterol and triglycerides), no significant effects were found. Therefore, the conclusion of this meta-analysis was that a high phenolic VOO provides small beneficial effects on systolic pressure and plasma oxidative status. No association between the intake of phenolic compounds from VOO and cardiovascular risk could be obtained.

In addition to this meta-analysis, two systematic reviews were published, both of them in 2019. Bahramsoltani *et al*., (2019) published a comprehensive review about dietary phenolic compounds and atherosclerosis and, even more recently, Schwingshackl *et al*., (2019) carried out a systematic review and network meta-analysis to assess the impact of different types of olive oil on cardiovascular risk factors.

The review by Bahramsoltani *et al*., (2019) included only six RCT, while the aforementioned meta-analysis by Hohmann and colaborators included eight. Of these 6 trials, only one reached a score of 3 on the Jadad Scale (Jadad *et al.*, 1996) which measures the methodological quality of a clinical trial from 0 (very poor) to 5 (rigurous) points. The others stayed at 2 or below. What was observed in the best designed study (Covas *et al.,* 2006) was an effect on the postprandial isoprostane F2 levels in plasma, which is a biomarker of oxidative stress. In this double-bind trial, 40 mL of three olive oils

with different phenolic contents were administered in a single dose: low (2.7 mg/kg), medium (164 mg/kg) and high (366 mg/kg). The authors observed that the concentration in total phenolic compounds in LDL increased in the postprandial period in a direct relationship with the phenolic content of the oils ingested. Moreover, plasma concentrations of tyrosol, hydroxytyrosol, and 3-O-methyl-hydroxytyrosol correlated with changes in the total phenolic compound contents in LDL. The other RCT included in the review also confirmed these results, as well as reduced lipid peroxidation (measured as 8-iso-PGF2α) and inflammation (assessed as intercellular adhesion molecule-1, ICAM-1).

The systematic review by Schwingshackl *et al*., (2019) included thirteen RCTs with 611 participants (mainly healthy) and compared refined olive oil, commercial olive oil (blend of refined and virgin olive oils), low-phenolic extra VOO and high-phenolic VOO. No differences for total cholesterol, HDL-cholesterol, triglycerides, and diastolic blood pressure were observed. However, the phenolic content positively correlated with a slight reduction in LDL-cholesterol (mean difference -0.14 mmol/L, 95%-CI: -0.28, -0.01) and oxidized LDL-cholesterol (standardized mean difference: -0.68, 95%-CI: -1.31, -0.04). Both, high- and low-phenolic VOO reduced systolic blood pressure compared to refined olive oil (range of mean difference: -2.99 to -2.87 mmHg). The authors concluded that high phenolic VOO may improve some cardiovascular risk factors, although the implications for public health were limited due to the overall low or moderate level of evidence provided and also because the duration of these RCTs was too short (≤12 weeks) and no data could be found for relevant outcomes such as cardiovascular events.

From these meta-analysis and systematic reviews, it can be concluded that the protective effect of VOO phenolics is related to blood pressure and LDL oxidation only. Nonetheless, although the oxidative hypothesis of atherosclerosis has been accepted for decades, the true contribution of LDL oxidation to cardiovascular disease is still unclear (Arsenault *et al*., 2017; Visioli *et al*., 2020). While the hypothesis is supported by hundreds of *in vitro* and animal studies, it does not explain why human trials with some antioxidants, such as vitamin E, did not provide sufficient convincing evidence for cardiovascular prevention (Sesso *et al*., 2008; Guallar *et al*., 2013). Possibly, the story is not as simple as it was believed. For instance, it has been proposed that LDL can become atherogenic even before oxidation. Modification of these lipoproteins begins with a desialylation and it is followed by a cascade of other physical and chemical alterations that increase LDL atherogenicity, including particle size reduction, increase in its density and negative electrical charge and loss of lipids (Summerhill *et al*., 2019). Therefore, the effects of VOO phenolics on cardiovascular disease via LDL modification are, as of today, dubious and there is need of more RCT that directly address the claim.

4. VOO PHENOLIC COMPOUNDS AND TYPE 2 DIABETES MELLITUS/METABOLIC SYNDROME

Accumulated data obtained from experimental models indicate that VOO phenolics have the potential to normalize metabolic syndrome and its pathophysiological complications, including diabetes. Diabetes and metabolic syndrome are linked to each other through insulin resistance, and subjects diagnosed with metabolic syndrome have a high risk of developing T2DM (Shin *et al.,* 2013). However, there are currently no RCT aimed at specifically assessing the effect of VOO phenolics on Type 2 diabetes (T2DM) patients, although two systematic reviews on the effects of these compounds on metabolic syndrome have been published (Chiva-Blanch and Badimon, 2017; Saibandith, 2017).

The review by Chiva-Blanch and Badimon (2017) was focused on human intervention trials administering phenolic-rich foods to patients with metabolic syndrome. They included a single RCT, carried out by Venturini *et al*., (2015), which administered extra VOO (10 mL/day). That trial reported an increase in the total radical-trapping antioxidant parameter (TRAP)/uric acid ratio, with no apparent effects on other markers of oxidative stress. On the other hand, the authors did not find changes in the lipid profile, plasma glucose, insulin resistance or blood pressure.

The systematic review by Saibandith *et al*., (2017) did assess the effects of VOO phenolics on components of the metabolic syndrome (i.e. glucose levels, blood pressure, central obesity, triglycerides and HDL-cholesterol). They summarized the current knowledge obtained from 18 clinical trials that were

not specifically aimed at this syndrome. Saibandith and collaborators confirmed the effects of VOO phenolics on systolic blood pressure observed in previous systematic reviews and meta-analyses, but they did not draw significant outcomes on obesity, triglycerides or HDL. Still, some reductions in plasma glucose and in biomarkers associated with glucose homeostasis were reported, but only in three of those trials. Surprisingly, the authors did not include the RCT by Venturini *et al*., (2015) that had been published a couple of years before, and therefore, they did not analyzed any RCTs involving patients with metabolic syndrome. The systematic review could only conclude that there was good evidence showing that, when consumed at appropriate doses, VOO phenolics may reduce blood pressure in hypertensive subjects and improve plasma glucose in pre-diabetic individuals.

It is noteworthy that the RCT involving patients with metabolic syndrome and published in 2016 by D'Amore *et al.,* was not included in both systematic reviews stated above. In that trial, 12 subjects with metabolic syndrome and 12 healthy controls received a single dose of 50 mL VOO from two olive varieties: *coratina*, providing 491 ppm of phenolic compounds, and *peranzana*, which had 270 ppm of phenolics. The most interesting result found in this study was the modification of the transcriptome of peripheral blood mononuclear cells, switching them to a less deleterious inflammatory phenotype. However, no relevant changes in the components of the metabolic syndrome were observed.

5. VOO PHENOLIC COMPOUNDS AND ALZHEIMER'S DISEASE

AD is pathologically characterized by substantial neuronal and synaptic losses, and decreased cognitive abilities, which are associated with cerebral deposits of amyloid-beta-enriched plaques and neurofibrillary aggregates of the Tau protein, as well as with chronic inflammation and oxidative stress (Rosales-Corral *et al*., 2015) elicited by the pathological activation of glial cells (Scimemi *et al*., 2013). Brain has revealed nowadays as an insulin-sensitive organ, where the hormone regulates important physiological processes, such as nutrient homeostasis, reproduction, cognition, and memory, and also exerts neurotrophic, neuromodulatory, and neuroprotective effects (Blazquez *et al*., 2014). Disturbances in insulin signaling in the brain may contribute to the development of several clinical entities, including T2DM and Alzheimer's disease. The close association between cerebral insulin resistance and Alzheimer's Disease brought some authors to propose the name "type 3 diabetes" for this illness (de la Monte and Wands, 2008).

For the moment, there are no RCTs available in which olive oil phenolics have been tested on Alzheimer's disease or other neurodegenerative disorders. Therefore, no systematic reviews or meta-analysis of RCT have been performed so far. All available information refers to observational studies and those carried out in animal models, which, as mentioned above, provide a low level of scientific evidence.

Regarding the potential neuroprotective effects of olive phenolics, it has been reported that dietary supplementation with oleuropein aglycone reduced the amount of amyloid-beta oligomers in the brain of Alzheimer's model mice (Luccarini *et al*., 2015), at the same time that they significantle improved their cognitive functions, presenting greater learning and memory capacities (Pantano *et al*., 2017). Oleuropein also enhanced the endogenous antioxidant response in the CA1 hippocampal area of rats suffering from colchicine-induced cognitive dysfunction, improving the redox status of glutathione, and increasing the activity of antioxidant enzymes, such as superoxide dismutase or catalase (Pourkhodadad *et al*., 2016). Similarly, hydroxytyrosol has been related to the amelioration of insulin resistance in the brain of mice (Kulas *et al*., 2020). In APP/PS1 mice, rodent models of AD, hydroxytyrosol improved cognitive function, increased the expression of antioxidant enzymes and phase 2 response genes, and reduced inflammatory factors in the brain of these animals (Peng *et al*., 2016).

Oleocanthal has also been associated with a reduction in amyloid-beta oligomers, as well as an attenuation of astrocytes activation and a reduction in systemic inflammation in neurons and astrocytes cells lines (Batarseh *et al*., 2017). Likewise, oleocanthal has demonstrated that it may modulate the astrocyte activation in TgSwDI mice, a model of Alzheimer's disease (Qosa *et al*., 2015).

6. VOO PHENOLIC COMPOUNDS AND CANCER

Only one systematic review about the effects of VOO phenolic compounds on cancer has

been published, which implies that there are no meta-analyses available and, in consequence, the highest level of scientific evidence cannot be reached. In their review, Fabiani *et al*., (2016) included 16 animal studies and 5 RCTs. Most of the animal studies confirmed the ability of secoiridoid compounds to inhibit carcinogenesis at both initiation and promotion/progression phases. However, all human intervention trials included in this review only investigated the effects of VOO phenolics on DNA damage and did not evaluate their effect on the incidence or development of any kind of cancer. These trials only reported determinations related to the oxidation of nucleic acids, in particular the concentrations of 8-oxo-7,8-dihydro-2'-deoxyguanosine, which is a marker of DNA oxidation. Between 10 and 182 subjects participated in these RCT, and the phenolic doses from VOO ranged from 10 to 592 mg/kg. Three of the five human trials showed that VOO phenolics reduced the 8-oxo-7,8-dihydro-2'-deoxyguanosine levels in urine, mitochondrial DNA of mononuclear cells and lymphocyte DNA. The other two trials failed to find a protective effect on DNA oxidation. Four of these studies were conducted by the same research group, and had similar experimental designs (Weinbrenner *et al.*, 2004; Hillestrøm *et al*., 2006; Machowetz *et al*., 2007; Romeu *et al*., 2016). Therefore, there is very little evidence relating the intake of VOO phenolic compounds with cancer. In fact, the authors suggested that further investigations are necessary to clarify the real chemopreventive potential of these compounds and that intervention studies on populations at high cancer risk are needed.

7. VOO PHENOLIC COMPOUNDS AND ARTHRITIS

There is currently only one RCT evaluating the effect of VOO phenolic compounds on arthritis (Takeda *et al.*, 2013). This trial was aimed at determining whether hydroxytyrosol intake could reduce knee pain in individuals with gonarthrosis (n=25). An extract of *Olea europaea* containing approximately 22% of hydroxytyrosol, at a dose of 11 mg/day for 4 weeks, was administrated to the participants. The conclusion of the trial was that the subjects informed a reduction in knee pain, as compared to the administration of a placebo.

8. CONCLUSIONS

The benefits of VOO phenolic compounds on health have been extensively investigated, and recent studies support the belief that these components may play a key role in the amelioration of pathophysiological conditions. In particular, studies on *in vitro* systems and animal models have shown that oleuropein derivatives, hydroxytyrosol and oleocanthal exert potent pharmacological activities on markers of cancer, atherosclerosis and metabolic diseases. More specifically, on those associated with inflammatory processes and oxidative stress. However, meta-analyses of RCT, from which the highest level of scientific evidence should be obtained, do not support those effects on humans, except for some markers of cardiovascular risk. In fact, the strongest piece of evidence available has been found for the ability of VOO phenolic compounds to protect lipoproteins from oxidation and to reduce systolic blood pressure in hypertensive individuals (Table 1).

Unfortunately, there is not enough high-level evidence at the moment to confirm that the intake of phenolic compounds isolated or as components of the VOO can be healthy (Table 2). No RCT have been carried out to assess the direct effect of VOO phenolics on diabetes or neurodegenerative diseases and very scarce information can be collected from the ones that have focused on osteoarthritis and cancer. Therefore, it is still necessary to develop double-bind RCT in extensive and diverse population groups, with different disorders and with doses of phenols adjusted to usual VOO consumptions, in order to provide a greater degree of scientific evidence before nutritional recommendations may be given to the general population.

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Table 1. Availability of studies and degree of evidence for the effect of virgin olive oil phenolics intake on different disorders according to evidence-based medicine.

Disorder	Meta-Analyses	Systematic reviews	RCT	In vivo (animals)	In vitro (cells)	Degree of evidence
Cardiovascular disease	Yes (1)	Yes (2)	Yes (13)	Yes	Yes	Medium (High por BP and ox LDL)
Cancer	No	Yes (1)	Yes (5)	Yes	Yes	Low
Alzheimer's disease	No	No	No	Yes	Yes	Very low
Arthritis	No	No	Yes (1)	Yes	Yes	Low
Diabetes mellitus	No	No	No	Yes	Yes	Low
Metabolic syndrome	No	Yes (2)	Yes (2)	Yes	Yes	Low (High for BP)

[a]Numbers in parentheses refer to the published number of studies of that kind. RCT, randomized controlled trial; BP, blood pressure; LDL, low-density lipoprotein.

Table 2. Summary of the studies cited in the text.

Disorder	Study design	n	Intervention	Dose (day)/time	Time	Main markers	References
CVD	RCT	22	2 olive oils (VOO vs ROO)	40 mL	7 weeks	TXB2, isoprostanes	Visioli *et al*., 2005
	RCT	200	3 olive oils (different PC)	25 mL	3 weeks	TG, HDL, oxLDL	Covas *et al*., 2006
	RCT	12	EVOO, OO and CO	50 mL	Postprandial	TXB2, LTB4	Bogani *et al*., 2007
	RCT	24	2 olive oils (different PC)	30 mg	8 weeks	BP, CRP, ox-LDL	Moreno-Luna *et al*., 2012
	Systematic review	6 RCT	Olive oils (different PC)	Various	Single-8 weeks	Lipids, oxidation, inflammation	Bahramsoltani *et al*., 2019
	Systematic review	13 RCT	Olive oils (different PC)	Various	3-12 weeks	TC, LDL, HDL, TG, ox-LDL, BP	Schwingshackl *et al*., 2019
	Meta-analysis	8 RCT	Olive oils (different PC)	Various	3-12 weeks	TC, LDL, HDL, TG, ox-LDL, BP	Hohmann *et al*., 2015
Cancer	RCT	12	3 olive oils (different PC)	25 mL	4 days	8-OHdG	Weinbrenner *et al*., 2004
	RCT	28	3 olive oils (different PC)	25 mL	3 weeks	Etheno–DNA adducts	Hillestrøm *et al*., 2006
	RCT	58	3 olive oils (different PC)	25 mL	3 weeks	8-Oxo-guanine	Machowetz *et al*., 2007
	RCT	33	2 olive oils (different PC)	25 mL	3 weeks	8-OHdG	Romeu *et al*., 2008
	Systematic review	5 RCT	2-3 olive oils (different PC)	25 mL. 50 g	4 days – 8 weeks	8-OHdG	Fabiani *et al*., 2016
Alzheimer's	Astrocyte cell line	N/A	Oleocanthal	5 µM	3-7 days	GLT1, GLUT1, IL-6	Batarseh *et al*., 2017
	Murine model (Tg-CRND8)	6/group	Oleuropein aglycone	50 mg/kg of diet	8 weeks	Aß42, pE3-Aß aggregation	Luccarini *et al*., 2015
	Murine model (TgSwDI)	6/group	Oleocanthal	6 mg/kg injection	4 weeks	Aß, IL-1ß	Qosa *et al*., 2015

Disorder	Study design	n	Intervention	Dose (day)/time	Time	Main markers	References
	Rat model	7/group	Oleuropein	10-20 mg/kg diet	10 days	SOD, Catalase, NO, MDA	Pourkhodadad *et al*., 2016
	Murine model (APP/PS1)	9/group	Hydroxytyrosol	5 mg/kg gavage	6 months	Cognitive, SOD, inflammation	Peng *et al*., 2016
	Murine model (Tg-CRND8)	6/group	Oleuropein aglycone	12.5 mg/kg of diet	8 weeks	Aß42 , pE3-Aß aggregation	Pantano *et al*., 2017
Arthritis	RCT	25	Hydroxytyrosol	11 mg/day	4 weeks	Knee pain	Takeda *et al*., 2013
MetS	RCT	102	Usual diet, EVOO, fish oil	10 mL/day	12 weeks	TRAP, uric acid	Venturini *et al*., 2015
	RCT	24	EVOO two varieties	50 mL	Postprandial	PBMC transcriptome	D'Amore *et al*., 2016
	Systematic review	1 RCT[b]	Usual diet, EVOO, fish oil	10 mL/day	12 weeks	TRAP, uric acid	Chiva-Blanch and Badimon, 2017
	Systematic review	18 RCT[c]	EVOO, olive leaf extract	Various	1 week -1 year	BP, HbA1C, Glucose insulin, WC	Saibandith, 2017

[a]8-OHdG, 8-hydroxy-deoxyguanosine; BP, blood pressure; CO, corn oil; CRP, C-reactive protein; EVOO, extra-virgin olive oil; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LTB4, leukotriene B4; OO, olive oil; ox-LDL, oxidized LDL; PC, phenolic content; RCT, randomized controlled trial; ROO, refined olive oil; TG, triglycerides; TXB2, thromboxane B2;

[a]Aß, amiloid beta; EVOO, extra-virgin olive oil; GLT1, glutamine transporter 1; GLUT1, glucose transporter 1; HbA1C, glycosylated hemoglobin; IL-1ß, interleukin 1 beta; IL-6, interleukin-6; MDA, malodialdehyde; MetS, metabolic syndrome; N/A, not applicable; NO, nitric oxide; PBMC, Peripheral blood mononuclear cellRCT, randomized controlled trial; SOD, superoxide dismutase; TRAP, peroxyl radical-trapping antioxidant potential; WC, waist circumference. [b]Review focused on phenolics from different sources. 1 RCT from VOO. [C] Review focused on MetS components, no RCT with MetS diagnosed subject

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