Critical Review

Modulation of Hepatic Lipid Metabolism by Olive Oil and its Phenols in Nonalcoholic Fatty Liver Disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease in western countries, being considered the hepatic manifestation of metabolic syndrome. Cumulative lines of evidence suggest that olive oil, used as primary source of fat by Mediterranean populations, may play a key role in the observed health benefits on NAFLD. In this review, we summarize the state of the art of the knowledge on the protective role of both major and minor components of olive oil on lipid metabolism during NAFLD. In particular, the biochemical mechanisms responsible for the increase or decrease in hepatic lipid content are critically analyzed, taking into account that several studies have often provided different and/or conflicting results in animal models fed on olive oil-enriched diet. In addition, new findings that highlight the hypolipidemic and the antisteatotic actions of olive oil phenols are presented. As mitochondrial dysfunction plays a key role in the pathogenesis of NAFLD, the targeting of these organelles with olive oil phenols as a powerful therapeutic approach is also discussed. © 2015 IUBMB Life, 67(1):9–17, 2015

Keywords: *lipogenesis; lipid metabolism; mitochondria; non-alcoholic fatty liver disease; oleic acid; olive oil; phenols*

Introduction

Nonalcoholic Fatty Liver Disease Definition and Risk Factors

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in western countries with an even higher incidence with respect to liver damage caused by hepatitis C virus or by alcohol abuse. It was first described in 1980 when Ludwig et al. (1) observed serious liver inflammation and steatosis in patients lacking a significant history of alcohol drinking. Nowadays, the definition of this disease includes mainly one characteristic: lipid content of >5–10% of the weight of the liver, in the absence of excessive consumption of alcohol or other liver diseases (2).

From the histological viewpoint, NAFLD encompasses a spectrum of conditions characterized by simple steatosis (nonalcoholic fatty liver, NAFL) to a more severe and treatment-resistant clinical status characterized by the appearance of inflammation, termed nonalcoholic steatohepatitis (NASH), which may in turn progresses to cirrhosis, hepatocarcinoma, and liver failure (2).

Two steps or "hits" have been proposed for the pathophysiology of NAFLD (3). The first hit is characterized by an intrahepatic triglyceride accumulation (owing to their increased synthesis, their decreased export, or both) and it is followed by a second step (or hit), which may lead to inflammation processes that likely involve: oxidative stress, cytochrome P450 activation, lipid peroxidation, mitochondrial dysfunction, cytokine production, and apoptosis (2,3).

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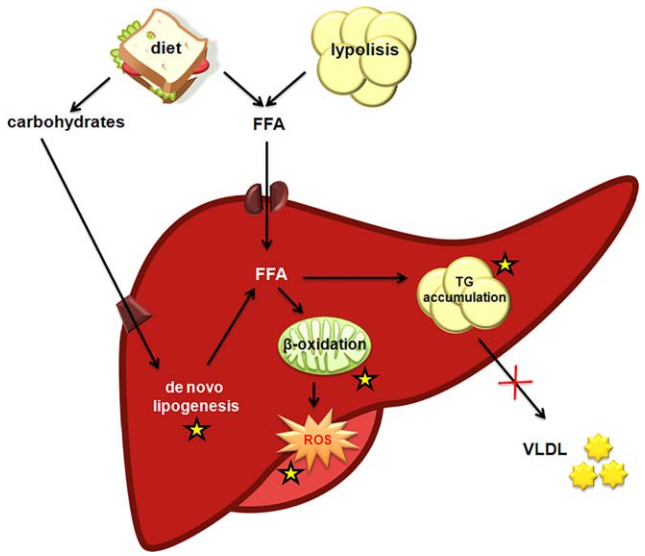

FIG 1 *Schematic representation of the metabolic changes occurring during the development of NAFLD.*

This chronic liver disease is quite diffused in the normal population, but there are many diagnoses of this disease in patients with hypertriglyceridemia, type-2 diabetes, and in obese people (4). On the basis of currently available data, it can be argued that even if NAFLD is associated with insulin resistance, it is unclear whether insulin resistance is a primitive feature of patients with NAFLD (5).

The Role of Lipid Metabolism in the Pathogenesis of NAFLD: An Overview

The increased hepatic accumulation of lipids observed in NAFLD patients can be stemmed from multiple factors. Increased lipolysis from the fat cells or increased intake of dietary fat, followed by enhancement of free fatty acids (FFA), can explain this phenomenon (6). In addition, *de novo* lipogenesis in the liver contributes greatly to hepatic steatosis (7). Finally, reduction in lipid disposal through an impairment of fatty acid β-oxidation (8) and triglyceride-rich lipoprotein secretion (very low density lipoprotein, VLDL) can also exacerbate this condition (9).

Figure 1 shows the main pathways involved in hepatic lipid metabolism; a perturbed balance between lipid synthesis (*de novo* lipogenesis and triglyceride synthesis) and lipid clearance (β-oxidation and VLDL secretion) leads to hepatic steatosis.

De novo lipogenesis implies a complex series of reactions starting in the mitochondrial matrix and continuing in the cytosol of hepatocytes. The main fuel for fatty acid synthesis is acetyl-CoA derived from sugar sources. As acetyl-CoA is formed in the mitochondrion, and *de novo* fatty acid synthesis occurs in the cytosol, the acetyl group must be exported, in the form of citrate, from the mitochondrial matrix into the cytosol of the cell before its conversion into fatty acids or cholesterol. This transport is catalyzed by the mitochondrial citrate carrier (CiC) (10).

Once acetyl-CoA reaches the cytosolic compartment, fatty acid synthesis begins with its conversion to malonyl-CoA in the reaction catalyzed by acetyl-CoA carboxylase (ACC). Next, the sequential extension is catalyzed by fatty acid synthase (FAS) which eventually leads to palmitic acid (16:0), which is the main product of *de novo* fatty acid synthesis.

Another biochemical pathway that plays a pivotal role in the onset and progression of hepatic fat accumulation is represented by triglyceride synthesis whose key enzyme is diglyceride acyltransferase (DGAT). This enzyme catalyzes the formation of triglycerides from diglycerides and the acyl-CoAs, which derive from the nonesterified fatty acids pool from adipose tissue, dietary fatty acids, and newly synthesized ones through *de novo* lipogenesis in the liver.

In normal conditions, *de novo* lipogenesis converts the excess of dietary carbohydrates into fatty acids that are then esterified for storage as triglycerides, which could later provide energy via mitochondrial fatty acid β-oxidation. On the other hand, during the onset of NAFLD, conditions such as hyperglycemia and hyperinsulinemia seem to be associated with the high rate of lipogenesis (7), resulting in a shift of cellular metabolism from fatty acid β-oxidation to triglyceride esterification, and hence increasing hepatic fat accumulation (2).

The liver does not enlarge indefinitely; the hepatocytes reach a new energetic steady state, whereby the increment of hepatic FFA uptake and synthesis is compensated by an increased hepatic removal of fatty acids (2). Carnitine palmitoyltransferase-I (CPT-I), the mitochondrial gateway for fatty acid entry into the matrix, is the main controller of the hepatic mitochondrial β-oxidation flux. The impairment in mitochondrial fatty acid oxidation owing to the downregulation of hepatic CPT-I is a crucial event in the pathogenesis of hepatic steatosis (8).

Besides the fatty acid accumulation, another important event occurs in NAFLD causing liver inflammation: the oxidative stress (11).

NAFLD: Mitochondria and Oxidative Stress

Oxidative stress is defined as an imbalance between the oxidant and the antioxidant systems of the organism, in favor of the oxidants. The oxidant systems are represented by free radicals, molecules containing unpaired electrons, mainly constituted by the so-called reactive oxygen species (ROS) (12). Targets for the free radicals are lipids, DNA, and proteins (12). If a fatty acid is damaged by the free radicals, then it becomes a free radical itself by setting up a chain reaction of lipid peroxidation (12), which destroys the cell membranes, inducing apoptosis and necrosis processes (13). Mitochondria are the major source of ROS; in particular, an increased food supply may cause a boost in mitochondrial ROS production, triggering a possible oxidative stress in the liver. During the development of NAFLD, the excessive liver intake of FFA increases the mitochondrial and peroxisomal fatty acid β-oxidation and promotes the induction of microsomal cytochromes CYP4A1 and CYP2E1. This results in elevated synthesis of ROS, and in turn

in lipid peroxidation (14). In addition, NAFLD generates serious problems at the level of the mitochondrial respiratory chain (MRC) and oxidative phosphorylation (OXPHOS), suggesting that NAFLD could be considered as a mitochondrial disease (15). In fact, MRC (mainly complexes I and III) is an important subcellular source of ROS, and as a result of its impairment a larger amount of ROS is produced, fatty acid β-oxidation and OXPHOS are severely inhibited, lipids are accumulated in the cytosol, confirming a relationship between steatosis and mitocondriopathies (16,17). It has also been shown that besides mitochondrial bioenergetic impairment, there are structural abnormalities of the organelle during NAFLD development. In particular, the inner mitochondrial membrane (and crests) as well as the intermembrane space increases in size to compensate for the reduction of mitochondrial activity (18). The mechanisms of this mitochondrial hypertrophy are unclear but it is believed that it is the first stage of a mitochondria "anomaly" (15).

Olive Oil Chemical Characteristics

A lower prevalence of chronic diseases such as certain types of cancers, or cardiovascular and hepatic disorders, has been demonstrated in countries of the Mediterranean region compared to other parts of the world (19,20). This reduced incidence has been partially attributed to the regular intake (25–50 mL/day) of olive oil, component of the Mediterranean diet. Nowadays, thanks to the flurry of studies on its healthy properties, olive oil is considered a functional food, which besides having a high level of monounsaturated fatty acids (MUFAs), contains multiple minor components with biological activities.

The averages of the main fatty acids in 100 g of olive oil are as follows: MUFA, ~70 g ($n-9$c oleic acid 18:1); saturated fatty acids, ~14 g (16:0 palmitic acid); and polyunsaturated fatty acids, ~8 g ($n-6$ linoleic acid 18:2 and $n-3$ alpha-linoleic acid 18:3) (21). MUFA include palmitoleic (C16:1), oleic ($n-9$c C18:1), elaidic ($n-9$t C18:1), and vaccenic acids ($n-11$c C18:1). In addition to a high MUFA content, olive oil contains a significant amount of antioxidants and phytochemicals; however, when refined or heated, olive oil loses these natural compounds (22). The phenolic fraction ranges from 50 to 800 mg/kg; this variability depends on several key factors, such as the cultivar, the climate, ripeness of the drupes at harvesting, and the processing system employed to produce the different types of olive oil: extra-virgin, virgin, olive oil, or pomace (23). Virgin olive oils (VOOs) are those obtained from the fruit of the olive tree solely by mechanical or other physical means under the conditions that do not lead to alteration of the oil. It must be underlined that as extra-VOOs (EVOOs) are obtained from once cold-pressed unfermented olives, they are characterized by a FFA content lower than 1% and the highest phenol levels (21). The minor components of olive oil are classified into two types: the unsaponifiable fraction, defined as the fraction extracted with solvents after the saponification of the oil, and the soluble fraction which includes the phenolic compounds (22).

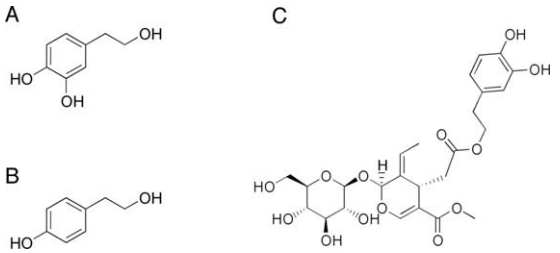

FIG 2 *Chemical structures of EVOO phenols. Chemical structures of hydroxtyrosol (A), tyrosol (B), and oleuropein (C).*

The simple phenolic compounds include tyrosol, hydroxytyrosol (3,4-dihydroxyphenylethanol), and phenolic acids such as vanillic and caffeic acids. The complex phenolic compounds are mainly tyrosol and hydroxytyrosol esters, oleuropein, and its aglycone. Oleuropein is the phenol that contributes primarily to the bitter taste of olives. In addition to the phenolic compounds described, newer information has revealed the presence of the lignan class of phenolics such as (+)−1-acetoxypinoresinol, (+)-pinoresinol, and (+)−1-hydroxypinoriesinol. For EVOO, the levels of these lignans can be as high as 100 mg/kg in the oils, but some variations do exist (24).

The highest concentrations of olive oil phenolic compounds, widely noted for their antioxidant activities, are those of oleuropein, hydroxytyrosol, and tyrosol (22,25). These three compounds are related structurally: hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol possesses an extra hydroxyl group in the meta-position. Oleuropein is an ester that consists of hydroxytyrosol and elenolic acid (Fig. 2).

Considering a dietary intake of olive oil within 25–50 mL/day, the ingested phenols have been estimated to be around 9 mg; at least 1 mg of them is derived or is constituted from free hydroxytyrosol and tyrosol, and 8 mg is related to their elenolic esters and also to the oleuropein- and ligstroside-aglycons (26). Bioavailability studies have demonstrated that hydroxytyrosol and tyrosol are dose dependently absorbed in animals and humans after olive oil ingestion, biotransformed, and accumulated in the body where they systemically exert biological effects (26).

Olive Oil Modulation of Hepatic Lipid Metabolism

Olive oil, often nicknamed "the liquid gold," exerts its healthy effects through two main components: MUFA and phenolic compounds. Regardless of which component is considered, the role of this fat on the modulation of hepatic lipid metabolism remains debated (Table 1). In olive oil-treated animals, a plethora of different effects on liver have been described: increase in the activities of ACC and FAS (27,28), decrease in the activity and the expression levels of CPT-I (28–30), or even no apparent effects on lipogenic (29,31) and oxidative enzymes (32). In some studies carried out in rodents, an olive oil-enriched diet induced fat accumulation in the liver (27–29,32–34). Conversely, other reports demonstrated that olive oil prevented the accumulation of liver triglycerides,

TABLE 1 ***Studies investigating the olive oil effects on hepatic lipid metabolism***

Study	*Experimental design*	*Oleic acid (%)*	*Phenols (mg/kg)*	*Effects*
Ruiz-Gutiérrez et al. (33)	Wistar rats fed for 12 weeks with a diet containing 10% of olive oil	79.20	n.d.	↑ Hepatic TG (with respect to fish oil)
Perona and Ruiz-Gutiérrez (34)	Spontaneously hypertensive rats fed for 12 weeks with a diet containing 10% of energy as virgin olive oil	79.22	n.d.	↑ Hepatic TG (with respect to a fat-free diet)
Portillo et al. (27)	Wistar rats fed for 4 weeks with a diet containing 40% of energy as olive oil	77.26	n.d.	↑ Hepatic TG (with respect to beef tallow) ↑ ACC and FAS activities (with respect to beef tallow)
Takeuchi et al. (28)	Wistar rats fed for 6 weeks with a diet containing 20% of olive oil	74.80	n.d.	↑ Hepatic TG (with respect to sunflower and fish oils) ↑ ACC activity (with respect to sunflower and fish oils) ↓ CPT-I activity (with respect to fish oil) = DGAT activity (with respect to sunflower and fish oils)
Deng et al. (38)	JCR:LA-cp (obese) rats fed for 2 weeks with a diet containing 40% of energy as olive oil	68	n.d.	↓ Hepatic TG ↓ ACC, FAS, and SREBP-I gene expression
Siculella et al. (31)	Wistar rats fed for 3 weeks with a diet containing 15% of olive oil	72.80	n.d.	= CiC gene expression (with respect to beef tallow)
Hernández et al. (39)	Wistar rats fed for 1 month with a diet containing 14% or 5% of olive oil	n.d.	n.d.	↓ Hepatic steatohepatitis in rats fed on 5% of olive oil as supplemented diet
Acín et al. (35)	Apoe$^{-/-}$ mice fed for 11 weeks with diet containing 10% of unsaponifiable fraction -enriched- EVOO	77.80/ 74.27	n.d.	↓ Hepatic steatohepatitis
Arbones-Mainar et al. (32)	Apoe$^{-/-}$ mice fed for 10 weeks with a diet containing 20% of EVOO	n.d.	90	↑ Hepatic TG (with respect to palm oil) = β-oxidation enzymes protein expression (with respect to palm oil)
Hussein et al. (37)	Sprague Dawley rats (weight, 0.45 mg/g) fed for 2 months with a NASH-inducing diet containing olive oil (0.45 mg/g rat weight)	71.26	n.d.	↓ Hepatic TG (with respect to NASH diet and NASH diet containing fish oil or butter) ↓ Hepatic steatohepatitis
Ferramosca et al. (29)	ICR mice fed for 8 weeks with a diet containing 7.5% of olive oil	27.40	n.d.	↑ Hepatic TG (with respect to week 0) = ACC, FAS, and CiC activities (with respect to week 0) ↓ CPT-I activity (with respect to week 0)
Priore et al. (30)	Wistar rats fed for 3 weeks with a diet containing 15% of olive oil	72.30	n.d.	↓ CPT-I gene expression (with respect to beef tallow)

Abbreviations: ACC = acetyl-CoA carboxylase; CiC = citrate carrier; CPT-I = carnitine palmitoyl transferase-I; DGAT = diglyceride acyltransferase; EVOO = extra-virgin olive oil; FAS = fatty acid synthase; n.d. = not determined; NASH = nonalcoholic steatohepatitis; TG = triglycerides.

lowering circulating lipid levels (35,36). These apparently conflicting data could depend on several factors: the animal model, the amount of dietary EVOO administered, its quality and composition, and the control group used.

An interesting study (37), carried out in rats with NAFLD, demonstrated that olive oil consumption decreases the accumulation of liver triglycerides. In particular, it has been suggested that olive oil may improve insulin resistance, increase the secretion of hepatic triglycerides as VLDL, and decrease the lipolytic flux from peripheral adipose tissue back to the liver (37). A similar recovery of the liver from fatty acid accumulation was previously observed in different models of steatotic rats fed on a balanced diet rich in olive oil (38,39). This effect is lost, however, when the diet contains a high percentage of olive oil or when dietary olive oil is supplied together with a great amount of cholesterol (40). Overall, the results indicate that in animal models olive oil provides protection against hepatic steatosis up to a certain fat intake. This would be especially true in low-cholesterol diets such as the traditional Mediterranean diet (35).

Further studies, carried out in humans, suggested that olive oil should be included in the diet of the patients with NAFLD as it could decrease insulin resistance and blood triglycerides by increasing the expression of hepatic fatty acid β-oxidation enzymes. This occurs through the activation of their corresponding genes by the transcription factor peroxisome proliferator-activated receptor-α (PPARα) (21,41). Alternatively, olive oil could downregulate lipogenic genes, through the inhibition of their master-regulator, the transcription factor sterol regulatory element-binding protein I (SREBP-I) (21,41).

A clear definition of the molecular events leading to the lipid clearance of a steatotic liver, consequent to olive oil administration, could be quite difficult owing to three important considerations: (i) liver is a multitasking organ that is involved in many different processes of lipid metabolism (such as fatty acid uptake, storage, conversion, oxidation, synthesis, and secretion); (ii) the pathophysiology of NAFLD remains to be fully elucidated; and (iii) olive oil is a complex matrix, a fat source of MUFA that retains all the lipophilic components of the fruit and sizeable amounts of phenolic compounds with several biological properties.

The beneficial effects of olive oil on NAFLD have been historically attributed to its high MUFA content as oleic acid is capable of reducing lipogenesis not only in liver (42), but also in other high-lipid-metabolizing tissues (43–45). However, EVOO is more than just a MUFA. Besides the large quantities of oleic acid, EVOO is rich in phenolic compounds. The previous research has suggested that the minor constituents of olive oil might have more effects on health than once believed (22). The biological activities of olive oil phenolic compounds have prompted several studies on their potential activity in the prevention and the treatment of NAFLD.

In an international study with 200 healthy male volunteers, Covas et al. (46) showed that EVOO phenols are able to increase plasma high density lipoprotein (HDL)-cholesterol levels as well as to reduce triglycerides level. *In vivo* experiments have demonstrated that oral administration of hydroxytyrosol (47) or oleuropein (48) to hypercholesterolemic rats significantly lowered the serum levels of total cholesterol, triglycerides, and low-density lipoprotein-cholesterol, and increased the serum level of high-density lipoprotein-cholesterol. Similarly, administration for 4 weeks of oleuropein- and hydroxytyrosol-rich extracts from olive leaves significantly reduced serum glucose and cholesterol levels in alloxan-induced diabetic rats (49). In addition, in mice fed on high-fat diets, oleuropein supplementation can attenuate liver steatosis by the Wnt10b- and fibroblast growth factor receptor 1-mediated signaling cascades involved in hepatic lipogenesis (50). Other studies confirmed the hepatoprotective effect of oleuropein, highlighting its preventive effect in the progression of NASH to hepatic fibrosis in mice fed a high-fat diet (51,52). These findings were further investigated *in vitro*, showing that the addition of oleuropein to steatotic hepatocytes decreased the number and size of lipid droplets in FFA-treated cells, and reduced intracellular triglyceride accumulation, through the inhibition of extracellular signal-regulated kinase (53). Very recently, a downregulation of fatty acid, cholesterol, and triglycerides syntheses, has also been observed in normal rat hepatocytes treated with isolated phenols (*i.e.*, hydroxytyrosol, tyrosol, and oleuropein) (54) or with an EVOO high phenol extract (HPE) (55). ACC and DGAT enzymatic activities, as well as AMP-activated protein kinase (AMPK), were involved in tuning down lipid synthesis (54,55). It is noteworthy that the effect on hepatic lipid synthesis induced by each tested phenol (54) is less powerful compared to that exerted by their mix as HPE, suggesting a synergistic action of phenols characterizing the EVOO extract (55). A similar short-term regulation of lipogenic enzymes was observed after the treatment of primary rat hepatocytes with resveratrol (a phenol mainly found in grapes and red wines) (56).

Olive Oil and NAFLD: A Focus on Mitochondria

Mitochondrial alterations as ultrastructural abnormalities, impaired biochemical pathways (β-oxidation, MRC, OXPHOS), increased oxidative stress, uncoupling, and apoptosis, are closely related to the pathogenesis of NAFLD (17). This cause–effect relationship has lead to the conviction that NAFLD could be considered a "mitochondrial disease" (15), and an increasing number of therapeutic approaches targeted to these organelles are going to be developed (57).

The efficacy of mitochondria-targeted therapy on NAFLD is provided by the administration of compounds that improve bioenergetics and reduce oxidative stress (58). For example, dietary curcumin, a phenolic compound of *Curcuma longa* largely used in the Indian cuisine, was effective in experimental NAFLD, by increasing mitochondrial antioxidants, lowering mitochondrial ROS and improving mitochondrial function (59).

But what about the potential effects of EVOO phenolic compounds on liver mitochondria during NAFLD? Currently, as far as we know, the literature on this topic is surprisingly

TABLE 2 *Olive oil, its phenols, and mitochondria*

Study	*Experimental design*	*Tissue/cell culture*	*Effects*
Takeuchi et al. (28)	Wistar rats fed for 6 weeks with a diet containing 20% of olive oil	Liver	↓ CPT-I activity (with respect to fish oil)
Arbones-Mainar et al. (32)	Apoe$^{-/-}$ mice fed for 10 weeks with a diet containing 20% of EVOO	Liver	= β-Oxidation enzymes protein expression (with respect to palm oil)
Ferramosca et al. (29)	ICR mice fed for 8 weeks with a diet containing 7.5% of olive oil	Liver	↓ CPT-I activity (with respect to week 0)
Hao et al. (66)	3T3-L1 -treated for 48 h with hydroxytyrosol at concentrations of 0.1, 1, 10, and 50 μmol/L	Human adipocytes	↑ Mitochondrial biogenesis ↑ Activities of Complexes I, II, III, and V activities and protein expression ↑ Mitochondrial oxygen consumption ↑ AMPK, CPT-I, and PPAR gene expression
Zhu et al. (69)	ARPE-19 treated with hydroxytyrosol (100 μM, 48 h) without and with exposure to acrolein (75 μM, 24 h)	Human retinal pigment epithelial cells	↑ Mitochondrial biogenesis ↑ Protein expression of Complexes I, II, III, and V protein expression
Priore et al. (30)	Wistar rats fed for 3 weeks with a diet containing 15% of olive oil	Liver	↓ CPT-I gene expression (with respect to beef tallow) ↓ CACT activity, gene, and protein expression (with respect to beef tallow)
Bullon et al. (68)	Wistar rats fed for 24 weeks with a diet containing 4% of virgin olive oil	Gingival and alveolar bone tissues	↑ Mitochondrial biogenesis ↑ MRC protein expression
Granados-Principal et al. (65)	Mammary tumor-bearing Sprague–Dawley rats injected for 6 weeks with hydroxytyrosol (0.5 mg/kg, 5 days/week) and/or doxorubicin (1 mg/kg/week)	Hearth	= Complexes I, III (Rieske subunit), and IV protein expression (with respect to doxorubicin) ↑ Complex II and complex III (CORE II subunit) protein expression (with respect to doxorubicin) = Complex I activity, ↑ complex IV activity (with respect to doxorubicin)
Signorile et al. (67)	NHDF-neo serum starved cells treated with 1 μM of hydroxytyrosol for different incubation time points of incubation	Neonatal normal human dermal fibroblasts	↑ Activities of Complexes I, III, and V activities and protein expression

Abbreviations: AMPK = AMP-activated protein kinase; CACT = carnitine-acylcarnitine translocase; CPT-I = carnitine palmitoyl transferase-I; MRC = mitochondrial respiratory chain; PPAR = peroxisome proliferator-activated receptor.

lacking although several studies have been published on other tissues or cellular models, or by using diets supplemented with EVOO (Table 2).

The effect of EVOO supplementation has also an impact on the mitochondrial pathways related to the hepatic accumulation of fat. The carnitine-acylcarnitine translocase (CACT) is a mitochondrial carrier that catalyzes the transport of fatty acids into the mitochondrial matrix where their β-oxidation occurs (30). A diet enriched with EVOO induced a decrease in the expression and activity of CACT (30). A mitochondrial carrier that plays an important role in the regulation of hepatic lipogenesis is CiC. Several studies have demonstrated that CiC activity and expression are regulated by nutritional factors (60–64). It is interesting to note that dietary EVOO has no effect on CiC activity and expression either in rats (31) and mice (29).

The findings of the past decade have shown that EVOO phenols, especially hydroxytyrosol, are able to counteract the ROS level and action by two main mechanisms: (i) stabilize radical molecules directly across the removal of an electron and (ii) activate the intracellular mechanisms that promote the increased levels of antioxidants already physiologically present in cells (65).

The hydroxytyrosol is an antioxidant molecule that enhances mitochondrial function, increases the expression and function of mitochondrial complexes I, II, III, and V, stimulates oxygen consumption, and decreases the content of FFAs in adipocytes 3T3-L1 (66). In quiescent-cultured human fibroblasts, characterized by a severely depressed functionality of complexes I, III, and V of the MRC, this important phenol is capable of restoring a proper activity of OXPHOS, acting at a transcriptional level (67).

In addition, hydroxytyrosol is capable of promoting mitochondrial function by stimulating the biogenesis of these organelles in 3T3-L1-cultured adipocytes (66). A similar effect was observed in the periodontal tissue of rats fed a VOO-rich diet (68), and in cultured retinal pigment epithelial cells (69).

It is noteworthy that many studies have focused on a single phenolic compound, the hydroxytyrosol, but it is equally important to learn more about the activity of all the other EVOO antioxidants or the crude extract.

Conclusions

NAFLD encompasses a range of metabolic disorders of the liver, and it depends on new lifestyle habits combining rich diet and lack of exercise. The progression of hepatic steatosis can stem from the increase of FFA uptake, *de novo* lipogenesis and triglyceride synthesis, and the decrease of triglyceride hydrolysis and fatty acid β-oxidation. Reduced triglyceride secretion via VLDL could also promote hepatic lipid accumulation. Recent findings, obtained *in vitro* and *in vivo*, have demonstrated that EVOO and its phenols have a regulative effect on hepatic lipid metabolism by reducing the lipogenic pathway, and thus attenuating liver steatosis.

As mitochondria are the key controllers of fatty acid removal that counteracts the excessive fat storage in hepatocytes, there are consistent lines of evidence that mitochondrial dysfunction plays a central role in the pathogenesis of NAFLD. Targeting these organelles with molecules useful to reverse the progression of NAFLD could be a powerful therapeutic approach. One possible candidate could be dietary EVOO for its high content in MUFA and phenolic compounds.

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