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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (12): Nonalcoholic fatty liver disease

Effects of resveratrol and other polyphenols in hepatic steatosis

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Abstract

Non-alcoholic fatty liver disease covers a wide spectrum of liver pathologies which range from simple steatosis to non-alcoholic steatohepatitis. Polyphenols are members of a very large family of plant-derived compounds that can have beneficial effects on human health, and thus their study has become an increasingly important area of human nutrition research. The aim of the present review is to compile published data concerning the effects of both isolated polyphenols as well as polyphenol extracts, on hepatocyte and liver fat accumulation under different steatosis-inducing conditions. The results reported clearly show that this group of biomolecules is able to reduce fat accumulation, but further studies are needed to establish the optimal dose and treatment period length. With regard to the potential mechanisms of action, there is a good consensus. The anti-lipidogenic effect of polyphenols is mainly due to reduced fatty acid and triacylglycerol synthesis, increased in fatty acid oxidation, and reduced of oxidative stress and inflammation. As a general conclusion, it can be stated that polyphenols are biomolecules which produce hepatoprotective effects. To date, these beneficial effects have been demonstrated in cultured cells and animal models. Thus, studies performed in humans are needed before these molecules can be considered as truly useful tools in the prevention of liver steatosis.

Key words: Polyphenols; Resveratrol; Quercetin liver; Steatosis; Non-alcoholic fatty liver disease

Core tip: Recently the beneficial effects of polyphenols in the prevention and treatment of liver steatosis have been reported. These biomolecules present hepatoprotective effects because they reduce liver fat accumulation, mainly by reducing lipogenesis and by increasing fatty acid oxidation, and decrease oxidative stress and inflammation, the main factors responsible for liver damage. To date, these beneficial effects have been demonstrated in cultured cells and animal models. Thus, studies performed in humans are needed before these molecules can be considered as truly useful tools in the prevention of liver steatosis.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver pathologies which range from simple steatosis to non-alcoholic steatohepatitis (NASH). NASH is characterized by steatosis plus features of cellular injury, such as inflammation and hepatocyte ballooning. Some patients with NAFLD develop liver fibrosis, with a proportion progressing to cirrhosis and its complications of liver failure, portal hypertension, and hepatocellular carcinoma. Currently, cirrhotic stage NAFLD represents the third or fourth most common indication for liver transplantation in the United States, and the second most common indication for liver transplantation in large transplantation centers[1-3]. In addition, the prevalence of NAFLD-related cirrhosis has markedly increased in recent years as the underlying liver disease among patients transplanted for hepatocellular carcinoma in the United States. These data reflect the high prevalence of NAFLD in the general population, putting a substantial proportion of individuals at risk of NAFLD-associated morbidity and mortality[4-6].

Current prevalence estimates for NAFLD range from 20% to 75% depending on ethnicity, body mass index (BMI) and the presence of others diseases such as diabetes mellitus or dyslipemia[7,8]. Data suggest that 10% to 30% of NAFLD patients meet the criteria for NASH, with an overall prevalence ranging from 3% up to 25%, depending on the population studied[9].

The long-term prognosis for individuals with NAFLD is not the same across the spectrum of the disease. Steatosis when not associated with cellular injury or fibrosis, follows a relatively benign clinical course, with an overall mortality similar to the general population of the same age and sex. For instance, < 1% of patients with simple steatosis progressed to cirrhosis or died from liver-related complication after a mean follow-up of 15 years in a pooled analysis of several reported series[6]. However, patients with NASH, particularly those with increased fibrosis, have a worse prognosis as compared to an age-and sex-matched population. The prevalence of cirrhosis and death related to liver complications is about 11% and 7%, respectively, in patients with NASH during the first 15 years of follow-up[5]. The most frequent etiology of NAFLD is overweight and obesity. More than 90% of patients with NAFLD are overweight or obese[10].

Drug-induced liver disease (for example, amiodarone, tamoxifen, corticosteroids, methotrexate, *etc.*), autoimmune or viral hepatitis, and cholestatic or metabolic/genetic liver disease can also cause NAFLD.

PHYSIOPATHOLOGY OF LIVER STEATOSIS

The exact pathogenic mechanisms of liver steatosis are not yet fully known. Steatosis, liver inflammation and fibrosis has been associated with an excessive triglyceride accumulation in the liver, insulin resistance and increases in visceral adipose tissue, mediated by increased free radical formation and free oxygen radical species, and modulated by genetic susceptibility[11,12]. There is evidence supporting the theory that these genetic factors account for considerable variability in susceptibility to NAFLD. Since the introduction of genome-wide association studies (GWASs) to investigate genomic variations, there have been significant advances in our understanding of human genome and its clinical effects over a range of diseases. A large number of single nucleotide polymorphisms (SNPs) related to NAFLD has been documented by candidate gene studies. The SNPs may increase or decrease the function of the target genes and their encoding proteins. Genes such as patatin-like phospholipase domain-containing protein 3 (PNPLA3), neurocan core protein (NCAN), glucokinase regulatory protein (GCKR) and lysophospholipase-like protein 1 (LYPLAL1) have been implicated in an increased risk of NAFLD[13]. Lipid peroxidation and free oxygen radical species can deplete antioxidant enzymes (glutathione, vitamin E, beta-carotene and vitamin C) and activate proinflammatory citokines, inflammatory mediators and activation of natural killer cells among others. Others factors as iron, leptin, adiponectin and resistin may contribute to the NAFLD. In the last years intestinal microbes have been implicated as a potential source of hepatotoxic oxidative injury. Intestinal bacterial overgrowth and increased intestinal permeability were observed in patients with NAFLD[14]. Mechanisms by which intestinal bacteria may contribute to hepatocellular injury include endotoxin production, deconjugation of bile salts and inactivation of hepatic lipotropes, such as choline.

As explained above, the most frequent etiology of NAFLD is overweight and obesity. In the following lines an attempt will be made to explain the steps involved in the progression of NAFLD to NASH under this metabolic situation. Adipocyte insensitivity to insulin (insulin resistance), frequently observed in obese patients, overrides the brake to lipolysis in adipose tissue, thus leading to the release of a large amount of free fatty acids. The excessive supply of free fatty acids to the liver is the primary mechanism of NAFLD production. Furthermore, increased amounts of insulin usually found under insulin resistance conditions produce a decrease in hepatic synthesis of apolipoprotein B-100 and the increase in hepatic synthesis of fatty acids. Consequently, the amount of triacylglycerols produced and stored in the liver increases.

NAFLD patients show increased prevalence of TNF-α 238 polymorphism, which induces the over-expression of TNF-α in adipose tissue and, in turn, alterations in the insulin receptor, which causes greater resistance to insulin[15]. Furthermore, several pro-inflammatory adipokines produced by adipose tissue are elevated in obese patients, and can contribute to systemic inflammation and liver damage. By contrast, adiponectin an anti-inflammatory adipokine which antagonizes excess lipid storage in the liver and protects it from inflammation and fibrosis[16], is reduced in these patients and is even lower in patients with hepatic steatosis or NASH.

Free fatty acids increase the expression of cytochrome P-450 2E1 (CYP 2E1)[17], an enzyme involved in the beta-oxidation of long-chain and very long-chain fatty acids, which causes the formation of reactive oxygen metabolites in the liver[18]. On the other hand, several long chain free fatty acids are metabolized in the peroxisomal beta-oxidation. This oxidation produces hydrogen peroxide in the presence of iron and causes hydroxyl radicals, which are reactive oxygen metabolites. This excess of reactive oxygen metabolites consumes antioxidant molecules, such as glutathione and vitamin E, in the liver and thereby generates an oxidative stress, which leads to lipid peroxidation[19]. In turn, lipid peroxidation causes a lesion of the membranes and organelles of hepatocytes, resulting in the phenomena of hepatocellular degeneration and necrosis. The injury caused by lipid peroxidation in mitochondria modifies their morphology (megamitochondria), alters the electron transfer along the respiratory chain and produces more reactive oxygen metabolites, which close the circle, thus generating more oxidative stress[19,20].

The end products of lipid peroxidation, malondialdehyde and 4-hydroxynonenal, have chemoattractant properties, activate proinflammatory cytokines (TNF-α, TGF-β, IL-6, IL-8) and stellate cell stimulatory collagen-producer in the liver. This results in a mixed lesion with hepatocyte degeneration and necrosis, fibrosis and inflammatory infiltrates, in addition to steatosis (NASH)[21]. Also, malondialdehyde and 4-hydroxynonenal covalently bind proteins and produce aggregates of proteins that promote immune response. Secondly, antibodies which can cause an antibody-mediated hepatocellular injury (autoimmune hepatitis) are produced[22]. The perpetuation of oxidative stress and lipid peroxidation causes sustained collagen production which in turn leads to a progression of fibrosis and thus to hepatic cirrhosis. Cirrhosis is the basis of the development of complications such as liver failure and hepatocellular carcinoma[21,23].

EFFECTS OF POLYPHENOLS ON HEPATIC STEATOSIS

Polyphenols are members of a very large family of plant-derived compounds that show an extensive variety of chemical structures. They are classified as flavonoids and non-flavonoids. Among the flavonoids, various groups can be distinguished: flavonols, flavan-3-ols, flavones, isoflavones, flavanones, proanthocyanidins and anthocyanidins. Non-flavonoids included stilbenes and phenolic acids[24].

Although not essentials as vitamins or minerals, polyphenols can have beneficial effects on human health, and thus their study has become an increasingly important area of human nutrition research. A large number of epidemiological studies have shown that the consumption of diets rich in fruits and vegetables is associated with a reduction in the risk of suffering chronic diseases, such as cardiovascular diseases, specific cancers or neurodegenerative diseases[25]. Recently the beneficial effects of polyphenols in the prevention and treatment of liver steatosis have been reported.

EFFECTS OF RESVERATROL ON HEPATIC STEATOSIS

Resveratrol (*trans*-3,4',5-trihydroxystilbene) is a stilbene occurring naturally in several plants and provided in the diet by various food stuffs such as grapes, berries, red wine and nuts. It is well known for its health benefits, such as improvement of insulin sensitivity and glucose tolerance, reduction of plasma lipids, enhancement and suppression of inflammation and oxidative stress[26]. In recent years, it has proved able to modify lipid metabolism, and more specifically to induce a reduction in liver triacylglycerol content. In this context, both *in vitro* and *in vivo* studies have been carried out in order to show its effects on the prevention and the treatment of liver steatosis (Tables 1 and 2).

In vitro studies

Few studies address the potential hepatoprotective action of resveratrol under *in vitro* conditions. In these studies different cell models have been used. In hepatocytes isolated from rat liver and incubated with 25 μmol/L resveratrol for 30 min, Gnoni and Paglialonga[27] observed 40% reduction in triacylglycerols and decreased acetyl-CoA carboxylase (ACC) activity, without changes in fatty acid synthase (FAS) activity. Based on these results, the authors suggested that resveratrol reduced *de novo* lipogenesis, thus decreasing the availability of fatty acids, and consequently the synthesis of triacylglycerols.

Wang *et al*[28] used a cell model of steatosis induced by palmitate treatment. This treatment induces maximal fat over-accumulation with minimal cytotoxicity. The inclusion of 40 μmol/L of resveratrol for 24 h in the incubation medium reduced triacylglycerol accumulation induced by palmitate almost to basal values, due to a decrease in both the expression and the activity of sterol regulatory element binding protein 1c (SREBP-1c), which is the most important transcription factor for *de*

Table 1 ***In vitro*** **studies performed with polyphenols**

Ref.	Animal model	Polyphenol dose and treatment period length	Effects	Mechanisms
Gnoni *et al*[27], 2009	HepG2 cells	25 μmol/L resveratrol 30 min	↓ Triacylglycerols	↓ ACC activity = FAS activity
Zang *et al*[29], 2006	HepG2 cells	10 μmol/L resveratrol 24 h	Prevent lipid accumulation	↑ phosphorylation AMPK (activation) in liver ↑ phosphorylation ACC (inhibition) in liver
Shang *et al*[30], 2008	HepG2 cells	50 μmol/L resveratrol 24 h	↓ Triacylglycerols	↑ phosphorylation AMPK liver (activation) ↓ mRNA de SREBP1c and FAS liver
Wang *et al*[28], 2009	Human HepG2 cells	40 μmol/L resveratrol 24 h	↓ Triacylglycerols	↓ SREBP 1c ↑ SIRT 1
Vidyashankar *et al*[53], 2013	Hep G2 cells	10 μmol/L quercetin 24 h	↓ Triacylglycerols ↓ Insuline resistance ↓ Oxidative stress ↑ Superoxide dismutase, catalase and glutathione peroxidase activities	
Guo *et al*[60], 2011	Hep G2 cells	Anthocyanin Cy-3-g 1, 10, 100 μmol/L 24 h	↓ Triacylglycerols	Inhibit translocation of GPAT1 ↓ GPAT, mtGPAT1 activity
Baselga-Escudero *et al*[61], 2012	FAO cells	Proanthocyanidins 10, 25, 50, 100 mg/L 1 h	↓ MiR-122 at 25,50, 100 mg/L ↓ MiR.122, FAS with time (1, 3 h. 25 mg/L)	25 mg/L, ↓ FAS (5 h) protein expression
Pil Hwang *et al*[62], 2013	Hep G2 cells	3-caffeoyl, 4-dihydrocaffeoylquinic acid 1, 3, 10 μmol/L 1 h	↓ Fat accumulation in a dose-dependent manner	↓ SREBP1c, FAS mRNA and protein expression = LXRα mRNA expression ↑ Activating AMPK ↑ SIRT1
Liu *et al*[65], 2011	HepG2 cells	Blueberry extract 20, 40, 60, 80 and 100 μg/mL 24 h	↓ Triacylglycerols accumulation 80 μg/m L ↓ 60% of triacylglycerols accumulation	
Lee *et al*[67], 2012	BALB/c normal liver cells	Extract of *Hibicus sabdariffa L* 0.05, 0.1, 0.5, 1 mg/mL 48 h	↑ Cell viability	↓ p-JNK and AIF, tBid and Bax protein expression ↓ Lipid peroxidation ↑ Catalase and GSH
	Steatosis produced by acetaminophen			
Wang *et al*[66], 2012	HepG2	Extract of *Ginkgo biloba* 200 μg/mL *in vitro* 24 h	↓ Triacylglycerols	↑ CPT-1a, ACO mRNA expression ↓ FAS, Acac-β mRNA expression ↑ CPT-1a protein expression

↓: Decrease; ↑: Increase. RSV: Resveratrol; ACC: Acetyl CoA carboxylase; FAS: Fatty acid synthase; AMPK: AMP-activated kinase; SREBP1c: Sterol regulatory element binding protein 1c; SIRT1: Sirtuin 1; Q: Quercetin; GPAT1: Glycerol-sn-3-phosphate acyltransferase 1; mtGPAT1: Mitochondrial glycerol-sn-3-phosphate acyltransferase 1; LXRα: Liver X receptor α; pJNK: Activated c-Jun N-terminal kinase; AIF: Apoptosis inducing factor; GSH: Glutathione; CPT-1a: Carnitine palmitoyltransferase 1a; ACO: Acyl-coenzyme A oxidase 1; Acacβ: Acetyl-coenzyme A carboxylase β.

novo lipogenesis, *via* deacetylase sirtuin 1 (SIRT1).

Other studies have been performed using human cells. With similar results to those observed in rat hepatocytes, Zang *et al*[29] showed that incubation of cultured human HepG2 hepatocytes exposed to high concentrations of glucose, with 10 μmol/L of resveratrol, a concentration lower than those used in other studies conducted in rat hepatocytes, for 24 h prevented lipid accumulation. The authors found a decreased activity of the lipogenic enzyme ACC. Moreover, they observed an activation of AMP-activated protein kinase (AMPK), and concluded that this activation was required for the lipid lowering effect of resveratrol. These results are in good accordance with the study published by Shang *et al*[30] where HepG2 hepatocytes were exposed to high concentrations of glucose and insulin to get a cell steatosis model. A dose of 50 μmol/L of resveratrol reduced triacylglycerol accumulation. This polyphenol prevented the decline of phosphorylated AMPK induced by steatosis, followed by the down-regulation of SREBP-1c and FAS.

In summary, the studies performed in different models of rodent and human hepatocyte steatosis demonstrate that resveratrol show anti-lipidogenic effects at doses in the range of 10-50 μmol/L. There is a good consensus concerning the mechanism of action underlying this effect because all the above mentioned reports

Table 2 ***In vivo*** **studies carried out with resveratrol**

Ref.	Animal model	Resveratrol dose and treatment period length	Effects	Mechanisms
Baur *et al*[31], 2006	Male C57BL/6NIA mice fed a high-fat diet	0.04% in the diet (estimated 22.4 mg/kg *bw* per day) 6 wk	↓ Lipid droplets in liver	↓ Acetylation status of PGC-1α protein in liver
		186 mg kg *bw* per day 6 wk	↑ Mitochondrial number	
Kasdallah-Grissa *et al*[39], 2006	Male Wistar rats fed a standard diet 3 g ethanol/kg *bw* per day	0.04% in the diet (estimated 300-450 mg/kg *bw* per day) 6 wk	↓ Oxidative stress ↓ Lipid peroxidation	
Ahn *et al*[32], 2008	Male C57BL6/J mice fed an atherogenic diet	0.0125% in the diet (estimated 10 mg/kg *bw* per day) 8 wk	↓Total lipids and triacylglycerols in liver Ameliorated necroinflammation	↓ Expression lipogenic enzymes ↑ Expression enzymes involved in fatty acid oxidation ↑ SIRT1 mRNA expression
Ajmo *et al*[40], 2008	Male C57BL/6J mice fed a low-fat diet Ethanol added to account 29% of total calories	200 mg/kg *bw* per day 400 mg/kg *bw* per day 4 wk	↓ Liver weight ↓ Lipid droplets ↓ Triacylglycerols	↓ SREBP-1c mRNA and protein expressions ↓ FAS, SCD, ACC, ME mRNA ↑ PGC-1α mRNA ↑ ACO, CPT-1a mRNA ↑ Fatty acid oxidation Activation AMPK/SIRT1 axis
Bujanda *et al*[37], 2008	Male Wistar rats Steatosis induced by feeding rats ad libitum for four days per week and then fasting them the remaining three days	10 mg/kg *bw* per day 4 wk	↓ Liver fat infiltration ↓ Oxidative stress ↓ ALT	
Kim *et al*[33], 2008	C57BL/6J mice a high fat diet	0.4% in the diet (estimated 400 mg/kg *bw* per day) 10 wk	↓ Liver weight ↓ Triacylglycerols	
Shang *et al*[30], 2008	Male Wistar rats fed a high-fat, high-sucrose diet	100 mg/kg *bw* per day 10 wk	↓ Triacylglycerols ↓ Lipid droplets	↑ AMPK phosphorylation (activation) ↓ SREBP-1c and FAS mRNA
Rivera *et al*[42], 2009	Male *fa/fa* Zucker rats fed standard diet	10 mg/kg *bw* per day 8 wk	↓ Triacylglycerols	↓ ACC activity AMPK activation
Cho *et al*[34], 2012	Male C57BL/6J mice fed a high-fat diet	0.005% in the diet (0.5 mg/kg *bw* per day) 10 wk	0.5 mg/kg *bw* per day ↓ Triacylglycerols ↓ Number and size of liver fat droplets	↓ PAP = Enzymes involved in fatty acid oxidation ↓ FAS and ME activities
		0.02% in the diet (2 mg/kg *bw* per day) 10 wk	2 mg/kg *bw* per day ↓ Triacylglycerols ↓ Number and size of liver fat droplets	↓ PAP = Enzymes involved in fatty acid oxidation
Gómez-Zorita *et al*[43], 2012	Male *fa/fa* Zucker rats fed a standard diet	15 mg/kg *bw* per day 45 mg/kg *bw* per day 6 wk	15 and 45 mg/kg *bw* ↓ Liver weight ↓ Triacylglycerols ↓ Oxidative stress 15 mg/kg *bw* ↓ Transaminases	= Lipogenic enzyme activity ↑ Activity of enzymes involved in fatty acid oxidation
Poulsen *et al*[35], 2012	Male Wistar rats fed a high-fat diet	100 mg/kg *bw* per day 8 wk	Normalized triacylglycerols No hepatic inflammation	↑ UCP2 ↑ Mitochondria number
Alberdi *et al*[36], 2013	Male Sprague-Dawley rats fed a high-fat, high-sucrose diet	30 mg/kg of *bw* per day 6 wk	↓ Triacylglycerols	↑ Activity of enzymes involved in fatty acid oxidation = FAS, G6PDH, ME activities ↑ Phosphorylated ACC/total ACC (inhibition) ↑ Phosphorylated AMPK/total AMPK (activation) = PPAR-α, SREBP-1c, SIRT1, PGC-1α, TFAM, COX2, and HNF-4α, ↓ Acetylated PGC-1α /total PGC-1α (activation)

Franco *et al*[38], 2013	Lactating Wistar rats	30 mg/kg per day 4 wk	↓ Triacylglycerols ↓ Oxidative stress

↓: Decrease; ↑: Increase. BW: Body weight; PGC-1α: Peroxisome proliferator-activated receptor-c coactivator 1 α; SIRT1: Sirtuin 1; SREBP-1c: Sterol regulatory element binding protein 1c; FAS: Fatty acid synthase; SCD: Stearoylcoenzyme A desaturase; ACC: Acetyl CoA carboxylase; ME: Malic enzyme; ACO: Acyl-coenzyme A oxidase 1; CPT-1a: Carnitine palmitoyltransferase 1a; AMPK: AMP-activated kinase; ALT: Alanine aminotransferase; PPAR-γ: Peroxisome proliferator-activated receptor γ; C/EBP: CCAAT/enhancer binding protein; LXR: Liver X receptor; PAP: Phosphatidate phosphohydrolase; UCP2: Mitochondrial uncoupling protein 2; G6PDH: Glucose-6-phosphate dehydrogenase; PPAR-α: Peroxisome proliferator-activated receptor α; TFAM: Mitochondrial transcription factor A; COX2: Cyclooxygenase 2; HNF-4 α: Hepatocyte nuclear factor 4 α.

show decreased *de novo* lipogenesis.

In vivo studies

The *in vivo* studies reported have been carried out in rodents (mice and rats) by using different models of liver steatosis (diet-induced steatosis, ethanol-induced steatosis, genetic steatosis, *etc.*). These studies have revealed that this polyphenol reduces liver weight and triacylglycerol content. To explain this effect several mechanisms of action have been proposed.

One of the most frequently used models of liver steatosis is high-fat feeding. The first study conducted in this rodent model was reported by Baur *et al*[31]. C57BL/6NIA mice were treated with 22.4 mg resveratrol/kg body weight per day for 6 wk. Histological examination of liver showed that resveratrol administration reduced the accumulation of large lipid droplets. In another cohort of mice supplemented with 186 mg resveratrol/kg body weight per day for 6 wk the authors observed that treated animals showed increased number of mitochondria than the controls. This effect was mediated by deacetylation, and thus activation, of PGC-1α the co-activator of PPAR-α, the transcription factor which regulates fatty acid oxidation.

Ahn *et al*[32] described that the addition of 0.0125% of resveratrol to an atherogenic diet (a type of high-fat diet) led to a reduction in total lipids and triacylglycerols in liver from C57BL/6J mice after 8 wk of treatment. This amount of resveratrol in the diet corresponded to a dose of 10 mg/kg body weight per day in this study, lower than that used by Baur *et al*[31]. Histological analysis of liver sections confirmed that resveratrol significantly ameliorated both hepatic steatosis and necroinflammation. These changes were accompanied by a reduction in the expression of genes related to lipogenesis and an increase in the expression of genes related to fatty acid oxidation. Moreover, resveratrol increased hepatic expression of SIRT1. Therefore, the authors suggested that the beneficial effects of resveratrol on liver lipid metabolism can be exerted by SIRT1 activation.

Kim *et al*[33] also reported a reduction in liver weight and triacylglycerols in C57BL/6J fed a high-fat diet supplemented with 0.4% resveratrol for 10 wk. This amount of resveratrol represented 400 mg/kg body weight per day in this study, a very high dose. Potential mechanisms of action for resveratrol concerning this action were not proposed in this study.

Cho *et al*[34] compared the effects of two doses of resveratrol in C57BL/6J mice fed a high-fat diet. Animals received 0.5 mg/kg body weight per day or 2 mg/kg body weight per day for 10 wk. Resveratrol significantly reduced hepatic triacylglycerol content, although not in a dose-dependent manner. Consistent with these results haematoxylin and eosin staining of liver sections indicated that resveratrol caused a marked decrease in the number and size of liver fat droplets. In this case the lower dose appeared to be more effective than the higher dose. In order to examine the mechanism of action of resveratrol under these experimental conditions the activity of hepatic lipid-regulating enzymes was assessed. The high dose of resveratrol significantly decreased the hepatic activity of fatty acid synthase and glucose-6-phosphate dehydrogenase, two lipogenic enzymes and both doses decreased the activity of phosphatidate phosphohydrolase, an enzyme that catalyzes the synthesis of triacylglycerols. The enzymes involved in fatty acid oxidation remained unchanged.

By using a high-fat feeding model, but in this case working with rats instead of mice, Shang *et al*[30] observed that resveratrol, at a dose of 100 mg/kg body weight per day, orally administered, reduced hepatic triacylglycerol content after 10 wk of treatment. This effect was confirmed by histopathological analysis. Moreover, rats treated with the polyphenol showed increased AMPK phosphorylation and reduced SREBP-1c and FAS gene expressions. The authors concluded that resveratrol protected the liver from NAFLD and that the activation of AMPK was involved in the mechanism underlying the reduction in triacylglycerol accumulation.

By using the same dose of resveratrol (100 mg/kg body weight per day), Poulsen *et al*[35] reported that resveratrol prevented liver triacylglycerol accumulation induced by high-fat feeding in rats after 8 wk of treatment. The semi-quantitative microscopical steatosis grading revealed severe microvesicular steatosis in the high-fat fed group, but only slight changes in rats treated with resveratrol. These findings were consolidated by chemical extraction of hepatic lipid, as the triglyceride content was significantly lower in resveratrol-treated rats than in control animals. This effect was related to the increase in liver mitochondria number. The authors also observed an increase in uncoupling protein 2 (UCP_2), as results that seems to be related to reduced oxidative stress.

Another study conducted in rats fed a high-fat diet, but using clearly lower dose of resveratrol, was that reported by Alberdi *et al*[36]. Rats receiving this polyphenol in the diet in amounts that assured a dose of 30 mg/kg body weight per day for 6 wk, showed increased activi-

ties of palmitoyl transferase 1a (CPT-1a) and acyl-CoA oxydase (ACO), two enzymes involved in fatty acid oxidation, and decreased activity of the lipogenic enzyme ACC. The conclusion was that resveratrol partially prevented the increase in liver fat by increasing fatty acid oxidation and reducing lipogenesis. The potential involvement of the AMPK/SIRT1 axis was proposed.

High-fat feeding is not the only model of dietary-induced steatosis. Bujanda *et al*[37] analyzed the effects of resveratrol in a model obtained by feeding rats *ad libitum* for four days per week and then fasting them the remaining three days. This cycle was repeated four times. After 4 wk of treatment, resveratrol, administered by the oral route through an orogastric catheter, at a dose of 10 mg/kg body weight per day significantly reduced fat infiltration in liver. Moreover, oxidative stress, which is believed to play an important role in the pathogenesis of NAFLD, was significantly reduced in resveratrol-treated rats. Finally, serum concentrations of alanine aminotransferase (ALT), an indicator of hepatocyte damage, were significantly reduced in treated rats.

Franco *et al*[38], worked with another model of steatosis in rat pups. At birth, lactating rats were randomly assigned to each one of these groups: early weaning group (pups from dams which were wrapped with a bandage to interrupt lactation in the last 3 d of lactation) or control group (dams whose pups had free access to milk throughout lactation for 21 d). Pups from the early weaning group developed fatty livers among other metabolic alterations. The administration of resveratrol, by gavage, at a dose of 30 mg/kg body weight per day for one month significantly reduced liver triacylglycerols and oxidative stress.

Ethanol-induced steatosis is another commonly used model of fatty liver. Kasdallah-Grissa *et al*[39] induced steatosis in rats by administering intraperitoneally 3 g ethanol/kg body weight, a dose which shows moderate toxicity. Rats were fed a diet supplemented with 0.5% of resveratrol for 6 wk. Taking body weight and food intake of the animals into account the dose of resveratrol was approximately 300-450 mg/kg body weight per day. Resveratrol treatment attenuated lipid peroxidation induced by ethanol which indicates that resveratrol reduced oxidative stress and thus showed hepatoprotective properties.

Along the same lines, Ajmo *et al*[40] reported that supplementation with resveratrol at doses of 200 and 400 mg/kg body weight per day protected against ethanol-induced steatosis, reducing liver weight and hepatic triacylglycerols in male C57BL/6J mice. With regard to the potential mechanisms of action underlying these effects, the authors reported that resveratrol reduced gene and protein expression of SREBP-1c. This change was paralleled by a reduction in FAS, stearoyl-CoA desaturase (SCD), ACC and malic enzyme (ME). These results demonstrate that reduction in lipogenesis was involved in the anti-lipidogenic effect of resveratrol. Futhermore, resveratrol increased mRNA levels of peroxisome proliferator-activated receptor-c coactivator 1 α (PGC-1α), ACO and CPT-1a. When fatty acid oxidation was measured, mice treated with resveratrol showed increased values, as expected. Finally, adiponectin, an adipokine that promotes fatty acid oxidation, was also increased. These results show that increased fatty acid oxidation also participated in the effect of resveratrol on liver fat reduction. All these effects were observed with both doses with no dose-response pattern. The authors suggested that this protective action of resveratrol was, in whole or in part, mediated throughout the up-regulation of SIRT1-AMPK signaling system.

Another model of liver steatosis is the *fa/fa* Zucker rat, a genetically obese rat, which shows many human metabolic syndrome features such as insulin resistance, dyslipidemia, hyperinsulinemia and hypertension. Peripheral insulin resistance in obese Zucker rats enhances the mobilization of peripheral fat and the serum level of free fatty acids. However, liver oxidation or utilization of free fatty acids is inhibited. Thus, the liver in this rodent model synthesizes an excess of triacylglycerols and oxidizes a small amount of fatty acids, leading to strong fat infiltration of the hepatic parenchyma[41].

By using this animal model, Rivera *et al*[42] demonstrated that the administration of resveratrol at a dose of 10 mg/kg body weight per day for 8 wk induced a decrease in liver triacylglycerol accumulation. The authors suggested that this effect was related to the increase of phosphorylation of AMPK and ACC.

In previous studies from our group, also conducted in obese Zucker rats, assessed the effect of two doses of resveratrol (15 and 45 mg/kg body weight) on liver triacylglycerol content as well as on the activity of enzymes involved in two key metabolic pathways in the control of hepatic fat accumulation, lipogenesis and fatty acid oxidation[43]. After 6 wk of treatment, liver weight and triacylglycerols were decreased, oxidative enzyme activities were increased and lipogenic enzyme activities remained unchanged in both resveratrol-treated groups, with no differences between them. These results suggest that the decrease in liver steatosis was due to increased fatty acid oxidation. In this study, resveratrol significantly decreased hepatic thiobarbituric acid reactive substances (TBARS) formation, indicating an antioxidant effect and protection from the oxidative stress induced by obesity and steatosis in Zucker rats. Also, the high dose was able to diminish the amount of oxidized glutathione (GSSG) as well as to increase the GSH/GSSG ratio, a sensitive and reliable measure of the overall level of oxidative stress. These results suggest that the glutathione redox state became less pro-oxidizing due to supplementation with resveratrol. However, the reactive oxygen species (ROS) scavenging enzyme superoxide dismutase (SOD) did not seem to be involved in the resveratrol-induced reduction of oxidative stress.

Summary

There is a general consensus concerning the positive ef-

fects of resveratrol on liver steatosis in animal models. The range of doses used in experiments carried out on mice has been very huge (0.5-400 mg/kg per day). All the doses analyzed have been shown to be effective. In the experimental design of the studies described above, while the lowest doses (0.5 and 2 mg/kg body weight per day) were used in longer treatments (10 wk), the highest doses (200-400 mg/kg body weight per day) were used for shorter periods (4 wk). Thus, based on the reported data it is not possible to known whether low doses would be effective over shorter periods of treatment. As far as rats are concerned, the range of doses used has also been huge, although less than in mice (10-450 mg/kg body weight per day). The treatment periods were similar to those used in mice (4-10 wk). Due to the fact that very low doses have not been used in rats it is not possible to know whether this animal species in less responsive to resveratrol than the mouse. All these results suggest that more studies are needed to establish the best combination of dose-experimental period.

In the vast majority of the experiments described in the present review resveratrol was administered to animals at the same time as the factor which induced liver steatosis (high-fat feeding, ethanol intake, *etc.*). This means that resveratrol is able to prevent liver steatosis; this effect was observed independently of the etiology of this metabolic situation. Moreover, this polyphenol also ameliorated steatosis when this alteration was prior to the treatment, as observed in genetically obese rats. These rats showed steatosis before being treated with resveratrol and then showed reduced liver fat accumulation. These facts demonstrate that resveratrol is useful not only in the prevention of liver steatosis but also in its treatment.

Only two of all the studies reported used more than one dose in the same experiment. In the case of Ajmo *et al*[40] who used 200 and 400 mg/kg body weight per day, no dose-response effect was found. By contrast, in the case of Cho *et al*[34], the low dose (0.5 mg/kg body weight per day) appeared to be more effective than the higher dose (2 mg/kg body weight per day), most likely a phenomenon of hormesis.

Altogether data reported concerning the mechanisms of action of resveratrol underlying its liver anti-lipidogenic effect demonstrate that this polyphenol decreases *de novo* fatty acid synthesis, as well as triacylglycerol synthesis, and increases fatty acid oxidation. Moreover, the reduction of oxidative stress also contributes to this positive effect. The activation of AMPK and SIRT1 mediates these changes.

Human studies

With regard to human beings, there is only one published study so far. Healthy, obese, male volunteers without a history of diabetes or any other disorder received 150 mg resveratrol/d or placebo for 1 mo in a randomized double-blind crossover design. Plasma ALT concentration was significantly lower after resveratrol treatment compared to the placebo group. Intrahepatic lipid content was lower after 30 d of resveratrol supplementation in comparison to placebo. This was paralleled by lower plasma ALT value, both indicating improved liver function[44].

EFFECTS OF QUERCETIN ON STEATOSIS

Quercetin is a natural polyphenol belonging to a group with a variable structure, known as flavonoids. It is found in onions, broccoli, tomatoes, apples and berries[45].

It has been reported that quercetin exhibits a wide range of biological functions, including antioxidant, anticarcinogenic and anti-inflammatory activities[46-50]. More recently, beneficial effects on blood pressure and heart disease have been described[50-52]. Since 2006, several studies have shown the interesting properties of this flavonoid in the prevention of liver steatosis (Tables 1 and 3).

In vitro studies

Vidyashankar *et al*[53] carried out a study with HepG2 cells rendered steatosis by incubation with oleic acid-bovine serum albumin complex. These cells were then treated with a dose of 10 μmol/L of quercetin for 24 h. Decreased triacylglycerol accumulation, insulin resistance and inflammatory cytokine secretion, and increased cellular antioxidants were observed. The study suggested that quercetin was an effective molecule reversing the symptoms of NAFLD.

In vivo studies

As in the case of resveratrol, different animal models have been used in *in vivo* studies. Ying *et al*[54] used gerbils fed a high-fat diet as a model of steatohepatitis, and treated them with three doses of quercetin, 15, 30 and 60 mg/kg body weight per day for 2 wk. The lowest dose was ineffective. By contrast, both 30 and 60 mg/kg body weight per day reduced liver triacylglycerols, liver lipid droplet size, serum transaminases and proinflammatory mediators, such as TNF-α and IL-6 in a dose-response pattern. Only the highest dose reduced liver collagen.

The rest of the studies reported were conducted in mice. Kobori *et al*[55] conducted an experiment in BALB/c mice showing streptozotocin (STZ)-induced diabetes. When quercetin was added to the diet at 0.5% for 2 wk, animals showed a reduction in oxidative stress, as well as in liver injury produced by STZ.

The same research group carried out another study by means of a different experimental design[56]. C57BL/6J mice were a high-fat diet supplemented with 5 g quercetin/kg diet for 20 wk. Treated mice showed a reduction in liver triacylglycerol accumulation. Moreover, the increase induced by the diet in the expression of peroxisome proliferator activated receptor γ (PPAR-γ), cluster of differentiation 36 (CD36), SREBP-1c and FAS, genes which promote lipid accumulation, was normalized.

Table 3 *In vivo* studies carried out with quercetin

Ref.	Animal model	Quercetin dose and treatment period length	Effects	Mechanisms
Kobori *et al*[55], 2009	BALB/c mice with STZ-induced diabetes and steatosis fed a standard diet	0.5% in the diet 2 wk	↓ Oxidative stress ↓ Liver damage	
Kobori *et al*[56], 2011	C57BL/6 J mice fed a high-fat diet	0.5% in the diet 20 wk	↓ Triacylglycerols ↓ Oxidative stress	Normalized gene expression of PPAR-γ, SREBP-1c, FAS, CD36 ↑ PPAR-α mRNA expression
Marcolin *et al*[58,59], 2012, 2013	Male C57BL/6J mice fed a diet deficient in methionine and choline	0.005% in the diet 4 wk	↓ Macro/micro vesicular steatosis ↓ Balloning ↓ Serum transaminases ↓ Oxidative stress ↓ DNA damage	↓ Proinflammatory ond profibritic gene expression
Panchal *et al*[57], 2012	Male Wistar rats fed a high-fat diet	0.8% in the diet (50 mg/kg *bw* per day) 8 wk	Attenuated steatosis	↑ Nrf2 ↓ NF-kβ ↑ CPT-1a
Jung *et al*[50], 2013	C57B1/6 mice fed a high-fat diet	0.025% in the diet 8 wk	↓ Liver weight ↓ Triacylglycerols ↓ Lipid droplet size	↓ FAS, Acaca, Apoa4, Abcg5, Fdft1 and GPAM mRNA expressions
Ying *et al*[54], 2013	Gerbils fed a high-fat diet	15, 30, 60 mg/kg *bw* per day 2 wk	All doses ↓ Triacylglycerols ↓ Lipid droplets size ↓ Serum transaminases 60 mg/kg *bw* per day ↓ Liver collagen	

↓: Decrease; ↑: Increase. STZ: Streptozotocin; PPAR-γ: Peroxisome proliferator activated receptor γ; SREBP-1c: Sterol regulatory element binding protein 1c; FAS: Fatty acid synthase; CD36: Cluster of differentiation 36; PPAR-α: Peroxisome proliferator activated receptor α; BW: Body weight; CPT-1a: Carnitine palmitoyltransferase 1a; Acaca: Acetyl-coenzyme A carboxylase α; Apoa4: Apolipoprotein A-Ⅳ; Abcg: ATP-binding cassette, subfamily G, member 5; Fdft1: Farnesyl-diphosphate farnesyltransferase 1; Gpam: Glycerol-3-phosphate acyltransferase, mitochondrial.

Another interesting effect was the increase in the expression of peroxisome proliferator activated receptor α (PPAR-α), a transcription factor which control fatty acid oxidation, reduced by high-fat feeding. Finally, oxidative stress was reduced by quercetin.

Panchal *et al*[57], also working with a model of steatosis induced by high-fat feeding, observed attenuated steatosis in rats treated for 8 wk with quercetin at a dose of approximately 50 mg/kg body weight per day (0.08% quercetin in the diet). The authors suggested that this effect was related to the down-regulation of NF-κB, a transcriptional factor that stimulates inflammation, and the up-regulation of Nrf2, which prevents oxidation. Moreover, quercetin treatment resulted in increased expression of CPT-1a, a key enzyme of fatty acid oxidation.

Jung *et al*[50] reported a reduction in liver weight due to a decrease in the amount of triacylglycerols and lipid droplets in C57BL/6J mice fed a high-fat diet supplemented with 0.025% of quercetin for 8 wk. In this study the authors also analyzed the effects of quercetin on the expression of genes related to lipid metabolism in liver, such as FAS (involved in *de novo* lipogenesis), Acetyl-coenzyme A carboxylase α (Acaca), apolipoprotein A-Ⅳ (Apoa4), ATP-binding cassette, subfamily G, member 5 (Abcg5), Fdft1, farnesyl-diphosphate farnesyltransferase 1 (Fdft1) (involved in the synthesis of saturated fatty acids) and glycerol-3-phosphate acyltransferase mitochondrial (GPAM), (involved in triacylglycerol synthesis and related to SREBP-1c gen). All these genes were down-regulated in quercetin-treated mice.

Marcolin *et al*[58] analyzed the effect of quercetin (0.005% in the diet) in the protection against steatosis induced in C57BL/6J mice fed a diet deficient in methionine and choline. In this study, a lower degree of steatosis and a reduction in transaminases and oxidative stress were observed in mice treated with quercetin for 4 wk. Moreover, proinflammatory and profibrotic gene expression was reduced. Later on, in the same cohort of animals, they observed a reduction in macro/microvesicular steatosis, ballooning and DNA damage induced by quercetin treatment[59].

Due to fact that the authors of the studies described above did not provide data concerning food intake, it is not possible to know the dose (mg/kg body weight per day) provided to animals in order to be compared with other reports.

Summary

The number of studies performed with quercetin is lower than that of studies carried out with resveratrol. The most commonly used animal model in these studies was mice. It is important to point out that a low dose of this polyphenol (0.005%) is able to prevent liver steatosis in a quite short experimental period (4 wk). The only study which analyzed different doses of quercetin[54] showed a dose response pattern. As far as the potential mechanisms of this polyphenol are concerned, quercetin similarly to resveratrol decreases de novo fatty acid synthesis.

Table 4 ***In vivo*** **studies carried out with other polyphenols**

Ref.	Animal model	Polyphenols dose and treatment period length	Effects	Mechanisms
Guo *et al*[60], 2011	Male KKAy mice fed a standard diet	Anthocyanin Cy-3-g 0.01% in the diet 12 wk	↓ Triacylglycerols ↓ Lipid droplets	↓ GPAT1 activity
Baselga-Escudero *et al*[61], 2012	Male Wistar rats fed a standard diet and 2.5 mL of lard oil/kg BW	Proanthocyanidins 250 mg/kg BW 3 h	↓ Triacylglycerols	↓ FAS mRNA ↑ miR-122 mRNA
Luo *et al*[64], 2012	Male C57BL/6 mice fed a methionine and choline-deficient high-fat diet	Theaflavin 30 mg/kg BW by intraperitoneal injection 48, 24, and 2 h before induction of steatosis by ischemia-reperfusion	↓ Cell ballooning ↓ Microvesicular and macrovesicular steatosis ↓ ALT ↓ Oxidative stress ↓ Hepatocyte apoptosis ↓ F4/80-positive cells (inflammatory cells)	
Yoshimura *et al*[63], 2013	KKAy mice fed a high-fat diet	Ellagic acid 0.1% in the diet 68 d	↓ Serum ALT, AST, ↓ Macrovesicular steatosis ↓Triacylglycerols	↑ FAS mRNA expression = Acaca, SREBP-1c mRNA expression = ACO mRNA expression ↑ CTP-1a, PPAR-α mRNA expression

↓: Decrease; ↑: Increase. GPAT1: Glycerol-sn-3-phosphate acyltransferase 1; BW: Body weight; FAS: Fatty acid synthase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Acaca: Acetyl-CoA carboxylase α; SREBP-1c: Sterol regulatory element binding protein 1c; ACO: Acyl-coenzyme A oxidase; CPT-1a: Carnitine palmitoyltransferase 1a; PPAR-α: Peroxisome proliferator activated receptor α.

EFFECTS OF OTHER POLYPHENOLS IN HEPATIC STEATOSIS

In addition to resveratrol and quercetin, other polyphenols such as antocyanin Cy-3-g, proanthocyanidins, teaflavin (a flavan-3-ol) and ellagic acid (a tannin) have studied as potential agent for both prevention and treatment of hepatic steatosis (Tables 1 and 4).

In vitro studies

Guo *et al*[60] performed a study in HepG2 cells incubated with 3 doses of anthocyanin cy-3-g (1, 10, 100 μmol/L) for 24 h. A reduction in triacylglycerol content was observed due to an inhibition in glycerol-sn-3-phosphate acyl transferase 1 (GPAT1), a key enzyme in the synthesis of triacylglycerols.

In FAO cells, a rat hepatoma cell line, Baselga-Escudero *et al*[61] studied the effect of proanthocyanidins on hepatic lipid metabolism. 25 mg/L of these polyphenols reduced FAS and miR-122 expression. miR-122 is a novel class of non-coding RNA that regulates genes involved in fatty acid and triacylglycerol synthesis. Moreover, the protein expression of FAS was also decreased.

Pil Hwang *et al*[62] conducted a study where HepG2 cells were treated with 3 doses (1, 3 and 10 μmol/L) of 3-Caffeoyl, 4-dihydrocaffeoylquinic acid for 1 h. The study showed that this polyphenol inhibited fat accumulation in a dose dependent manner due to a decrease in *SREBP-1c* and *FAS* gene and protein expressions, through the activation of AMPK and SIRT1.

In vivo studies

After analyzing the *in vitro* effects of anthocyanin Cy-3g, Guo *et al*[60] conducted an *in vivo* study with KKAy mice fed a standard diet supplemented with 100 mg/kg of this anthocyanin or not, for 12 wk. The study showed a reduction in triacylglycerol content and lipid droplets due to an inhibition in translocation of GPAT1 and a reduction in the synthesis of triacylglycerols. These results are in good accordance with their own results obtained in cultured cells.

In the same animal model, but using a high-fat diet supplemented with 0.1% ellagic acid or not for 68 d, Yoshimura *et al*[63] observed a decreased in serum free fatty acids, triacylglycerol, ALT, aspartate transaminase (AST) and resisitin concentrations, without changes in leptin and adiponectin. Moreover, a decrease in macrovesicular steatosis and hepatic triacylglycerol was shown. CPT-1a and PPAR-α mRNA expression was increased but Acaca, SREBP-1c and ACO mRNA expression remained unchanged. Surprisingly, the mRNA expression of FAS was increased.

Luo *et al*[64] carried out a study with C57BL/6 mice fed a methionine and choline deficient high-fat diet and treated with 30 mg of theaflavin /kg body weight by intraperitoneal injections 2, 24 and 48 h before induction of steatosis by ischemia-reperfusion. They observed that theoflavin reduced cell ballooning, micro and macrovesicular steatosis, hepatocyte apoptosis, oxidative stress and inflammatory cells in liver, as well as serum transaminase concentrations.

Baselga-Escudero *et al*[61] conducted a study with Wistar rats fed *ad libitum* with a standard diet. The rats were orally gavaged with lard oil (2.5 mL/body weight) or 250 mg of proantocyanindins dissolved in the lard oil. After 3 h a reduction in serum and hepatic triacylglycerol content

was observed. Similar to what these authors observed in cultured cells, increased miR-122 mRNA levels accompanied by decreased FAS mRNA levels were found.

Summary

It is not possible to compare the above described studies because important differences in terms of type of polyphenol used, dose and animal model exist among them. Nevertheless, the reported results show that all the polyphenols tested, belonging to different families, show an anti-lipidogenic effect. As in the case of resveratrol, the potential mechanisms that justify this effect are decreased synthesis of fatty acids and triacylglycerols and increased fatty acid oxidation.

EFFECTS OF POLYPHENOL EXTRACTS IN HEPATIC STEATOSIS

In addition to studies conducted with individual polyphenols, several works have used polyphenol extracts with different origins and compositions. Although using polyphenol extracts makes it quite complicated to assign the beneficial effects observed to a specific molecule, they have two clear advantages. On the one hand, they better mimic the real situation in our dietary patterns. On the other hand, additive or synergic effects can be observed. In this context, sometimes combinations of molecules present beneficial effects that are not shown when they are administrated separately (Tables 1 and 5).

In vitro studies

There are few studies analyzing the effect of polyphenols extracts *in vitro*. Liu *et al*[65] studied the effect of blueberry phenolic compounds (anthocyanins, flavanols) at different doses (20-120 μmol/L) in HepG2 cells for 24 h. An inhibitory effect was observed in triacylglycerols accumulation in a dose-dependent manner. The maximum inhibitory value (approximately 60%) was reached at a concentration of 80 μmol/L.

Wang *et al*[66] analyzed the effect of 200 μmol/L of a *Ginkgo biloba* extract (quercetin, kaempfenol) for 24 h in HepG2 cells. This extract reduced triacylglycerols content due to the up-regulation of CPT-1a, ACO, FAS and Acetyl-CoA carboxylase β (Aca-β) gene expression and CPT-1a protein expression.

Lee *et al*[67] studied the effect of an extract of *Hibiscus sabdariffa L.* (including 8.83% protocatechuic acid, 9.97% catechin, 10.23% epigallocatechin, 20.20% epigallocatechin gallate, 18.10 caffeic acid) in BALB/c liver cells damaged with acetaminophen. Cells were treated with different doses of this polyphenol extract (0.05, 0.1, 0.5 or 1 mg/mL) for 48 h. The results showed that *Hibiscus sabdariffa L.* reduced triacylglycerol content. Moreover, it was able to eliminate the release of intermembrane proteins and to reduce cell death.

In vivo studies

Lee *et al*[67], in the study described in the previous section, also conducted an *in vivo* experiment in BABL/c mice showing liver damage produced by acetaminophen. Mice were treated with 3 doses of extract of *Hibicus sabdariffa L.* (0.01, 0.02 or 0.03% in the diet) for 2 wk. The study showed that this polyphenol extract reduced transaminases in a dose-dependent manner, and oxidative stress. Moreover, decreased liver damage and steatosis were shown by histopathological analysis.

Beltrán-Debón *et al*[68] administered an aqueous extract of *Aspalathus linearis L.* (rooibos; aspalathin, orientin, rutin) (10 g/L drinking water) for 14 wk to LDLr-/- mice, which is a model of metabolic alterations that resembles human metabolic syndrome in some aspects. This extract reduced serum triacylglycerols and free fatty acids. Moreover, histopathological analysis showed that steatosis degree was also lower in mice supplemented with rooibos extract.

Db/db mice fed a standard diet were used in the study reported by Tsuruta *et al*[69]. Animals were supplemented with an extract of *Nelumbo nucifera L.* (lotus root; proanthocyanidins, carechin, gallocatechin) at a dose of 0.5% in the diet for 3 wk. Lotus root extract reduced liver weight by 15% and triacylglycerol accumulation by 62%, but these changes did not reach statistical significance. Transaminases, which are hepatic injury markers, also tended to be lower (-24% ALT and -17% AST). In addition, the activity of lipogenic enzymes FAS and ME were decreased and CPT-1a, an enzyme related to fatty acid oxidation, remained unchanged.

In order to assess the effect of green tea, Axling *et al*[70] carried out a study with C57BL/6J mice fed a high-fat diet supplemented with 4% for 11 and 22 wk. Green tea (catechins) reduced serum triacylglycerols and ALT. In liver, this polyphenol extract reduced weight and the amount of triacylglycerols. All these results were observed during both treatment periods. In order to analyze the potential mechanisms of action, gene expression of SREBP-1c, PPAR-γ and ACC was assessed, but only in the group treated for 22 wk. All these genes showed down-regulation induced by green tea.

The effects of an extract of grape skin were tested by Park *et al*[71]. C57BL/6J mice were fed a high-fat diet supplemented with 0.15% of this extract for 10 wk. They showed decreased serum free fatty acids and leptin and increased adiponectin concentrations. With regard to liver, a reduction in triacylglycerols was observed. In order to determine the potential mechanisms of action, gene expression and activity of enzymes related to hepatic triacylglycerol metabolism were analyzed. Both the expression and the activity of enzymes involved in *de novo* lipogenesis, such as FAS, glucose-6-phosphate dehydrogenase (G6PDH) and ME and triacylglycerol synthesis, such as phosphatidate phosphohydrolase (PAP), were decreased. Accordingly, gene expression of PPAR-γ was also reduced. CPT-1a and PPAR-α mRNA levels, as well as β-oxidation were increased. Surprisingly, no changes were observed in CPT-1a activity.

Feillet-Coudray *et al*[72] carried out a study using Wistar rats fed a high-fat diet supplemented (0.2%) with

Table 5 ***In vivo*** **studies carried out with polyphenol extracts**

Ref.	Animal model	Polyphenols extract, dose and treatment period length	Effects	Mechanisms
Feillet-Coudray *et al*[72], 2009	Male Wistar rats fed a high-fat diet	Provinol®, a polyphenol extract obtained from red wine 0.2% in the diet 6 wk	↓ Macroesteatosis ↓ Lipid droplets ↓ Lipid peroxidation	
Aoun *et al*[73], 2010	Male Wistar rats fed a high fat diet	Provinol®, a polyphenol extract obtained from red wine 0.2% in the diet 6 wk	↓ Triacylglycerols ↓ Macroesteatosis = Fatty acid composition	↑ SIRT protein expression = SCD1, pAMPK, SREBP-1c, FAS, HNF-4 α, PGC1α and CPT-1a protein expression
Beltrán-Debón *et al*[68], 2011	LDLr-/- mice fed a standard diet	*Aspalathus linearis L.* (rooibos) 10 g/L drinking water 14 wk	Lower steatosis degree	↑ AMPK protein expression
Tsuruta *et al*[69], 2011	*db/db* mice fed a standard diet	*Nelumbo nucifera L.* (lotus root) 0.5% in the diet 3 wk	↓ 15% Liver weight (tendency) ↓ 62%Triacylglycerols (tendency) ↓ Transaminases (tendency)	= CPT-1a activity ↓ FAS, ME activity
Axling *et al*[70], 2012	C57BL/6 J mice fed a high fat diet	Green tea 4% in the diet 11 and 22 wk	↓ Liver weight ↓ Triacylglycerols ↓ Serum ALT	↓ SREBP-1c, PPAR-γ, ACC mRNA (22 wk)
Lee *et al*[67], 2012	BABL/c mice fed a standard diet Steatosis produced by acetaminophen	Extract of *Hibicus sabdariffa L* 0.01%, 0.02% or 0.03% in the diet 2 wk	↓ Liver damage ↓ Liver steatosis ↓ Serum ALT, AST (dose dependent) ↓ Oxidative stress	↓ p-JNK and AIF, tBid and Bax protein expression
Wang *et al*[66], 2012	Male Wistar rats fed a high-fat diet	Extract of *Ginkgo biloba* 0.25% in the diet 12 wk	↓ Triacylglycerols	↑ CPT-1a activity ↑ CPT-1a, Acaa1, Slc25a20, Hadh, ACO, PPAR-α, RXR-α mRNA expression, ↓ FAS mRNA expression
Park *et al*[71], 2013	C57BL/6 J mice fed a high-fat diet	Extract of grape skin 0.15% in the diet (160 mg/kg *bw* per day) 10 wk	↓ Triacylglycerols ↓ Serum leptin ↑ Serum adiponectin	↓ FAS, SCD1, PAP ↑CPT-1a, PPAR-α mRNA expressions and activities ↓ PPAR-γ, ↑ PPAR-α, CPT-1a mRNA expressions ↑ β oxidation, = CPT-1a activity
Yui *et al*[74], 2013	OLETF rats fed a standard diet	*Humulus lupulus L.* (hop pomace) 1% in the diet 70 d	↓Liver weight (tendency) ↓ Triacylglycerols (tendency)	↓ *de novo* lipogenesis = ACO, CPT-1a activity

↓: Decrease; ↑: Increase. SIRT: Sirtuin 1; SCD1: Stearoyl-CoA desaturase; AMPK: AMP-activated kinase; pAMPK: Phosphorylated AMP-activated kinase; SREBP-1c: Sterol regulatory element binding protein 1c; FAS: Fatty acid synthase; HNF-4α: Hepatocyte nuclear factor 4 α; PGC-1α: Peroxisome proliferator-activated receptor-c coactivator 1α; CPT-1a: Carnitine palmitoyltransferase 1a; ME: Malic enzyme; ALT: Alanine aminotransferase; PPAR-γ: Peroxisome proliferator activated receptor γ; ACC: Acetyl CoA carboxylase; pACC: Phosphorylated acetyl CoA carboxylase; AST: Aspartate aminotransferase; p-JNK: Activated c-Jun N-terminal kinase; AIF: Apoptosis inducing factor; Acaa1: acetyl-coenzyme A acyltransferase 1; Slc25a20: Solute carrier family 25, member 20; Hadh: Hydroxyacyl-coenzyme A dehydrogenase; ACO: Acyl-coenzyme A oxidase; PPAR-α: Peroxisome proliferator activated receptor α; RXR-α: Retinoid X receptor α; PAP: Phosphatidate phosphohydrolase.

of Provinol® (46.0% proanthocyanidols, 21.0% prodelphinidol, 6.1% anthocyanins, 3.8% cathechin, 3.0 % epicatechin gallate, 1.8% OH cinnamid acid, 1.4% quercetol; 0.15% resveratrol, 0.09 free anthocyanins) which is a polyphenol extract obtained from red wine for 6 wk. The histological analysis revealed a decrease in macroesteatosis and fat droplets in treated animals. A reduction in hepatic lipid peroxidation was also observed. The potential mechanisms of action underlying these effects were further reported by this research group[73]. Provinol® increased protein expression of SIRT1, without changing SCD1, SREBP-1c, FAS, hepatocyte nuclear factor 4 α (HNF-4α), PGC-1α, CPT-1a and phosphorylated AMPK. Moreover, a decrease in phosphorylated ACC was observed. Therefore, the authors suggested that the reduction in liver triacylglycerol accumulation was, at least in part, regulated by the inhibition of ACC, the limiting enzyme in *de novo* lipogenesis, probably through the activation of SIRT1 deacetylase.

Finally, Yui *et al*[74] carried out a study with OLEF rats fed a standard diet supplemented with 1% *Humulus lupulus L.* (hop pomace; flavonoids, procyanidins) for 70 d. Liver weight and hepatic triacylglycerol content showed a tendency towards reduced values. A reduction in *de novo* lipogenesis was observed, without changes in ACO and CPT-1a activity.

Summary

All the polyphenol extracts analyzed were able to reduce liver fat accumulation. As expected, the mechanisms underlying this effect were those reported in studies carried out with isolated polyphenols.

CONCLUSION

As a general conclusion, it can be stated that polyphenols are biomolecules which present hepatoprotective effects because they reduce liver fat accumulation and decrease oxidative stress and inflammation, the two main factors responsible for liver damage. To date, these beneficial effects have been demonstrated in cultured cells and animal models. Thus, studies performed in humans are needed before these molecules can be considered as truly useful tools in the prevention of liver steatosis.

REFERENCES

1 **Baffy G**, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012; **56**: 1384-1391 [PMID: 22326465 DOI: 10.1016/j.jhep.2011.10.027]

2 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

3 **Agopian VG**, Kaldas FM, Hong JC, Whittaker M, Holt C, Rana A, Zarrinpar A, Petrowsky H, Farmer D, Yersiz H, Xia V, Hiatt JR, Busuttil RW. Liver transplantation for nonalcoholic steatohepatitis: the new epidemic. *Ann Surg* 2012; **256**: 624-633 [PMID: 22964732 DOI: 10.1097/SLA.0b013e31826b4b7e]

4 **Dam-Larsen S**, Becker U, Franzmann MB, Larsen K, Christoffersen P, Bendtsen F. Final results of a long-term, clinical follow-up in fatty liver patients. *Scand J Gastroenterol* 2009; **44**: 1236-1243 [PMID: 19670076 DOI: 10.1080/00365520903171284]

5 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

6 **Angulo P**. Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? *Hepatology* 2010; **51**: 373-375 [PMID: 20101746 DOI: 10.1002/hep.23521]

7 **Lazo M**, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, Koteish A, Brancati FL, Clark JM. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013; **178**: 38-45 [PMID: 23703888 DOI: 10.1093/aje/kws448]

8 **Caballería L**, Pera G, Rodríguez L, Auladell MA, Bernad J, Canut S, Torán P. Metabolic syndrome and nonalcoholic fatty liver disease in a Spanish population: influence of the diagnostic criteria used. *Eur J Gastroenterol Hepatol* 2012; **24**: 1007-1011 [PMID: 22668875 DOI: 10.1097/MEG.0b013e328355b87f]

9 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

10 **Angulo P**, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, Haflidadottir S, Day CP, George J. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 782-789.e4 [PMID: 23860502 DOI: 10.1053/j.gastro.2013.06.057]

11 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]

12 **Hijona E**, Hijona L, Arenas JI, Bujanda L. Inflammatory mediators of hepatic steatosis. *Mediators Inflamm* 2010; **2010**: 837419 [PMID: 20300479 DOI: 10.1155/2010/837419]

13 **Anstee QM**, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 645-655 [PMID: 24061205 DOI: 10.1038/nrgastro.2013.182]

14 **Podrini C**, Borghesan M, Greco A, Pazienza V, Mazzoccoli G, Vinciguerra M. Redox homeostasis and epigenetics in non-alcoholic fatty liver disease (NAFLD). *Curr Pharm Des* 2013; **19**: 2737-2746 [PMID: 23092327 DOI: 10.2174/1381612811319150009]

15 **Valenti L**, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A, Taioli E, Fiorelli G, Fargion S. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **122**: 274-280 [PMID: 11832442 DOI: 10.1053/gast.2002.31065]

16 **Buechler C**, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* 2011; **17**: 2801-2811 [PMID: 21734787 DOI: 10.3748/wjg.v17.i23.2801]

17 **Weltman MD**, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 1998; **27**: 128-133 [PMID: 9425928 DOI: 10.1002/hep.510270121]

18 **Rao MS**, Reddy JK. Peroxisomal beta-oxidation and steatohepatitis. *Semin Liver Dis* 2001; **21**: 43-55 [PMID: 11296696 DOI: 10.1055/s-2001-12928]

19 **García-Monzón C**, Martín-Pérez E, Iacono OL, Fernández-Bermejo M, Majano PL, Apolinario A, Larrañaga E, Moreno-Otero R. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. *J Hepatol* 2000; **33**: 716-724 [PMID: 11097478 DOI: 10.1016/S0168-8278(00)80301-3]

20 **Pérez-Carreras M**, Del Hoyo P, Martín MA, Rubio JC, Martín A, Castellano G, Colina F, Arenas J, Solis-Herruzo JA. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* 2003; **38**: 999-1007 [PMID: 14512887 DOI: 10.1053/jhep.2003.50398]

21 **Angulo P**, Lindor KD. Non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2002; **17** Suppl: S186-S190 [PMID: 12000605 DOI: 10.1046/j.1440-1746.17.s1.10.x]

22 **Pessayre D**, Berson A, Fromenty B, Mansouri A. Mitochondria in steatohepatitis. *Semin Liver Dis* 2001; **21**: 57-69 [PMID: 11296697 DOI: 10.1055/s-2001-12929]

23 **Chitturi S**, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001; **21**: 27-41 [PMID: 11296694 DOI: 10.1055/s-2001-12927]

24 **Del Rio D**, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* 2013; **18**: 1818-1892 [PMID: 22794138 DOI: 10.1089/ars.2012.4581]

25 **Landete JM**. Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. *Crit Rev Food Sci Nutr* 2012; **52**: 936-948 [PMID: 22747081 DOI: 10.1080/10408398.2010.513779]

26 **Frémont L**. Biological effects of resveratrol. *Life Sci* 2000; **66**: 663-673 [PMID: 10680575]

27 **Gnoni GV**, Paglialonga G. Resveratrol inhibits fatty acid and triacylglycerol synthesis in rat hepatocytes. *Eur J Clin*

Invest 2009; **39**: 211-218 [PMID: 19260951 DOI: 10.1111/j.1365-2362.2008.02077.x]
28 **Wang GL**, Fu YC, Xu WC, Feng YQ, Fang SR, Zhou XH. Resveratrol inhibits the expression of SREBP1 in cell model of steatosis via Sirt1-FOXO1 signaling pathway. *Biochem Biophys Res Commun* 2009; **380**: 644-649 [PMID: 19285015 DOI: 10.1016/j.bbrc.2009.01.163]
29 **Zang M**, Xu S, Maitland-Toolan KA, Zuccollo A, Hou X, Jiang B, Wierzbicki M, Verbeuren TJ, Cohen RA. Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes* 2006; **55**: 2180-2191 [PMID: 16873680]
30 **Shang J**, Chen LL, Xiao FX, Sun H, Ding HC, Xiao H. Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. *Acta Pharmacol Sin* 2008; **29**: 698-706 [PMID: 18501116 DOI: 10.1111/j.1745-7254.2008.00807.x]
31 **Baur JA**, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006; **444**: 337-342 [PMID: 17086191 DOI: 10.1038/nature05354]
32 **Ahn J**, Cho I, Kim S, Kwon D, Ha T. Dietary resveratrol alters lipid metabolism-related gene expression of mice on an atherogenic diet. *J Hepatol* 2008; **49**: 1019-1028 [PMID: 18930334 DOI: 10.1016/j.jhep.2008.08.012]
33 **Kim S**, Jin Y, Choi Y, Park T. Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice. *Biochem Pharmacol* 2011; **81**: 1343-1351 [PMID: 21439945 DOI: 10.1016/j.bcp.2011.03.012]
34 **Cho SJ**, Jung UJ, Choi MS. Differential effects of low-dose resveratrol on adiposity and hepatic steatosis in diet-induced obese mice. *Br J Nutr* 2012; **108**: 2166-2175 [PMID: 22414733 DOI: 10.1017/s0007114512000347]
35 **Poulsen MM**, Larsen JØ, Hamilton-Dutoit S, Clasen BF, Jessen N, Paulsen SK, Kjær TN, Richelsen B, Pedersen SB. Resveratrol up-regulates hepatic uncoupling protein 2 and prevents development of nonalcoholic fatty liver disease in rats fed a high-fat diet. *Nutr Res* 2012; **32**: 701-708 [PMID: 23084643 DOI: 10.1016/j.nutres.2012.08.004]
36 **Alberdi G**, Rodríguez VM, Macarulla MT, Miranda J, Churruca I, Portillo MP. Hepatic lipid metabolic pathways modified by resveratrol in rats fed an obesogenic diet. *Nutrition* 2013; **29**: 562-567 [PMID: 23274094 DOI: 10.1016/j.nut.2012.09.011]
37 **Bujanda L**, Hijona E, Larzabal M, Beraza M, Aldazabal P, García-Urkia N, Sarasqueta C, Cosme A, Irastorza B, González A, Arenas JI. Resveratrol inhibits nonalcoholic fatty liver disease in rats. *BMC Gastroenterol* 2008; **8**: 40 [PMID: 18782455 DOI: 10.1186/1471-230X-8-40]
38 **Franco JG**, Lisboa PC, Lima NS, Amaral TA, Peixoto-Silva N, Resende AC, Oliveira E, Passos MC, Moura EG. Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning. *J Nutr Biochem* 2013; **24**: 960-966 [PMID: 22959054 DOI: 10.1016/j.jnutbio.2012.06.019]
39 **Kasdallah-Grissa A**, Mornagui B, Aouani E, Hammami M, Gharbi N, Kamoun A, El-Fazaa S. Protective effect of resveratrol on ethanol-induced lipid peroxidation in rats. *Alcohol Alcohol* 2006; **41**: 236-239 [PMID: 16517551 DOI: 10.1093/alcalc/agh256]
40 **Ajmo JM**, Liang X, Rogers CQ, Pennock B, You M. Resveratrol alleviates alcoholic fatty liver in mice. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G833-G842 [PMID: 18755807 DOI: 10.1152/ajpgi.90358.2008]
41 **Argilés JM**. The obese Zucker rat: a choice for fat metabolism 1968-1988: twenty years of research on the insights of the Zucker mutation. *Prog Lipid Res* 1989; **28**: 53-66 [PMID: 2682670 DOI: 10.1016/0163-7827(89)90007-6]
42 **Rivera L**, Morón R, Zarzuelo A, Galisteo M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol* 2009; **77**: 1053-1063 [PMID: 19100718 DOI: 10.1016/j.bcp.2008.11.027]
43 **Gómez-Zorita S**, Fernández-Quintela A, Macarulla MT, Aguirre L, Hijona E, Bujanda L, Milagro F, Martínez JA, Portillo MP. Resveratrol attenuates steatosis in obese Zucker rats by decreasing fatty acid availability and reducing oxidative stress. *Br J Nutr* 2012; **107**: 202-210 [PMID: 21733326]
44 **Timmers S**, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, Moonen-Kornips E, Hesselink MK, Kunz I, Schrauwen-Hinderling VB, Blaak EE, Auwerx J, Schrauwen P. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* 2011; **14**: 612-622 [PMID: 22055504 DOI: 10.1016/j.cmet.2011.10.002]
45 **Yoshino J**, Conte C, Fontana L, Mittendorfer B, Imai S, Schechtman KB, Gu C, Kunz I, Rossi Fanelli F, Patterson BW, Klein S. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. *Cell Metab* 2012; **16**: 658-664 [PMID: 23102619 DOI: 10.1016/j.cmet.2012.09.015]
46 **Somerset SM**, Johannot L. Dietary flavonoid sources in Australian adults. *Nutr Cancer* 2008; **60**: 442-449 [PMID: 18584477 DOI: 10.1080/01635580802143836]
47 **Bischoff SC**. Quercetin: potentials in the prevention and therapy of disease. *Curr Opin Clin Nutr Metab Care* 2008; **11**: 733-740 [PMID: 18827577 DOI: 10.1097/MCO.0b013e32831394b8]
48 **Zhang R**, Yao Y, Wang Y, Ren G. Antidiabetic activity of isoquercetin in diabetic KK -Ay mice. *Nutr Metab* (Lond) 2011; **8**: 85 [PMID: 22133267 DOI: 10.1186/1743-7075-8-85]
49 **Chirumbolo S**. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. *Inflamm Allergy Drug Targets* 2010; **9**: 263-285 [PMID: 20887269 DOI: 10.2174/187152810793358741]
50 **Jung CH**, Cho I, Ahn J, Jeon TI, Ha TY. Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. *Phytother Res* 2013; **27**: 139-143 [PMID: 22447684 DOI: 10.1002/ptr.4687]
51 **Perez-Vizcaino F**, Duarte J, Jimenez R, Santos-Buelga C, Osuna A. Antihypertensive effects of the flavonoid quercetin. *Pharmacol Rep* 2009; **61**: 67-75 [PMID: 19307694]
52 **Han JJ**, Hao J, Kim CH, Hong JS, Ahn HY, Lee YS. Quercetin prevents cardiac hypertrophy induced by pressure overload in rats. *J Vet Med Sci* 2009; **71**: 737-743 [PMID: 19578281]
53 **Vidyashankar S**, Sandeep Varma R, Patki PS. Quercetin ameliorate insulin resistance and up-regulates cellular antioxidants during oleic acid induced hepatic steatosis in HepG2 cells. *Toxicol In Vitro* 2013; **27**: 945-953 [PMID: 23348005 DOI: 10.1016/j.tiv.2013.01.014]
54 **Ying HZ**, Liu YH, Yu B, Wang ZY, Zang JN, Yu CH. Dietary quercetin ameliorates nonalcoholic steatohepatitis induced by a high-fat diet in gerbils. *Food Chem Toxicol* 2013; **52**: 53-60 [PMID: 23123425 DOI: 10.1016/j.fct.2012.10.030]
55 **Kobori M**, Masumoto S, Akimoto Y, Takahashi Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Mol Nutr Food Res* 2009; **53**: 859-868 [PMID: 19496084 DOI: 10.1002/mnfr.200800310]
56 **Kobori M**, Masumoto S, Akimoto Y, Oike H. Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. *Mol Nutr Food Res* 2011; **55**: 530-540 [PMID: 21462320 DOI: 10.1002/mnfr.201000392]
57 **Panchal SK**, Poudyal H, Brown L. Quercetin ameliorates

cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. *J Nutr* 2012; **142**: 1026-1032 [PMID: 22535755 DOI: 10.3945/jn.111.157263]
58 **Marcolin E**, San-Miguel B, Vallejo D, Tieppo J, Marroni N, González-Gallego J, Tuñón MJ. Quercetin treatment ameliorates inflammation and fibrosis in mice with nonalcoholic steatohepatitis. *J Nutr* 2012; **142**: 1821-1828 [PMID: 22915297 DOI: 10.3945/jn.112.165274]
59 **Marcolin E**, Forgiarini LF, Rodrigues G, Tieppo J, Borghetti GS, Bassani VL, Picada JN, Marroni NP. Quercetin decreases liver damage in mice with non-alcoholic steatohepatitis. *Basic Clin Pharmacol Toxicol* 2013; **112**: 385-391 [PMID: 23331460 DOI: 10.1111/bcpt.12049]
60 **Guo H**, Li D, Ling W, Feng X, Xia M. Anthocyanin inhibits high glucose-induced hepatic mtGPAT1 activation and prevents fatty acid synthesis through PKCζ. *J Lipid Res* 2011; **52**: 908-922 [PMID: 21343633 DOI: 10.1194/jlr.M013375]
61 **Baselga-Escudero L**, Bladé C, Ribas-Latre A, Casanova E, Salvadó MJ, Arola L, Arola-Arnal A. Grape seed proanthocyanidins repress the hepatic lipid regulators miR-33 and miR-122 in rats. *Mol Nutr Food Res* 2012; **56**: 1636-1646 [PMID: 22965541 DOI: 10.1002/mnfr.201200237]
62 **Pil Hwang Y**, Gyun Kim H, Choi JH, Truong Do M, Tran TP, Chun HK, Chung YC, Jeong TC, Jeong HG. 3-Caffeoyl, 4-dihydrocaffeoylquinic acid from Salicornia herbacea attenuates high glucose-induced hepatic lipogenesis in human HepG2 cells through activation of the liver kinase B1 and silent information regulator T1/AMPK-dependent pathway. *Mol Nutr Food Res* 2013; **57**: 471-482 [PMID: 23349077 DOI: 10.1002/mnfr.201200529]
63 **Yoshimura Y**, Nishii S, Zaima N, Moriyama T, Kawamura Y. Ellagic acid improves hepatic steatosis and serum lipid composition through reduction of serum resistin levels and transcriptional activation of hepatic ppara in obese, diabetic KK-A(y) mice. *Biochem Biophys Res Commun* 2013; **434**: 486-491 [PMID: 23583377 DOI: 10.1016/j.bbrc.2013.03.100]
64 **Luo XY**, Takahara T, Hou J, Kawai K, Sugiyama T, Tsukada K, Takemoto M, Takeuchi M, Zhong L, Li XK. Theaflavin attenuates ischemia-reperfusion injury in a mouse fatty liver model. *Biochem Biophys Res Commun* 2012; **417**: 287-293 [PMID: 22155236 DOI: 10.1016/j.bbrc.2011.11.102]
65 **Liu Y**, Wang D, Zhang D, Lv Y, Wei Y, Wu W, Zhou F, Tang M, Mao T, Li M, Ji B. Inhibitory effect of blueberry polyphenolic compounds on oleic acid-induced hepatic steatosis in vitro. *J Agric Food Chem* 2011; **59**: 12254-12263 [PMID: 21999238 DOI: 10.1021/jf203136j]
66 **Wang SD**, Xie ZQ, Chen J, Wang K, Wei T, Zhao AH, Zhang QH. Inhibitory effect of Ginkgo biloba extract on fatty liver: regulation of carnitine palmitoyltransferase 1a and fatty acid metabolism. *J Dig Dis* 2012; **13**: 525-535 [PMID: 22988926 DOI: 10.1111/j.1751-2980.2012.00627.x]
67 **Lee CH**, Kuo CY, Wang CJ, Wang CP, Lee YR, Hung CN, Lee HJ. A polyphenol extract of Hibiscus sabdariffa L. ameliorates acetaminophen-induced hepatic steatosis by attenuating the mitochondrial dysfunction in vivo and in vitro. *Biosci Biotechnol Biochem* 2012; **76**: 646-651 [PMID: 22484925]
68 **Beltrán-Debón R**, Rull A, Rodríguez-Sanabria F, Iswaldi I, Herranz-López M, Aragonès G, Camps J, Alonso-Villaverde C, Menéndez JA, Micol V, Segura-Carretero A, Joven J. Continuous administration of polyphenols from aqueous rooibos (Aspalathus linearis) extract ameliorates dietary-induced metabolic disturbances in hyperlipidemic mice. *Phytomedicine* 2011; **18**: 414-424 [PMID: 21211952 DOI: 10.1016/j.phymed.2010.11.008]
69 **Tsuruta Y**, Nagao K, Kai S, Tsuge K, Yoshimura T, Koganemaru K, Yanagita T. Polyphenolic extract of lotus root (edible rhizome of Nelumbo nucifera) alleviates hepatic steatosis in obese diabetic db/db mice. *Lipids Health Dis* 2011; **10**: 202 [PMID: 22067945 DOI: 10.1186/1476-511X-10-202]
70 **Axling U**, Olsson C, Xu J, Fernandez C, Larsson S, Ström K, Ahrné S, Holm C, Molin G, Berger K. Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. *Nutr Metab* (Lond) 2012; **9**: 105 [PMID: 23181558 DOI: 10.1186/1743-7075-9-105]
71 **Park HJ**, Jung UJ, Lee MK, Cho SJ, Jung HK, Hong JH, Park YB, Kim SR, Shim S, Jung J, Choi MS. Modulation of lipid metabolism by polyphenol-rich grape skin extract improves liver steatosis and adiposity in high fat fed mice. *Mol Nutr Food Res* 2013; **57**: 360-364 [PMID: 23109491 DOI: 10.1002/mnfr.201200447]
72 **Feillet-Coudray C**, Sutra T, Fouret G, Ramos J, Wrutniak-Cabello C, Cabello G, Cristol JP, Coudray C. Oxidative stress in rats fed a high-fat high-sucrose diet and preventive effect of polyphenols: Involvement of mitochondrial and NAD(P)H oxidase systems. *Free Radic Biol Med* 2009; **46**: 624-632 [PMID: 19135522 DOI: 10.1016/j.freeradbiomed.2008.11.020]
73 **Aoun M**, Michel F, Fouret G, Casas F, Jullien M, Wrutniak-Cabello C, Ramos J, Cristol JP, Coudray C, Carbonneau MA, Feillet-Coudray C. A polyphenol extract modifies quantity but not quality of liver fatty acid content in high-fat-high-sucrose diet-fed rats: possible implication of the sirtuin pathway. *Br J Nutr* 2010; **104**: 1760-1770 [PMID: 20673376 DOI: 10.1017/S0007114510002850]
74 **Yui K**, Uematsu H, Muroi K, Ishii K, Baba M, Osada K. Effect of dietary polyphenols from hop (Humulus lupulus L.) pomace on adipose tissue mass, fasting blood glucose, hemoglobin A1c, and plasma monocyte chemotactic protein-1 levels in OLETF rats. *J Oleo Sci* 2013; **62**: 283-292 [PMID: 23648402]

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