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Polyphenols and non-alcoholic fatty liver disease: impact and mechanisms

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Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic component of the metabolic syndrome and its prevalence is rapidly increasing due to its strong association with insulin resistance and obesity. At present, given that NAFLD is highly prevalent and therapies are limited, much attention is focused on identifying effective dietary strategies for the prevention and treatment of the disease. Polyphenols are a group of plant bioactive compounds whose regular consumption have been associated with a reduction in the risk of a number of metabolic disorders associated with NAFLD. Here we review the emerging and relatively consistent evidence from cell culture and rodent studies showing that select polyphenols positively modulate a variety of contributors to the NAFLD phenotype, through diverse and complementary mechanisms of action. In particular, the reduction of *de novo* lipogenesis (via sterol regulatory element-binding protein 1c) and increased fatty acid β -oxidation, presumably involving AMP-activated protein kinase activation, will be discussed. The indirect antioxidant and anti-inflammatory properties of polyphenols which have been reported to contribute to the amelioration of NAFLD will also be addressed. In addition to a direct study of the liver, rodent studies have provided insight into the impact of polyphenols on adipose tissue function and whole body insulin sensitivity, which are likely to in part modulate their impact on NAFLD development. Finally an overview of the limited data from clinical trials will be given along with a discussion of the dose extrapolation from animal studies to human subjects.

Flavonoids: Steatosis: Sterol regulatory element-binding protein 1c: PPAR α : Insulin resistance: Obesity

The term non-alcoholic fatty liver disease (NAFLD) refers to a condition defined by ectopic fat accumulation in the form of TAG in the liver, when it accounts for more than 5% of the total organ weight. NAFLD encompasses a wide spectrum of liver damages, ranging from simple TAG accumulation in hepatocytes (steatosis) to non-alcoholic steatohepatitis (NASH), which is characterised by the additional presence of inflammation and tissue injury^(1,2). NASH can lead to fibrosis, which can progress to cirrhosis with a high risk of liver failure and hepatocellular carcinoma⁽³⁾. NAFLD is a major public health issue in industrialised countries⁽³⁾, with an estimated prevalence in the general population of 20–

30%⁽²⁾. Most NAFLD patients are clinically asymptomatic with approximately 10–25% progressing to NASH and 5–8% of those will be susceptible to develop cirrhosis within 5 years. Furthermore, it has been reported that 12.8% of patients with liver cirrhosis will develop hepatocellular carcinoma within 3 years⁽⁴⁾.

NAFLD is considered to be the hepatic component of the metabolic syndrome, which is characterised by insulin resistance, obesity (>90% of NAFLD patients are overweight), hyperinsulinaemia, dyslipidaemia and hypertension^(3,5,6). Besides it is a significant risk factor for CVD, which is the most prevalent clinical feature of NAFLD⁽⁶⁾.

Abbreviations: AMPK, AMP-activated protein kinase; CPT, carnitine palmitoyl transferase 1; FA, fatty acids; HFD, high fat diet; HSE, *Hibiscus sabdariffa* L. extract; MCD, methionine and choline deficiency; JNK, c-Jun N-terminal kinase; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; Nrf2, nuclear factor-erythroid 2-related factor 2; SIRT-1, sirtuin 1; SREBP-1c, sterol regulatory element-binding protein 1c.

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Although a persistent elevation of plasma transaminase enzymes can be used as an early indication of liver damage, the accurate diagnosis of NAFLD presence and severity is not possible by routine blood tests. For an accurate and sensitive diagnosis of NAFLD a liver biopsy accompanied by histological staining and NAFLD activity scoring is considered the gold standard, but its use in clinical practice is limited by its invasive nature^(2,7,8).

At present, NAFLD due to its high prevalence and pathological consequences, represents an important economic burden for European countries⁽⁹⁾. However, to date, there is no licensed medication or surgical procedure for NAFLD. Lifestyle strategies such as dietary and exercise regimens focused on weight reduction and insulin sensitisation have been the primary therapeutic approach⁽³⁾. Although these strategies have been shown to be efficacious in randomised controlled trials, at a population level, due to poor compliance, they have had a limited impact on NAFLD incidence and severity⁽³⁾. Therefore there is a great need to identify effective approaches for NAFLD management.

Polyphenols are found ubiquitously in plants and their regular consumption has been associated with a reduction in the risk of a number of metabolic diseases, including obesity, insulin resistance, hypertension and CVD^(10,11). New evidence supports the idea that a polyphenol-rich diet may have an important role in the prevention and treatment of NAFLD. The purpose of the present review is to consider the efficacy of polyphenols in NAFLD and to discuss the key molecular mechanisms which modulate their potential clinical benefits.

Non-alcoholic fatty liver disease pathophysiology

NAFLD has a complex pathophysiology, which is described by the two-hit hypothesis⁽⁷⁾. In this model, the first hit describes the accumulation of fatty acids (FA) and TAG in hepatocytes leading to steatosis, which results from multiple mechanisms such as: (a) increased hepatic delivery and uptake of FA associated with increased lipolysis in adipose tissue and/or increased intake of dietary fat; (b) decreased FA oxidation; (c) increased hepatic *de novo* lipogenesis; and (d) decreased hepatic lipid export via VLDL^(7,12). The inability to regulate lipid partitioning leads to the second hit, whereby an overwhelmed FA β -oxidation produces mitochondrial dysfunction which increases reactive oxygen species resulting in sustained oxidative stress and a depletion of the antioxidant defences^(13,14). FA intermediates and a compromised oxidative status activates Kupffer cells producing inflammatory mediators, and dysregulated insulin action leading to the progression from benign steatosis to NASH^(3,13). Finally, chronic inflammation and oxidative stress induce hepatocyte apoptosis and injury which activates stellate cells which are central to the progression to liver fibrosis^(3,14).

Polyphenols: chemical structures and sources

Phenolic compounds are secondary metabolites of plants which are present in high amounts in fruits, vegetables, cereals and beverages such as red wine, tea or coffee. More than 8000 structures have been identified ranging from compounds with at least one aromatic ring with one or two hydroxyl groups, to polymers of up to fifty units with multiple hydroxyl groups. Generally, all phenolic compounds are commonly referred to as polyphenols, despite a group of them having only one aromatic ring. Polyphenols are divided into two main categories, namely flavonoids and non-flavonoids, based on the number of phenol rings and the way in which these rings interact⁽¹⁵⁾.

Flavonoids have a common basic structure of fifteen carbons ($C_6-C_3-C_6$) with two aromatic carbon rings (A and B rings) connected by a three-carbon bridge (C ring). Flavonoids may be sub-classified according to the degree of oxidation of the C-ring, the hydroxylation pattern of the ring structure and the substitution of the three-position into: (a) flavonols (e.g. quercetin and kaempferol) whose sources include onions and broccoli, (b) flavones (e.g. luteolin, apigenin) found in celery and parsley, (c) isoflavones (e.g. genistein and daidzein) found in leguminous plants and in particular soybeans and soya products, (d) flavanones (e.g. naringenin and hesperitin) abundant in citrus fruits, wine and herbs such as oregano, (e) anthocyanidins (e.g. cyanidin and peonidin) found in berry fruits and red wine, and (f) flavanols (e.g. (+)-catechin, (-)-epicatechin, epigallocatechin) abundant in cocoa and green tea^(11,15) (Fig. 1).

Non-flavonoids may be sub-classified into phenolic acids and stilbenes. Phenolic acid includes hydroxybenzoic acids (C_6-C_1) and hydroxycinnamic acids (C_6-C_3). Hydroxybenzoic acids (e.g. gallic acid) are found in pomegranate and raspberries. Hydroxycinnamic acids (e.g. caffeic acid) can be found in coffee beans and blueberries. Stilbenes have a $C_6-C_2-C_6$ structure. Resveratrol which is the main stilbene, can be found as *cis* or *trans* isomers as well as conjugated derivatives in grapes and red wine^(11,15) (Fig. 1).

Polyphenols have been identified as powerful antioxidants *in vitro*⁽¹⁶⁾. However, given their extensive metabolism and relatively low tissue concentrations, their *in vivo* preventative properties are considered largely independent of conventional antioxidant activities⁽¹⁶⁾. The ability of polyphenols to exert antioxidant properties *in vivo* depends on the extent of their phases 1 and 2 biotransformation and conjugation products during absorption in the gastrointestinal tract and post-absorption primarily in the liver. Although a full overview of polyphenols metabolism and its regulation is beyond the scope of the current review (see Rodriguez-Mateos *et al.*⁽¹¹⁾ for an extensive review), knowledge about their bio-kinetics (the composite of their distribution, biotransformation and elimination) alluded to throughout, is essential to understand the bioactivity of polyphenols *in vivo*⁽¹¹⁾.

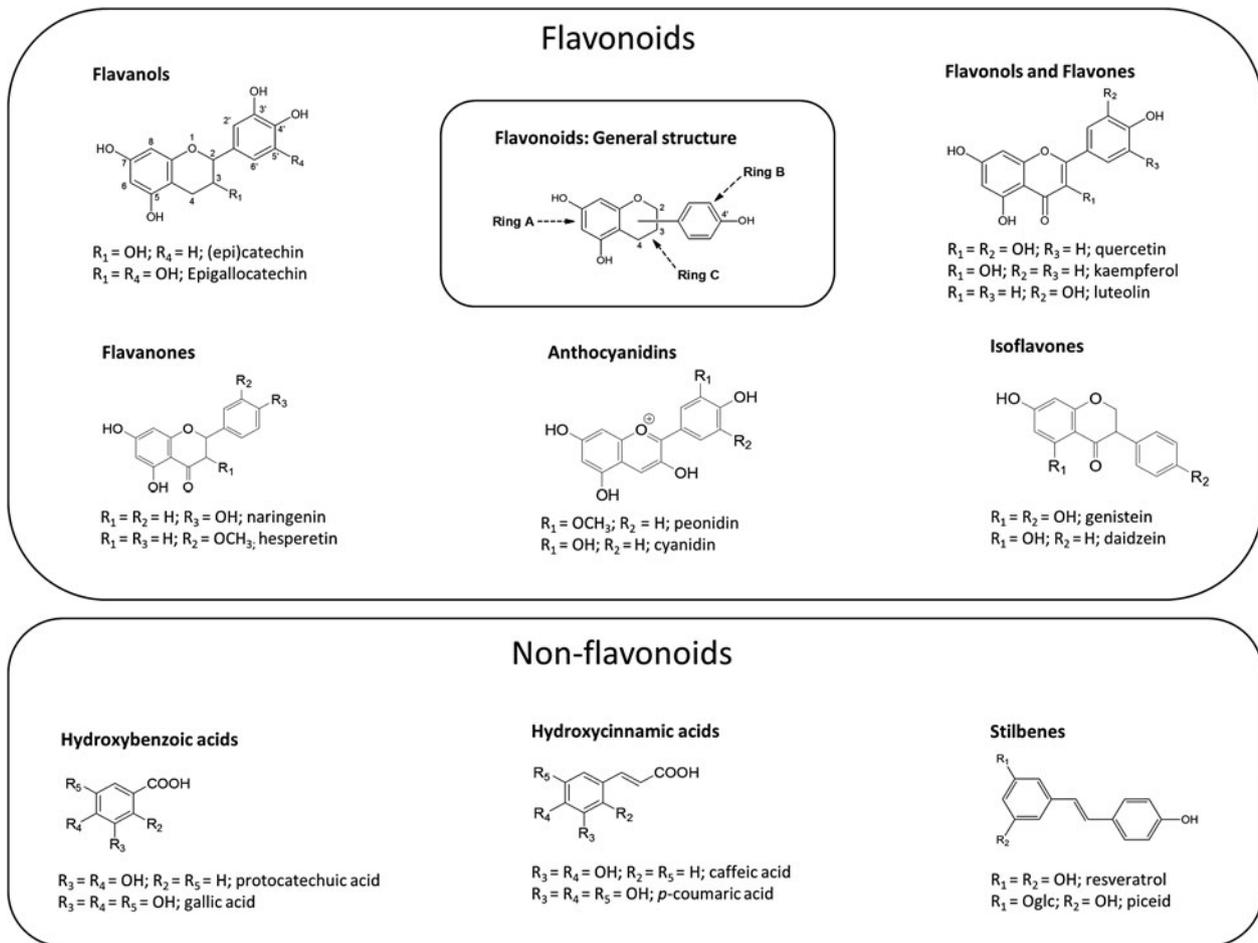


Fig. 1. Polyphenol structures.

In vitro studies

Cell culture studies constitute a useful tool to elucidate the molecular mechanisms of action of polyphenols in the prevention of steatosis. Primary cultures of human hepatocytes are the optimal cell culture model for studying determinants of NAFLD. However their widespread use is limited by logistical factors such as liver samples availability. The main alternative model is the human hepatocyte-derived cell line, HepG2.

Palmitic (16 : 0) and oleic (18 : 1n-9) acids are the most abundant FA in the liver of both normal subjects and NAFLD patients⁽¹⁷⁾ and have been used (generally in a bovine serum complex) to induce lipid accumulation in HepG2 and reproduce the key cellular features of human NAFLD⁽¹⁷⁻¹⁹⁾. In addition, steatosis in HepG2 cells has been induced by high concentrations of glucose (25–30 mM)^(20,21) which through a multistep process, including glycolysis and the Krebs Cycle generates acetyl-CoA, a key substrate for *de novo* lipogenesis⁽⁷⁾.

Pure polyphenol compounds and polyphenol-rich extracts have been tested in both these *in vitro* models of steatosis (Table 1). Most studies are concordant with the fact that a range of polyphenols reduce hepatocellular TAG accumulation induced by FA^(18,19,22-24) or by high

glucose concentrations^(20,21,25) with a range of reported mechanisms, including an inhibition of lipogenesis and a promotion of FA catabolism (Fig. 2).

Sterol regulatory element-binding protein 1c (SREBP-1c) is the most important transcription factor regulating genes involved in FA synthesis and TAG metabolism in the liver⁽²⁶⁾. A number of *in vitro* studies with polyphenols have shown a down-regulation of SREBP-1c and its main targets in lipogenesis^(20,22,24). In particular, Liu *et al.*⁽²²⁾ reported that luteolin induced a reduction of palmitate-stimulated lipid accumulation in HepG2 cells associated with decreased SREBP-1c and FA synthase gene expression and an attenuation of the activity of acetyl-CoA carboxylase. Acetyl-CoA carboxylase and FA synthase play an essential role in *de novo* lipogenesis converting the acetyl-CoA into palmitate that subsequently is esterified into TAG in the liver. Similar reduced expression of SREBP-1c and FA synthase were reported using a chlorogenic acid derivative (3-caffeoyl, 4-dihydrocaffeoylquinic acid) and rutin (quercetin-3-*O*-rutinoside) in a high glucose-stimulated and oleic-stimulated lipid accumulation HepG2 cell model, respectively^(20,24). In addition, treatment with 3-caffeoyl, 4-dihydrocaffeoylquinic acid, luteolin and rutin induced an activation (by

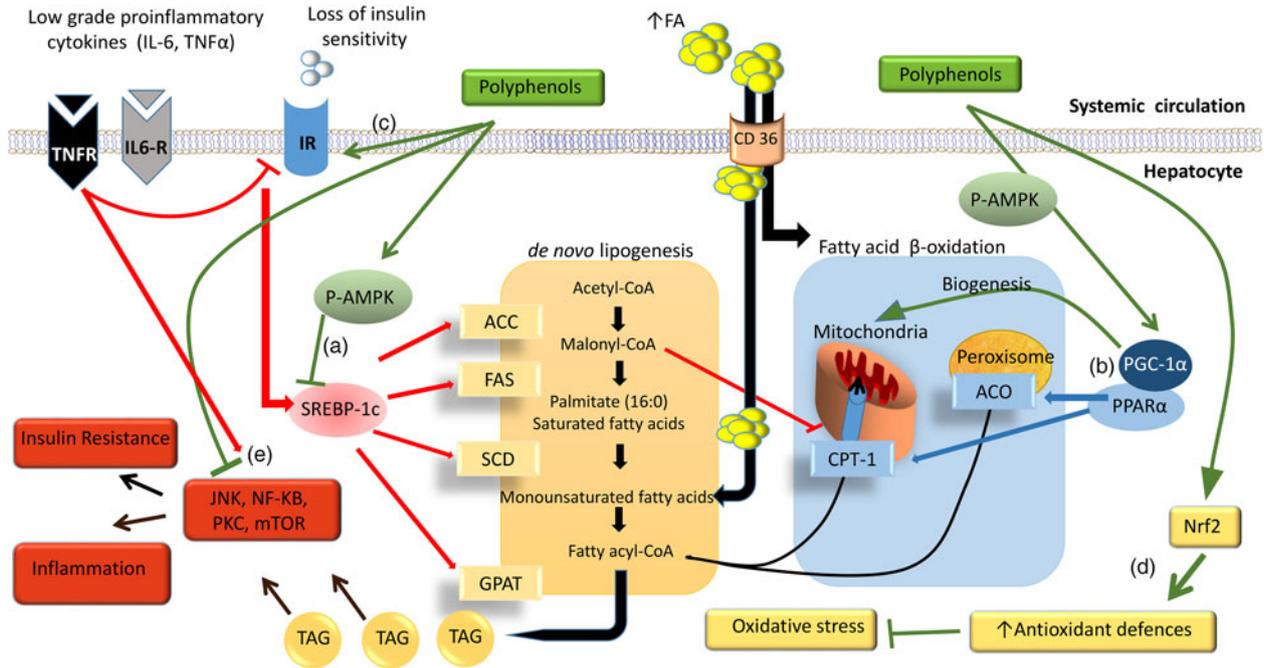


Fig. 2. (Colour online) Possible mechanisms underlying the effect of polyphenols in non-alcoholic fatty liver disease (NAFLD). Polyphenols may prevent cellular damage in hepatocytes associated with NAFLD through different mechanism of action including: (a) reducing *de novo* lipogenesis through sterol regulatory element-binding protein 1c (SREBP-1c) down-regulation, (b) increasing β -fatty acid (FA) oxidation by PPAR α up-regulation, (c) improving insulin sensitivity (d) reducing oxidative stress through increasing the antioxidant defence levels via nuclear factor-erythroid 2-related factor 2 (Nrf2), (e) attenuating the inflammatory pathways. Presumably SREBP-1c down-regulation and PPAR α up-regulation are modulated by AMPK activation (by phosphorylation). TNFR, TNF α receptor; IL6-R, IL-6 receptor; IR, insulin receptor; CD36, cluster of differentiation 36/FA translocase; p-AMPK, phosphorylated AMP-activated protein kinase α ; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD, stearoyl-CoA desaturase; GPAT, glycerol-3-phosphate acyltransferase; CPT-1, carnitine palmitoyl transferase 1; ACO, acyl-CoA oxidase; PGC-1, PGC1 α , PPAR γ coactivator-1 α ; JNK, c-Jun N-terminal kinase; PKC, protein kinase C; mTOR, mammalian target of rapamycin.

phosphorylation) of AMP-activated protein kinase (AMPK), a well-known inhibitor of SREBP-1c and hence of lipogenesis^(20,22,24). Sirtuin 1 (SIRT-1) activation by polyphenols represents a downstream regulator of AMPK⁽²⁷⁾. Pil *et al.*⁽²⁰⁾ found that 3-caffeoyl, 4-dihydrocaffeoylquinic acid treatment increased SIRT-1 activity, suggesting that SIRT-1 may be involved in the AMPK-dependent reduction in SREBP-1c and FA synthase expression induced by polyphenols. Cyanidin-3-O- β -glucoside also attenuated *de novo* lipogenesis through an alternative pathway, increasing protein kinase C ζ activity and suppressing mitochondrial glycerol-3-phosphate acyltransferase 1 activation, the rate limiting enzyme which controls the first step of TAG synthesis from palmitate⁽²⁵⁾.

In the liver, PPAR α plays a pivotal role in FA metabolism by up-regulating the expression of numerous genes involved in FA oxidation as well as other processes which regulate cellular FA status such as receptor mediated FA uptake and lipoprotein assembly and secretion⁽²⁸⁾. As a consequence, activation of PPAR α is associated with decreased hepatic fat storage⁽⁷⁾. Oxidation of FA occurs within the mitochondria, peroxisomes and the endoplasmic reticulum and is regulated mainly through key rate limiting enzymes such

as carnitine palmitoyl transferase 1 (CPT-1) and acyl-CoA oxidase. In the outer membrane of mitochondria, (CPT-1) mediates the transfer of FA from the cytosol into the mitochondria prior to β -oxidation and acyl-CoA oxidase catalyses the first rate-limiting step in peroxisomal β -oxidation^(7,13). Procyanidin B1 (an epicatechin-(4 β →8)-catechin dimer) suppressed palmitic-stimulated lipid accumulation in HepG2 cells through an up-regulation of the acyl-CoA oxidase and (CPT-1) mRNA expression⁽¹⁹⁾. In addition to inhibiting lipogenesis, luteolin induced (CPT-1) gene expression in HepG2 challenged with palmitate⁽²²⁾. Furthermore, rutin increased PPAR α protein levels which was associated with a reduction in the lipid load in HepG2 cells⁽²⁴⁾.

It is well known that a number of polyphenols can indirectly act as antioxidants by inducing phase II antioxidant defences enzymes^(29–31). There is evidence suggesting that the antioxidant response can alleviate the cellular damage induced by oxidative stress during the progression of NAFLD⁽¹⁴⁾. Accordingly, Vidyashankar *et al.*⁽¹⁸⁾ reported that quercetin induced an increase in the activity of antioxidant cellular defences, such as catalase, glutathione peroxidase and superoxide dismutase and an increase of reduced glutathione levels. Likewise,

Table 1. Cell culture studies investigating the impact of polyphenols on non-alcoholic fatty liver disease

Polyphenol	Concentration	System studied	Effects	References
Luteolin	10–20 μM	HepG2 cells	↓TAG ↑p-AMPK α , ↑p-ACC protein expression ↓SREBP-1c, ↓FAS, ↑CPT-1 gene expression ↓ROS	(22)
Procyanidin B1	10–30 μM	HepG2 cells	↓TAG ↑ACO, ↑CPT-1 gene expression	(19)
3-caffeoyl, 4-dihydrocaffeoylquinic acid	1–10 μM	HepG2 cells	↓TAG ↓SREBP-1c, ↓FAS gene and protein expression ↑p-AMPK, ↑p-ACC protein expression ↑SIRT-1 activity	(20)
Quercetin	10 μM	HepG2 cells	↓TAG ↑Glucose uptake ↓Lipid peroxidation ↓DNA fragmentation ↑GSH/GSSG ratio ↓TNF α and IL-8 cytokines ↑CAT, ↑GPx, ↑SOD activities ↑Albumin ↓ALT	(18)
Rutin	100–200 μM	HepG2 cells	↓TAG ↑p-AMPK α , ↑PPAR α protein expression ↓SREBP-1c protein expression, ↓HMGCR, ↓GPAT, ↓FAS, ↓ACC gene expression ↑SOD, ↑GPx, ↑CAT protein expression	(24)
Cyanidin-3-O- β -glucoside	1–100 μM	HepG2 cells	↓TAG ↑PKC ζ protein phosphorylation and activity ↓GPAT translocation ↓mtGPAT activity	(25)
	50 μM	Primary hepatocytes of mice fed a HFD	↓death cell ↓ROS ↓Caspase-3,-9 protein and activity ↓Bax protein ↓JNK signalling, ↑AKT signalling	(32)
Chinese blueberry polyphenols-rich extract	20–100 $\mu\text{g/ml}$	HepG2 cells	↓TAG	(23)
Polyphenol extract of <i>Hibiscus sabdariffa</i> L.	0.05–1 mg/ml	BALB/c normal liver cells	↓Death cell, ↓Loss of mitochondrial membrane potential ↓p-JNK, ↓Bax, ↓tBid protein expression ↓MDA, ↑GSH levels ↑CAT activity	(74)

Arrow indicates an increase (↑) or decrease (↓) in the levels of gene expression, protein concentrations or activity.

p-AMPK α , phosphorylated AMP-activated protein kinase α ; ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyl transferase 1; FAS, fatty acid synthase; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element-binding protein 1c; p-ACC, phosphorylated ACC; SIRT-1, sirtuin-1; GSH, reduced glutathione; GSSG, oxidised glutathione; TNF α , tumor necrosis factor α ; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; ALT, alanine aminotransferase; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; GPAT, glycerol-3-phosphate acyltransferase; mtGPAT, mitochondrial GPAT; PKC, protein kinase C; p-JNK, phosphorylated, c-Jun N-terminal kinase; MDA, malondialdehyde; HFD, high-fat diet.

rutin attenuated the cellular oxidative stress induced by oleic acid through raised superoxide dismutase, glutathione peroxidase and catalase protein levels which was associated with an increase in PPAR α protein levels⁽²⁴⁾. A sustained oxidative stress can induce hepatocyte

apoptosis and accentuate the transition from simple steatosis to NASH. Jiang *et al.*, showed that cyanidin-3-O- β -glucoside reduced oxidative stress and the apoptotic pathway activation induced by hyperglycaemia, preventing mitochondrial dysfunction through modulation of

phosphatidylinositol-3-kinase/protein kinase B and c-Jun N-terminal kinase (JNK) -signalling pathways⁽³²⁾.

Animal *in vivo* studies

Animal models of NAFLD can be divided in three major categories: those caused by a genetic mutation, by a dietary or pharmacological manipulation, or a combination of both models⁽³³⁾. The choice of model results in variability in the characteristics and severity of the NAFLD phenotype and its aetiological basis, with careful selection needed in order to address the specific research question in a meaningful way. For example, two of the most widely used dietary models of NASH, the high fat diet (HFD) and the methionine and choline deficiency (MCD) models display important differences in their metabolic characteristics. Although both present significant steatosis, mice fed a HFD develop obesity and insulin resistance which are characteristic of NAFLD and NASH in human subjects. Conversely mice fed a MCD exhibit atypical (for human subjects) weight loss and low serum insulin and leptin levels. However, the MCD model produces a more pathological form of NAFLD characterised by severe inflammation, oxidative stress, mitochondrial dysfunction, apoptosis and fibrogenesis, features which are only induced to a limited extent when using the HFD model⁽³³⁾. For evaluation of the efficacy of dietary approaches in NAFLD, the HFD may be chosen when evaluating the ability to prevent NAFLD development or for ameliorating steatosis, whereas the MCD model may be more appropriate to assess the therapeutic potential to reverse NASH associated liver injury.

Several studies have revealed that different subclasses of polyphenols ameliorate the severity and metabolic consequences of NAFLD in animal models. In general, liver biopsies (using haematoxylin/eosin staining) accompanied by semi-quantitative NAFLD activity scoring have shown that pure polyphenols or polyphenolic extracts reduced liver TAG accumulation^(34–38). However, the underlying molecular mechanisms associated with reduced steatosis are variable and dependant on the choice of animal model and the dose of phenolic compound of interest (Table 2 and Fig. 2).

Adipokine amelioration

NAFLD has been correlated with visceral adiposity and dysregulation of a variety of adipokines⁽³⁹⁾. Increased serum leptin levels are found in NAFLD patients and are correlated with the severity of hepatic steatosis⁽⁴⁰⁾. Adiponectin has been recently reported to hamper the excess lipid storage in the liver and decreased levels of this adipokine are observed in NASH patients⁽⁴¹⁾. In HFD-fed mice, dietary intake of the isoflavone genistein has been shown to reduce hepatic steatosis and adiposity. This 'anti-adiposity' effect has been associated with a modulation of adipokines gene expression, reducing leptin levels and increasing adiponectin levels in the adipose tissue⁽⁴²⁾. Likewise, in the HFD-fed mice model,

polyphenol-rich grape extract supplementation ameliorated abnormal plasma leptin and adiponectin levels which were associated with a reduction in NEFA⁽⁴³⁾. Collectively these results suggest that polyphenols could partially prevent the hepatic steatosis associated with obesity through improved regulation of adipokines.

Improvement of insulin sensitivity and de novo lipogenesis reduction

Postprandial insulin secretion promotes hepatic glucose uptake, and glycogen synthesis inhibits gluconeogenesis and stimulates *de novo* lipogenesis through SREBP-1c activation⁽¹³⁾. In obese-hyperinsulinaemic mice, insulin signalling fails to decrease gluconeogenesis but still stimulates lipogenesis through SREBP-1c up-regulation, producing liver hypertriglyceridaemia and hyperglycaemia⁽⁴⁴⁾. Using different NAFLD rodent models, resveratrol, genistein and an anthocyanin rich *Hibiscus sabdariffa* L. extract (HSE) have been shown to reduce insulin levels^(21,38,45) along with reducing *de novo* lipogenic gene and protein expression and their master regulator SREBP-1c^(38,42,43,45–47). In addition, in nSREBP-1c transgenic C57/BL6 male mice, which show severe insulin resistance and develop NASH, an epigallocatechin-3-gallate supplement improved insulin sensitivity and promoted the functional recovery of insulin receptor substrate-1⁽³⁴⁾.

Enhancement of β -fatty acid oxidation

An imbalance between lipogenesis and FA oxidation is central to the development and progression of steatosis/NASH. In this regard, an increase in the liver SREBP-1c:PPAR α ratio, due to an up-regulation of SREBP-1c and/or down-regulation of PPAR α , has been proposed to favour the development of steatosis in obese patients with NAFLD⁽⁴⁸⁾. In mice fed an HFD, anthocyanin-rich juice supplementation stimulated PPAR α up-regulation in parallel with a down-regulation of *de novo* lipogenic genes expression in the liver⁽⁴⁹⁾. Supplementation with isoflavones reduced liver steatosis by up-regulating genes involved in FA β -oxidation and down-regulating genes associated with lipogenesis in the adipose tissue⁽⁴²⁾. Vitaglione *et al.*⁽⁵⁰⁾ have also reported an up-regulation of PPAR α gene expression and a higher rate of β -oxidation in the liver of rats with NASH supplemented with coffee polyphenols extract as a mechanism to reduce fat deposition in the liver. In addition, resveratrol supplementation in rats fed a high fat-high sucrose diet activated PPAR γ co-activator 1 α , a co-factor of PPAR α in the induction of mitochondrial oxidative metabolism, associated with an increase in β -FA oxidation⁽⁵¹⁾.

Adenosine monophosphate-activated protein kinase as a key regulator in non-alcoholic fatty liver disease prevention

There is evidence that activation of AMPK is a central target for the effects of polyphenols in metabolic disorders related to NAFLD⁽⁵²⁾. Consistent with this

Table 2. Rodent studies evaluating the impact of polyphenols on non-alcoholic fatty liver disease

Polyphenol/extract	Intervention (dose and period)	Animal model	Mechanisms	References
EGCG	0.05–0.1 % of EGCG in water 12 weeks	Nuclear SREBP-1c transgenic C57BL/6J male mice	<ul style="list-style-type: none"> ↓Steatosis ↓Hepatocyte ballooning ↓Mallory-Denk body ↓ALT, TAG, total cholesterol and phospholipids in serum ↓Oxidative DNA damage ↓Nuclear SREBP-1c protein level in adipose tissue ↑IRS-1 and p-IRS1 protein level ↓p-AKT, pIKKβ and p-NF-κB protein level 	(34)
Genistein	1 g/kg diet 2 g/kg diet 4 g/kg diet 12 weeks	C57BL/6J mice fed a (36 %) HFD	<ul style="list-style-type: none"> ↓Body weight ↓Fat mass ↓Total lipids, TAG and total cholesterol in liver and serum ↑HDL-C and ↓NEFA in serum ↓Serum ALT ↓Hypertrophy of adipocytes ↑PPARα, ↑AMPK, ↓ACC2 gene expression ↓SREBP-1c, ↓PPARγ, ↓LXRα gene expression ↓Leptin, ↓TNFα, ↑adiponectin gene expression (adipose tissue) 	(42)
	4 mg/kg bw 40 mg/kg bw 160 mg/kg bw (injected/d) (5 d)	Neonatal male Sprague–Dawley rats fed a (60 %) HFD	<ul style="list-style-type: none"> ↓Body weight ↓Steatosis, ↓TAG ↓Hepatic inflammation ↓Hepatocyte apoptosis ↓Plasma Insulin and ALT ↑Plasma Glucagon ↓FAS, ↓SREBP-1c, ↓TNFα gene and protein expression ↑PPARα, ↑CPT-1 gene and protein expression 	(45)
	4 mg/kg bw/d 8 mg/kg bw/d 12 weeks	Male Sprague–Dawley rats fed a (60 %) HFD	<ul style="list-style-type: none"> ↓ALT and AST ↓TBARS ↓ inflammation score ↓serum/liver TNFα and IL-6 cytokines and mRNA ↓p-JNK and ↑ IκB protein levels ↓ NF-κB nuclear translocation 	(35)
Quercetin	50 mg/kg/d 4 weeks	Male C57BL/6 mice fed a methionine- and choline-deficient diet	<ul style="list-style-type: none"> ↓Steatosis, inflammation and ballooning –score ↓ALT and AST ↓TBARS ↑CAT activity 	(36)
	50 mg/kg/d 2 weeks 4 weeks	Male C57BL/6 mice fed a methionine- and choline-deficient diet	<ul style="list-style-type: none"> ↓Steatosis, inflammation and ballooning –score ↓αSMA (fibrosis marker) ↓TBARS ↓ALT and AST ↓TBARS ↓TLR-4, ↓p-JNK and ↓NF-κB protein-levels ↓Pro-inflammatory and pro-fibrotic gene expression 	(62)
Resveratrol	100 mg/kg bw/d 8 weeks	Male Wistar rats fed a (60 %) HFD	<ul style="list-style-type: none"> ↓TAG, ↓steatosis ↑Mitochondria content in liver tissue ↑UCP2 	(75)

Table 2. (Cont.)

Polyphenol/extract	Intervention (dose and period)	Animal model	Mechanisms	References
Resveratrol	100 mg/kg bw/d 10 weeks	Male Wistar rats fed a (59 %) HFD	↓Body weight ↓TAG ↓Serum Insulin ↑p-AMPK protein level ↓SREBP-1c and ↓FAS gene expression	(21)
	10 mg/kg bw/d 4 weeks	Male Wistar rats. Steatosis induced by feeding rats <i>ad libitum</i> a high carbohydrate-fat-free diet for 4 d per week and then fasting them the remaining 3 d (4 weeks)	↓Steatosis ↓TNF α ↓Lipid peroxidation ↓NOS, ↑CAT, ↑SOD and ↑GPx liver-activities	(60)
	30 mg/kg bw/d 6 weeks	Male Sprague-Dawley rats fed an HF-HS diet (6 weeks)	↓TAG ↑ACO and ↑CPT-1 activity ↑p-AMPK and ↑p-ACC protein levels ↑PGC1 α activation	(51)
	15 mg/kg bw/d 45 mg/kg bw/d 6 weeks	Male Zucker (<i>fa/fa</i>) rats fed a standard diet	↓Body and liver weight ↓Fat mass ↓Hepatic TAG and ↓Steatosis ↓Total cholesterol, ↓HDL- and non-HDL-cholesterol (serum) ↑Serum insulin ↓Serum AST, ALT and ALP ↓TBARS ↑GSH:GSSG ratio ↑ACO and ↑CPT-1 activity	(37)
	30 mg/kg/d bw 60 d	Male FVB/N mice fed a (61 %) HFD	↓Body weight ↓Total cholesterol, ↓TAG, ↑HDL-cholesterol, ↓Transaminases, ↓insulin plasma levels ↓ACC, ↓PPAR γ , ↓SREBP-1c gene expression ↓IL-6, ↓TNF α , ↓NF- κ B gene expression ↑SIRT-1 gene expression	(46)
Resveratrol	50 mg/kg bw 4 weeks	C56BL/6J wild-type and autophagic mediator ULK1 heterozygous knockout, fed a (23 %) HFD for 8 weeks plus 4 weeks of resvestrol and HFD	↓Steatosis ↓Hepatic fibrosis ↓Body weight ↓ALT and AST ↓Insulin resistance ↓MDA ↓SREBP-1c gene expression ↑Adiponectin gene expression ↑GPx gene expression ↓IL-6↓TNF α gene expression ↓NF- κ B activity, ↑I κ B α protein level ↑Autophagic pathways	(59)
Polyphenol extract from red grapes	2 g/Kg diet 6 weeks	Male Wistar rats fed a high fat-high sucrose diet	↓Steatosis, ↓TAG ↑SIRT-1, ↑p-ACC protein level	(76)
Polyphenol-rich grape skin extract	160 mg/kg bw/d 10 weeks	C57BL/6J mice fed a (20 %) HFD	↓Body weight ↓Fat mass ↓Adiposity and hepatic steatosis ↓Hepatic cholesterol and TAG ↓ChREBP, ↓PPAR γ , ↓SCD1 ↓FAS, ↓ACAT, ↑PPAR α , ↑CPT gene expression ↑ β -oxidation, FAS, ME activity ↓Leptin, ↑adiponectin, ↓NEFA in plasma	(43)

Coffee polyphenols extract	4.2 mg of polyphenols/d 8 weeks	Male Wistar rats fed a (58 %) HFD for 4 weeks plus 8 weeks with coffee polyphenols extract and HFD	↓ALT plasma ↓Lipid droplets and inflammatory infiltration ↓TNF α ↑PPAR α and Adipo R2 gene expression ↑GSH/GSSG ratio ↓MDA ↑FRAP in serum ↑IL-4 and IL-10 ↓IL-1a and IL-1b	(50)
Roibos (<i>Aspalathus linearis</i>) polyphenol extract	2.5 g of polyphenols/l of water. 14 weeks	Male C57BL/6J LDLr ^{-/-} mice fed with chow diet and HFD (20 % fat and 0.25 % cholesterol)	↓Steatosis, ↓TAG ↓Macrophage infiltration ↓Cholesterol and NEFA in serum ↑p-AMPK protein level ↑FAS protein level (adipose tissue)	(53)
Lotus root polyphenolic extract	4.46 g of polyphenols/kg diet 3 weeks	Male db/db mice (C57BLKS/J Iar ⁺ +Lepr ^{db} /+Lepr ^{db})	↓Liver weight and TAG ↓FAS and ME activity:	(47)
Polyphenol extract of <i>Hibiscus sabdariffa</i> L.	22 mg of polyphenols/kg/d 10 weeks	Male C57BL/6J mice deficient in LDL receptor fed a high-fat, high cholesterol diet (22 % fat and 0.32 % cholesterol)	↓Body weight ↓TAG, ↓Steatosis ↓Adipocyte size in adipose tissue ↓Insulin resistance ↓miR-103, ↓miR-107 and ↑miR-122 expression in liver ↓FAS, ↑p-AMPK protein levels ↓SREBP-1c gene expression Q3 G accumulates in cells and tissues	(38)
Anthocyanin rich-orange juice	0.34 mg anthocyanin/d 12 weeks	C57BL/6J mice fed a (60 %) HFD	↓Steatosis ↓Body weight ↓TAG ↓Total cholesterol ↓Insulin resistance ↑PPAR α , ↑ACO gene expression ↓LXR α , ↓FAS and ↓GPAT1 gene expression	(49)

Arrow indicates an increase (↑) or decrease (↓) in the levels of expression or activity.

EGCG, epigallocatechin-3-gallate; ALT, alanine aminotransferase; SREBP-1c, sterol regulatory element-binding protein 1c; IRS-1, insulin receptor substrate-1; p-IRS-1, phosphorylated IRS-1; p-AKT, phosphorylated AKT; p-IKK β , phosphorylated inhibitor of κ B kinase β subunit; HFD, high fat diet; AMPK, adenosine monophosphate-activated protein kinase; ACC, acetyl-CoA carboxylase; LXR α , liver X receptor α ; FAS, fatty acid synthase; CPT-1, carnitine palmitoyl transferase; TBARS, thiobarbituric acid reactive substances; p-JNK, phosphorylated, c-Jun N-terminal kinase; κ B, inhibitor of κ B; AST, aspartate aminotransferase; CAT, catalase; α SMA, α -smooth muscle actin-positive cells; TLR, toll-like receptor; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; NOS, nitric oxide synthase, SOD, superoxide dismutase; GPx, glutathione peroxidase; ALP, alkaline phosphatase; UCP2, uncoupling protein 2; PGC1 α , PPAR γ coactivator-1 α ; GSH, reduced glutathione; GSSG, oxidised glutathione; ACO, acyl-Coenzyme A oxidase; SIRT-1, sirtuin-1; p-ACC, phosphorylated ACC, ChREBP, carbohydrate-responsive element-binding protein; SCD-1, stearoyl-CoA desaturase; ACAT, acyl-CoA:cholesterol acyltransferase; ME, malic enzyme; G6PD, glucose-6-phosphate dehydrogenase; MDA, malondialdehyde; FRAP, ferric reducing antioxidant power; miR, microRNA; Q3G, quercetin 3-O- β -D-glucuronide; GPAT1, glycerol-3-phosphate acyltransferase 1.

assumption, Beltran-Debón *et al.*^(38,53) have demonstrated that HSE and Rooibos extracts can prevent steatosis through AMPK activation in LDL receptor deficient mice (LDLr^{-/-}) fed a high fat–high cholesterol diet. Similarly, other studies have reported that the preventative effect of resveratrol on liver fat accumulation, through up-regulation of FA oxidation and down-regulation of lipogenesis, was at least in part mediated by the activation of the AMPK/SIRT-1 axis^(21,51). It has also been reported that AMPK in the liver enhances the ratio between β -oxidation and lipogenesis, via SREBP-1c down-regulation⁽⁵⁴⁾ and a promotion of mitochondrial content and function⁽²⁷⁾. Furthermore, AMPK stimulates β -FA oxidation indirectly through inhibition of acetyl-CoA carboxylase which synthesises

malonyl-CoA from acetyl-CoA⁽⁵⁵⁾. Malonyl-CoA has been described as an allosteric inhibitor of carnitine palmitoyl transferase 1⁽⁵⁶⁾. Therefore, acetyl-CoA carboxylase inactivation by AMPK reduces TAG synthesis but also enhances the FA influx to the mitochondria for β -FA oxidation⁽⁵⁵⁾. In consequence, the activation of AMPK by polyphenols has emerged as an important target in the prevention of NAFLD.

Antioxidant defences mechanisms prevent non-alcoholic fatty liver disease progression

NAFLD is characterised by oxidative stress and a redox imbalance generated in part as a consequence of insulin resistance and an accumulation of FA in

hepatocytes^(3,13). Elevated free radicals, lipid peroxidation and reduced antioxidants have been observed in NAFLD patients and animals models⁽¹³⁾. Nuclear factor-erythroid 2-related factor 2 (Nrf2) is the main transcription factor which maintains cellular redox status through downstream modulation of antioxidant defences genes⁽¹⁴⁾. It has been recently reported that Nrf2 knock-out mice (Nrf2^{-/-}) fed a HFD developed a more severe steatosis and inflammation than wild-type Nrf2 mice^(57,58) which indicates the hepato-protective role of Nrf2. It is widely accepted that numerous polyphenols can activate Nrf2 which in turn, induce a variety of antioxidant defence enzymes which would result in reduced oxidative stress^(29,30). Consistent with this statement, supplementation with quercetin, resveratrol and genistein have been reported to reduce lipid peroxidation in both the liver^(35–37,59,60) and serum⁽³⁵⁾ of NAFLD animals. Gomez-Zorita *et al.*⁽³⁷⁾ also reported a raised reduced-glutathione:oxidised glutathione ratio level and Bujanda *et al.*⁽⁶⁰⁾ an increase in the catalase, superoxide dismutase and glutathione peroxidase enzymatic activities in the liver of the NAFLD animals fed with resveratrol.

Anti-inflammatory effect preventing non-alcoholic fatty liver disease onset and progression

Inflammation is one of the main hallmarks of the progression from steatosis to NASH. It has been proposed that obesity promotes a systemic chronic low-grade inflammation which contributes to the development of metabolic disorders such as NAFLD⁽⁴⁾. TNF α and IL-6 are two of the main pro-inflammatory cytokines involved in the onset and progression of NAFLD which are secreted initially in the adipose tissue and later in the liver by Kupffer cells^(5,39). It has been described that the interaction of TNF α with its receptor inhibits insulin receptors and activates NF- κ B transcription factor and JNK pathways⁽¹²⁾. In addition increased hepatic and circulating TNF α and IL-6 levels have been observed in patients with NAFLD^(7,12). Recently, it has been proposed that a HFD can alter gut microbiota speciation and metabolism which e.g. via alterations in lipopolysaccharide production, can influence not only gastrointestinal, but also systemic inflammation⁽⁶¹⁾. In rodent models supplementation with different polyphenols reduced the inflammatory profile in the serum/liver induced by HFD or MCD contributing to the amelioration of fatty liver dysfunction^(35,46,50,59,62). In particular, studies using genistein, quercetin and resveratrol suggested that this anti-inflammatory effect was achieved through the repression of NF- κ B translocation or gene expression^(35,46,62) as well as a diminution in the JNK phosphorylation protein levels^(35,62). Adiponectin is also involved in the anti-inflammatory response^(7,39). The enhanced adiponectin secretion and gene expression induced by polyphenol-rich grape extract⁽⁴³⁾ and genistein⁽⁴²⁾ (see earlier) may also contribute to reduced hepatic inflammation and ultimately the progression of NAFLD.

Clinical trials

To the best of our knowledge, only five human randomised controlled trials (all with a double-blinded placebo-controlled design) focused on polyphenols and NAFLD, have been published to date (Table 3). Three were undertaken with 500 and 600 mg resveratrol for 12 weeks^(63,64) or 3000 mg for 8 weeks⁽⁶⁵⁾. The other two studies were carried out using an HSE (about 150 mg polyphenols)⁽⁶⁶⁾ or a bayberry juice (500 ml equivalent to 1350 mg polyphenols)⁽⁶⁷⁾ for 12 and 4 weeks, respectively. Four out of the five studies have reported a significant impact of intervention on select characteristics of NAFLD. Chang *et al.* reported that anthropometric measures (body weight, BMI and waist:hip ratio) were significantly lower (1.4, 1.33 and 1.09 %, respectively) following intervention with HSE⁽⁶⁶⁾ but no changes were observed with bayberry juice⁽⁶⁷⁾. For the two clinical trials using a similar dose of resveratrol (500 and 600 mg) only one observed a reduction in anthropometric measurements. This apparent discrepancy is likely due to the fact that in one of the trials resveratrol intervention was accompanied by a change in lifestyle with patients advised to follow physical activity guidelines⁽⁶³⁾. With regard to hepatic function, two of the resveratrol interventions reduced the serum alanine transaminase concentrations by 15 %^(63,64) although no reduction was detected in the studies with other polyphenol extracts^(66,67). In addition, one of the interventions with resveratrol and the HSE showed a reduction in serum total- and LDL-cholesterol⁽⁶⁴⁾ and NEFA⁽⁶⁶⁾. A significant reduction in the homeostasis model assessment insulin resistance index associated with lower serum glucose levels following resveratrol supplementation was also reported⁽⁶⁴⁾. The clinical trials using the bayberry juice and resveratrol reported anti-inflammatory effects, with a reduction in serum cytokines (TNF α , IL-6 and IL-8)^(63,64,67) and increased serum adiponectin levels⁽⁶⁴⁾. In addition, one of the interventions with resveratrol reported a reduction in NF- κ B activity in the peripheral blood mononuclear cells⁽⁶³⁾.

None of the clinical trials conducted liver biopsies and therefore had histological data on the severity of NAFLD. Instead non-invasive approaches such as semi-quantitative liver ultrasound examinations were carried out. Employing this approach, Chang *et al.* reported that HSE supplementation significantly reduced (by about 15 %) the liver damage score⁽⁶⁶⁾ and among the clinical trials with resveratrol, only the one accompanied by a change in lifestyle observed a significant reduction in steatosis^(63,64). Finally, the non-beneficial effect of resveratrol observed at the higher supplementation dose⁽⁶⁵⁾ is likely due to a hormesis phenomenon, characterised by a low-dose stimulation and inhibition and a potentially detrimental effect at high-dose, which has been described for a number of bioactive compounds including resveratrol⁽⁶⁸⁾.

Doses of polyphenols: from animals studies to clinical trials

As discussed earlier, animal studies have been widely employed to assess the effects of a variety of polyphenols

Table 3. Clinical trials carried out with polyphenols in non-alcoholic fatty liver disease (NAFLD) subjects

Polyphenol/extract	Intervention dose	Study design	Subjects	Effects	References
Polyphenol extract of <i>Hibiscus sabdariffa L.</i>	150 mg polyphenols/d	Randomised double-blind placebo-controlled parallel study, 12 weeks	Male or non-pregnant females with BMI ≥ 27 kg/m ² , untreated fatty liver	↓Body weight ↓BMI ↓serum NEFA ↓Liver steatosis =ALT and AST ↓Albumin	(66)
Bayberry juice	1350 mg polyphenols/d	Randomised double-blind placebo-controlled, crossover study, 4 weeks	Subjects between 18–25 years with BMI ≥ 23.1 kg/m ² , and NAFLD diagnosis by ultrasonographic examination	=Anthropometric characteristics =HOMA-IR ↓Carbonyl group protein ↓TNF α ↓IL-8 ↓Polypeptide-specific antigen ↓Cytokeratin-18 fragment	(67)
Resveratrol	500 mg/d	Randomised double-blind placebo-controlled study, 12 weeks intervention, with a change in lifestyle	Patients > 18 years with evidence of NAFLD defined by ultrasonographic and fibroscan examination and high ALT levels	↓Body weight ↓BMI ↓Waist circumference ↓ALT ↓Steatosis ↓IL-6 ↓TNF α ↓CRP ↓NF-KB ↓Cytokeratin-18 fragment	(63)
	600 mg/d	Randomised double-blind, placebo-controlled trial, 12 weeks	Adult NAFLD patients confirmed by ultrasound examination, aged 20–60 years	=Anthropometric characteristics =Steatosis ↓ALT and AST ↓Glucose ↓HOMA-IR ↓Total and LDL cholesterol ↓TNF α ↑Adiponectin ↓Cytokeratin-18 fragment	(64)
	3000 mg/d	Double-blind randomised placebo-controlled, parallel study, 8 weeks	Males with BMI ≥ 25 kg/m ² and waist circumference ≥ 90 cm and evidence of steatosis by ultrasound examination	=Anthropometric characteristics =Steatosis =HOMA-IR ↑ALT and AST	(65)

Arrow indicates increase (↑) and decrease (↓) in the levels of expression or activity. HOMA-IR, homeostasis model assessment insulin resistance index; TNF α , tumour necrosis factor alpha; CRP, C-reactive protein; AST: aspartate aminotransferase; ALT, alanine aminotransferase

in NAFLD. However, the majority of these studies have used supra-physiological doses of compounds, with little consideration given to human equivalent doses⁽⁶⁹⁾. Taking resveratrol as an example, most of the pre-clinical studies in rats have employed doses ranging from 10 to 100 mg/kg body weight. Following allometric scaling calculations⁽⁶⁹⁾, such doses would equate to 97 and 970 mg resveratrol for a 60 kg person, although an estimated consumption in human subjects is only about 0.93 mg/d⁽⁷⁰⁾. Therefore from a dose perspective the majority of the rodent scientific literature provides little insight into

the likely benefits of dietary sourced resveratrol in human NAFLD, although such higher doses may be achievable through the consumption of resveratrol rich supplements.

However, the estimated intake of total polyphenols in Western populations is about 1–2 g/d with other polyphenols occurring in much higher amounts in the diet than resveratrol⁽⁷¹⁾, with most plant sources consisting of a combination of different compounds which collectively may have a much greater impact on liver health relative to the effect of each one in isolation. Thus,

more studies assessing possible additive and synergistic effects of polyphenol combinations commonly found in the diet are needed.

Conclusion

NAFLD is the major cause of chronic liver disease in Western countries and currently about 2–5 % of the population have NASH which is predicted to double by 2050^(72,73). As NAFLD is essentially a condition of overnutrition, and as there is a current lack of effective therapies, there is a great need to identify dietary approaches for NAFLD prevention and treatment. Taken together, the current cell and animal evidence suggests that a number of polyphenols could prevent steatosis and its progression to NASH. The mechanisms underlying such observations are likely to include improved adipokine regulation and insulin sensitivity, a decline in *de novo* lipogenesis (via SREBP-1c) and an increase in FA β -oxidation activity which would reduce the lipid load in the liver. Recent insights have proposed that the activation of the AMPK/SIRT-1 axis is the common trigger for the regulation of all these molecular processes. However, more experiments are required to verify this hypothesis. In addition, the indirect antioxidant and anti-inflammatory effects exerted by polyphenols are also likely to make a significant contribution to the amelioration of NAFLD. But to date results from clinical studies are limited and often shown a subtle effect in comparison with animal models. Further research in rodents and human subjects using dietary achievable doses of individual polyphenols or select combinations are needed.

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Conflict of Interest

None.

Authorship

I. R. R. wrote the manuscript and D. V. and A. M. M. critically reviewed, contributed to, and approved the final manuscript.

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