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 its strong association with insulin resistance and obesity. At present, given than NAFLD is highly prevalent and therapies are limited, much attention is focussed on identifying effective dietary strategies for the prevention and treatment of the disease. Polyphenols are a group of plants 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* SREBP-1c) and increased fatty acid β-oxidation, presumably involving AMPK activation, will be discussed. The 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 humans.

Flavonoids: Steatosis: SREBP-1c: PPARα: Insulin resistance: Obesity

Introduction

The term "non-alcoholic fatty liver disease" (NAFLD) refers to a condition defined by ectopic fat accumulation in the form of triglycerides (TG) in the liver, when it accounts for more than 5% of total organ weight. NAFLD encompasses a wide spectrum of liver damages, ranging from simple TG 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 Western industrialized 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 five years. Furthermore, Milic *et al.*, 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 (MS), which is characterised by insulin resistance, obesity (>90% of NAFLD patients are overweight), hyperinsulinemia, dyslipidaemia and hypertension[3,5,6]. NAFLD has emerged as a significant risk factor for CVD, which is the most prevalent clinical feature of NAFLD[6].

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/NASH a liver biopsy accompanied by histological staining and NAFLD activity scoring (NAS) 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 focussed 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 highlight the efficacy of polyphenols in NAFLD and discuss the key molecular mechanisms which modulate their potential clinical benefits.

NAFLD 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 TG 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 visceral and subcutaneous 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 (ROS) 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 hepatocytes 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 50 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. These compounds are divided into two main categories, namely flavonoids and non-flavonoids, based on the number of phenols rings and the way in which these rings interact[15].

Flavonoids have a common basic structure of 15 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 3-position into: a) flavonols (e.g., quercetin, 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 soy products, d) flavanones (e.g., naringerin, hesperitin) abundant in citrus fruits, wine and herbs such as oregano, e) anthocyanidins (e.g., cyanidin, peonidin) found in berry fruits and red wine, and f) flavanols (e.g., (+)-catechin, (-)-epicatechin, epigalocatechin) abundant in cocoa and green tea[11,15] (Figure 1).

Non-flavonoids may be sub-classified into phenolic acids and stilbenes. Phenolic acid includes hydroxybenzoic acid (C_6-C_1) and hydroxycinnamic acids (C_6-C_3). Hydroxybenzoic acids (e.g., gallic acid, ellagic 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_3-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] (Figure 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 phase 1 and 2 biotransformation and conjugation 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].

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 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 (18:1n9) 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[17,18,19]. In addition, steatosis in HepG2 cells has been induced by high concentrations of glucose (25-30mM)[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 polyphenols 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 TG accumulation induced by fatty acids[18,19,22,23,24] 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 (Figure 2).

Sterol regulatory element-binding protein 1c (SREBP-1c) is the most important transcription factor regulating genes involved in fatty acid synthesis and TG 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 fatty acid synthase (FAS) gene expression and an attenuation of the activity of acetyl-coenzyme A carboxylase (ACC). ACC and FAS play an essential role in *de novo* lipogenesis converting the acetyl-CoA into palmitate that subsequently is esterified into TG in the liver. Similar reduced expression of SREBP-1c and FAS were reported using a chlorogenic acid derivate (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

phosphorylation) of the adenosine monophosphate-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 FAS expression induced by polyphenols. Cyanidin-3-*O*-β-glucoside (C3G) also attenuated *de novo* lipogenesis through an alternative pathway, increasing PKC ζ activity and suppressing mitochondrial glycerol-3-phosphate acyltransferase 1 (mtGPAT1) activation, the rate limiting enzyme which controls the first step of TG synthesis from palmitate[25].

In the liver, PPARα plays a pivotal role in FA metabolism by up-regulating the expression of numerous genes involved in FA oxidation as well as numerous other processes which regulate cellular FA status such as receptor mediated FA uptake and lipoprotein assembly and secretion[28]. As a consequence, activation of PPARα is associated with decreased hepatic fat storage[7]. Oxidation of FAs occurs within the mitochondria, peroxisomes and the endoplasmic reticulum (ER) and is regulated mainly through key rate limiting enzymes such as carnitine palmitoyl transferase 1 (CPT1) and acyl-CoA oxidase (ACO). In the outer membrane of mitochondria, CPT1 mediates the transfer of FAs from the cytosol into the mitochondria prior to β-oxidation and ACO catalyses the first rate limiting step in peroxisomal β-oxidation[7,13]. Procyanidin B1 (an epicatechin-(4β→8)-catechin dimer) suppressed palmitic-stimulated lipid accumulation in HepG2 cells through an up-regulation of the ACO and CPT1 mRNA expression[19]. In addition to inhibiting lipogenesis, luteolin, induced CPT1 gene expression in HepG2 challenged with palmitate[22]. Furthermore, rutin increased PPARα 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[29,30,31]. 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 (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and an increase of reduced glutathione (GSH) levels. Likewise, rutin attenuated the cellular oxidative stress induced by oleic acid through raised SOD, GPx and CAT protein levels which was associated with an increase in PPARα 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 C3G reduced oxidative stress and the apoptotic pathway activation induced by hyperglycaemia preventing mitochondrial dysfunction through modulation of PI3K/AKT and 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) display important differences in their metabolic characteristics. Although both models present significant steatosis, mice fed a HFD develop obesity and insulin resistance which are characteristic of NAFLD and NASH in humans. In the other hand, mice fed a MCD exhibit atypical (for humans) 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 NAS scoring have shown that pure polyphenols or polyphenolic extracts reduced liver TG accumulation[34,35,36,37,38]. 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 Figure 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-obesity" 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, glycogen synthesis, inhibits gluconeogenesis and stimulates *de novo* lipogenesis through SREBP-1c activation[13]. In obese-hyperinsulinemic mice, insulin signalling fails to decrease gluconeogenesis but still stimulates lipogenesis through SREBP-1c up-regulation, producing liver hypertriglyceridemia and hyperglycemia[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 [38,42,43,45,46,47]. In addition, in nSREBP-1c transgenic C57/BL6 male mice, which show severe insulin resistance and develop NASH, a epigallocatechin-3-gallate supplement improved insulin sensitivity and promoted the functional recovery of insulin receptor substrate-1 (IRS-1)[34].

Enhancement of β-fatty acid oxidation: An imbalance between lipogenesis and fatty acid 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 a 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 fatty acid 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 coactivator 1α (PGC1-α), a co-factor of PPAR-α in the induction of mitochondrial oxidative metabolism, associated with an increase in β-fatty acid oxidation[51]

AMPK as a key regulator in NAFLD 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 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 in liver fat accumulation, through up-regulation of fatty acid 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-fatty acid oxidation indirectly through inhibition of ACC which synthetises malonyl-CoA from acetyl-CoA[55]. Malonyl-CoA has been described as an allosteric inhibitor of CPT-1[56]. Therefore, ACC inactivation by AMPK reduces TG synthesis but also enhances the fatty acids influx to the mitochondria for β-fatty acid 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 NAFLD 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 knockout 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, induces 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[35,36,37,59,60] and serum[35] of NAFLD animals. Gomez-Zorita *et al.*[37] also reported a raised GSH/GSSG ratio level and Bujanda *et al.*[60] an increase in the CAT, SOD and GPx enzymatic activities in the liver of the NAFLD animals fed with resveratrol.

Anti-inflammatory effect preventing NAFLD 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 interaction of TNF-α with its receptor (TNFR) inhibits insulin receptors and activates NF-κB 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 LPS 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-κB 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]. Then, the enhanced adiponectin secretion and gene expression induced by polyphenol-rich grape extract[43] and genistein[42] (see above) 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 500mg and 600mg of resveratrol for 12 weeks[63,64] or 3000mg for 8 weeks[65]. The other two studies were carried out using a HSE (about 150 mg of polyphenols)[66] or a bayberry juice (500mL equivalent to 1350 mg of 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 characteristics (body weight, body mass index 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 600mg) 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 with 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 alanine transaminase liver enzyme (ALT) in serum by 15%[63,64] although no reduction were 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 (HOMA-IR) 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 (TNFα, IL-6 and IL-8)[63,64,67] and increased serum adiponectin levels[64]. In addition, one of the intervention with resveratrol reported a reduction in NF-κB 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%) liver score damage[66] and among the clinical trial with resveratrol, only the one accompanied with

a change in the lifestyle observed a significantly 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, characterized by a low-dose stimulation and inhibition and a potentially detrimental effect at high-dose, which has been described for a number bioactive compounds including resveratrol[68].

Doses of polyphenols: from animals studies to clinical trials

As discussed above, animal studies have been widely employed to assess the effects of a variety of polyphenols in NAFLD. However, the majority of these studies have used supra-physiological doses of compounds which are often found in low concentrations in the diet, 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 of body weight. Following allometric scaling calculations[69], such doses would equate to 97 and 970 mg of resveratrol for a 60 kg person. It should be pointed out that resveratrol is present in the diet in low amounts, with an estimated consumption in humans of about 0.93mg/day[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/day with other polyphenols occurring in much higher amounts in the diet than resveratrol[71], with most plant sources comprised 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 over-nutrition, 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 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 antioxidant and anti-inflammatory effect 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 to animal models. Further research in rodents and humans using dietary achievable doses of individual polyphenols or select combinations are needed.

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Conflict of interest

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Authorship

IRR wrote the manuscript and DV and AMM critically reviewed, contributed to, and approved the final manuscript.

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Figures caption

Figure 1: Polyphenol structures.

Figure 2: Possible mechanisms underlying the preventative effect of polyphenols in NAFLD. Polyphenols may prevent cellular damage in hepatocytes associated with NAFLD through different mechanism of action including: a) reducing *de novo* lipogenesis through SREBP-1c down-regulation, b) increasing β-fatty acid oxidation by PPAR-α up-regulation, c) improving insulin sensitivity d) reducing oxidative stress through increasing the antioxidant defence levels *via* Nrf2, e) attenuating the inflammatory pathways. Presumably SREBP-1c down-regulation and PPAR-α up-regulation are modulated by AMPK activation (by phosphorylation). TNFR, TNFα receptor; IL6-R, IL-6 receptor; IR, insulin receptor; FA, fatty acids; CD36, cluster of differentiation 36/FA translocase; p-AMPK, phosphorylated AMP-activated protein kinase α; SREBP-1c, sterol regulatory element-binding protein 1c; 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-Coenzyme A; PGC-1, PGC1α, PPAR-γ coactivator-1α; Nrf2, nuclear factor-erythroid 2-related factor 2; JNK, c-Jun N-terminal kinase; PKC, protein kinase C; mTOR, mammalian target of rapamycin.