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Special Article

Therapeutic potential of green tea in nonalcoholic fatty liver disease

Christopher Masterjohn and Richard S Bruno

Nonalcoholic fatty liver disease (NAFLD) is a constellation of progressive liver disorders that are closely related to obesity, diabetes, and insulin resistance and may afflict over 70 million Americans. NAFLD may occur as relatively benign, nonprogressive liver steatosis, but in many individuals it may progress in severity to nonalcoholic steatohepatitis, fibrosis, cirrhosis, and liver failure or hepatocellular carcinoma. No validated treatments currently exist for NAFLD except for weight loss, which has a poor long-term success rate. Thus, dietary strategies that prevent the development of liver steatosis or its progression to nonalcoholic steatohepatitis are critically needed. Green tea is rich in polyphenolic catechins that have hypolipidemic, thermogenic, antioxidant, and anti-inflammatory activities that may mitigate the occurrence and progression of NAFLD. This review presents the experimental evidence demonstrating the hepatoprotective properties of green tea and its catechins and the proposed mechanisms by which these targeted dietary agents protect against NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) describes a constellation of asymptomatic liver diseases that are thought to afflict over 70 million Americans.[1] No validated therapies for NAFLD currently exist beyond weight loss, which is well known to have a poor long-term success rate.[2] Green tea (*Camellia sinensis*) is a functional food rich in polyphenolic catechins (Figure 1).[3] Its consumption is associated with a lower risk of mortality due to cardiovascular disease and all causes[4] as well as lower concentrations of hepatological biomarkers.[5] Experimental evidence supports a role of green tea or its catechins in protecting against NAFLD by regulating energy homeostasis and decreasing oxidative stress and inflammatory responses.[6,7] Thus, this review provides an overview of the prevalence and etiology of NAFLD and describes the multifaceted bioactivities of green tea and its catechins that may protect against this potentially debilitating disorder. While the protective effects of green tea extract (GTE) against obesity and diabetes have been comprehensively reviewed elsewhere,[8,9] this review provides the first integration of these findings with the hepatoprotective potential of GTE in the prevention and treatment of NAFLD.

NAFLD: PRIMARILY AN OBESITY-RELATED DISORDER

NAFLD is closely related to obesity,[10,11] insulin resistance,[12] and diabetes.[13,14] It is histologically similar to alcoholic fatty liver disease, but patients have no significant history of alcohol use or abuse.[15] NAFLD progresses from relatively benign simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis; it potentially contributes to liver-related morbidity or mortality due to liver failure or hepatocellular carcinoma.[16–18] Patients with elevated serum aminotransferases are often referred for liver biopsy,[19] but aminotransferases are neither sensitive nor specific for NAFLD,[19,20] and no better biomarkers currently exist. Thus, the lack of validated

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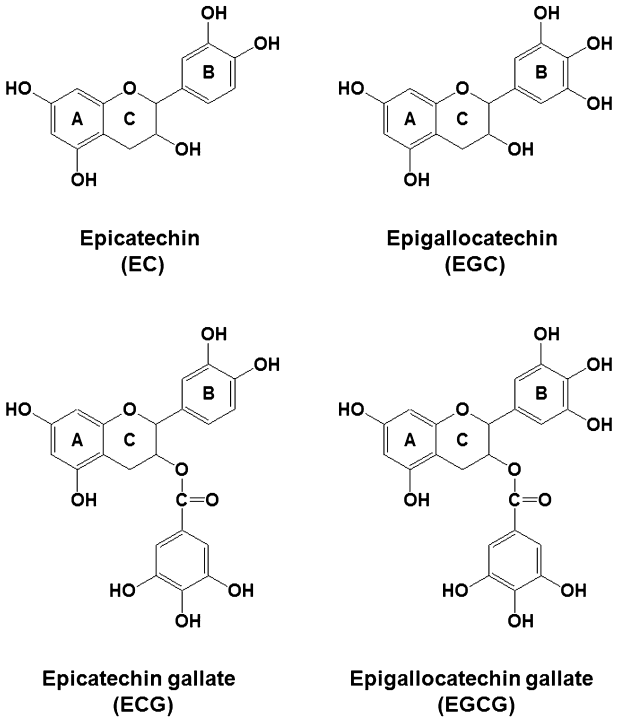

Figure 1 **Catechins (flavan-3-ols) are polyphenolic flavonoids that consist of a 2-phenyl benzopyran basic flavonoid structure with di- (epicatechin and ECG) or tri- (EGC and EGCG) hydroxy substitutions on the B ring and a 5,7-meta-dihydroxy substitution on the A ring and either an additional hydroxyl group (epicatechin and EGC) or a gallate ester (ECG and EGCG) at the C-3 position of the C ring.** Adapted from Wang and Ho.[93]

biomarkers and the relatively asymptomatic presentation of NAFLD strongly suggest that it is likely underdiagnosed. Although early estimates suggested approximately 40 million Americans may be afflicted with NAFLD, based on extrapolations from rates of obesity and diabetes,[21] more recent estimates based on population studies using magnetic resonance imaging[22] suggest that over 70 million Americans may have NAFLD.[1]

Classic studies in animal models of obesity due to hypothalamic damage[23] or genetics[24] produced fatty liver. Zelman[25] published the first case series of obese patients with NAFLD in 1952 after initially observing the disease in a hospital aide who consumed ≥20 bottles/day of sweetened soft drinks. However, NAFLD remained understudied and largely undiagnosed until Ludwig et al.[15] coined the term NASH in 1980. Autopsy[10,11] and ultrasonography studies[26,27] conducted since then have confirmed the relation between obesity and NAFLD, showing that rates of liver steatosis and NASH are 5–16-fold greater in obese individuals compared with lean individuals and that up to 76% of obese individuals may have liver steatosis.

The association between diabetes and fatty liver was recognized in the 1930s.[28] Studies from the modern scientific era have confirmed high rates of NAFLD in type I and type II diabetics[13,29] as well as the correlation between liver steatosis and insulin resistance independent of body mass index and intra-abdominal fat, even in nondiabetic individuals.[12] Albeit to a lesser extent than obesity and diabetes, lipodystrophy,[30] hepatotoxic drugs,[31] intestinal bypass surgery,[32,33] choline-deficient total parenteral nutrition,[34] abetalipoproteinemia, and Wilson's disease[15] can also cause NAFLD. Although there is no accurate means to track the longitudinal prevalence of NAFLD, the prevalence of diagnosed diabetes in the United States has increased from 5% to 8% since 1988,[35] and the prevalence of obesity has increased from 13% to 34% since 1960.[36] Thus, it is likely that the prevalence of NAFLD has closely paralleled the growing rates of diabetes and obesity.

Whether NAFLD is the cause or consequence of insulin resistance and obesity is controversial.[37] However, the independent importance of NAFLD has been supported by epidemiological studies showing that NAFLD is an independent predictor of cardiovascular disease.[13,38] Among type 1 diabetics, those with NAFLD were 7–10-fold more likely to have cardiovascular disease, and NAFLD was associated with cardiovascular disease independently of traditional risk factors.[13] In a 5-year prospective cohort study of apparently healthy Japanese, those with NAFLD at baseline were 4-fold more likely to develop cardiovascular disease.[38] When NAFLD and metabolic syndrome were combined in multivariate analysis, NAFLD persisted as an independent predictor of cardiovascular disease but metabolic syndrome did not.[38] These observations clearly emphasize the need to investigate hepatoprotective properties and not only the anti-obesogenic and antidiabetogenic properties of novel dietary agents.

TWO-HIT THEORY OF NASH

Liver biopsy is the gold standard approach for diagnosing the presence and severity of NAFLD.[39] Confirmation of hepatic steatosis upon histological examination requires lipid accumulation in >5% of hepatocytes. Histological grading of steatosis progresses as lipid accumulation increases from grade 1 (5–33%) to grade 2 (33–66%) and grade 3 (>66%).[40] Histological examination also allows characterization of hallmark features of NASH, including inflammatory cell infiltration, hepatocyte ballooning, necrosis, Mallory's hyaline, and fibrosis.[40]

The observation that simple steatosis does not always progress to more advanced stages of NAFLD[41] led to the "two-hit" hypothesis, wherein steatosis represents a "first hit" that may in and of itself be benign, but increases the vulnerability of the liver to a "second hit" in the form of

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oxidative or inflammatory insults that trigger the progression to NASH.[42] Increased *de novo* lipogenesis (DNL),[43,44] increased circulation of nonesterified fatty acids (NEFA) released from adipose,[45] decreased β-oxidation,[46] and impaired secretion of hepatic triglyceride[47] contribute to the first hit. Hepatic lipid peroxidation[48,49] due to the greater presence of oxidizable fat in steatotic livers[50] and to the greater generation of reactive oxygen and nitrogen species from increased microsomal ω-oxidation of excess fatty acids,[51,52] mitochondrial dysfunction,[53–56] and inflammatory cell activation[57] causes the progression from liver steatosis to NASH. Thus, dietary strategies aimed at increasing β-oxidation and hepatic triglyceride export while decreasing DNL, adipose lipolysis, oxidative stress, and inflammation should effectively mitigate the development of hepatic steatosis and its progression to NASH. These strategies can be tested in several available animal models of diet- or genetic-induced NAFLD.

ANIMAL MODELS OF NAFLD

The mechanisms responsible for NAFLD are still not fully understood. Comprehensive studies in humans with NAFLD are also precluded by the lack of validated biomarkers and the requirement for performing highly invasive liver biopsies to assess the presence and severity of the disease. Thus, animal models are needed to define the mechanisms leading to NAFLD and to assess the potential efficacy of novel dietary interventions. Unfortunately, no single model system fully recapitulates the etiology of NAFLD. Moreover, those that most closely resemble human NAFLD also involve comorbidities such as obesity and diabetes, making it difficult to define the hepatoprotective activities of green tea independent of its effects against obesity and diabetes. These complexities emphasize that a model, whether it be genetic or diet-induced, be chosen in a hypothesis-dependent manner. As animal models of NAFLD have been extensively reviewed elsewhere,[58–60] the focus below will be on model systems often used to examine the protective effects of green tea or its catechins in the development or progression of NAFLD.

Diet-induced models of NAFLD

Studies examining the hepatoprotective effects of GTE or its catechins have successfully used diet-induced and genetic models of NAFLD. Common diet-induced models include diets high in fat (>60% of energy), high in fructose (60% wt/wt), or deficient in methionine and choline. High-fat diets administered by gavage,[61] intragastrically,[62,63] or in liquid form[64] induce NASH within 3 weeks, provided that the fat content is high (~70%) and the primary source is corn oil. In contrast, lard-based diets containing 60% of energy as fat induce liver steatosis within 8 weeks, but evidence of NASH is limited,[65] and rats fed 45% of energy as coconut oil or butter do not develop liver steatosis even after 14 weeks.[66] Thus, the quantity and quality of dietary lipid are important considerations. These diets effectively induce obesity,[65] insulin resistance,[64] and CYP2E1 expression,[64] but the quantity of fat needed to induce NAFLD exceeds that of diets from NASH patients,[67] and, consequently, this suppresses DNL,[65] whereas DNL is increased in NAFLD patients.[44]

Dietary models of high-fructose feeding are intended to mimic the 30% increase in total fructose and the 100-fold increase in free fructose consumption due to high-fructose corn syrup[68] as well as the relation between soft-drink-derived fructose and NAFLD.[69,70] High-fructose diets cause hypertriglyceridemia, insulin resistance, and steatosis within 5 weeks in mice.[71] These effects result from greater DNL, both because fructose acts as a lipogenic substrate[72] and because it increases the expression of the lipogenic transcription factor sterol regulatory element binding protein 1c (SREBP-1c) and the lipogenic genes fatty acid synthase and steroyl-CoA desaturase-1.[73] Fructose also decreases lipoprotein lipase activity,[74] which may explain why plasma triglycerides are greater in NASH patients despite impaired hepatic triglyceride secretion.[47] Fructose intakes needed to induce these effects are clearly greater than those of NASH patients.[67] Likewise, fructose feeding does not induce overt NASH,[71] and the inflammation that does ensue is typically periportal,[75] whereas NASH patients primarily exhibit centrilobular inflammation.[15] This model also fails to induce obesity,[71] but the propensity to become obese is greater when rodents are switched to a high-fat diet.[76]

NAFLD in rodents can also be induced using a methionine- and choline-deficient (MCD) diet.[77] These nutrients are required for phosphatidylcholine synthesis, thereby making them essential for hepatic triglyceride secretion.[78] An MCD diet containing sucrose as the primary carbohydrate allows for the induction of liver steatosis, hepatocyte ballooning, inflammation, and histological evidence of NASH, whereas a starch-based MCD diet does not induce these effects.[72] MCD diets reduce the expression of lipogenic genes, but the inclusion of dietary fructose increases DNL.[72] MCD induces liver steatosis and NASH regardless of lard or olive oil as the lipid source.[79] In addition, substituting carbohydrate, coconut oil, or beef tallow for corn oil offers no protection against steatosis, apoptosis, or elevated serum aminotransferases, but these modifications attenuate hepatic lipid peroxidation and NASH.[48] MCD decreases body

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mass and adiposity and increases insulin sensitivity.[80,81] The choline-deficient, high-fat diet is a variant that also fails to induce obesity and insulin resistance.[82,83] Thus, the MCD model fails to recapitulate the key comorbidities found in association with human NAFLD. In addition, although humans often consume choline at levels below the recommended intakes,[84] the severe deficiencies of these nutrients caused by the MCD diet are not frequently observed in developed countries, which may limit extrapolations to the current epidemic of obesity-associated NAFLD. Nevertheless, choline-deficient models are useful for studying the hepatoprotective properties of a dietary agent independent of obesity and insulin resistance.

Genetic models of NAFLD

Models of leptin deficiency and leptin resistance are commonly used to rapidly induce NAFLD and mirror many of the pathogenic events observed in human NAFLD patients. Leptin-deficient mice ($Lep^{ob/ob}$) develop severe macrovesicular steatosis by 8–10 weeks of age, and lipopolysaccharide administration induces NASH within hours.[49,57,85,86] Similar to NAFLD patients, *ob/ob* mice are obese, insulin resistant, hyperlipidemic, and have elevated aminotransferases despite the obvious difference that obese humans have greater, rather than decreased, levels of leptin.[57,87] *Ob/ob* mice also do not develop fibrosis[88] because leptin deficiency suppresses norepinephrine, a profibrogenic factor,[89] which is potentially advantageous for studies seeking a homogeneous induction of NAFLD and the evaluation of targeted dietary strategies having hypolipidemic and/or antioxidant activity. Leptin-resistant mice (*db/db*) and obese Zucker rats (*fa/fa*) have mutations in the long-form leptin receptor and spontaneously develop obesity, diabetes, and liver steatosis.[57,90] They also have short-form leptin receptors, thereby allowing the development of fibrosis.[90,91] These models mimic human leptin resistance, although leptin resistance in humans is partial rather than complete, and leptin-resistant humans do not always develop diabetes.[87] Nonetheless, *db/db* mice and *fa/fa* rats are appropriate models if the hypothesis being tested requires fibrosis and/or a more severe insulin-resistant phenotype. Lastly, mice overexpressing adipose SREBP-1c spontaneously develop liver steatosis, lobular inflammation, hepatocyte ballooning, and pericellular fibrosis secondary to decreased white adipose tissue and circulating leptin.[92] Thus, these mice have lipodystrophy, not obesity-associated NAFLD. Because they develop NASH spontaneously, the model is more appropriate for the investigation of a NASH-specific hypothesis than for the examination of the independent contributions of "first- and second-hit" mechanisms.

GREEN TEA PROTECTS AGAINST NAFLD

History and composition of GTE

Tea was first cultivated in China and then Japan, but its commercial cultivation spread to Indonesia, the Indian subcontinent, and Europe during the 15th–17th centuries, and it is now consumed worldwide more frequently than any other beverage except water.[93] Green tea, oolong tea, and black tea are all derived from the leaves and buds of *Camellia sinensis*, but they vary in their polyphenol content as a result of differences in post-harvest processing.[93] Green tea is not fermented, whereas black tea is fermented and oolong tea is partially fermented.[93] During the production of green tea, the leaves are either steamed (Japanese sen-cha) or pan-fried (Chinese) before rolling and drying to inactivate polyphenol oxidase and peroxidase, which otherwise oxidize flavonols to theaflavins and thearubigins to provide the flavor characteristics of black tea.[93]

Catechins (flavan-3-ols) are the major polyphenols present in green tea and constitute 30–42% of the solid weight of the brewed tea.[3] The major tea catechins include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Figure 1).[3] Each of these consists of a 2-phenyl benzopyran flavonoid structure with di- (EC, ECG) or tri- (EGC, EGCG) hydroxy substitutions on the B ring and a 5,7-meta-dihydroxy substitution on the A ring, while ECG and EGCG are also esterified to the trihydroxy benzoate, gallic acid, at the 3-position. EGCG is the predominant catechin in green tea, accounting for 50–75% of the total catechin content, but the less abundant catechins have greater bioavailability in humans, mice, and rats.[94–96] Consequently, they reach concentrations similar to those of EGCG in liver and plasma.[94–96]

A comprehensive review of green tea catechin pharmacokinetics[97] has led to the understanding that maximal plasma catechin concentrations (~0.10–4.4 μM) occur approximately 1–2.7 h after ingestion of green tea, that plasma catechin half-lives are approximately 1.5–5.7 h following green tea ingestion, and that catechins are extensively biotransformed through phase II reactions (i.e., glucuronidation, sulfation, and O-methylation). Thus, given the generally low oral bioavailability and rapid elimination of catechins, the benefits of green tea may only be realized when consumed frequently (≥5–10 cups/day), as suggested by epidemiological studies,[4,5] although the concurrent ingestion of sucrose and vitamin C may enhance catechin bioavailability.[98] Green tea also contains other minor constituents such as flavonol glycosides (e.g., quercetin, kaempferol, myricetin; 2–3% dry wt/wt) and caffeine (2.5–4% dry wt/wt).[3] Since variations in many condi-

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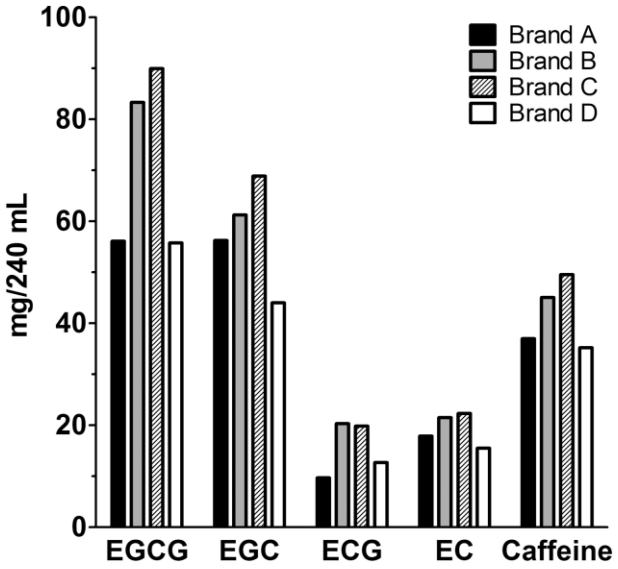

Figure 2 **Catechin and caffeine levels of commercially available green tea products.** Green tea infusions were prepared by steeping 2.2 g tea in 240 mL of boiling water for 5 min. The brewed tea was diluted 1:3 with 2% glacial acetic acid, centrifuged (1,950 × *g*, 10 min), and the supernatant was analyzed by high performance liquid chromatography-UV as described.[166]

tions, including geographical location, soil composition, climate, and tea processing, may lead to different catechin and caffeine contents of GTE preparations, experimental studies should always verify and report the composition of the GTE used. For example, high-performance liquid chromatography analysis of the catechin and caffeine contents of several commercially available green teas indicates that EGCG content ranges from 56 to 90 mg/cup and the caffeine content varies from 35 to 50 mg/cup (Figure 2). Further complicating matters is that the quantity and proportion of other catechins also differs between various green tea products.

Models used to study the hepatoprotective mechanisms of GTE

The hepatoprotective mechanisms of GTE in NAFLD have been examined under various conditions, including in vitro models, cultured cells, tissue models, and in vivo models. When evaluating these models for their physiological relevance, it is important to take into account the pharmacokinetics and biotransformation of GTE catechins and the potential bioactivity of catechin metabolites, which have been extensively reviewed.[97,99] Maximal catechin concentrations after oral administration of GTE are typically in the approximate range of 0.1–4.4 μM.[97] Catechins undergo substantial biotransformation by colonic microflora, including hydrolysis of gallate moieties, hydrogenation, dehydroxylation, oxidation, and ring cleavage. These metabolites circulate in plasma and urine and exhibit antioxidant, anti-inflammatory, and enzyme inhibition activities.[97,99] The in vivo effects of GTE are likely to be mediated through the combined action of these metabolites and their parent compounds. By contrast, most in vitro and ex vivo studies have been conducted with individual catechins and, less commonly, individual metabolites. This review therefore emphasizes in vivo observations specifically related to NAFLD and uses other forms of evidence to better define the in vivo mechanisms while emphasizing in vitro works using catechins at physiological concentrations (≤10 μM). There is also emerging evidence that various GTE catechins may act synergistically,[100] complicating the interpretation of different in vivo studies that use isolated catechins such as EGCG or mixed catechins in varying proportions. Independently conducted studies using GTE, EGCG, and a microbially fermented GTE low in EGCG provide indirect evidence of the independent or additive actions of catechins. However, caution is needed in interpreting these outcomes since the experimental models differed and no studies to date have directly compared the effects of these dietary agents on NAFLD.

GTE: protective against NAFLD in various animal models

Epidemiological evidence suggests that green tea consumption is associated with lower plasma levels of aminotransferases, triglycerides, and atherogenic lipoproteins,[5] as well as a lower risk of cardiovascular and all-cause mortality.[4] The close relation of these factors to NAFLD strongly supports that green tea or its catechins would also protect against NAFLD in humans. Consistent with previous work using EGCG in an ischemia/reperfusion model of liver injury,[101] subsequent studies provided the first evidence that GTE protects against hepatic steatosis and injury in the *ob/ob* mouse model of obesity-triggered NAFLD.[6] It has also been shown that GTE protects against NAFLD in a high-fat, choline-deficient, nitrite-injection model[82] and that EGCG protects against NAFLD in high-fat[102,103] and SREBP-1c overexpression[104] models. These studies have shown that GTE and its catechins protect against both steatosis and liver injury, as well as their progression to NASH (Table 1). The high-fat, choline-deficient model is particularly important because it does not induce obesity or insulin resistance in rodents.[82,83] GTE may, therefore, have hepatoprotective effects in NAFLD (Figure 3) that are independent of its protective effects against obesity and diabetes, which are described in greater detail below.

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Table 1 **Summary of the protective effects of green tea extract and green tea catechins on liver steatosis and nonalcoholic steatohepatitis in experimental models of nonalcoholic fatty liver disease.**

Stage	Model system	Treatment	Duration	Effect	Reference
Liver steatosis	Leptin-deficient (*ob/ob*) mice	1–2% GTE*	6 weeks	↓ Serum ALT and AST ↓ Hepatic total lipid and triglyceride	Bruno et al. [2008][6]
	Leptin-deficient (*ob/ob*) mice	0.5–1% GTE*	6 weeks	↓ Serum ALT ↓ Hepatic total lipid, triglyceride, cholesterol	Park et al. [2011][7]
	Mice fed high-fat (60% kcal) diet	3.2% EGCG	16 weeks	↓ Hepatic triglyceride	Bose et al. [2008][103]
	Mice overexpressing adipose SREBP-1c	1 g/L EGCG	12 weeks	↓ Histological grading of steatosis	Ueno et al. [2009][104]
	Rats fed high-fat (60% kcal) diet	1 g/L EGCG	6 weeks	↓ Histological grading of steatosis	Kuzu et al. [2008][102]
	Rats fed choline-deficient, high-fat diet,‡ i.p. nitrite	3% low-EGCG fermented GTE†	14 weeks	↓ Hepatic triglyceride	Nakamoto et al. [2009][82]
NASH	Rats fed high-fat (60% kcal) diet	1 g/L EGCG	6 weeks	↓ Hepatic inflammatory cell infiltration	Kuzu et al. [2008][102]
	Mice overexpressing adipose SREBP-1c	1 g/L EGCG	12 weeks	↓ Hepatocyte ballooning and Mallory-Denk bodies	Ueno et al. [2009][104]
	Rats fed choline-deficient, high-fat diet,‡ i.p. nitrite	3% low-EGCG fermented GTE†	14 weeks	↓ Histological evidence of fibrosis	Nakamoto et al. [2009][82]
	Leptin-deficient (*ob/ob*) mice	0.5-1% GTE	6 weeks	↓ Hepatic inflammatory cell infiltration	Chung et al. [2011][158]

* 30% total catechins (wt/wt), 48% EGCG, 31% epigallocatechin, 13% epicatechin gallate, 8% epicatechin.
† 19% total catechins, 54% epigallocatechin, 37% gallocatechin, 6% epicatechin gallate, 3% EGCG.
‡ Fat content not reported.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; EGCG, epigallocatechin gallate; GTE, green tea extract; i.p., intraperitoneal; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SREBP-1c, sterol regulatory element binding protein-1c.

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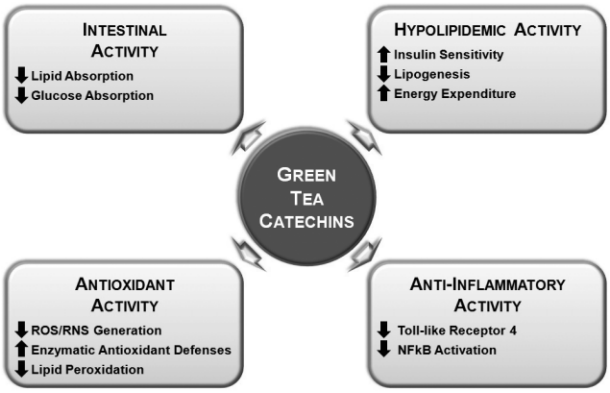

Figure 3 **Hepatoprotective activities of green tea in NAFLD.**

GTE: protective effects against the "first hit" of NASH

Five-week-old *ob/ob* mice and their lean littermates were fed diets containing 0%, 1%, or 2% GTE for 6 weeks.[6] GTE at 1% was chosen on the basis that it is equivalent to approximately 7 servings/day of green tea, an amount similar to that consumed by Japanese adults having a lower risk of cardiovascular mortality[4] and lower plasma levels of hepatic injury markers.[5] GTE at 2% reflects greater amounts of green tea consumed in many regions of the world[5] and was chosen to evaluate a potential dose-dependent effect. Obese (*ob/ob*) mice developed marked steatosis and elevated serum aminotransferases, consistent with their well-established phenotype.[105] GTE at 1% and 2% reduced adipose mass by 33% and 41%, respectively, without affecting food intake. GTE at 1% and 2% also dose dependently decreased hepatic total lipid by 22% and 40%, respectively, whereas both dietary levels similarly decreased hepatic triglyceride by 20–35%, plasma alanine aminotransferase by 30–41%, and aspartate aminotransferase by 22–33%.

Others similarly reported that dietary EGCG supplementation (3.2%, wt/wt) decreased the incidence of fatty liver from 95% to 18% and normalized hepatic triglyceride in mice fed a high-fat diet for 16 weeks.[103] EGCG provided in the drinking water (1 g/L) decreased histological steatosis grading in rats fed a high-fat diet for 6 weeks[102] and in mice overexpressing SREBP-1c and fed a standard rodent diet for 12 weeks.[104] A microbially fermented GTE (3%, wt/wt) containing primarily ECG and gallocatechin, but low amounts of EGCG, was also effective in reducing hepatic triglyceride levels in rats fed a choline-deficient, high-fat diet for 10 weeks.[82] GTE has several protective bioactivities that would be expected to prevent liver steatosis, which include decreasing lipid and carbohydrate absorption, decreasing DNL and adipose lipolysis, increasing β-oxidation and thermogenesis, and improving insulin sensitivity.

Ability of GTE to decrease dietary lipid and carbohydrate absorption. In mice fed a high-fat diet for 16 weeks, the addition of 3.2% EGCG decreased intestinal lipid absorption, as evidenced by a 2.5-fold increase in fecal lipid.[103] These hypolipidemic effects paralleled reductions in NAFLD and decreases in body mass. GTE most likely decreases lipid absorption through the actions of EGCG by inhibiting pancreatic lipase[106] and intestinal phospholipase A_2[107] and by increasing the size of lipid emulsions, which decreases their surface area.[108] Acute administration of EGCG also decreases glucose absorption by 20% in rats,[109] most likely by competitive inhibition of intestinal glucose transporters.[110] Thus, GTE may protect against liver steatosis by decreasing the absorption of dietary lipid and lipogenic substrates.

Ability of GTE to decrease circulating nonesterified fatty acids and de novo lipogenesis. Park et al. have demonstrated that feeding of GTE at 0.5–1% for 6 weeks protects against liver steatosis in *ob/ob* mice by decreasing the expression of lipogenic genes.[7] Interestingly, it was found that GTE downregulated the expression of SREBP-1c, fatty acid synthase, and steroyl-CoA desaturase-1 in adipose tissue but not in liver. In addition, adipose mRNA levels of hormone-sensitive lipase were decreased, as were serum NEFA concentrations.[7] This suggests that GTE suppresses adipose lipogenesis and hormone-sensitive lipase-mediated lipolysis, thereby reducing the flux of NEFA to the liver, where it would otherwise be esterified and stored as triglyceride, thereby contributing to hepatic steatosis. In contrast to the findings of Park et al.,[7] 10 μM but not 1 μM of EGCG increased hormone-sensitive lipase mRNA in 3T3-L1 adipocytes,[111] and 0.2–0.5% EGCG fed to C57BL/6J mice consuming a high-fat (45% kcal) diet also increased hormone-sensitive lipase mRNA.[112] These differences may result from differences in dose, the animal models used, or the effects of mixed catechins in GTE compared with purified EGCG. Nevertheless, despite increased lipolytic gene expression, EGCG decreased plasma NEFA due to greater β-oxidation and downregulated SREBP-1c and fatty acid synthase mRNA, in agreement with the findings of Park et al.[7] In a model of fructose feeding to ovariectomized rats, GTE at 0.5–1% reduced hepatic triglyceride by up to 73%, which was accompanied by decreases in the expression of hepatic SREBP-1c, fatty acid synthase, and steroyl-CoA desaturase-1.[73] Thus, GTE may protect against NAFLD by suppressing adipose lipolysis and by decreasing hepatic and adipose lipogenesis.

Ability of GTE to increase β-oxidation and thermogenesis. Mice fed a high-fat diet containing 0.5% of highly concentrated GTE (92% total polyphenols, 73% total catechins) for 11 months exhibited reductions in adipose

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mass and hepatic triglyceride accumulation of 79% and 75%, respectively.[113] GTE administration caused a small (~6%) but significant decrease in food intake and 2.8-fold greater hepatic β-oxidation. In a separate study, mice fed EGCG at 1% for 4 weeks had 44% lower adipose mass without any alterations in food intake.[114] In this study, fecal energy increased significantly (~5%), and the expression of hepatic uncoupling protein 2 increased by twofold, suggesting that EGCG uncoupled mitochondrial phosphorylation and increased thermogenesis. Likewise, a randomized crossover study showed that acute administration of GTE to humans increased 24-h energy expenditure by 2.8% and decreased the respiratory quotient by 2.5% compared with a control trial using a placebo with equivalent caffeine content.[115] Thus, GTE may protect against NAFLD by upregulating β-oxidation and thermogenesis, leading to increased energy expenditure.

Ability of GTE to improve insulin sensitivity. GTE improves systemic insulin sensitivity in both fructose- and starch-fed rats,[116–118] which may contribute to its protective effect against liver steatosis. Although insulin stimulates DNL, NAFLD is paradoxically associated with both increased DNL[44] and systemic insulin resistance.[12] This paradox occurs because hepatic insulin resistance is selective, since insulin fails to suppress gluconeogenesis but continues to promote DNL.[119] As a result of greater gluconeogenesis, hepatic glucose export increases. Pancreatic insulin secretion then increases in order to maintain normoglycemia, which further increases rates of hepatic DNL.[119] A further paradox occurs in NAFLD in that secretion of hepatic triglyceride is decreased, but plasma triglyceride is increased.[47] This likely results from reduced clearance of triglyceride from plasma, mediated by decreases in insulin-stimulated lipoprotein lipase activity in adipose tissue.[74,117] In contrast to the selectivity of hepatic insulin resistance, adipose insulin resistance can result from decreased cell surface insulin receptor expression and insulin receptor substrate 1 phosphorylation as well as other changes that lie upstream from the bifurcation of the insulin signaling pathway that occurs in hepatic insulin resistance.[120,121]

GTE appears to resolve both adipose and hepatic insulin resistance.[116–118,122] GTE decreases plasma glucose and insulin in fructose-fed Sprague-Dawley rats[73,117] while simultaneously decreasing hepatic DNL,[73] which is consistent with a reversal of selective hepatic insulin resistance. GTE increases gene expression of hepatic glucose transporters and insulin receptor substrate 2 in fructose-fed rats,[118] but EGCG also suppresses gluconeogenesis independent of insulin signaling in mice and in primary mouse hepatocytes by activating adenosine monophosphate-activated kinase.[122,123] Thus, it is unclear whether GTE normalizes hepatic insulin sensitivity per se or normalizes the associated phenotype through insulin-independent mechanisms. The effects of GTE on adipose insulin sensitivity are much clearer. In both fructose- and starch-fed Sprague-Dawley rats, 12 weeks' administration of 0.5% GTE in the drinking water decreased fasting and postprandial insulin and increased adipocyte insulin-binding activity and insulin-mediated glucose uptake.[116,117] In the fructose-fed rats, GTE abolished fasting and postprandial hyperglycemia and increased expression of adipose glucose transporter type 4 protein.[117] These results suggest that GTE reverses adipose insulin resistance by increasing cell surface expression of the insulin receptor and are consistent with findings that GTE decreases hormone-sensitive lipase mRNA, serum NEFA, and serum triglyceride in *ob/ob* mice.[7]

The effects of EGCG on plasma glucose and insulin have been studied in obese Zucker *fa/fa* rats and *db/db* mice, with conflicting results. Dietary EGCG decreased plasma glucose and free fatty acids in *fa/fa* rats without affecting plasma insulin or triglyceride.[124] This is consistent with findings in rat hepatoma H4IIE cells showing downregulation of gluconeogenic and lipogenic gene expression.[124] However, high concentrations of EGCG (50–100 μM) were used, and the effects of more physiologically relevant concentrations (<10 μM) were not examined. In the same report, EGCG also decreased plasma glucose and triglyceride while increasing plasma insulin in *db/db* mice.[124] It was suggested, on the basis of prior studies in vitro,[125,126] that EGCG may increase insulin concentrations by improving pancreatic function consistent with an antioxidant mechanism. Intraperitoneal injection of EGCG also decreased plasma glucose, insulin, and triglyceride in obese Zucker rats, but this was associated with a decrease in food intake.[127] Thus, EGCG appears to alleviate diabetes in *fa/fa* rats and *db/db* mice, but this effect is not mediated by decreases in plasma insulin unless food intake is affected. Thus, it remains unclear whether the effects of EGCG on insulin in these models differ from those of GTE in Sprague-Dawley rats because of the dose, the animal models used, or the effects of mixed catechins in GTE compared with purified EGCG. Nonetheless, the differences between *fa/fa* rats and *db/db* mice indicate that the effect of EGCG on insulin is at least partly model-dependent.

Additional work is also needed to define whether GTE increases insulin sensitivity directly or whether this effect is secondary to changes in macronutrient absorption and energy expenditure. EGCG decreases insulin-dependent glucose uptake in adipocytes at physiologically relevant concentrations (0.1–10 μM).[128–130] Some,[129] but not all[130] of these studies have shown EGCG to impair basal glucose uptake as well. Indeed, EGCG has been shown to decrease[129] or increase[131,132] glucose uptake in skeletal muscle at concentrations of 1 nM–10 μM. In

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humans, GTE improved glucose tolerance when provided simultaneously with an oral glucose tolerance test, but it impaired glucose tolerance when provided 1 h beforehand.[129] However, glucose tolerance improved when GTE was provided 1 h before glucose administration and simultaneously with polyethylene glycol in order to inhibit catechin absorption.[129] Thus, these data suggest that in vivo improvements in insulin sensitivity resulting from GTE administration may be secondary to decreased carbohydrate absorption in the intestine.

Collectively, GTE likely protects against the "first hit" of NAFLD by reducing dietary lipid and carbohydrate absorption, decreasing lipogenesis, increasing energy expenditure by upregulating β-oxidation and thermogenic responses, and increasing insulin sensitivity. Many of these effects are likely to be interdependent on one another and may even be secondary to reductions in hepatic steatosis. For example, the lipotoxicity that occurs during liver steatosis could compromise mitochondrial β-oxidation or insulin signaling. Thus, future studies are needed to elucidate the cause-and-effect pathways mediating the hepatoprotective effects of GTE by administering GTE through routes that bypass the intestinal tract, or by using enzyme inhibitors and siRNA systems to disrupt specific pathways. Such studies will improve understanding of the etiology of NAFLD, the optimal GTE formulation, and the hepatoprotective effects of GTE against NAFLD.

GTE: protective effects against the "second hit" of NASH

Rats fed a high-fat diet for 6 weeks developed marked hepatic inflammatory cell infiltration, which was reduced by 1 g/L EGCG provided in the drinking water.[102] The same dose of EGCG provided to mice overexpressing SREBP-1c for 12 weeks reduced histological evidence of hepatocyte ballooning and Mallory-Denk bodies.[104] EGCG protected against inflammation in these models but did not protect against fibrosis. By contrast, 3% microbially fermented GTE containing primarily ECG and gallocatechin had no effect on inflammation in rats fed a high-fat, choline-deficient diet and given daily intraperitoneal injections of nitrite, but it normalized fibrosis as evidenced by histological findings.[82] Whether these differences are due in part to the different catechins used is presently unclear, but these improvements are at least partly model-dependent because EGCG has been shown to reduce fibrosis in other in vivo models of liver damage.[133] Furthermore, EGCG decreases the proliferation of cultured hepatic stellate cells,[134,135] their matrix metalloproteinase-dependent invasion of collagenous membranes,[136] and their expression of fibrogenic genes.[134,135,137,138] GTE also has several protective functions that would be expected to prevent the progression from steatosis to NASH, which include its direct and indirect antioxidant activities as well as its anti-inflammatory activities.

Ability of GTE to protect against oxidative stress. Hepatic oxidative stress, as evidenced by greater lipid peroxidation levels, is a centrally implicated pathogenic event contributing to the progression of liver steatosis to NASH.[48,49] This is clearly exemplified by studies demonstrating that α- and γ-tocopherols retard hepatic lipid peroxidation and the progression of lipopolysaccharide-triggered NASH in *ob/ob* mice.[49] Other studies also demonstrate that *ob/ob* mice, even in the absence of a lipopolysaccharide challenge, have greater hepatic malondialdehyde, decreased total and reduced glutathione, and decreased activities of hepatic antioxidant enzymes, including mitochondrial and cytosolic superoxide dismutases, catalase, and glutathione peroxidase.[7] Dietary supplementation of GTE normalized malondialdehyde and glutathione concentrations as well as the activities of both superoxide dismutase isoforms to the levels of lean littermate controls and increased the activity of glutathione peroxidase. Improvements in hepatic glutathione levels were accompanied by greater mRNA expression of glutamyl cysteine ligase, supporting the possibility that GTE may have activated the nuclear factor erythroid 2 (Nrf2) pathway, which regulates the transcription of genes related to the cellular antioxidant defense. Others have shown protection against NASH mediated by GTE or its catechins to be associated with decreased hepatic lipid peroxidation and increased plasma superoxide dismutase activity in the high-fat, choline-deficient model,[82] decreased hepatic lipid peroxidation and increased hepatic glutathione in the high-fat diet model,[102] and decreased oxidative DNA damage in the SREBP-1c overexpression model.[104] GTE may decrease oxidative stress either directly, by scavenging reactive oxygen and nitrogen species, or indirectly, by activating the Nrf2 pathway.

Ability of catechins to directly scavenge reactive oxygen and nitrogen species. Catechins derived from green tea directly scavenge reactive oxygen and nitrogen species, including peroxyl radicals,[139] superoxide,[140] singlet oxygen,[140] hydroxyl radicals,[141] and peroxynitrite.[142] Catechins also protect linoleate from peroxidation synergistically with vitamins C and E: vitamin C recycles catechins, and catechins in turn recycle vitamin E.[143] The number of hydroxyl groups contributes to the hydrogen-donating capacity of catechins by allowing delocalization of the unpaired electron of the resultant phenoxyl radical.[140] Consequently, the radical-scavenging activity of a catechin is proportional to its number of hydroxyl groups and proceeds in order from highest to lowest as

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ECG≈EGCG>EGC>EC.[139] By contrast, the presence of a 3-gallate ester or a 5′-unsubstituted B-ring contributes to the capacity of a catechin to inhibit the nitration of tyrosine residues.[142] Indeed, EGCG and ECG more effectively scavenge peroxynitrite, as suggested by increases in the optical detection of a nitrophenol.[142]

GTE and EGCG generate reactive oxygen species (ROS) when incubated with copper in vitro[144–146] and when incubated with cultured cells at concentrations ≥50 μM.[147–150] In PC12 cells, 50–800 μM EGCG increased ROS and 400–800 μM increased lipid peroxidation, whereas a more physiologically relevant concentration (10 μM) improved antioxidant status by increasing glutathione concentrations.[147] The latter is consistent with in vivo findings that GTE at 0.5–1% (wt/wt) improves antioxidant status and protects against oxidative stress responses,[7] as these dietary levels would be expected to result in plasma EGCG below 1 μM.[94] Whether these protective effects of GTE in vivo result from the direct antioxidant effects of catechins is unknown, since the methods for measuring oxidized catechins as markers of ROS scavenging are not yet available under in vivo conditions. Given the low bioavailability of GTE catechins,[94–96] it is likely that the antioxidant activities of GTE are largely mediated indirectly.

Ability of catechins to upregulate gene transcription of antioxidant defenses. Limited evidence suggests that GTE catechins may exert indirect antioxidant effects by activating the Nrf2 pathway.[151] Nrf2 is a transcription factor that activates the antioxidant response element, which lies in the promoter region of a number of genes that encode proteins critical to the cellular defense against oxidative stress.[152] Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1) under basal conditions, which prevents its translocation to the nucleus.[153] Endogenous ROS or exogenous electrophiles oxidize the thiol groups of Keap1, allowing Nrf2 to translocate to the nucleus, where it regulates gene transcription. Numerous studies have shown that EGCG increases the expression of genes known to be regulated by Nrf2, such as glutathione *S*-transferase, glutathione peroxidase, quinone reductase, glutamyl cysteine ligase, and heme oxygenase-1.[154] EGCG provided by oral gavage (200 mg/kg) altered the expression of 228 genes, including glutamyl cysteine ligase, more than 2-fold in wild-type mice but not in Nrf2 knockout mice.[151] The finding that GTE increases glutamyl cysteine ligase mRNA in *ob/ob* mice[7] provides indirect evidence that GTE may activate the Nrf2 pathway at physiologically relevant levels. The mechanisms by which GTE catechins activate the Nrf2 pathway remain unclear. The autooxidation and dimerization of catechins in vitro generates superoxide and hydrogen peroxide,[146,155] while catechins themselves directly form adducts with protein thiols.[156] These effects would be expected to induce nuclear translocation of Nrf2. Catechins may also induce the phosphorylation of Nrf2 by activating the extracellular-related kinase pathway,[157] although the role of phosphorylation in Nrf2 translocation is controversial.[153]

Ability of GTE to protect against inflammation. Diagnosis of NASH requires histological evidence of inflammatory cell infiltration, hepatocyte ballooning, necrosis, Mallory's hyaline, and fibrosis.[40] Thus, NASH is, by definition, an inflammatory condition. Moreover, the fact that some experimental models for inducing liver steatosis will rapidly induce NASH upon cotreatment with an exogenous mediator of inflammation such as lipopolysaccharide emphasizes the centrality of inflammation in its etiology.[57] EGCG protects against NASH in the adipose SREBP-1c overexpression model by decreasing the expression of hepatic phosphorylated Akt, phosphorylated inhibitor of nuclear factor kappa-B (NFκB), and phosphorylated NfκB.[104] Consistent with these findings, recently completed studies found that *ob/ob* mice developed severe liver steatosis with moderate inflammation that was attenuated by GTE.[158] Greater inflammatory cell activation in obese mice was evidenced by greater hepatic expression of inducible nitric oxide synthase, NADPH oxidase, and myeloperoxidase, whereas GTE normalized the expression and activity of these inflammatory enzymes to the level of lean controls.

The anti-inflammatory effects of GTE may result through interactions between galloylated catechins and the 67-kDa laminin receptor (67LR).[159] Indeed, EGCG at physiologically relevant concentrations (1 μM) binds to 67LR in mouse macrophages and thereby downregulates Toll-like receptor 4 expression and upregulates Toll-interacting protein, which is a negative regulator of Toll-like receptor 4.[159] Consequently, EGCG blocks the phosphorylation and degradation of inhibitor of NFκB, the nuclear translocation of the p50 and p65 subunits of NFκB, the phosphorylation and activation of mitogen-activated protein kinases, and the protein expression of tumor necrosis factor-α, interleukin-6, inducible nitric oxide synthase, and cyclooxygenase-2, all of which are otherwise induced by lipopolysaccharide.[159] These effects are enhanced by all-*trans* retinoic acid, which upregulates 67LR transcription,[160] suggesting that vitamin A and green tea catechins may additively protect against inflammatory responses. ECG may also bind to and activate 67LR,[161] but catechins that are not galloylated do not exhibit such effects.[162] Thus, GTE may regulate inflammatory responses through direct cell surface receptor interactions.

Collectively, GTE likely prevents the progression from steatosis to NASH by directly scavenging reactive oxygen and nitrogen species, by upregulating the tran-

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scription of genes related to the cellular antioxidant defense, and by directly suppressing inflammatory responses by interacting with the 67LR. Nevertheless, the precise mechanisms mediating these responses remain unclear. Additional work is warranted using Nrf2 knockout mice and specific disruption of the 67LR pathway. These studies, along with the development of sensitive analytical methods to measure oxidized catechins, will more comprehensively expand the knowledge of the anti-inflammatory and antioxidant effects of GTE.

JUSTIFICATION FOR HUMAN CLINICAL TRIALS TO EVALUATE GREEN TEA

Epidemiological evidence suggests that green tea consumption is associated with lower circulating levels of aminotransferases, triglyceride, and atherogenic lipoproteins[5] as well as a lower risk of cardiovascular and all-cause mortality,[4] factors closely associated with NAFLD. No randomized, controlled, clinical trials have yet tested the hepatoprotective effects of GTE in human NAFLD. The available data from animal studies are promising and warrant the implementation of clinical trials, which are needed to verify the hepatoprotective effects of GTE in humans before GTE can be recommended as a useful prophylactic against or treatment of NAFLD in humans. Since it is unclear at this time whether GTE protects against NAFLD directly or indirectly through its protective effects against obesity and diabetes, care should be taken to independently test the hepatoprotective effects of GTE in individuals with and without underlying comorbidities.

SAFETY OF GREEN TEA

At least 36 cases of idiosyncratic hepatoxicity have been reported in humans using weight-loss products that include GTE or its catechins as part of their formulation.[163] Hepatotoxicity has been observed in mice fed an acute intragastric dose of 1,500 mg/kg EGCG and in mice that received 2–7 days of 500 mg/kg EGCG/day.[164] These data suggest interindividual differences in catechin metabolism or that supraphysiological intakes of green tea catechins can evoke liver injury. However, dechallenge/rechallenge evidence has only been provided in seven human case reports, and even in those cases, product contamination cannot be ruled out definitively. The doses that produce hepatotoxicity in mice are markedly outside of the therapeutic range. Moreover, a 3-week placebo-controlled study in overweight men found that high doses of green tea polyphenols (714 mg/day) are well tolerated and do not adversely affect routine clinical chemistries for liver and kidney function or cardiovascular risk biomarkers.[165] Thus, evidence for idiosyncratic adverse reactions to green tea or its catechins in humans is currently very limited, and additional work is needed to identify the safe upper limit.

CONCLUSION

NAFLD is closely associated with obesity, diabetes, and insulin resistance. It currently afflicts a significant proportion of Americans and its prevalence is expected to increase, consistent with the current obesity epidemic that affects two-thirds of Americans.[77] Since no validated treatments exist for NAFLD, it remains critically important to develop and validate novel dietary strategies that can prevent, attenuate, or reverse the development of hepatic steatosis and its progression to NASH. Evidence from in vitro systems and animal models suggests that green tea catechins are likely to prevent steatosis by decreasing intestinal lipid and carbohydrate absorption, by decreasing adipose lipolysis and both adipose and hepatic DNL, by stimulating hepatic β-oxidation and thermogenesis, and by improving insulin sensitivity. Furthermore, catechins are likely to prevent the progression from liver steatosis to NASH through their antioxidant and anti-inflammatory properties. To date, no randomized, controlled trials in humans with NAFLD have examined the potentially beneficial effects of green tea. Such studies are warranted, given that obesity dramatically increases the risk for NAFLD and the obesity epidemic is expected to remain problematic throughout the 21st century. These critical studies are necessary to provide direct evidence that green tea, either in a prophylactic or therapeutic manner, can effectively mitigate the development and/or progression of NAFLD in humans.

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Declaration of interest. The authors have no relevant interests to declare.

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