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Journal of Nutritional Biochemistry 22 (2011) 393–400
Journal of Nutritional Biochemistry

Green tea extract attenuates hepatic steatosis by decreasing adipose lipogenesis and enhancing hepatic antioxidant defenses in *ob*/*ob* mice☆

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Received 30 November 2009; received in revised form 10 March 2010; accepted 16 March 2010

Abstract

Excess hepatic lipid accumulation and oxidative stress contribute to nonalcoholic fatty liver disease (NAFLD). Thus, we hypothesized that the hypolipidemic and antioxidant activities of green tea extract (GTE) would attenuate events leading to NAFLD. Obese mice (*ob*/*ob*; 5 weeks old, $n=38$) and their lean littermates ($n=12$) were fed 0%, 0.5% or 1% GTE for 6 weeks. Then, hepatic steatosis, oxidative stress and inflammatory markers were measured. Obese mice, compared to lean controls, had greater hepatic lipids and serum alanine aminotransferase (ALT). GTE at 1% lowered ($P<.05$) hepatic lipids and ALT in obese mice. The GTE-mediated attenuation in hepatic steatosis was accompanied by decreased mRNA expression of adipose sterol regulatory element-binding protein-1c, fatty acid synthase, stearoyl CoA desaturase-1, and hormone-sensitive lipase and decreased serum nonesterified fatty acid concentrations. Immunohistochemical data indicated that steatotic livers from obese mice had extensive accumulation of tumor necrosis factor-α (TNF-α), whereas GTE at 1% decreased hepatic TNF-α protein and inhibited adipose TNF-α mRNA expression. Hepatic total glutathione, malondialdehyde and Mn- and Cu/Zn-superoxide dismutase activities in obese mice fed GTE were normalized to the levels of lean littermates. Also, GTE increased hepatic catalase and glutathione peroxidase activities, and these activities were inversely correlated with ALT and liver lipids. Collectively, GTE mitigated NAFLD and hepatic injury in *ob*/*ob* mice by decreasing the release of fatty acids from adipose and inhibiting hepatic lipid peroxidation as well as restoring antioxidant defenses and decreasing inflammatory responses. These findings suggest that GTE may be used as an effective dietary strategy to mitigate obesity-triggered NAFLD.

Keywords: Green tea; NAFLD; Lipogenesis; Lipolysis; Oxidative stress; Inflammation

1. Introduction

Hepatic steatosis and nonalcoholic steatohepatitis (NASH) belong to a group of fatty liver diseases collectively termed *nonalcoholic fatty liver disease* (NAFLD). NAFLD is associated with obesity and is relatively asymptomatic [1]. Its growing prevalence has paralleled the obesity epidemic that currently affects two thirds of Americans [1,2]. Indeed, 10–24% of the general population is estimated to be afflicted, with up to 74% of obese adults and 53% of obese children having some form of NAFLD [1].

The mechanisms leading to NASH remain unclear, but the "two-hit" mechanism was proposed to describe the events contributing to its development [3]. Hepatic steatosis is the "first hit," which is characterized by obesity and dysregulated lipid metabolism including increased *de novo* lipogenesis (DNL) and adipose lipolysis that lead to excess hepatic lipid accumulation [4]. Steatotic livers are highly vulnerable to oxidative stress-mediated "second hits" that induce lipid peroxidation, hepatic injury and inflammation, which provoke the progression toward NASH [3]. Also, the proinflammatory cytokine tumor necrosis factor-α (TNF-α) plays a central role in the etiology of NASH by stimulating hepatic lipogenesis and adipose lipolysis [5]. Furthermore, it triggers hepatic mitochondrial dysfunction and oxidative stress that induce liver injury [5].

Currently, there are no established treatments for NAFLD beyond comorbidity and weight management [1]. However, weight loss has a poor long-term success rate [6]. Thus, a need exists to validate approaches that ameliorate the development and progression of hepatic steatosis, oxidative stress, and inflammation in association with NAFLD. Epidemiological studies suggest that green tea (*Camellia sinensis*) consumption decreases mortality from cardiovascular disease [7] and that it may protect against liver disorders [8]. The health benefits of green tea are attributed to its catechins, which have hypolipidemic [9] and antioxidant [10] actions that may mitigate the "multiple hits" of NAFLD. Green tea extract (GTE) decreased the expression of hepatic lipogenic genes including sterol element binding protein-1c (SREBP-1c) and its downstream target genes, fatty acid

☆ This study was supported by grants to RSB from the National Research Initiative (2007-02303) from the USDA Cooperative State Research, Education and Extension Service and the USDA-HATCH (CONS00802) program.
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doi:10.1016/j.jnutbio.2010.03.009

synthase (FAS) and stearoyl-CoA desaturase (SCD-1) in fructose-fed ovariectomized rats [11]. GTE also inhibited hepatic steatosis and injury in obese (*ob*/*ob*) mice [12] and attenuated lipid peroxidation and mitochondrial reactive oxygen species (ROS) production in rats fed choline-deficient, high-fat diets and administered nitrite [13]. Epigallocatechin gallate (EGCG), the principal green tea catechin, decreased adiposity and hepatic lipid accumulation [14] and reduced the expression of adipogenic genes [15] in mice fed a high-fat diet. EGCG also inhibited hepatotoxicity by decreasing lipid peroxidation and inflammation in carbon tetrachloride-treated mice [16].

Despite these hypolipidemic and antioxidant activities, the extent to which GTE improves antioxidant status and oxidative stress responses has not been well studied in *ob*/*ob* mice, a commonly used model for examining the mechanisms leading to NAFLD [17,18]. *Ob*/*ob* mice are well suited for assessing the therapeutic response to green tea because they become obese and develop severe hepatic steatosis by 8 to 10 weeks of age [12]. They also generate higher amounts of ROS [19] and have greater hepatic lipid peroxidation levels [20]. Thus, studies in *ob*/*ob* mice enable our ability to define the hepatoprotective activities of GTE against oxidative stress responses implicated in obesity-triggered NAFLD. Indeed, our laboratory described that 6-week feeding of GTE at 1% or 2% similarly decreased histologic evidence of hepatic steatosis in obese (*ob*/*ob*) mice [12]. Thus, the present study was conducted to better define the lipid-lowering and antioxidant-related mechanisms by which GTE protects against NAFLD and to determine the lowest effective dietary GTE level that mitigates the "multiple hits" of NAFLD in this animal model. We hypothesized that GTE would attenuate obesity-triggered NAFLD by decreasing hepatic and adipose lipogenesis as well as oxidative stress and inflammatory responses implicated in NAFLD. To this end, we fed 5-week-old *ob*/*ob* mice diets containing 0%, 0.5% or 1% GTE for 6 weeks, which corresponds to the age that they develop hepatic steatosis and oxidative stress [12]. We then examined the expression of lipogenic and lipolytic genes and markers of hepatic steatosis, antioxidant defenses, lipid peroxidation and inflammation.

2. Materials and methods

2.1. Materials

All HPLC-grade solvents and chemicals were purchased from Fisher-Scientific (Fair Lawn, NJ, USA) except diethylenetriamine-pentaacetic acid, 1,1,3,3-tetramethoxypropane (TPP) and thiobarbituric acid (TBA), which were from Sigma-Aldrich (St Louis, MO, USA).

2.2. Study design

The study protocol was approved by the Institutional Care and Use Committee at the University of Connecticut. Male obese (*ob*/*ob*) mice (n=38; 5 weeks old) and their C57BL/6J lean littermates (n=12) were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and housed individually in an environmentally controlled room with a 12-h light–dark cycle. After 1 week acclimation, obese mice (n=12–13/group) were assigned randomly to the following treatments for 6 weeks: (A) a modified AIN-93G diet [21] containing no GTE, (B) the same diet containing 0.5% GTE (w/w) or (C) the same diet containing 1% GTE. All lean mice were fed the basal diet without GTE because our prior studies indicated that lean mice do not develop NAFLD under these conditions and were otherwise unaffected by GTE [12]. Animal body weights were recorded weekly and food intake was measured daily. The basal diet was purchased from Dyets (Bethlehem, PA, USA) and had egg white substituted for casein. Powdered GTE was provided by Unilever BestFoods (Englewood, NJ, USA) and contained 30% (w/w) total catechins [12]. GTE at 1% is equivalent to ~7 servings/day of green tea in humans (extrapolated on the basis of energy consumption) and was shown to effectively attenuate hepatic steatosis and injury in obese (*ob*/*ob*) mice [12]. GTE at 0.5% was chosen to determine whether a lower dietary level of GTE protects against NAFLD. After 6 weeks of feeding, all mice were sacrificed under isoflurane following 10–12 h fasting. Blood was collected from the retroorbital sinus and serum obtained by centrifugation. Liver and adipose (retroperitoneal and epididymal) were harvested, snap-frozen, and stored at −80°C. Portions of liver and retroperitoneal adipose were collected into RNALater (Ambion Inc., Austin, TX, USA) for real-time PCR analysis.

2.3. Hepatic injury and lipids

Serum alanine aminotransferase (ALT) activity was measured using a spectrophotometric clinical assay according to the manufacturer's instructions (ThermoElectron; Waltham, MA, USA). Hepatic total lipid was determined gravimetrically after overnight extraction with chloroform/methanol [12]. Hepatic total cholesterol, triglyceride (Thermo Electron, Waltham, MA, USA) and nonesterified fatty acid (NEFA; Wako Diagnostics; Richmond, VA, USA) were measured using enzymatic kits as described [12]. Serum cholesterol and NEFA were also measured using the aforementioned kits in accordance with the manufacturer's instructions.

2.4. Hepatic lipid peroxidation

Hepatic malondialdehyde (MDA) was extracted [22] and measured [23] by HPLC-FL. In brief, homogenates prepared in KCl were derivatized (1 h, 100°C) with TBA, extracted with butanol, and injected onto a Beckman Gold 126 HPLC system (Beckman Coulter; Fullerton, CA, USA) equipped with a Jasco FP-2020^{+} detector (Easton, MD, USA; 532/553 nm, excitation/emission). Samples were separated isocratically at 0.9 ml/min on a Luna C18(2) column (250×4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA) using 60:40 methanol and phosphate buffer. MDA was quantified against standards prepared in parallel from TPP. MDA was normalized to hepatic protein, which was measured using a Bradford micro-assay (Bio-Rad Laboratories; Hercules, CA, USA).

2.5. Hepatic TNF-α

TNF-α localization was visualized by immunohistochemistry. Formalin-fixed, paraffin-embedded liver sections (4–5 μm) were incubated with rabbit polyclonal antibodies against TNF-α (Abcam; Cambridge, MA, USA) followed by horseradish peroxidase-conjugated goat antirabbit IgG (Vector Laboratories; Burlingame, CA, USA). Sections were stained with DAB substrate, counterstained with hematoxylin, and then images (400×) were captured with an Olympus BX41 microscope (Center Valley, PA, USA). Hepatic TNF-α was quantified by ELISA using homogenates prepared in RIPA buffer [24] and analyzed according to the manufacturer's instructions (eBioscience, San Diego, CA, USA).

2.6. Hepatic glutathione

Liver GSH and GSSG were measured simultaneously using HPLC-Boron Doped Diamond (BDD) detection (ESA Biosciences, Chelmsford, MA, USA). Homogenates prepared in PBS were mixed 1:1 with 10% perchloric acid (PCA). Supernatants were stored at −80°C following centrifugation and analyzed within 1 month. Samples were thawed gently, placed on the refrigerated autosampler maintained at 4°C and were separated isocratically (0.6 ml/min) on a Shiseido C18 column (250×3.0 mm, 5 μ; Tokyo, Japan) using 25 mM sodium dihydrogen phosphate and 1.4 mM 1-octanesulfonic acid prepared in 6% acetonitrile (pH 2.65). Thiols were detected at an electrochemical potential setting of 1400 mV and a 5021A conditioning cell (400 mV) was placed immediately before the BDD. GSH and GSSG were quantified from standards prepared in PCA.

2.7. Hepatic enzymatic antioxidant defenses

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione *S*-transferase (GST) activities were measured from homogenates prepared in potassium phosphate buffer and analyzed spectrophotometrically according to the manufacturer's instructions (Cayman Chemical; Ann Arbor, MI, USA). Liver enzyme activities were normalized to hepatic protein as described above.

2.8. Quantitative real-time PCR

Total RNA was extracted using TRIzol (Invitrogen; Carlsbad, CA, USA) following the manufacturer's protocol. Reverse transcription for cDNA synthesis and quantitative real-time PCR analysis were performed as described [25]. Primers were designed according to the GenBank database using Primer Express software (Table 1). 18S was used to normalize the data.

2.9. Statistical analysis

Data (means±S.E.) were analyzed using GraphPad Prism (Version 4.03; GraphPad Software, Inc.; San Diego, CA, USA). One-way ANOVA with Newman–Keuls's post-test was used to evaluate group mean differences. Regression analysis was used to evaluate associations between variables. Analyses were considered statistically significant at an α level of $P<.05$.

Table 1
Primers used for RT-PCR

Gene	Forward primer	Reverse primer
SREBP-1c	5′-GGCCGAGATGTGCGAACT-3′	5′-CCCGGGAAGTCACTGTCTTG-3′
FAS	5′-TCCTGGAACGAGAACACGATCT-3′	5′-GAGACGTGTCACTCCTGGACTTG-3′
SCD-1	5′-CAGTGCCGCGCATCTCT-3′	5′-CCCGGGATTGAATGTTCTTG-3′
HSL	5′-CTCCTATGACCTACGGGAAGGA-3′	5′-TCAGATTTTGCCAGGCTGTTG-3′
TNF-α	5′-GGCTGCCCCGACTACGT-3′	5′-ACTTTCTCCTGGTATGAGATAGCAAAT-3′
GCLc	5′-GGCCACTATCTGCCCAATTG-3′	5′-CACGTAGCCTCGGTAAAATGG-3′
GCLm	5′-ACATTGAAGCCCAGGATTGG-3′	5′-CTCTTCACGATGACCGAGTACCT-3′
18s rRNA	5′-CGCTTCCTTACCTGGTTGAT-3′	5′-GAGCGACCAAAGGAACCATA-3′

GCLc, catalytic glutamate cysteine ligase; GCLm, modulatory glutamate cysteine ligase.

3. Results

3.1. GTE inhibited body weight and adipose mass gain

Following dietary treatment, obese controls weighed more than their lean littermates (Table 2). Obese mice fed GTE at 1% gained less body weight relative to obese controls, which occurred without affecting food intake (Table 2). Liver and adipose masses were greater in obese controls, whereas mice fed GTE at 1% had decreased organ masses compared to obese controls. Body weight correlated ($P<.05$) with adipose ($r=.893$) and liver ($r=.719$) mass, suggesting that the GTE-mediated decrease in body weight was attributed to the reduction in adiposity and liver mass, independent of food intake.

3.2. GTE inhibited hepatic steatosis and injury

Obese mice, compared to lean controls, had greater hepatic total lipid due to increases in triglyceride and total cholesterol concentrations (Table 3). GTE at 1% decreased hepatic total lipid and triglyceride, whereas both GTE levels decreased hepatic cholesterol. Obese mice had greater serum cholesterol and NEFA compared to lean mice. GTE at 1% lowered serum NEFA and cholesterol compared to those from obese controls. Serum ALT activity was greater in obese than in lean controls, whereas both GTE levels lowered serum ALT (Table 3) supporting that GTE decreased hepatic injury. Serum ALT was also correlated with hepatic lipids, suggesting that hepatic steatosis induces liver injury (Table 4).

3.3. GTE-mediated attenuation in hepatic steatosis was accompanied by improved adipose lipogenic gene expression

We examined the expression of genes regulating lipogenesis to better define how GTE inhibits hepatic steatosis. In obese mice, the expression of SREBP-1c, FAS, and SCD-1 was significantly up-regulated in liver and adipose (Fig. 1A–B and their expression levels at the liver were unaffected by GTE. Contrasting the liver, GTE at 1% decreased the expression of adipose SREBP-1c, whereas both dietary levels of GTE decreased adipose FAS and SCD-1 mRNA levels (Fig. 1B). GTE at 0.5% and 1% also decreased adipose hormone-sensitive lipase (HSL) expression to levels observed in lean controls. Collectively, these data suggest that GTE suppressed adipose lipogenesis and HSL-mediated lipolysis. Accordingly, GTE likely attenuated hepatic steatosis by decreasing the availability of adipose-derived NEFA to the liver where it would be otherwise esterified, stored as triglyceride, and exacerbate liver steatosis.

3.4. GTE decreased hepatic inflammation by attenuating hepatic and adipose TNF-α

Hepatic steatosis is associated with increased hepatic TNF-α, which stimulates lipogenesis and lipolysis [26] as well as induces hepatic dysfunction [5]. Immunohistochemical data indicated that obese mice had more intense staining for TNF-α, which was localized predominately around the central and portal veins (Fig. 2A). This inflammatory response was attenuated by GTE. ELISA analysis demonstrated that hepatic TNF-α protein was greater in obese controls and lower in obese mice fed GTE at 1% (Fig. 2B). Hepatic TNF-α mRNA was unaffected by obesity and GTE (Fig. 2C). However, adipose TNF-α mRNA expression mice was ~16-fold greater in obese controls than in lean controls and was normalized to that of lean mice by 1% GTE. Also, hepatic TNF-α protein correlated with hepatic lipid, serum ALT, and adipose mass ($r=.493–.591$, $P<.05$; Table 4).

3.5. GTE inhibited hepatic steatosis-triggered hepatic lipid peroxidation

NAFLD is associated with increased lipid peroxidation that induces hepatic dysfunction [3]. Hepatic MDA was 40% greater in obese control mice, and GTE normalized it to the levels observed in lean mice (Fig. 3). Hepatic MDA correlated with serum ALT and hepatic lipids (Table 4). Thus, the antioxidant activities of GTE inhibited hepatic injury partly by ameliorating lipid peroxidation.

3.6. GTE restored hepatic glutathione and increased the expression of genes responsible for glutathione synthesis

Consistent with hepatic oxidative stress, obese controls had lower hepatic total GSH (tGSH) (Table 5), which was attributed to 16% and 22% decreases in GSH and GSSG, respectively. GTE at both levels increased GSH, GSSG and tGSH to the levels observed in lean mice. Hepatic tGSH was also inversely correlated to serum ALT (Table 4). Collectively, these data suggest that hepatic steatosis and hepatic

Table 2
Body composition and food intake in lean and obese mice fed GTE[1]

	Lean+0% GTE	Obese+0% GTE	Obese+0.5% GTE	Obese+1% GTE	ANOVA
Initial body mass (g)	21.15±0.64[a]	29.41±1.01[b]	28.28±0.90[b]	28.02±1.00[b]	<0.05
Final body mass (g)	32.34±0.45[a]	42.86±0.66[c]	41.17±1.21[c]	34.99±1.01[b]	<0.05
Liver mass (g)	1.11±0.07[c]	2.47±0.11[a]	2.72±0.14[a]	2.08±0.12[b]	<0.05
Adipose mass[2] (g)	1.97±0.09[c]	4.05±0.09[a]	4.07±0.19[a]	3.18±0.13[b]	<0.05
Food intake (g/day)	3.55±0.09[a]	3.86±0.04[b]	3.88±0.07[b]	3.74±0.04[b]	<0.05

[1] Means±S.E. Values in a row not sharing a common letter differ ($P<.05$).
[2] Sum of epididymal and retroperitoneal adipose mass.

Table 3
Hepatic and serum lipids and serum ALT activity in lean and obese mice fed GTE[1]

	Lean+0% GTE	Obese+0% GTE	Obese+0.5% GTE	Obese+1% GTE	ANOVA
Hepatic total lipid (mg/g liver)	134.3±3.6^{c}	308.2±4.8^{a}	295.5±11.4^{ab}	267.0±8.8^{b}	<0.05
Hepatic triglyceride (μmol/g liver)	41.32±1.17^{c}	61.96±2.17^{a}	58.40±2.22^{ab}	53.64±1.24^{b}	<0.05
Hepatic total cholesterol (μmol/g liver)	7.09±0.17^{c}	10.79±0.42^{a}	9.08±0.59^{b}	9.45±0.34^{b}	<0.05
Hepatic NEFA (μmol/g liver)	19.41±1.10	17.98±1.01	18.58±1.20	19.04±1.05	>0.05
Serum total cholesterol (mmol/L)	3.45±0.09^{c}	6.72±0.25^{a}	6.06±0.33^{ab}	5.56±0.31^{b}	<0.05
Serum NEFA (mmol/L)	1.09±0.04^{c}	1.52±0.10^{a}	1.37±0.07^{ab}	1.23±0.07^{bc}	<0.05
Serum ALT activity (U/L)	44.15±8.23^{c}	323.42±25.16^{a}	260.70±24.22^{b}	241.95±20.82^{b}	<0.05

[1] Means±S.E. Values in a row not sharing a common letter differ (P<.05).

injury were accompanied by decreased glutathione status, which was restored by GTE along with improvements in oxidative stress.

To define how hepatic steatosis and GTE altered GSH status, we examined the expression of hepatic GCL, the rate-limiting enzyme for GSH biosynthesis [27]. Although hepatic tGSH was lower in obese controls, the expression of GCL subunits (GCLc and GCLm) were unaffected (Table 5). However, GTE at 1% increased the expression of GCLc and GCLm to levels greater than those in obese and lean controls. Thus, steatotic livers had decreased glutathione status due to oxidative stress rather than impaired GSH biosynthesis and GTE improved glutathione levels, at least in part, by increasing its biosynthesis.

3.7. GTE enhanced hepatic enzymatic antioxidant defenses

The GTE-mediated improvements in oxidative stress and inflammatory responses were further defined by evaluating hepatic enzymatic antioxidant activities. Cu/Zn- and Mn-SOD activities were significantly lower (Fig. 4) in obese controls supporting that steatotic livers are vulnerable to ROS-mediated insults. GTE at both levels normalized the CuZn- and Mn-SOD activities to those of lean mice. CAT was lower in obese mice, whereas GTE increased it. Also, GPx activity was lower in obese mice and GTE at both levels increased its activity. Hepatic GST and GR activities were increased in obese mice and only GTE at 1% further increased GST activity.

Inverse correlations between hepatic lipids and activities of Cu/Zn-SOD, CAT and GPx (Table 4) further suggest that steatotic livers were vulnerable to oxidative insults leading to the "second hit" of NAFLD. Correlative evidence (Table 4) also suggests that impaired peroxidative enzyme and mitochondrial SOD activities contributed to hepatic oxidative stress, inflammation and injury that may exacerbate NAFLD.

4. Discussion

The present study demonstrates that GTE protects against NAFLD by decreasing adipose lipogenesis and lipolysis, which reduces the delivery of adipose-derived NEFA to the liver. Data also demonstrate that GTE decreases oxidative stress and inflammatory responses known to contribute to the development of NAFLD and that GTE restores the level of hepatic tGSH and enzymatic antioxidant activities, which are likely to protect against hepatic lipid peroxidation and hepatic injury. In addition, GTE attenuates the expression of hepatic TNF-α protein and adipose TNF-α mRNA that are otherwise increased by NAFLD. Lastly, the data indicate dose-dependent effects of GTE such that a lower GTE level (0.5%) decreases oxidative stress and hepatic injury, but a higher level (1%) is needed to ameliorate hepatic steatosis and inflammatory responses. Collectively, these data provide new evidence that GTE mitigates NAFLD in *ob*/*ob* mice by decreasing the release of fatty acids from adipose and inhibiting hepatic lipid peroxidation as well as restoring antioxidant defenses and decreasing proinflammatory responses.

The GTE-mediated reductions in hepatic lipid accumulation and adiposity are consistent with our prior work [12]. As expected, in

Table 4
Correlations between hepatic steatosis and oxidative stress markers

	ALT	MDA	TNF-α	TL	TG	TC	tGSH	Cu/Zn-SOD	Mn-SOD	CAT	GPx	GR	GST
MDA	.358[a]	1.000											
	.027[b]	.000											
TNF-α	.493	.294	1.000										
	.003	.073	.000										
TL	.895	.383	.507	1.000									
	.000	.012	.001	.000									
TG	.777	.359	.500	.785	1.000								
	.000	.019	.001	.000	.000								
TC	.634	.379	.449	.680	.658	1.000							
	.000	.013	.004	.000	.000	.000							
tGSH	−.339	−.255	−.248	−.283	−.189	−.220	1.000						
	.043	.117	.139	.076	.244	.185	.000						
Cu/Zn-SOD	−.267	−.306	−.311	−.329	−.387	−.402	−.093	1.000					
	.106	.046	.051	.031	.010	.008	.569	.000					
Mn-SOD	−.246	−.075	−.412	−.232	−.216	−.174	.086	.206	1.000				
	.142	.645	.010	.139	.170	.284	.603	.196	.000				
CAT	−.375	−.344	−.177	−.489	−.503	−.495	.376	.414	.125	1.000			
	.038	.040	.317	.002	.002	.003	.029	.011	.473	.000			
GPx	−.785	−.112	−.589	−.794	−.716	−.628	.170	.502	.219	.407	1.000		
	.000	.521	.000	.000	.000	.000	.336	.002	.213	.023	.000		
GR	.564	.203	.300	.591	.474	.324	.151	−.047	−.241	−.112	−.298	1.000	
	.000	.187	.054	.000	.001	.032	.341	.761	.115	.498	.066	.000	
GST	.407	.152	−.021	.406	.238	.276	.187	−.096	−.006	−.237	−.185	.459	1.000
	.019	.390	.906	.013	.163	.108	.283	.577	.972	.200	.318	.004	.000

TL, hepatic total lipid; TG, hepatic triglyceride; TC, hepatic total cholesterol.
[a] Pearson correlation coefficient.
[b] P value.

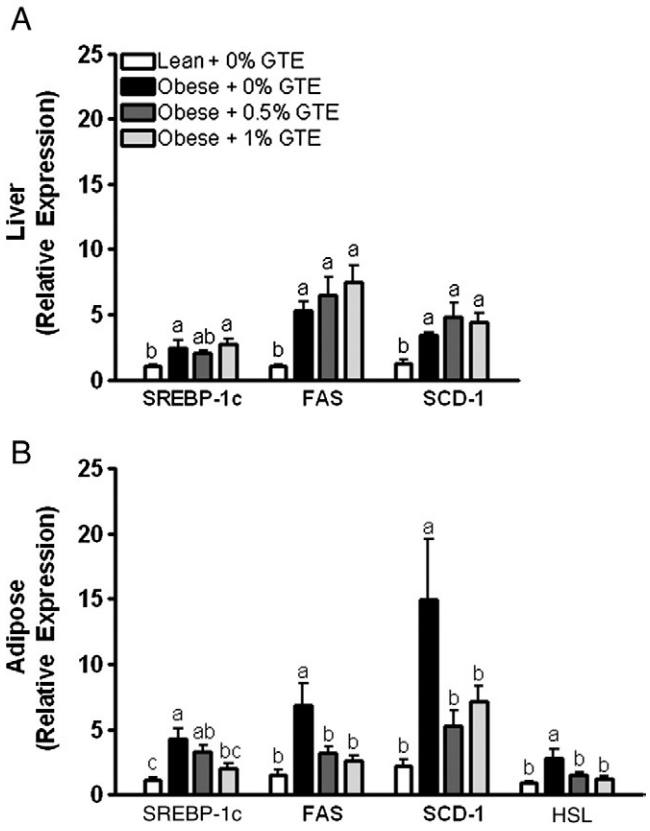

Fig. 1. Expression of hepatic (A) and adipose (B) lipid metabolism genes from lean and obese mice fed GTE for 6 weeks (means±SE; n=12–13 mice per group). RNA was isolated using TRIzol and reverse transcribed by MMLV reverse transcriptase for RT-PCR analysis using the primers described (Table 1). Groups without a common letter are significantly different (P<.05).

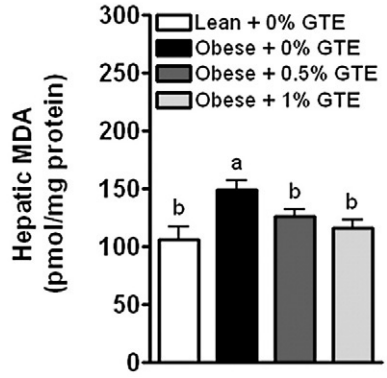

Fig. 3. Hepatic MDA in lean and obese mice fed GTE for 6 weeks (means±S.E., n=12–13 mice per group). Homogenates were incubated with TBA, extracted with butanol, and analyzed by HPLC-FL. Groups without a common letter are significantly different (P<.05).

obese mice, the expression of SREBP-1c, FAS and SCD-1 was up-regulated in adipose and liver. GTE suppressed adipose expression of these lipogenic genes, whereas it did not affect their expression in the liver despite a significant decrease in hepatic lipid accumulation in GTE-fed mice. Previously, GTE was shown to decrease the expression of these lipogenic genes in livers of fructose-fed ovariectomized rats [11]. These contrasting findings may be explained, in part, by differences in animal models and/or dietary treatment. Regardless, our findings indicate that GTE decreases adiposity in obese mice, which may be attributed to decreases in lipogenesis. GTE also decreases the expression of adipose HSL mRNA and reduces serum NEFA levels. Collectively, these data strongly suggest that GTE concomitantly suppresses adipose lipogenic and lipolytic activities. This in turn would decrease the flux of NEFA from adipose to the liver

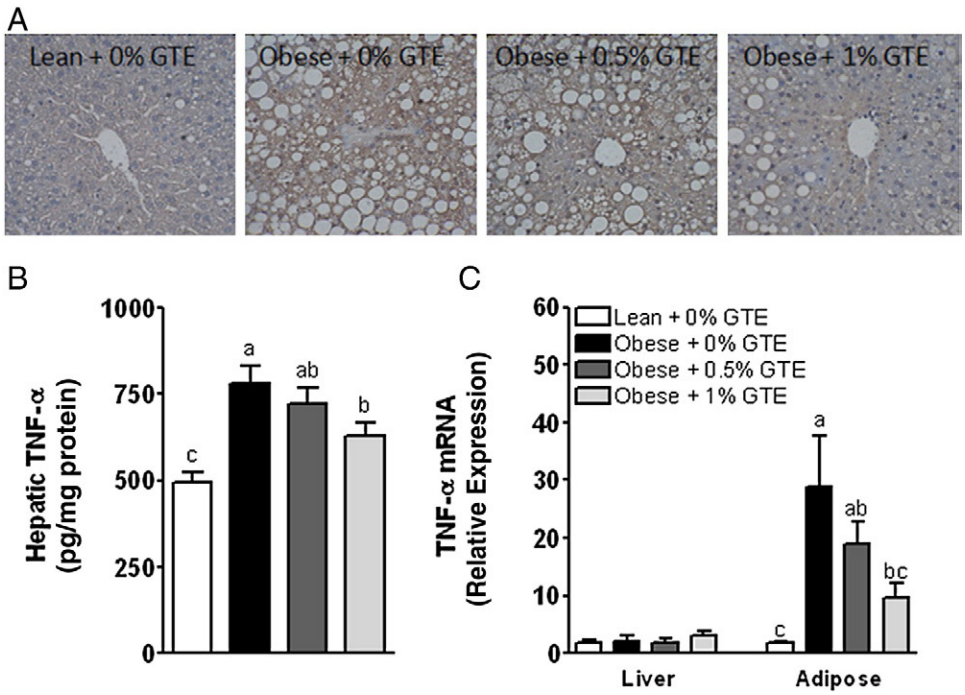

Fig. 2. Evaluation of hepatic TNF-α localization, hepatic TNF-α protein concentration, and liver and adipose TNF-α mRNA expression in lean and obese mice fed GTE for 6 weeks. (A) Formalin-fixed, paraffin-embedded sections were incubated with rabbit polyclonal antibodies against TNF-α, followed by incubation with HRP-conjugated goat antirabbit IgG, and visualized with DAB substrate after hematoxylin counterstaining. Representative images were captured at 400×. (B) Liver homogenates prepared in RIPA buffer were used for ELISA following centrifugation. (C) RT-PCR was performed as described above. Values are means±S.E. (n=12–13 mice per group). Groups without a common letter are different (P<.05).

Table 5
Hepatic glutathione and expression of glutathione synthesis genes in lean and obese mice fed GTE[1]

	Lean+0% GTE	Obese+0% GTE	Obese+0.5% GTE	Obese+1% GTE	ANOVA
Total GSH (nmol/g liver)	4765.1±126.4[a]	3984.7±321.2[b]	4570.0±132.0[a]	4927.5±134.8[a]	<0.05
GSH (nmol/g liver)	4544.6±122.6[a]	3821.3±321.5[b]	4354.2±127.4[a]	4714.0±133.9[a]	<0.05
GSSG (nmol/g liver)	111.7±5.1[a]	86.7±5.0[b]	106.6±5.4[a]	107.1±4.6[a]	<0.05
GCLc (relative expression)	0.91±0.09[b]	0.97±0.07[b]	1.34±0.30[ab]	1.88±0.28[a]	<0.05
GCLm (relative expression)	1.23±0.09[b]	1.40±0.09[b]	2.10±0.56[ab]	2.90±0.42[a]	<0.05

[1] Means±S.E. Values in a row not sharing a common letter differ (P<.05).

where it would be otherwise esterified to triglyceride, contributing to hepatic steatosis.

Consistent with our findings, *ob*/*ob* mice were shown to have enhanced adipose DNL and lipolysis [28]. Using labeled water, the synthesis of palmitate and triglyceride in adipose and net adipose lipolysis rates were greater in *ob*/*ob* mice compared to lean controls [28]. This was also accompanied by greater adipose FAS mRNA expression. GTE has also been shown to decrease adipose enzymes that regulate fatty acid synthesis and adipose mass in *ob*/*ob* mice fed high fat diets [29]. Furthermore, EGCG decreases adipose mass and the expression of lipogenic genes including SREBP-1c and FAS in mice fed a high-fat diet [15]. Other studies indicate that TNF-α induces hepatocyte SREBP-1 maturation [30] and stimulates hepatic lipid accumulation [26]. Our data here showed that GTE decreased hepatic TNF-α protein levels that were associated with increases in hepatic lipid content. These observations suggest the possibility that via a TNF-α-mediated pathway, GTE may suppress SREBP-1 maturation, and hence, lipogenesis in the liver. Further study is needed to define whether GTE post-transcriptionally regulates lipogenic genes.

The protective mechanisms of GTE on hepatic steatosis are likely to be multifaceted. GTE is also known to exert hypolipidemic activity by inhibiting intestinal lipid absorption [31]. GTE reduces luminal emulsification, hydrolysis and micellar solubilization of lipids [32] and decreases pancreatic lipase and phospholipase A_2 activities [31,33]. Studies in rodents also show that GTE markedly increases fecal lipid excretion [34]. Thus, GTE-mediated reductions in hepatic steatosis and obesity are likely to be, at least in part, regulated at the intestinal level thereby decreasing lipid bioavailability to adipose and liver.

NAFLD is also associated with oxidative stress and inflammatory responses that exacerbate hepatic lipid peroxidation and injury [3]. *Ob*/*ob* mice having steatotic livers are known to produce higher levels of ROS [19]. Green tea has been suggested to protect against ROS produced by ethanol toxicity by preventing decreases in enzymatic antioxidant activities [35]. However, most studies examining the antioxidant effects of green tea or its catechins have been conducted under in vitro conditions. Thus, no information is available on the antioxidant activity of GTE in the *ob*/*ob* model of NAFLD. Our data showed that obese mice have elevated hepatic MDA that was accompanied by decreases in ROS-detoxifying enzyme activities and tGSH. Moreover, MDA levels were positively correlated with hepatic lipids and ALT and inversely correlated with Cu/Zn-SOD and CAT. In addition, CAT and GPx were inversely related to ALT suggesting that the GTE-induced increases in these peroxidative enzyme activities decreased hepatic injury. Thus, these findings provide evidence that GTE restores hepatic enzymatic and non-enzymatic antioxidant defenses and reduces lipid peroxidation and hepatic injury in *ob*/*ob* mice.

Green tea has been shown to decrease lipid peroxidation and mitochondrial ROS production in choline-deficient rats fed a high-fat diet and injected with nitrite to induce NASH [13]. Green tea catechins have also been suggested to function as antioxidants that scavenge ROS [10]. Their antioxidant function is also mediated by inhibiting redox-sensitive transcription factors including nuclear factor kappa B

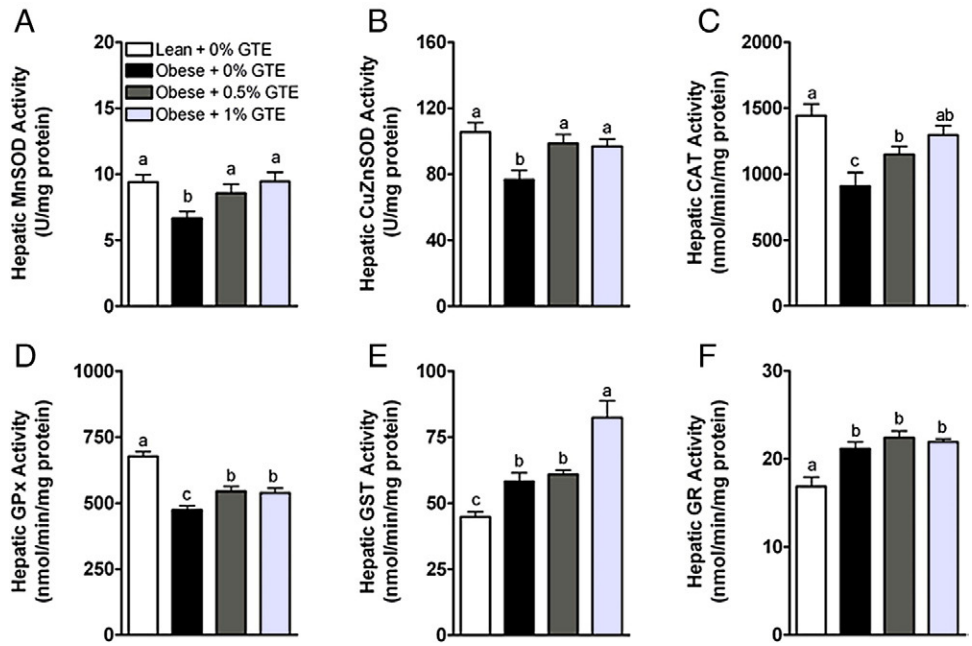

Fig. 4. Hepatic activities of (A) Mn-SOD, (B) Cu/Zn-SOD, (C) CAT, (D) GPx, (E) GST, and (F) GR in lean and obese mice fed GTE for 6 weeks (means±S.E., n=12–13 mice per group). Homogenates were prepared in phosphate buffer, centrifuged, and the supernatant used to measure enzyme activities. Groups without a common letter are significantly different (P<.05).

(NFκB), inhibiting prooxidant enzymes including inducible nitric oxide synthase, inducing antioxidant defenses (i.e., SOD, GPx, GST), and increasing glutathione synthesis [36]. Our findings are consistent with the known antioxidant activities of GTE. Whether these antioxidant activities of GTE are direct or indirect is unknown since the methods for measuring oxidized catechins as markers of ROS scavenging are not yet available under in vivo conditions. However, given the limited bioavailability of green tea catechins [37], it is likely that the antioxidant activities of GTE are largely mediated in an indirect manner yet to be defined. It is possible that the induction of these antioxidant defenses occurs downstream from the transcriptional activation of NF-E2-related factor-2 (Nrf2) via its interaction with the antioxidant-response element (ARE) [38]. Indeed, EGCG has been shown to increase Nrf2 nuclear translocation and ARE transcriptional activity in vitro [36] and up-regulate Nrf2 protein expression in a mouse model [39]. These GTE-mediated antioxidant activities are important because membrane peroxidation induces cellular damage and activates NFκB-mediated responses that induce proinflammatory cytokine expression including TNF-α [3], which contribute to NAFLD [40]. EGCG has been shown to decrease TNF-α production in vitro by blocking NFκB activation via the inhibition of IkB kinase [41,42]. Our data indicate GTE decreases the expression of hepatic TNF-α protein and adipose TNF-α mRNA suggesting that anti-inflammatory effects of GTE may be due to the suppression of NFκB activation. Additional studies are warranted to define the GTE-mediated mechanisms that improve antioxidant defenses and anti-inflammatory responses in NAFLD.

To further define the antioxidant role of GTE in ameliorating NAFLD-mediated oxidative stress, we examined hepatic glutathione. Hepatic glutathione is regulated by its synthesis, utilization, and export [43] and tGSH can be decreased by oxidative stress such as that mediated by NAFLD [44]. In the present study, obese mice have lower hepatic GSH and GSSG concentrations without alterations in hepatic GCL expression, and hepatic tGSH was inversely correlated with hepatic injury. Oxidative stress is thought to shift glutathione redox status toward lower GSH and higher GSSG levels. Obese control mice had altered activities of glutathione-dependent enzymes such that GST and GR activities were greater whereas GPx activity was decreased. Thus, the net effect of these altered activities and greater ROS levels would be expected to decrease hepatic GSH and GSSG levels. Moreover, increased ROS is known to decrease GPx, CAT, and SOD activities [45,46]. Of importance is our observation that GTE restores hepatic tGSH with increases in hepatic GCL expression, suggesting that GTE may increase GSH biosynthesis. Thus, glutathione status may be important to the regulation of NAFLD-mediated oxidative stress and hepatic injury.

Our data here and a previous report [12] indicate that GTE at 1% (equivalent to approximately seven servings/day in humans) or higher levels are necessary to attenuate hepatic steatosis. The beneficial effects of GTE at 1% are in agreement with epidemiologic observations suggesting that green tea (≥5–10 servings/day) protects against liver disorders and cardiovascular disease in humans [7,8]. However, GTE at a lower level (0.5%) did not prevent hepatic steatosis, although it effectively attenuated hepatic injury, lipid peroxidation and restored hepatic antioxidant enzymes activities. Further studies in NAFLD patients are needed to determine the optimal GTE intake to prevent or treat hepatic steatosis. Controversy exists regarding the potential hepatotoxicity of GTE or its catechins. Case reports in humans [47] and mice provided EGCG at excessively high levels (750 mg/kg body weight) [48] suggest interindividual differences in catechin metabolism or that supraphysiological intakes of green tea polyphenols can evoke liver injury. This contradicts the findings from obese mouse models where dietary supplementation of GTE up to 2% [12] as well as oral and intraperitoneal administration of EGCG (85 mg/kg body mass) [49] attenuates biomarkers of liver injury. Likewise, a 3-week placebo-controlled, parallel study in overweight men demonstrates that supplementation of green tea polyphenols (714 mg/day) does not adversely affect routine clinical chemistries for liver and kidney function or cardiovascular risk biomarkers [50]. Thus, additional work is needed to identify the safe upper limit for the consumption of green tea and/or its catechins.

It should also be noted that the obese (*ob*/*ob*) mouse model used here does not fully recapitulate the events regulating the development and progression of NAFLD in humans [51]. Thus, this model has potential limitations because most humans with NAFLD express leptin [51] and leptin deficiency inhibits fibrogenesis because it suppresses norepinephrine, a profibrogenic factor [52]. However, the *ob*/*ob* mouse model mimics the hepatic pathology, greater oxidative stress, and dysregulated metabolism that are observed in humans with NAFLD. Using this experimental model, the present study clearly demonstrated the hepatoprotective activities of GTE in regulating events leading to the development of hepatic steatosis and NASH without the potential confounding effects of more advanced stages of NAFLD.

In conclusion, GTE mitigates hepatic injury by inhibiting hepatic lipid accumulation and reducing oxidative damage and inflammation in an obese mouse model of NAFLD. These GTE effects appear to be dose-dependent and are likely mediated via alterations in adipose lipid metabolism and improvements in hepatic anti-inflammatory responses and antioxidant defenses. Further studies are warranted to determine whether GTE may be recommended as an effective strategy for preventing and/or treating obesity-triggered NAFLD in humans.

References

[1] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–31.
[2] Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999–2004. JAMA 2006;295:1549–55.
[3] Day CP. Pathogenesis of steatohepatitis. Best Pract Res Clin Gastroenterol 2002;16:663–78.
[4] Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab 2008;34:643–8.
[5] Carter-Kent C, Zein NN, Feldstein AE. Cytokines in the pathogenesis of fatty liver and disease progression to steatohepatitis: implications for treatment. Am J Gastroenterol 2008;103:1036–42.
[6] Ayyad C, Andersen T. Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999. Obes Rev 2000;1:113–9.
[7] Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, et al. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA 2006;296:1255–65.
[8] Imai K, Nakachi K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. BMJ 1995;310:693–6.
[9] Babu PV, Liu D. Green tea catechins and cardiovascular health: an update. Curr Med Chem 2008;15:1840–50.
[10] Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 2003;133:3275S–84S.
[11] Shrestha S, Ehlers SJ, Lee JY, Fernandez ML, Koo SI. Dietary green tea extract lowers plasma and hepatic triglycerides and decreases the expression of sterol regulatory element-binding protein-1c mRNA and its responsive genes in fructose-fed, ovariectomized rats. J Nutr 2009;139:640–5.
[12] Bruno RS, Dugan CE, Smyth JA, DiNatale DA, Koo SI. Green tea extract protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. J Nutr 2008;138:323–31.
[13] Nakamoto K, Takayama F, Mankura M, Hidaka Y, Egashira T, Ogino T, et al. Beneficial effects of fermented green tea extract in a rat model of non-alcoholic steatohepatitis. J Clin Biochem Nutr 2009;44:239–46.
[14] Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS. The major green tea polyphenol, (−)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. J Nutr 2008;138:1677–83.
[15] Lee MS, Kim CT, Kim Y. Green tea (−)-epigallocatechin-3-gallate reduces body weight with regulation of multiple genes expression in adipose tissue of diet-induced obese mice. Ann Nutr Metab 2009;54:151–7.
[16] Chen JH, Tipoe GL, Liong EC, So HS, Leung KM, Tom WM, et al. Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. Am J Clin Nutr 2004;80:742–51.
[17] Diehl AM. Lessons from animal models of NASH. Hepatol Res 2005;33:138–44.
[18] Koteish A, Mae Diehl A. Animal models of steatohepatitis. Best Pract Res Clin Gastroenterol 2002;16:679–90.

[19] Yang S, Zhu H, Li Y, Lin H, Gabrielson K, Trush MA, et al. Mitochondrial adaptations to obesity-related oxidant stress. Arch Biochem Biophys 2000;378: 259–68.
[20] de Oliveira CP, de Lima VM, Simplicio FI, Soriano FG, de Mello ES, de Souza HP, et al. Prevention and reversion of nonalcoholic steatohepatitis in OB/OB mice by *S*-nitroso-*N*-acetylcysteine treatment. J Am Coll Nutr 2008;27:299–305.
[21] Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[22] Lykkesfeldt J. Determination of malondialdehyde as dithiobarbituric acid adduct in biological samples by HPLC with fluorescence detection: comparison with ultraviolet-visible spectrophotometry. Clin Chem 2001;47:1725–7.
[23] Thiele JJ, Traber MG, Polefka TG, Cross CE, Packer L. Ozone-exposure depletes vitamin E and induces lipid peroxidation in murine stratum corneum. J Invest Dermatol 1997;108:753–7.
[24] Zhao J, Chen H, Li Y. Protective effect of bicyclol on acute alcohol-induced liver injury in mice. Eur J Pharmacol 2008;586:322–31.
[25] Rasmussen HE, Blobaum KR, Park YK, Ehlers SJ, Lu F, Lee JY. Lipid extract of *Nostoc commune* var. *sphaeroides* Kutzing, a blue-green alga, inhibits the activation of sterol regulatory element binding proteins in HepG2 cells. J Nutr 2008;138: 476–81.
[26] Endo M, Masaki T, Seike M, Yoshimatsu H. TNF-alpha induces hepatic steatosis in mice by enhancing gene expression of sterol regulatory element binding protein-1c (SREBP-1c). Exp Biol Med (Maywood) 2007;232:614–21.
[27] Lu SC. Regulation of glutathione synthesis. Mol Aspects Med; 2008.
[28] Turner SM, Roy S, Sul HS, Neese RA, Murphy EJ, Samandi W, et al. Dissociation between adipose tissue fluxes and lipogenic gene expression in ob/ob mice. Am J Physiol Endocrinol Metab 2007;292:E1101–1109.
[29] Kim HJ, Jeon SM, Lee MK, Jung UJ, Shin SK, Choi MS. Antilipogenic effect of green tea extract in C57BL/6J-Lep ob/ob mice. Phytother Res 2009;23:467–71.
[30] Lawler Jr JF, Yin M, Diehl AM, Roberts E, Chatterjee S. Tumor necrosis factor-alpha stimulates the maturation of sterol regulatory element binding protein-1 in human hepatocytes through the action of neutral sphingomyelinase. J Biol Chem 1998;273:5053–9.
[31] Koo SI, Noh SK. Green tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid-lowering effect. J Nutr Biochem 2007;18: 179–83.
[32] Shishikura Y, Khokhar S, Murray BS. Effects of tea polyphenols on emulsification of olive oil in a small intestine model system. J Agric Food Chem 2006;54: 1906–13.
[33] Wang S, Noh SK, Koo SI. Green tea catechins inhibit pancreatic phospholipase A(2) and intestinal absorption of lipids in ovariectomized rats. J Nutr Biochem 2006;17: 492–8.
[34] Raederstorff DG, Schlachter MF, Elste V, Weber P. Effect of EGCG on lipid absorption and plasma lipid levels in rats. J Nutr Biochem 2003;14:326–32.
[35] Skrzydlewska E, Ostrowska J, Stankiewicz A, Farbiszewski R. Green tea as a potent antioxidant in alcohol intoxication. Addict Biol 2002;7:307–14.
[36] Na HK, Kim EH, Jung JH, Lee HH, Hyun JW, Surh YJ. (−)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells. Arch Biochem Biophys 2008;476:171–7.
[37] Lambert JD, Sang S, Yang CS. Biotransformation of green tea polyphenols and the biological activities of those metabolites. Mol Pharm 2007;4:819–25.
[38] Na HK, Surh YJ. Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. Food Chem Toxicol 2008;46:1271–8.
[39] Yuan JH, Li YQ, Yang XY. Protective effects of epigallocatechin gallate on colon preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-f] quinoline in mice. Mol Med 2008;14:590–8.
[40] Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343–50.
[41] Yang F, Oz HS, Barve S, de Villiers WJ, McClain CJ, Varilek GW. The green tea polyphenol (−)-epigallocatechin-3-gallate blocks nuclear factor-kappa B activation by inhibiting I kappa B kinase activity in the intestinal epithelial cell line IEC-6. Mol Pharmacol 2001;60:528–33.
[42] Chen PC, Wheeler DS, Malhotra V, Odoms K, Denenberg AG, Wong HR. A green tea-derived polyphenol, epigallocatechin-3-gallate, inhibits IkappaB kinase activation and IL-8 gene expression in respiratory epithelium. Inflammation 2002;26:233–41.
[43] Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983;52:711–60.
[44] Das KS, Balakrishnan V, Mukherjee S, Vasudevan DM. Evaluation of blood oxidative stress-related parameters in alcoholic liver disease and non-alcoholic fatty liver disease. Scand J Clin Lab Invest 2008;68:323–34.
[45] Blum J, Fridovich I. Inactivation of glutathione peroxidase by superoxide radical. Arch Biochem Biophys 1985;240:500–8.
[46] Escobar JA, Rubio MA, Lissi EA. Sod and catalase inactivation by singlet oxygen and peroxyl radicals. Free Radic Biol Med 1996;20:285–90.
[47] Mazzanti G, Menniti-Ippolito F, Moro PA, Cassetti F, Raschetti R, Santuccio C, et al. Hepatotoxicity from green tea: a review of the literature and two unpublished cases. Eur J Clin Pharmacol 2009;65:331–41.
[48] Lambert JD, Kennett MJ, Sang S, Reuhl KR, Ju J, Yang CS. Hepatotoxicity of high oral dose (−)-epigallocatechin-3-gallate in mice. Food Chem Toxicol 48:409-16.
[49] Fiorini RN, Donovan JL, Rodwell D, Evans Z, Cheng G, May HD, et al. Short-term administration of (−)-epigallocatechin gallate reduces hepatic steatosis and protects against warm hepatic ischemia/reperfusion injury in steatotic mice. Liver Transpl 2005;11:298–308.
[50] Frank J, George TW, Lodge JK, Rodriguez-Mateos AM, Spencer JP, Minihane AM, et al. Daily consumption of an aqueous green tea extract supplement does not impair liver function or alter cardiovascular disease risk biomarkers in healthy men. J Nutr 2009;139:58–62.
[51] Larter CZ, Yeh MM. Animal models of NASH: getting both pathology and metabolic context right. J Gastroenterol Hepatol 2008;23:1635–48.
[52] Oben JA, Roskams T, Yang S, Lin H, Sinelli N, Li Z, et al. Norepinephrine induces hepatic fibrogenesis in leptin deficient ob/ob mice. Biochem Biophys Res Commun 2003;308:284–92.