



Published in final edited form as:

J Nutr. 2008 September ; 138(9): 1677–1683.

The Major Green Tea Polyphenol, (–)-Epigallocatechin-3-Gallate, Inhibits Obesity, Metabolic Syndrome, and Fatty Liver Disease in High-Fat–Fed Mice^{1,2}

Mousumi Bose^{3,5}, Joshua D. Lambert^{3,6}, Jihyeung Ju³, Kenneth R. Reuhl⁴, Sue A. Shapses⁵, and Chung S. Yang^{3,5,*}

³Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, Piscataway, NJ 08854

⁴Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, Piscataway, NJ 08854

⁵Graduate Program of Nutritional Sciences, Rutgers, the State University of New Jersey, New Brunswick, NJ 08901

Abstract

In this study, we investigated the effects of the major green tea polyphenol, (–)-epigallocatechin-3-gallate (EGCG), on high-fat–induced obesity, symptoms of the metabolic syndrome, and fatty liver in mice. In mice fed a high-fat diet (60% energy as fat), supplementation with dietary EGCG treatment (3.2 g/kg diet) for 16 wk reduced body weight (BW) gain, percent body fat, and visceral fat weight ($P < 0.05$) compared with mice without EGCG treatment. The BW decrease was associated with increased fecal lipids in the high-fat–fed groups ($r^2 = 0.521$; $P < 0.05$). EGCG treatment attenuated insulin resistance, plasma cholesterol, and monocyte chemoattractant protein concentrations in high-fat–fed mice ($P < 0.05$). EGCG treatment also decreased liver weight, liver triglycerides, and plasma alanine aminotransferase concentrations in high-fat–fed mice ($P < 0.05$). Histological analyses of liver samples revealed decreased lipid accumulation in hepatocytes in mice treated with EGCG compared with high-fat diet-fed mice without EGCG treatment. In another experiment, 3-mo-old high-fat–induced obese mice receiving short-term EGCG treatment (3.2 g/kg diet, 4 wk) had decreased mesenteric fat weight and blood glucose compared with high-fat–fed control mice ($P < 0.05$). Our results indicate that long-term EGCG treatment attenuated the development of obesity, symptoms associated with the metabolic syndrome, and fatty liver. Short-term EGCG treatment appeared to reverse preexisting high-fat–induced metabolic pathologies in obese mice. These effects may be mediated by decreased lipid absorption, decreased inflammation, and other mechanisms.

Introduction

The rates of obesity, defined as $\text{BMI} \geq 30 \text{ kg/m}^2$, have increased dramatically in the United States in the past 20 y and especially in the last 10 y (1). There is a strong positive association between obesity and type II diabetes, cardiovascular disease, and hypertension (2). These associations describe the metabolic syndrome, a clustering of risk factors including abdominal

¹Supported by NIH grant CA88961 (to C.S.Y.), American Institute for Cancer Research grant 05A047 (to J.D.L.), NIH grant CA125780 (to J.D.L.), and by facilities supported by NIEHS Center Grant ES05022.

²Author disclosures: M. Bose, J. D. Lambert, J. Ju, K. R. Reuhl, S. A. Shapses, and C. S. Yang, no conflicts of interest.

* To whom correspondence should be addressed. E-mail: csyang@rci.rutgers.edu.

⁶Present address: Department of Food Science, Pennsylvania State University, University Park, PA 16802.

obesity, insulin resistance, and dyslipidemia. Metabolic syndrome is also often characterized by chronic inflammation and hepatic steatosis (3).

Green tea is consumed worldwide, especially in East Asian countries. Green tea contains caffeine and polyphenolic compounds known as catechins. The most abundant catechin found in green tea is (–)-epigallocatechin-3-gallate (EGCG),⁷ which has been suggested to be responsible for many of the potential health effects of tea (4,5).

In 1999, Dulloo et al. (6) found that administration of a green tea extract significantly increased energy expenditure and fat oxidation in a group of young males. Since then, several clinical trials have reported the effects of tea preparations on increasing energy expenditure, fat oxidation, weight loss, fat mass, and weight maintenance after weight loss (7–9). Nevertheless, whether these effects are due to catechins or caffeine has yet to be resolved. Several studies in rodent models showed that green tea extract decreased weight gain and body fat gain (10,11). In 2005, it was reported that treatment with TEAVIGO, a green tea extract containing ≥94% EGCG and ≤0.1% caffeine, significantly reduced body weight (BW) and body fat in different strains of mice fed a high-fat diet (12,13).

In addition to its weight loss effects, there are studies that have suggested that tea consumption may alleviate other metabolic abnormalities related to obesity. Several clinical investigations showed that tea treatment reduced fasting blood glucose and improved glucose tolerance in healthy and diabetic subjects (14,15). A recent clinical trial reported that when healthy subjects were given an extract of green, black, and mulberry tea concurrently with a high-starch and -lipid meal, carbohydrate absorption was significantly blunted (16).

There are several studies describing the beneficial effects of tea constituents in animal models of the metabolic syndrome (15,17). One study reported that oral administration of EGCG for 3 wk significantly reduced blood pressure and increased insulin sensitivity in spontaneously hypertensive rats (18). A recent study by Wolfram et al. (19) reported that TEAVIGO treatment significantly improved glucose tolerance and insulin sensitivity in *db/db* mice.

Hepatic steatosis (or nonalcoholic fatty liver disease) and chronic low-grade inflammation are 2 conditions that are associated with obesity and the metabolic syndrome (3,20). Green tea consumption has been shown to be inversely correlated with liver damage (a consequence of progressive hepatic steatosis) and with markers of inflammation in humans (21,22). In high-fat-fed C57BL/6J and leptin-deficient *ob/ob* obese mice, green tea treatment reduced liver lipids and markers of liver damage (10,23).

The effects of physiologically relevant doses of EGCG on obesity and high-fat-induced pathologies associated with the metabolic syndrome have not been systematically studied. The goals of this study were to determine the effect of dietary EGCG on weight gain, markers of insulin resistance, hepatic steatosis, and inflammatory markers in a high-fat diet-induced mouse model for obesity and the metabolic syndrome. We studied both the long-term effects of EGCG in growing high-fat-fed mice and the short-term effects of EGCG in 3-mo-old obese mice.

⁷Abbreviations used: ALT, alanine aminotransferase; BW, body weight; EGCG, (–)-epigallocatechin-3-gallate; HF, high-fat; HFE, high-fat plus EGCG; HOMA-IR, homeostasis model assessment for insulin resistance; IL-6, interleukin-6; LF, low-fat; MCP-1, monocyte chemoattractant protein 1.

Materials and Methods

Chemicals and diets

EGCG was a gift from Mitsui Norin. Commercial low-fat diet (LF; 10% energy as fat), high-fat diet (HF; 60% energy as fat), and high-fat plus EGCG (HFE; 60% energy as fat, 3.2 g EGCG/kg diet) diet (Table 1) were prepared by Research Diets. Briefly, each ingredient was weighed (within 0.5% of amount needed) and blended to homogeneity in the appropriate powdered diet. The mixture was then formed into equally sized pellets and placed into a temperature- and humidity-controlled room to remove the moisture from the diet.

Diet treatment

Long-term dietary administration of EGCG (Expts. 1 and 2)—All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee at Rutgers University (Piscataway, NJ). One hundred male C57BL/6J mice (age 5–6 wk) from either the departmental breeding colony or Jackson Laboratories were used in 2 long-term experiments. The first long-term treatment (Expt. 1, $n = 38$, 6 wk old) was designed to determine the effects of EGCG on BW and body fat in high-fat-fed mice. The 2nd long-term treatment (Expt. 2, $n = 56$, 5 wk old) was designed to confirm the findings on BW and fat from Expt. 1 and also to determine the effects of EGCG on fecal lipids, hyperglycemia, insulin resistance, markers of inflammation, and fatty liver caused by a high-fat diet. In both studies, mice were fed a LF, HF, or HFE diet for 16 wk. Based on allometric scaling, the presently used dose of 3.2 g/kg diet in mice corresponds to 10 200-mL cups of green tea (containing 2 g tea leaves per cup) per day for an average person requiring 8374 kJ (2000 kcal)/d. BW and food intake (on a per-cage basis) were measured weekly. For fecal lipid measurements from Expt. 2, cage bedding was changed during wk 10 and feces were collected from the cages the following day.

Short-term dietary treatment of EGCG (Expt. 3)—To determine the short-term effects of EGCG on high-fat-fed mice (Expt. 3), 5- to 6-wk-old male C57BL/6J mice were fed either a LF ($n = 9$) or HF ($n = 18$) diet for 9 wk, after which, 10 mice from the HF group were randomly picked and switched to the HFE diet. Mice were treated with diets for an additional 4 wk. BW and food intake (on a per cage basis) were measured weekly.

Blood glucose

On the day of blood glucose measurement, food was removed and the cage bedding was changed (to minimize coprophagy) 7–8 h before measurement. Blood was collected from the tail vein and glucose concentrations were measured with the OneTouch Basic glucose monitor (LifeScan). Blood glucose (from food-deprived mice) was measured 6 times throughout the course of Expt. 2. For Expt. 3, blood glucose was measured 3 times throughout the course of the study, twice before mice were transferred to the HFE diet (at wk 4 and 7 of study) and once at the end of the study (at wk 13).

Tissue harvesting

For Expt. 1, mice were food deprived for 6 h prior to killing after 16 wk of treatment. Mice were weighed and killed by CO₂ overdose. Total percent body fat was measured postmortem by dual-energy X-ray absorptiometry using a PIXImus densitometer (Lunar GE Medical Systems). Visceral adipose tissues (mesenteric, epididymal, and retroperitoneal depots) were harvested, rinsed, and weighed.

For Expt. 2, mice were food deprived and killed as described above. Whole blood was obtained by cardiac puncture. Visceral adipose tissues (mesenteric, epididymal, and retroperitoneal

depots) and liver were harvested, rinsed, and weighed. Fatty liver was initially diagnosed by altered coloration (pink color due to lipid accumulation) and later confirmed by histological analyses (criteria described below). One lobe of the liver was fixed in 10% formalin, the rest was snap-frozen on dry ice. Plasma was isolated by centrifugation at $700 \times g$ at room temperature; 15 min, and snap-frozen on dry ice. All frozen samples were stored at -80°C .

For Expt. 3, all mice from the LF, HF, and HFE groups were weighed and killed by CO_2 overdose after 13 wk of treatment. Visceral adipose tissue (mesenteric, epididymal, and retroperitoneal depots) were harvested, rinsed, and weighed.

Biochemical analyses of plasma samples

All samples analyzed in food-deprived mice were from Expt. 2. Plasma insulin was determined by ELISA (Millipore). Plasma cholesterol was determined by a Cholesterol E kit (Wako Diagnostics). Plasma monocyte chemoattractant protein 1 (MCP-1) was determined by ELISA (R&D Systems). Plasma tumor necrosis factor- α and interleukin-6 (IL-6) were determined by ELISA (Invitrogen). Plasma alanine aminotransferase (ALT) concentrations were determined spectrophotometrically using the ALT DiscretePak from Catatchem.

Calculation of homeostasis model assessment of insulin resistance

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the final blood glucose and insulin determinations in food-deprived mice from Expt. 2. This method is widely used to estimate insulin resistance in humans and animal models (24). The following formula was applied (25):

$$\text{Insulin resistance} = \frac{\text{glucose (mmol} \cdot \text{L}^{-1}) \times \text{insulin (mU} \cdot \text{L}^{-1})}{22.5}$$

Fecal lipid analyses

For determination of fecal lipids, feces (0.9–1.3 g) collected during wk 10 of Expt. 2 were weighed, added to 3–4 mL deionized water, and allowed to sit at 4°C overnight. The following day, the feces mixture was homogenized by vortexing. Following vortexing, lipids were extracted with methanol:chloroform (2:1, v:v) using a previously described method (26). The lipophilic layer from the extraction was collected and dried under vacuum. Total lipids were measured gravimetrically.

Measurement of liver triglycerides

For determination of hepatic triglycerides in mice from Expt. 2, liver tissue (50–100 mg) was homogenized in 2 mL isopropanol with a Polytron disrupter. The homogenate was centrifuged at $2000 \times g$; 10 min, and the supernatant was collected. Triglycerides in the supernatant were measured with a Triglyceride H kit (Wako Diagnostics).

Liver histopathology

Embedded liver tissue blocks from Expt. 2 were cut into 6-micron sections and stained with hematoxylin and eosin. Mounting medium and cover slips were placed on the slides, which were then left to dry overnight. A diagnosis of fatty liver was made based on the presence of macro- or microvesicular fat in $>5\%$ of the hepatocytes in a given slide.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism software. Oneway ANOVA with Tukey's post hoc test was used for statistical analysis of BW gain, body fat, and biochemical measurements. For analysis of BW and blood glucose over the treatment time period, repeated-

measures ANOVA with Bonferroni's post hoc test was used. A chi-square test was performed for analysis of fatty liver incidence. Pearson correlation coefficient was used for the correlation analysis between fecal lipids and BW change. All data are presented as means \pm SEM. Significance was assigned at $P < 0.05$.

Results

Effect of long-term EGCG treatment on food intake, BW, and body fat

Daily food intake per cage did not differ among groups in either long-term experiment (data not shown). For Expt. 1 (Fig. 1A) and Expt. 2 (Fig. 1C), HF mice were significantly heavier than LF mice by wk 3–4 of treatment. These trends remained throughout the course of the treatment period. By the end of the 16-wk treatment period, HFE mice weighed significantly less than the HF mice (Fig. 1A,C). BW gain was 33–41% lower in HFE mice (Expt. 1: 14.02 ± 2.67 g; Expt. 2, 19.21 ± 2.06 g) compared with HF mice (Expt. 1, 23.80 ± 0.11 g; Expt. 2, 30.48 ± 0.90 ; $P < 0.05$).

Results from the dual-energy X-ray absorptiometry analysis showed that HF mice exhibited significantly increased percent body fat ($49.95 \pm 0.98\%$) compared with LF mice ($19.16 \pm 1.36\%$). HFE mice had significantly lower percent body fat (to $44.77 \pm 2.06\%$) compared with HF mice. HF mice had significantly increased total visceral adipose weight compared with LF mice in both Expts. 1 (Fig. 1B) and 2 (Fig. 1D). For Expt. 1, EGCG treatment reduced the weight of the mesenteric adipose depot (19% decrease) and retroperitoneal adipose depot (21% decrease) in HF mice (Fig. 1B). In Expt. 2, EGCG treatment reduced total visceral adipose tissue weight by 37% in HF mice ($P < 0.05$; Fig. 1D). This was attributable to weight decreases in the mesenteric (48% decrease), epididymal (28% decrease), and retroperitoneal (34% decrease) depots ($P < 0.05$ for all depots; Fig. 1D).

Effect of long-term EGCG treatment on blood glucose, plasma insulin, and HOMA-IR index

In Expt. 2, HF mice had higher blood glucose compared with LF mice by wk 3 of treatment ($P < 0.05$, Fig. 1E). By wk 10, HFE mice had lower blood glucose than HF mice (25% decrease; Fig. 1E) and this trend was significant over the 16-wk treatment period.

HF mice had increased plasma insulin concentrations compared with LF mice ($P < 0.05$; Table 2) and HFE mice had reduced plasma insulin compared with HF mice (61% reduction; Table 2). HOMA-IR calculations (using the final blood glucose and insulin concentrations) showed that the HF diet increased the HOMA-IR index compared with LF mice ($P < 0.05$; Table 2), whereas HFE mice exhibited a 76% decrease compared with HF mice ($P < 0.05$; Table 2).

Effect of long-term EGCG treatment on plasma cholesterol, inflammatory markers, and fecal lipids

HF mice had significantly higher plasma cholesterol and MCP-1 concentrations than LF mice in Expt. 2 (Table 2). EGCG treatment attenuated the elevation in cholesterol (by 24%) and MCP-1 (by 69%) in HF mice (Table 2; $P < 0.05$). Plasma tumor necrosis factor- α and IL-6 were not detectable in mice (using ELISA methods) from any of the treatments (data not shown).

At wk 10 of Expt. 2, fecal lipid concentrations did not differ between the HF (17.73 ± 1.42 mg lipids/g feces) and LF (11.33 ± 1.35 mg lipids/g feces) groups but were greater in HFE mice (43.23 ± 10.77 mg lipids/g feces) compared with either LF or HF mice ($P < 0.05$). The increase in fecal lipids was inversely correlated with the decrease in BW in mice from high-fat-fed groups ($r^2 = 0.521$; $P < 0.05$).

Effect of long-term EGCG treatment on liver pathology

HF mice had significantly increased liver size (relative to BW) compared with LF mice in Expt. 2. These mice also had a significantly higher incidence of fatty liver (Table 2; Fig. 2A), higher liver triglycerides (>2-fold of LF concentrations), and elevated plasma ALT concentrations (6-fold of LF concentrations). EGCG treatment reduced incidence of fatty liver ($P < 0.05$; Table 2; Fig. 2A), liver size ($P < 0.05$; 22% decrease), liver triglycerides ($P < 0.05$; 69% decrease), and plasma ALT concentrations ($P < 0.05$; 67% decrease) in HF mice (Table 2).

Histopathological analyses showed increased lipid deposition in the livers of HF mice (Fig. 2C) compared with those of LF mice (Fig. 2B). Microvesicular fat was present throughout the liver in HF mice; however, it had a central lobular distribution. The periportal zone was relatively free of accumulated lipids. Livers from HFE (Fig. 2D) mice showed a marked decrease in liver lipid accumulation compared with HF mice.

Effect of short-term EGCG treatment on food intake, BW, body fat, and blood glucose

Daily food intake per cage did not differ among groups in Expt. 3. After 9 wk of a LF or HF diet, HF mice were significantly heavier than the LF mice ($P < 0.05$; Fig. 3A). EGCG treatment for 4 wk tended to decrease BW gain (Fig. 3A,B).

After 9 wk of LF or HF diet, HF mice had increased total visceral fat weight compared with LF mice ($P < 0.05$; Fig. 3C). EGCG treatment for 4 wk decreased the weight of the mesenteric depot by 36% compared with HF-fed mice ($P < 0.05$; Fig. 3C).

By wk 4 of the study, HF mice had elevated blood glucose concentrations compared with LF mice ($P < 0.05$; Fig. 3D). At wk 13 (after 4 wk of EGCG treatment), HF mice still had higher blood glucose concentrations than LF mice ($P < 0.05$; respectively, Fig. 3D), but the HFE-treated mice had 22% lower blood glucose than HF mice ($P < 0.05$; Fig. 3D).

Discussion

In the present study, 16-wk dietary EGCG treatment significantly decreased BW in mice fed a high-fat diet. This was evident in both long-term experiments (Expts. 1 and 2). The BW gain was larger in Expt. 2 compared with Expt. 1; this may have been due to the fact that the mice in Expt. 2 were younger at the start of treatment.

Previous studies have shown that EGCG treatment (5–10 g EGCG/kg diet) reduced BW in mice fed a high-fat diet (12,13). The presently used dose of 3.2 g EGCG/kg diet in mice, which corresponds to 10 200-mL cups of green tea (containing 2 g tea leaves per cup) per day, may represent a more attainable dose than those previously reported in weight loss studies with animal models. Future studies are needed to determine whether there is a dose-response effect of EGCG on weight gain produced by a high-fat diet.

We found that 16-wk EGCG treatment also significantly decreased total percent body fat and visceral body fat weight. The decrease in visceral body fat by EGCG was apparent in the mesenteric, epididymal, and retroperitoneal depots, with the largest decrease occurring in the mesenteric depot. These decreases in body fat may be due to EGCG inhibiting lipid absorption or increasing fat oxidation. Indeed, we found that long-term EGCG treatment increased fecal lipids with EGCG treatment compared with high-fat-fed control mice, supporting the hypothesis that EGCG decreased lipid absorption.

Although there was no significant change in BW gain or total visceral fat weight in diet-induced obese mice treated with EGCG for 4 wk, there was significant decrease in the weight of the mesenteric depot of visceral fat. Previous studies showed that EGCG significantly reversed

total body fat percentage, subcutaneous fat weight, and epididymal fat weight in high-fat-fed mice (12,13). Our study clearly demonstrated the effects of EGCG on the mesenteric and retroperitoneal adipose depots. Previously, weight loss studies in humans showed that visceral adipose tissue is more metabolically active than subcutaneous adipose tissue, suggesting the increased risk for metabolic syndrome by adipose tissue is depot specific (27). This finding is supported by studies that showed visceral fat reduction was more effective at decreasing risk for metabolic syndrome than reduction of the subcutaneous depot in obese humans (28). A recent study in high-fat-fed mice found that mesenteric adipose tissue produced significantly higher levels of MCP-1 compared with the subcutaneous, epididymal, or renal depots in obese mice, which may be related to increased risk for related metabolic conditions (29). Our data, showing significantly lower plasma MCP-1 by EGCG treatment in high-fat-fed mice, suggest that EGCG-mediated decreases in mesenteric and retroperitoneal adipose tissue weight may play a role in the effects of EGCG on high-fat-induced inflammation and the development of metabolic syndrome.

The metabolic syndrome is a grouping of pathological conditions related to obesity and insulin resistance. We found that EGCG significantly decreased blood glucose, insulin, and insulin resistance in high-fat-fed mice. Furthermore, short-term treatment with EGCG reversed the effects of a high-fat diet on blood glucose. A previous study showed that EGCG (10 g TEAVIGO/kg diet) reduced plasma insulin in high-fat-fed C57BL/6J mice (19). However, these measurements were taken in the fed state; an effect of variable food intake on insulin levels in previous studies cannot be ruled out.

The effects of EGCG on insulin resistance in our study are at least partially attributable to the observed decrease in weight gain and body fat. There may, however, be some direct effects of EGCG on improvement of glucose homeostasis. Previous studies showed that green tea supplementation increased muscle glucose transporter protein expression in insulin-resistant rats (17). A study by Koyama et al. (30) found that EGCG treatment (1.5 g/kg diet) for 7 d significantly decreased the expression of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in the livers of mice. Studies have also shown decreased expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase by EGCG in rat hepatoma cells (5,31). These studies suggest that green tea and EGCG improve insulin sensitivity and glucose homeostasis, in part by directly increasing glucose disposal into the muscle and decreasing gluconeogenesis in the liver.

The most interesting finding in our study was that EGCG treatment decreased high-fat-induced hepatic steatosis. This was demonstrated histologically and biochemically. The decreased plasma ALT by EGCG also reflects decreased high-fat-induced hepatocyte injury.

The effect of EGCG on hepatic steatosis may contribute to its effects on insulin resistance. Hepatic steatosis is associated with insulin resistance in humans and studies in rodent models have shown that suppression of hepatic steatosis improved hepatic insulin sensitivity (32). These effects may be mediated through the activation of protein kinase C, which is stimulated by fatty acids and contributes to the phosphorylation of the insulin receptor substrate at Ser-307, resulting in inhibition of normal insulin signaling (32,33). EGCG has been shown to both inhibit protein kinase C and increase insulin receptor substrate-mediated downstream signaling in cell line studies (31).

On the other hand, the effects of EGCG on insulin resistance may partially contribute to the prevention of hepatic steatosis. Insulin resistance causes increased plasma FFA and enhances liver uptake of fatty acids, which are then stored as triglycerides (34). It is possible that EGCG improves insulin sensitivity and consequently reduces the risk for hepatic steatosis. Further

studies are needed to determine the causal relationship between insulin resistance and hepatic steatosis, and the role of EGCG in attenuating both of these conditions.

We also observed that EGCG decreased plasma MCP-1 in high-fat-fed mice and this is a new finding, to our knowledge. A previous study found that EGCG treatment reduced MCP-1 expression in endothelial cells exposed to phorbol 12-myristate 13-acetate, a chemical inducer of inflammation (35). A recent study by Kanda et al. (36) showed that MCP-1 is involved in the recruitment of macrophages into the adipose tissue, which leads to chronic inflammation. It is now widely accepted that obesity-induced inflammation plays a key role in the development of metabolic syndrome (37). Kanda et al. (36) reported that MCP-1 contributed to the development of insulin resistance. The results from the present study suggest that reduction of MCP-1 may be one mechanism by which EGCG reduces insulin resistance. On the other hand, the elevated MCP-1 may be a result of the increased body fat caused by the high-fat diet and the reduction of MCP-1 by EGCG treatment may be secondary to the decrease in adipose tissue. Future studies are needed to clearly determine whether EGCG elicits its metabolic effects by attenuation of inflammation.

The present study showed that EGCG significantly reduced plasma total cholesterol that was elevated by a high-fat diet containing cholesterol (Table 2). These results are consistent with previous studies that showed that orally administered EGCG decreased plasma cholesterol and inhibited cholesterol absorption from the intestine of rodents (38). The volume of plasma collected at harvest was a limiting factor in these studies; future studies can determine whether EGCG improves other plasma lipids related to metabolic syndrome, such as plasma triglycerides and HDL-cholesterol.

These studies show that a physiologically relevant dose of dietary EGCG reduces the development of obesity, hyperglycemia, insulin resistance, hypercholesterolemia, and hepatic steatosis in high-fat-fed mice. They also suggest that EGCG treatment may reverse the effects of a high-fat diet on BW and blood glucose. These effects may be related to decreased fat absorption and antiinflammatory effects mediated by EGCG. Other possible mechanisms such as decreased fatty acid synthesis and increased fatty acid oxidation need further investigation. It will also be interesting to determine how EGCG restores glucose homeostasis and attenuates fatty liver disease. Finally, it will be important to clearly determine whether our presently observed effects occur in humans and whether EGCG or green tea consumption can be used as a tool to prevent the development of obesity and its comorbidities.

Literature Cited

1. Healthy weight, overweight, and obesity among U.S. adults. This data brief discusses weight trends based on the NHANES. 2005 [2006 Jan 25]. Available from: <http://www.cdc.gov/nchs/data/nhanes/databriefs/adultweight.pdf>
2. Rossner S. Obesity: the disease of the twenty-first century. *Int J Obes Relat Metab Disord* 2002;26 (Suppl 4):S2–4. [PubMed: 12457290]
3. Daskalopoulou SS, Mikhailidis DP, Elisaf M. Prevention and treatment of the metabolic syndrome. *Angiology* 2004;55:589–612. [PubMed: 15547646]
4. Lambert JD, Yang CS. Mechanisms of cancer prevention by tea constituents. *J Nutr* 2003;133:S3262–7.
5. Wolfram S, Wang Y, Thielecke F. Anti-obesity effects of green tea: from bedside to bench. *Mol Nutr Food Res* 2006;50:176–87. [PubMed: 16470636]
6. Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Vandermander J. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr* 1999;70:1040–5. [PubMed: 10584049]
7. Kovacs EM, Lejeune MP, Nijs I, Westerterp-Plantenga MS. Effects of green tea on weight maintenance after body-weight loss. *Br J Nutr* 2004;91:431–7. [PubMed: 15005829]

8. Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, Tokimitsu I. Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr* 2005;81:122–9. [PubMed: 15640470]
9. Rumppler W, Seale J, Clevidence B, Judd J, Wiley E, Yamamoto S, Komatsu T, Sawaki T, Ishikura Y, et al. Oolong tea increases metabolic rate and fat oxidation in men. *J Nutr* 2001;131:2848–52. [PubMed: 11694607]
10. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. *Int J Obes Relat Metab Disord* 2002;26:1459–64. [PubMed: 12439647]
11. Ikeda I, Hamamoto R, Uzu K, Imaizumi K, Nagao K, Yanagita T, Suzuki Y, Kobayashi M, Kakuda T. Dietary gallate esters of tea catechins reduce deposition of visceral fat, hepatic triacylglycerol, and activities of hepatic enzymes related to fatty acid synthesis in rats. *Biosci Biotechnol Biochem* 2005;69:1049–53. [PubMed: 15914933]
12. Klaus S, Pultz S, Thone-Reineke C, Wolfram S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *Int J Obes (Lond)* 2005;29:615–23. [PubMed: 15738931]
13. Wolfram S, Raederstorff D, Wang Y, Teixeira SR, Elste V, Weber P. TEAVIGO (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. *Ann Nutr Metab* 2005;49:54–63. [PubMed: 15735368]
14. Hosoda K, Wang MF, Liao ML, Chuang CK, Iha M, Clevidence B, Yamamoto S. Antihyperglycemic effect of oolong tea in type 2 diabetes. *Diabetes Care* 2003;26:1714–8. [PubMed: 12766099]
15. Tsuneki H, Ishizuka M, Terasawa M, Wu JB, Sasaoka T, Kimura I. Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. *BMC Pharmacol* 2004;4:18. [PubMed: 15331020]
16. Zhong L, Furne JK, Levitt MD. An extract of black, green, and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. *Am J Clin Nutr* 2006;84:551–5. [PubMed: 16960168]
17. Wu LY, Juan CC, Hwang LS, Hsu YP, Ho PH, Ho LT. Green tea supplementation ameliorates insulin resistance and increases glucose transporter IV content in a fructose-fed rat model. *Eur J Nutr* 2004;43:116–24. [PubMed: 15083319]
18. Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim JA, Quon MJ, Montagnani M. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. *Am J Physiol Endocrinol Metab* 2007;292:E1378–87. [PubMed: 17227956]
19. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Riegger C, Weber P. Epigallocatechin gallate supplementation alleviates diabetes in rodents. *J Nutr* 2006;136:2512–8. [PubMed: 16988119]
20. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006;43:S99–112. [PubMed: 16447287]
21. Imai K, Nakachi K. Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases. *BMJ* 1995;310:693–6. [PubMed: 7711535]
22. Steptoe A, Gibson EL, Vuononvirta R, Hamer M, Wardle J, Rycroft JA, Martin JF, Erusalimsky JD. The effects of chronic tea intake on platelet activation and inflammation: a double-blind placebo controlled trial. *Atherosclerosis* 2007;193:277–82. [PubMed: 17010979]
23. 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. [PubMed: 18203899]
24. Konrad D, Rudich A, Schoenle EJ. Improved glucose tolerance in mice receiving intraperitoneal transplantation of normal fat tissue. *Diabetologia* 2007;50:833–9. [PubMed: 17334653]
25. Mlinar B, Marc J, Janez A, Pfeifer M. Molecular mechanisms of insulin resistance and associated diseases. *Clin Chim Acta* 2007;375:20–35. [PubMed: 16956601]
26. Tsujita T, Takaichi H, Takaku T, Aoyama S, Hiraki J. Antiobesity action of epsilon-polylysine, a potent inhibitor of pancreatic lipase. *J Lipid Res* 2006;47:1852–8. [PubMed: 16723640]

27. Zamboni M, Armellini F, Turcato E, Todesco T, Bissoli L, Bergamo-Andreis IA, Bosello O. Effect of weight loss on regional body fat distribution in premenopausal women. *Am J Clin Nutr* 1993;58:29–34. [PubMed: 8317385]
28. Park HS, Lee K. Greater beneficial effects of visceral fat reduction compared with subcutaneous fat reduction on parameters of the metabolic syndrome: a study of weight reduction programmes in subjects with visceral and subcutaneous obesity. *Diabet Med* 2005;22:266–72. [PubMed: 15717873]
29. Yu R, Kim CS, Kwon BS, Kawada T. Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. *Obesity (Silver Spring)* 2006;14:1353–62. [PubMed: 16988077]
30. Koyama Y, Abe K, Sano Y, Ishizaki Y, Njelekela M, Shoji Y, Hara Y, Isemura M. Effects of green tea on gene expression of hepatic gluconeogenic enzymes in vivo. *Planta Med* 2004;70:1100–2. [PubMed: 15549673]
31. Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M, Granner DK. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *J Biol Chem* 2002;277:34933–40. [PubMed: 12118006]
32. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, Shulman GI. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 2004;279:32345–53. [PubMed: 15166226]
33. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 2006;55(Suppl 2):S9–15. [PubMed: 17130651]
34. Utzschneider KM, Kahn SE. Review: the role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2006;91:4753–61. [PubMed: 16968800]
35. Hong MH, Kim MH, Chang HJ, Kim NH, Shin BA, Ahn BW, Jung YD. (–)-Epigallocatechin-3-gallate inhibits monocyte chemotactic protein-1 expression in endothelial cells via blocking NF-kappaB signaling. *Life Sci* 2007;80:1957–65. [PubMed: 17379255]
36. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 2006;116:1494–505. [PubMed: 16691291]
37. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation* 2005;111:1448–54. [PubMed: 15781756]
38. 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. [PubMed: 12873714]

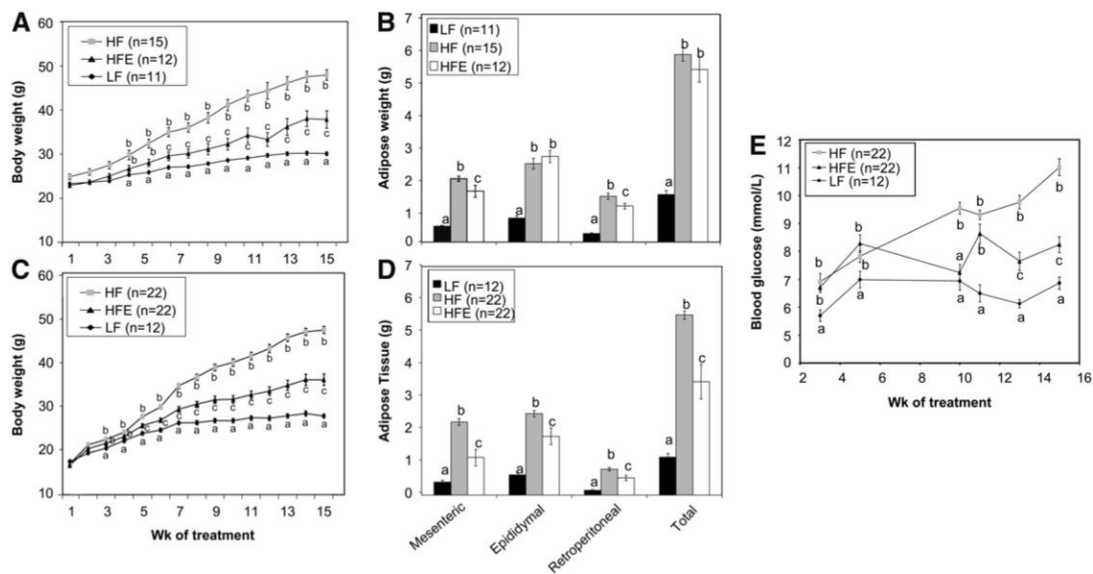


FIGURE 1. Effect of long-term EGCG treatment (16 wk) on BW (A,C), visceral fat weight (B,D), and blood glucose (E) in high-fat-fed C57BL/6 mice. Blood glucose was measured in food-deprived mice. Data are means \pm SEM. Labeled means at a time without a common letter differ, $P < 0.05$.

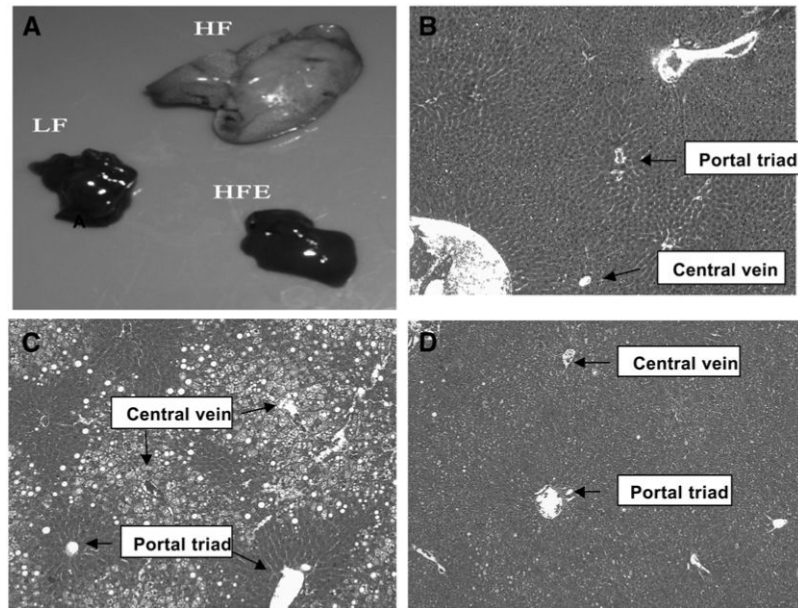


FIGURE 2. Effect of long-term EGCG treatment (16 wk) on fatty liver in high-fat-fed C57BL/6J mice. Gross examination of liver samples from LF, HF, and HFE mice (A). Histological examination of liver samples from LF (B), HF (C), and HFE (D) mice, shown at 5 \times magnification.

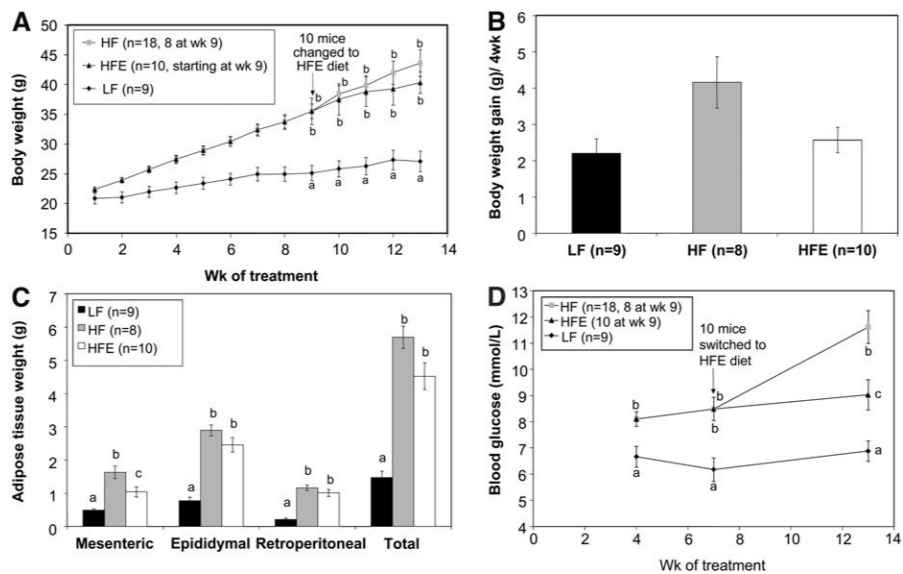


FIGURE 3. Effect of short-term EGCG treatment (4 wk) on BW (A,B), visceral fat weight (C), and blood glucose (D) in high-fat-fed C57BL/6J mice. Blood glucose was measured in food-deprived mice. Data are means \pm SEM. Labeled means at a time without a common letter differ, $P < 0.05$ (Expt. 3).

TABLE 1

Composition of experimental diets

	LF	HF	HFE
Macronutrient composition			
Protein, % of energy	20.0	20.0	20.0
Carbohydrate, % of energy	70.0	20.0	20.1
Fat, % of energy	10.0	60.0	59.9
Energy, MJ/kg ¹	15.9	21.8	21.8
Ingredient, g/kg			
Casein	189.6	258.4	257.6
L-Cystine	2.8	3.9	3.9
Corn starch	298.5	0.0	0.0
Maltodextrin	33.2	161.5	161.0
Sucrose	331.7	88.9	88.6
Cellulose	47.4	64.6	64.4
Lard	19.0	316.6	315.6
Soybean oil	23.7	32.3	32.2
Mineral mix ¹	9.5	12.9	12.9
Vitamin mix ²	9.5	12.9	12.9
Choline bitartrate	1.9	2.6	2.6
EGCG	0.0	0.0	3.2

¹ Mineral mix adds the following components (per g mineral mix): sodium chloride, 259 mg; magnesium oxide, 41.9 mg; magnesium sulfate, 257.6 mg; chromium K sulfate, 1.925 mg; cupric carbonate, 1.05 mg; sodium fluoride, 0.2 mg; potassium iodate, 0.035 mg; ferric citrate, 21 mg; manganous carbonate, 12.25 mg; ammonium molybdate, 0.3 mg; sodium selenite, 0.035 mg; zinc carbonate, 5.6 mg.

² Vitamin mix adds the following components (per g vitamin mix): retinyl acetate, 0.8 mg; cholecalciferol, 1.0 mg; DL- α -tocopheryl acetate, 10.0 mg; menadione sodium bisulfite, 0.05 mg; biotin, 0.02 mg; cyanocobalamin, 1 μ g; folic acid, 0.2 mg; nicotinic acid 3 mg; calcium pantothenate, 1.6 mg; pyridoxine-HCl, 0.7 mg; riboflavin, 0.6 mg; thiamin HCl, 0.6 mg.

TABLE 2

Effect of long-term EGCG treatment on plasma biomarkers and liver pathology in C57BL/6J mice fed a high-fat diet for 16 wk (Expt. 2)¹

	LF	HF	HFE
<i>n</i>	11	21	21
Plasma biomarkers ²			
Insulin, <i>pmol/L</i>	74.13 ± 5.17 ^a	867.24 ± 100 ^b	353.44 ± 86.21 ^a
HOMA-IR	3.12 ± 0.37 ^a	74.47 ± 6.47 ^b	17.41 ± 12.48 ^a
Cholesterol, <i>mmol/L</i>	2.63 ± 0.15 ^a	5.57 ± 0.13 ^b	4.21 ± 0.16 ^c
Plasma MCP-1, <i>pmol/L</i>	0.86 ± 0.15 ^a	6.70 ± 0.99 ^b	2.04 ± 0.33 ^a
Liver pathology			
Liver wt/BW, <i>g/g</i>	0.036 ± 0.0006 ^a	0.045 ± 0.002 ^b	0.035 ± 0.001 ^a
Fatty liver incidence	0/12 ^a	21/22 ^b	4/22 ^a
Liver triglycerides, <i>μmol/g tissue</i>	49.69 ± 7.17 ^a	200.76 ± 15.24 ^b	61.37 ± 21.34 ^a
Plasma ALT, <i>U/L</i>	13.23 ± 1.50 ^a	105.21 ± 15.58 ^b	35.25 ± 5.47 ^a

¹Data are presented as means ± SEM. Means in a row with superscripts without a common letter differ, *P* < 0.05.

²Blood samples were taken in food-deprived mice.