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Green tomato extract attenuates high-fat-diet-induced obesity through activation of the AMPK pathway in C57BL/6 mice

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Abstract

Obesity is a risk factor for numerous metabolic disorders. Recently, natural compounds that may be beneficial for improving obesity have received increasing attention. In this study, we investigated whether red and green tomato extracts attenuate high-fat-diet-induced obesity in C57BL/6 mice. The mice were maintained on a normal diet (ND) or high-fat diet (HFD) for 4 weeks and then fed ND, HFD, HFD plus 2% red tomato extract (RTE) or HFD plus 2% green tomato extract (GTE) for 13 weeks. The weekly food intakes among the groups were not significantly different. Body weight of mice fed HFD plus GTE was significantly decreased to the level of mice fed ND, but the body weight was only slightly reduced in mice fed HFD plus RTE. Epididymal adipose tissue and liver weights were significantly decreased in mice fed HFD plus GTE compared to those in HFD. Serum total cholesterol and low-density lipoprotein cholesterol levels in mice fed GTE were modestly reduced, and liver total cholesterol level was strongly decreased in HFD plus GTE-fed mice compared to that in HFD-fed mice. Adenosine-monophosphate-activated protein kinase (AMPK) and acetyl-CoA carboxylase phosphorylation in liver from HFD plus GTE-fed mice was significantly elevated, and HMG-CoA reductase expression was also significantly decreased. GTE strongly decreased the expression of peroxisome proliferator-activated receptor gamma, CCAAT/enhancer-binding protein alpha and perilipin in the adipose tissue of mice fed HFD plus GTE. Our results indicate that the antiobesity effects of GTE may be associated with activation of the AMPK pathway.

Keywords: Tomato; C57BL/6 mice; Obesity; AMPK

1. Introduction

Obesity is a risk factor for various metabolic disorders such as type II diabetes, hypertension and coronary heart disease [1–3]. Obesity results from excessive growth and expansion of adipose tissue due to an imbalance between energy intake and expenditure [4].

Adenosine-monophosphate-activated protein kinase (AMPK), a key enzyme of energy metabolism, regulates glucose and lipid uptake, storage and utilization in adipose tissue, liver, skeletal muscle, heart, pancreatic β cells and brain [5,6]. AMPK is phosphorylated and then inactivates metabolic enzymes involved in fatty acid and cholesterol synthesis, such as acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGCR) [7–9]. AMPK is also involved in the regulation of peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer-binding protein alpha (C/EBPα), which are the central regulators of adipogenesis [10].

Recently, natural compounds that may be beneficial for reducing the pathogenesis of obesity and metabolic syndrome have been investigated. Green tea extract containing epigallocatechin gallate decreased body weight and fat mass and suppressed blood cholesterol, glucose and triglyceride levels in mice [11–14].

Tomato is known to have lipid-lowering effects and antioxidant activities. Red tomato paste decreased the serum levels of total cholesterol and low-density lipoprotein (LDL) cholesterol and increased the high-density lipoprotein (HDL) cholesterol levels in hamsters fed a high-cholesterol diet. The tomato paste also decreased the plasma levels of malondialdehyde and increased the activities of superoxide dismutase, catalase and glutathione peroxidase in hamsters [15]. Tomatine, a major component of green tomato, decreased serum LDL cholesterol through the formation of tomatine-cholesterol complex, which was subsequently excreted in the feces; however, tomatine did not change HDL cholesterol levels in hamsters fed a high-cholesterol diet [16]. Red tomato contains lycopene, β-carotene, anthocyanins, caffeic acid, chlorogenic acid and flavonoids such as kaempferol, naringenin and quercetin [17]. Unlike red tomato, green tomato contains high levels of

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glycoalkaloids, such as α-tomatine and dehydrotomatine, and low levels of carotenoids [18–21].

In this study, we investigated whether red and green tomato extracts could improve high-fat-diet-induced obesity in C57BL/6 mice and elucidated its mechanism.

2. Materials and methods

2.1. Preparation and analysis of tomato extracts

Red and green tomatoes were extracted with water for 1 h at 25°C, respectively, using an ultrasonic apparatus and then concentrated under vacuum to yield red tomato extract (RTE) and green tomato extract (GTE). For comparison of RTE and GTE, the contents of dehydrotomatine, α-tomatine and trigonelline were measured by high-performance liquid chromatography (HPLC) analysis.

For measurement of dehydrotomatine and α-tomatine, tomato extract was extracted with chloroform/methanol (MeOH) (2:1, v/v), and the solvent was evaporated. Then, 0.2 N HCl and 2% NH_4OH were added. This mixture was centrifuged at 9,800 *g* for 10 min at 4°C. The supernatant was reconstituted in 2% NH_4OH and MeOH and evaporated at 45°C. Dehydrotomatine and α-tomatine in tomato extract were quantified by HPLC (Dionex, Ultimate 3000, USA) under the following conditions: column, C18 (250×4.6 mm, 5 μm, Phenomenex Co., Torrance, CA, USA); column temperature, 30°C; detector wavelength, UV 200 nm; flow rate, 1.0 ml/min; and mobile phase, MeOH. The retention times of dehydrotomatine and α-tomatine were 5.3 min and 5.8 min, respectively.

For measurement of trigonelline, the tomato extract was reconstituted in water and used for HPLC analysis. Trigonelline in tomato extract was quantified under the following conditions: column, Hypersil Gold column (250×4.6 mm, 5 μm, Phenomenex Co.); column temperature, 30°C; detector wavelength, UV 268 nm; flow rate, 1.0 ml/min; and mobile phase, MeOH/water (4:6, v/v). The retention time of trigonelline was 3.1 min.

The contents of dehydrotomatine, α-tomatine and trigonelline in tomato extract were determined from the liner regression equation of the calibration graph. Values are expressed as the mean±standard deviation (S.D.). Data were analyzed using an unpaired *t* test. *P* values less than .05 were considered statistically significant. The contents of α-tomatine and trigonelline in GTE were significantly increased compared to those in RTE (Table 1).

2.2. Animal treatment

The study protocol was approved by the Animal Care and Use Committee of Chungbuk National University. Male C57BL/6 mice (n=40; 4 weeks old) were purchased from Central Lab. Animal, Inc. (Seoul, South Korea). All mice were housed in a room with controlled temperature (21°C–23°C), humidity (55%–60%) and lighting (12-h light/dark cycle), and given water *ad libitum*. After acclimation for 1 week, mice were randomly divided into four groups (n=10/group) and fed normal diet (7 g% fat) or high-fat diet (24 g% fat) for 4 weeks. The mice were then fed normal diet, high-fat diet, high-fat diet plus 2% RTE (20 g/kg diet) or high-fat diet plus 2% GTE (20 g/kg diet) for another 13 weeks. The experimental diet (Table 2) was purchased from Research Diets, Inc. (New Brunswick, NJ, USA). At the end of the experimental period, mice were anesthetized, blood was collected, and organs (epididymal adipose tissue, liver, brain, kidney and lung) were excised. The organs were rinsed with physiological saline solution, weighed and stored at −80°C.

2.3. Measurement of serum biochemical parameters

All mice were euthanized using diethyl ether after 15-h fast. Blood was collected from abdominal vena cava and centrifuged at 3,500 *g* for 10 min at 4°C. The contents of serum total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride were determined using Hitachi Modular (Hitachi, Ltd., Tokyo, Japan).

2.4. Hepatic lipid extraction and measurement

Lipids were extracted from liver as described by Freedman et al. [22]. Mouse liver (100 mg) was homogenized in 0.5 ml of 1 M NaCl. Liver tissue homogenate was extracted with 3 ml of chloroform/methanol (2:1) plus 0.5 ml of 1 M NaCl. The organic phase was collected, dried and resuspended in 0.5 ml of Triton X-100/methanol (2:1). The total cholesterol and triglyceride levels were determined using the enzymatic method by Hitachi Modular (Hitachi, Ltd., Tokyo, Japan).

Table 1
Components of tomato extracts

Unit: μg/g

	RTE	GTE
Dehydrotomatine	7.3±7.1	9.7±4.8
α-Tomatine	36.5±14.4	86.7±27.2*
Trigonelline	2,981.0±45.1	3,355.0±25.3***

Values are expressed as mean±S.D. *P<.05, ***P<.001; significantly different from the HFD plus RTE group.

Table 2
Composition of experimental diets

Unit: g%

	ND	HFD	HFD+RTE	HFD+GTE
Formula				
Protein	20	24	24	24
Carbohydrate	64	41	41	41
Fat	7	24	24	24
Ingredient				
Casein	20	23.3	23.3	23.3
L-Cystine	0.3	0.3	0.3	0.3
Corn starch	39.7	8.5	8.5	8.5
Maltodextrin	13.2	11.7	11.7	11.7
Sucrose	10	20.1	20.1	20.1
Cellulose	5	5.8	5.8	5.8
Soybean oil	7	2.9	2.9	2.9
Lard	0	20.7	20.7	20.7
Cholesterol	0	0.5	0.5	0.5
Mineral mixture	3.5	1.2	1.2	1.2
Dicalcium phosphate	0	1.5	1.5	1.5
Calcium carbonate	0	0.6	0.6	0.6
Potassium citrate	0	1.9	1.9	1.9
Vitamin mixture	1	1.2	1.2	1.2
Choline bitartrate	0.3	0.2	0.2	0.2
RTE	0	0	2	0
GTE	0	0	0	2

2.5. Western blot analysis

Epididymal adipose tissue and liver were homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% sodium dodecylsulfate (SDS), 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The homogenate was centrifuged at 18,300*g* for 30 min at 4°C, and the supernatant was collected. The total protein concentration of each lysate was measured with BCA Protein Assay Reagent (Pierce, Rockford, IL, USA). Proteins in the lysates were electrophoretically separated in 7.5% to 12.5% SDS polyacrylamide gel and then transferred to polyvinylidene difluoride membranes (GE Healthcare Life Sciences, Piscataway, NJ, USA). The membranes were blocked in 5% bovine serum albumin (Roche Diagnostics, Mannheim, Germany) overnight at 4°C and then incubated overnight at 4°C with the following primary antibodies: PPARγ, C/EBPα, perilipin, phospho-acetyl-CoA carboxylase (p-ACC), phospho-AMPKα (p-AMPK), HMGCR and β-actin. Antibodies against PPARγ, C/EBPα, perilipin, HMGCR and β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and those against p-AMPK and p-ACC were obtained from Cell Signaling Technology (Beverly, MA, USA). The membranes were next incubated with horseradish-peroxidase-conjugated secondary antibodies overnight at 4°C. The bands were visualized with enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, UK), and the intensities of the bands were quantified in Scion Image for Windows.

2.6. Cell culture and adipocyte differentiation induction

3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM), bovine calf serum (BCS) and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA, USA). Insulin was obtained from Roche Diagnostics (Mannheim, Germany), and 3-isobutyl-1-methylxanthine, dexamethasone and trigonelline were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tomatine was obtained from Chromadex (Irvine, CA, USA). 3T3-L1 preadipocytes were cultured in DMEM containing 10% BCS at 37°C in 5% CO_2 incubator. To induce differentiation, 2-day postconfluent preadipocytes were incubated in differentiation medium containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone and 1 μg/ml insulin for 2 days. The medium was then changed to DMEM containing 10% FBS and 1 μg/ml insulin, and the cells were cultured for 2 days. The cells were then incubated in DMEM supplemented with 10% FBS for 2 more days [23].

2.7. Oil Red O staining

Oil Red O dye was purchased from Sigma Chemical Co. (St. Louis, MO, USA). After the induction of adipocyte differentiation, the cells were washed with phosphate-buffered saline and fixed at room temperature with 10% formalin for 1 h. 3T3-L1 cells

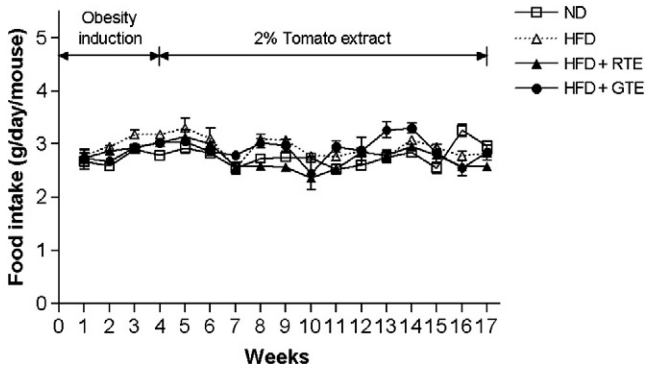

Fig. 1. Effect of tomato extracts on food intake in high-fat-diet-induced obese mice. C57BL/6 mice were fed normal or high-fat diets for 4 weeks. The animals were then treated with 2% RTE or 2% GTE for 13 weeks. The food intake per mouse was recorded every other day. Values are mean±S.E. of 10 mice.

were stained at room temperature with Oil Red O for 1 h and washed three times with distilled water. For quantitative analysis, Oil Red O staining was dissolved in isopropanol, and the optical density was measured at λ=490 nm with a Molecular Devices enzyme-linked immunosorbent assay reader (Sunnyvale, CA, USA).

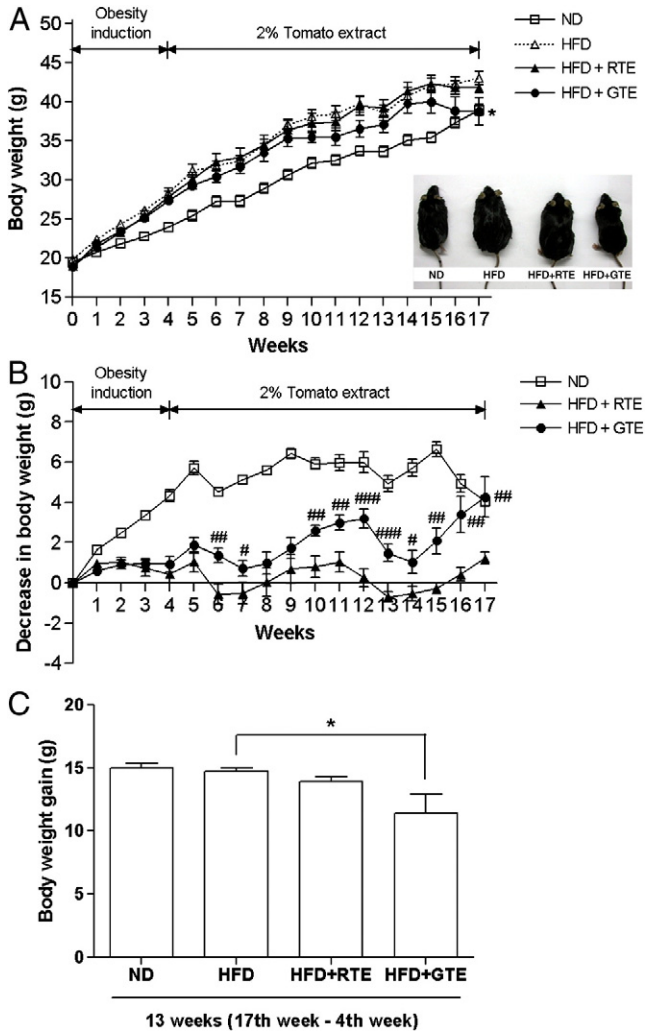

Fig. 2. Effect of tomato extracts on body weight in high-fat-diet-induced obese mice. (A) Body weight was measured weekly. (B) Decrease in body weight was measured weekly. (C) Body weight gain was measured from the 4th week until the 17th week. Values are mean±S.E. (n=10). $^{*}P$<.05; significantly different from the HFD group. $^{\#}P$<.05, $^{\#\#}P$<.01, $^{\#\#\#}P$<.001; significantly different from the HFD plus RTE group.

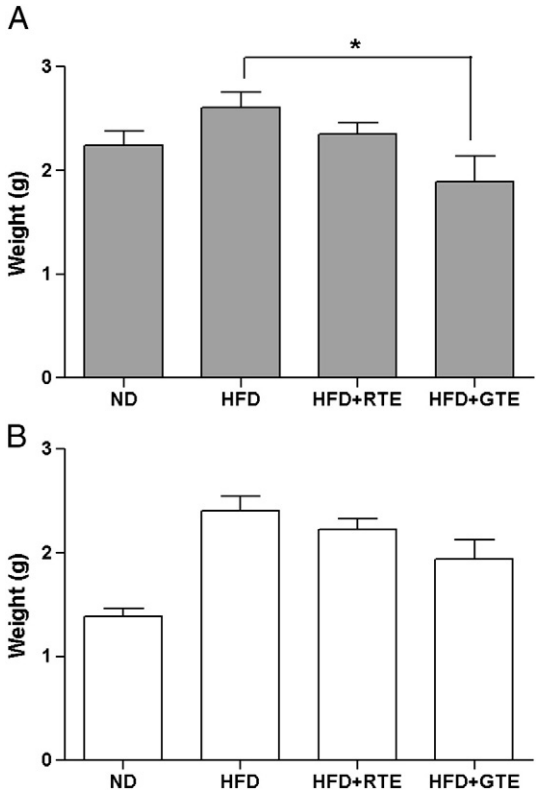

Fig. 3. Effect of tomato extracts on organ weights in high-fat-diet-induced obese mice. (A) Epididymal adipose tissue and (B) liver were obtained from the mice after fasting for 15 h at the end of study. Values are expressed as mean±S.E. (n=10). $^{*}P$<.05, significantly different from the HFD group.

2.8. Statistical analysis

Values are expressed as the mean±standard error (S.E.). Statistical significance was determined by a one-way analysis of variance with Newman-Keuls Multiple Comparison test. P values less than .05 were considered statistically significant.

3. Results

3.1. Invariability of food intake in mice during study period

C57BL/6 mice were randomly divided into four groups (n=10). The first group was fed normal diet (ND), the second was fed high-fat diet (HFD), and the other two groups were fed HFD plus 2% RTE or 2% GTE. Food intake was measured every other day. The weekly food intakes among the groups were not significantly different throughout the experimental period (Fig. 1). The average food intake during the experimental period in mice fed ND, HFD, HFD plus RTE or HFD plus GTE was 2.7±0.4 g, 2.9±0.5 g, 2.8±0.4 g or 2.9±0.5 g, respectively. Mice fed the tomato extracts did not show any pathological signs or abnormalities during the feeding period.

3.2. Inhibitory effect of GTE on body weight gain

At the fourth week, the body weight of mice fed HFD was significantly increased compared with that of mice fed ND (Fig. 2A). The body weight in the group fed ND was 23.9±0.5 g, while that in the three groups fed HFD was 28.3±0.7 g, 27.8±0.7 g and 27.3±0.4 g at the fourth week. GTE supplement in mice fed HFD suppressed further gains in body weight throughout the remainder of the study. Indeed, the body weight of mice fed HFD plus GTE was gradually

Table 3
Effect of tomato extracts on biochemical parameters of serum and hepatic lipid contents in high-fat-diet-induced obese mice

Unit: mg/dl

	ND	HFD	HFD+RTE	HFD+GTE
Serum				
Total cholesterol	171.9±14.6	231.7±16.5	224.2±22.6	217.3±22.4
LDL-cholesterol	16.6±1.3	28.2±2.0	25.4±2.8	23.5±3.7
HDL-cholesterol	72.9±6.3	90.3±6.0	89.1±7.6	87.7±5.6
Triglyceride	22.7±1.2	18.7±1.6	18.2±1.8	17.0±1.1
Liver				
Total cholesterol	95.4±2.2	213.2±26.1	183.7±19.0	98.9±12.2***
Triglyceride	1687±79.0	1805±63.7	1807±31.4	1586±195.3

Serum and liver were collected from the mice after fasting for 15 h at the end of study; lipids were then analyzed. Values are expressed as mean±S.E. of 10 mice. ***P<.001, significantly different from the high-fat diet group.

decreased, and the body weight difference between HFD and HFD plus GTE was also gradually increased during weeks 9–17, except for weeks 13–15. At weeks 9, 10, 11, 12, 16 and 17, the body weights of mice fed HFD plus GTE was reduced by approximately 4.6%, 6.8%, 7.7%, 8%, 8% and 9.9%, respectively, compared to those in mice fed HFD. However, the body weight of mice fed HFD plus RTE was not changed compared with that of mice fed HFD. Decrease in body weight in mice fed GTE was statistically significant during weeks 10–17 compared to that in RTE-fed mice (Fig. 2B). Final body weights of mice fed ND, HFD, HFD plus RTE and HFD plus GTE were 38.9±0.7 g, 43.0±0.9 g, 41.8±0.7 g and 38.8±1.8 g, respectively. Body weight gain in mice fed HFD plus GTE was reduced by about 22.5% compared to that in mice fed HFD (Fig. 2C).

3.3. Inhibitory effect of GTE on adipose and liver mass gain

Brain, kidney and lung weights in mice were not changed among the groups. However, epididymal adipose tissue weight from mice fed HFD plus GTE was significantly decreased by approximately 27.5% compared to that in mice fed HFD (Fig. 3A). Liver weight in mice fed HFD significantly increased by 42.2% compared to that in ND-fed mice, and GTE decreased liver weight by 19.5% (Fig. 3B). HFD plus RTE slightly reduced epididymal adipose tissue and liver weights compared to those in HFD-fed mice. Adipose and liver masses correlated with body weight in the mice fed tomato extracts, particularly GTE, suggesting that the GTE-mediated decrease in body weight could be attributed to a reduction in adipose and liver masses, independent of food intake.

3.4. Effect of GTE on lipid profile in serum and liver

Serum total cholesterol and LDL-cholesterol levels in mice fed GTE were reduced by 6.2% and 16.7%, respectively, compared to those in HFD-fed mice (Table 3). RTE supplement in mice fed HFD decreased total cholesterol and LDL-cholesterol levels by 3.2% and 9.9%, respectively, compared to those in mice fed HFD. Serum HDL-cholesterol and triglyceride levels were not different among HFD, HFD plus RTE and HFD plus GTE groups.

Total cholesterol level in liver from HFD-fed mice increased by approximately 55.3% compared with that from mice fed ND (Table 3). GTE supplement in HFD-fed mice significantly decreased the liver total cholesterol level by about 53.6%, to a level similar to that in mice fed ND. RTE supplement in HFD-fed mice reduced total cholesterol level by about 13.8% compared to that in HFD. HFD plus GTE reduced the liver triglyceride level by 12.1% compared to that in HFD, although it was not significant compared to the level in HFD-fed mice.

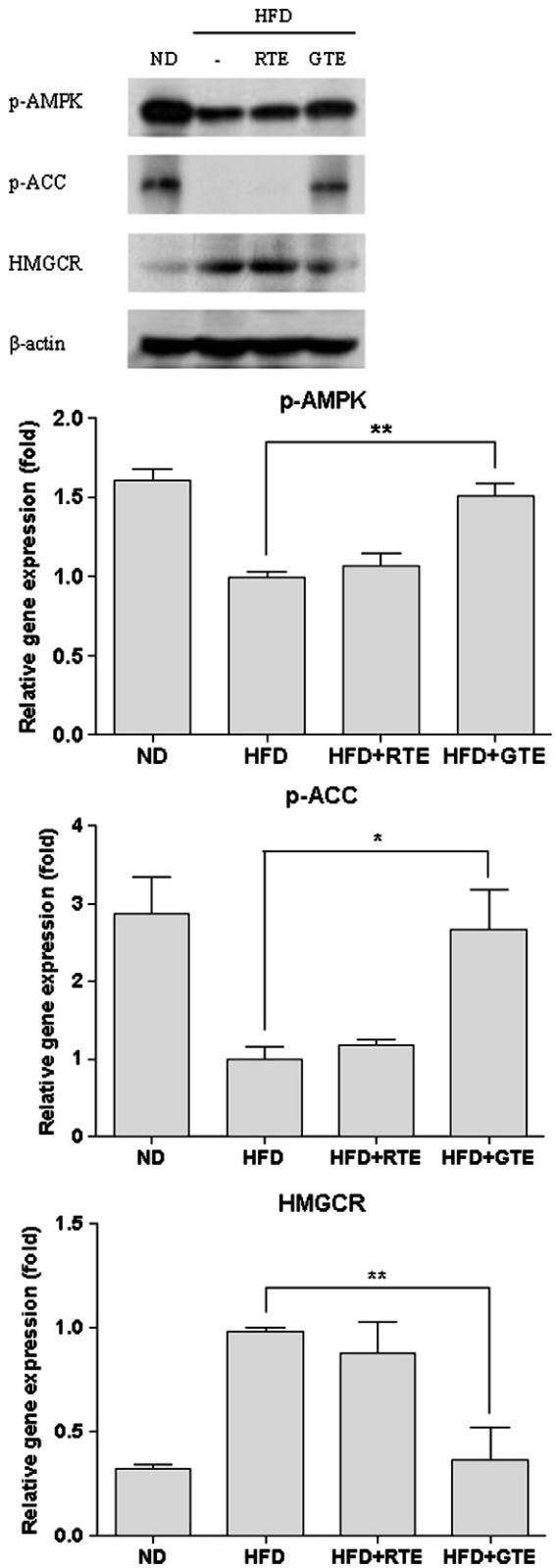

Fig. 4. Effect of tomato extracts on the expression of energy and lipid metabolism genes in liver. Liver was homogenized and the lysates were subjected to Western blot analysis for p-AMPK, p-ACC and HMGCR. The intensity of each band was quantified by the Scion Image for Window Program. Values are expressed as mean±S.E. *P<.05, **P<.01; significantly different from the HFD group.

3.5. Effect of GTE on the expression of enzymes involved in fatty acid and cholesterol synthesis in liver

In this study, the expression of p-AMPK, p-ACC and HMGCR, which are involved in lipid synthesis, was assessed in liver of mice fed HFD plus tomato extracts. HFD suppressed the phosphorylation of AMPK and ACC, and GTE supplement in HFD-fed mice restored AMPK and ACC phosphorylation (Fig. 4). HMGCR expression significantly decreased in mice fed HFD plus GTE compared to that in HFD-fed mice. The expression of p-AMPK and p-ACC in mice fed HFD plus GTE increased by approximately 0.5-fold and 1.7-fold, respectively, compared with those in mice fed HFD, and HMGCR expression in mice fed HFD plus GTE decreased by about 0.6-fold. Phosphorylation of AMPK and ACC was slightly increased in mice fed HFD plus RTE, and HMGCR expression was also mildly decreased.

3.6. Inhibitory effect of GTE on expression of adipocyte marker proteins in adipose tissue

PPARγ and C/EBPα play essential roles in adipocyte differentiation through transcription of various genes responsible for fat transport and accumulation. PPARγ regulates perilipin gene expression in adipocytes, and perilipin participates in storage and mobilization of lipids. The expression of PPARγ, C/EBPα and perilipin in adipose tissue of mice fed HFD was elevated by 0.5-fold, 0.5-fold and 0.5-fold, respectively, compared with those of normal mice (Fig. 5). PPARγ, C/EBPα and perilipin expressions were strongly decreased in mice fed HFD plus GTE compared to those in HFD-fed mice. GTE supplement in mice fed HFD decreased PPARγ, C/EBPα and perilipin expressions by approximately 0.8-fold, 0.9-fold and 1.0-fold, respectively, compared with those of mice fed HFD. RTE supplement significantly reduced PPARγ, C/EBPα and perilipin expressions to a lesser degree than GTE. The expressions of PPARγ, C/EBPα and perilipin in adipose tissue of mice fed HFD plus RTE were reduced by 0.5-fold, 0.6-fold and 0.7-fold, respectively, compared with those of mice fed HFD. These results suggest that the GTE-mediated decrease in adipose tissue weight could be attributed to a reduction in expression of adipocyte marker proteins.

3.7. Inhibitory effect of tomatine on adipocyte differentiation in 3T3-L1 cells

Adipocyte differentiation has often been a target for antiobesity strategies. Confluent 3T3-L1 preadipocytes initiate the expression of differentiation-related transcription factors when the cells are exposed to adipogenic inducers containing 3-isobutyl-1-methylxanthine, dexamethasone and insulin. The cells then become spherical, accumulate lipid droplets and develop into fully differentiated cells. In this study, we examined whether tomatine and trigonelline (Fig. 6A), the compounds derived from tomato extracts, modulate the differentiation of 3T3-L1 preadipocytes into mature adipocytes. Tomatine (10 μM) strongly decreased the formation of lipid droplets, and trigonelline (300 μM) mildly reduced the fat accumulation (Fig. 6B and C). The relative fat contents of the adipocytes treated with 10 μM tomatine or 300 μM trigonelline were 21.4%±11.6% or 76.6%±5.3%, respectively, of the control. These results suggest that tomatine may be responsible for GTE-mediated decrease in body weight.

4. Discussion

Obesity is a risk factor for type II diabetes, coronary heart disease, hypertension and metabolic syndrome [1–3]. Tomato is a food source that contains various compounds with health benefits, and previous studies have shown that tomato intake reduces the incidence of coronary heart disease and platelet aggregation in type II diabetes [24,25]. In this study, we investigated whether RTE and GTE could

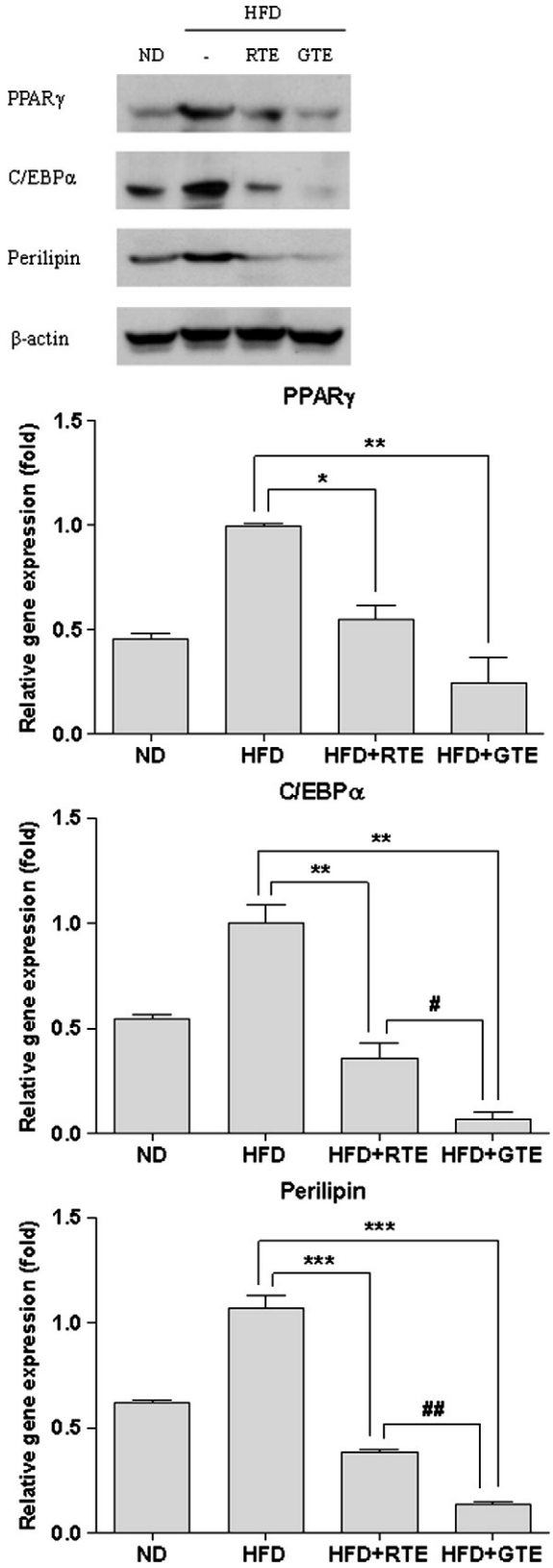

Fig. 5. Effect of tomato extracts on the expression of adipocyte marker proteins in adipose tissue. Epididymal adipose tissue was homogenized and the lysates were subjected to Western blot analysis for PPARγ, C/EBPα and perilipin. The intensity of each band was quantified by the Scion Image for Window Program. Values are expressed as mean±S.E. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$; significantly different from the HFD group. $^{\#}P<.05$, $^{\#\#}P<.01$; significantly different from the HFD plus RTE group.

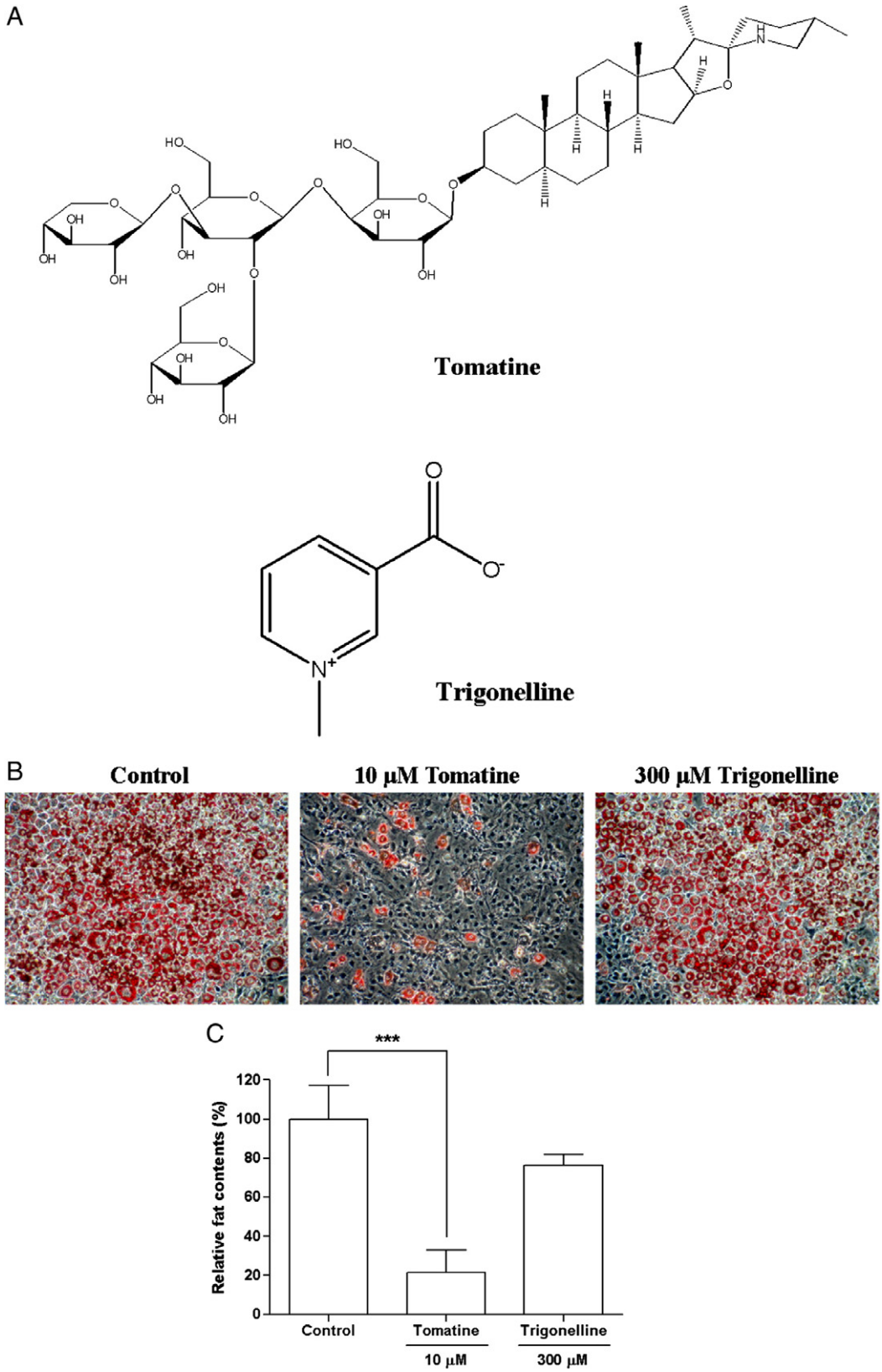

Fig. 6. Effect of tomatine and trigonelline on adipocyte differentiation. (A) Chemical structures of tomatine and trigonelline. (B) 3T3-L1 preadipocytes were cultured in the differentiation medium containing 10 μM tomatine or 300 μM trigonelline for 6 days. The cells were stained with Oil Red O and photographed (100×). (C) The stained fat content was quantified, and the percentages presented for the adipocytes treated with tomatine or trigonelline are relative to the control (100%). All values are presented as the mean±S.D. of three experiments performed in triplicate. Statistical significance: ***P<.001.

improve HFD-induced obesity in C57BL/6 mice and explored their mechanism. C57BL/6 mice were maintained on ND or HFD for 4 weeks and then fed ND, HFD, HFD plus RTE or HFD plus GTE for another 13 weeks. The weekly food intakes among the groups were not different from each other. Body weight of mice fed HFD plus GTE was reduced to that of mice fed ND when measured at the end of study, but the body weight in mice fed HFD plus RTE was only slightly reduced. Similarly, green tomato feeding in cholesterol-fed hamsters reduced body weight gain more than red tomato feeding [26]. Consistent with the results in body weight change, GTE supplement obviously reduced the weights of epididymal adipose tissue and liver in HFD-fed mice, but RTE supplement modestly lowered the organ weights. These results suggest that the GTE-mediated decrease in body weight was due to a reduction in adipose tissue and liver weight, independent of food intake.

In hamsters, green tomato lowered the cholesterol diet-induced levels of plasma cholesterol, LDL-cholesterol and triglyceride, and red tomato decreased the levels to a lesser degree [26]. Unlike red tomato, green tomato contains high amounts of α-tomatine, dehydrotomatine and trigonelline and low amounts of carotenoids [18–21]. A tomatine-enriched diet significantly decreased plasma LDL-cholesterol levels in hamsters fed a high-fat, high-cholesterol diet [16]. Tomatine also reduced serum levels of cholesterol and triglyceride and liver cholesterol levels in cholesterol-fed rats [27]. Trigonelline decreased the serum and liver levels of cholesterol and triglyceride in nonobese type 2 diabetic Goto–Kakizaki rats [28]. In accordance with these studies, we found that serum cholesterol and LDL-cholesterol levels and liver triglyceride level in mice fed GTE were further reduced compared with those in mice fed RTE. GTE supplement also strongly reduced liver cholesterol levels in HFD-fed mice compared with RTE supplement. Thus, GTE supplement is much more effective in reducing cholesterol levels than RTE supplement.

The AMPK pathway is involved in the regulation of body weight, systemic glucose homeostasis, lipid metabolism, mitochondrial biogenesis and insulin signaling [5]. Once AMPK is activated, lipogenesis in liver is inhibited, which consequently inhibits fat accumulation [5,29]. Phosphorylation of AMPK inactivates metabolic enzymes such as ACC and HMGCR [7–9]. AMPK phosphorylation in C57BL/6 mice is inhibited by HFD [29–31]. In this study, GTE supplement restored AMPK phosphorylation and inhibited the activities of lipogenic enzymes such as ACC and HMGCR in livers of HFD-fed mice. RTE supplement in HFD-fed mice also slightly increased AMPK phosphorylation and mildly decreased ACC and HMGCR activation. Phosphorylated AMPK inhibits PPARγ and C/EBPα, which are mainly found in adipose tissue and act as key transcription factors during adipogenesis and lipogenesis [10,32,33]. Perilipin, a PPARγ target gene, participates in storage and mobilization of lipids [34]. We found that GTE supplement strongly decreased the expression of PPARγ, C/EBPα and perilipin in the adipose tissue and that RTE significantly lowered the expression of these proteins to a lesser degree than GTE.

Adipocyte differentiation is closely associated with obesity. In this study, tomatine, one of the major compounds in GTE, potently inhibited fat accumulation during adipocyte differentiation. Thus, this result indicates that tomatine may be responsible for the GTE-induced reduction of body weight.

In conclusion, GTE attenuated HFD-induced obesity through activation of the AMPK pathway in C57BL/6 mice, and GTE may be a potential candidate for antiobesity drugs.

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