Research Article

Korean Chungtaejeon tea extract attenuates weight gain in C57BL/6J–Lep ob/ob mice and regulates adipogenesis and lipolysis in 3T3–L1 adipocytes

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ABSTRACT

OBJECTIVE: Traditional Korean Chungtaejeon (CTJ) tea is a type of fermented tea, which has received increasing attention in recent years because of its purported health benefits. The present study was designed to investigate the effect and mechanism of CTJ tea extract on body weight gain using C57BL/6J-Lep ob/ob mice and 3T3-L1 adipocytes, respectively.

METODS: The effects of CTJ on cell viability, lipid accumulation, and expression of protein and mRNA were measured in 3T3-L1 adipocytes by using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, oil red O staining, Western blotting, and reverse transcriptase-polymerase chain reaction analyses. C57BL6J-Lep ob/ob mice were administered with CTJ (200 or 400 mg/kg body weight) for ten weeks. Then, body weight, food intake, total cholesterol, and triglyceride were measured in ob/ob mice.

RESULTS: CTJ tea extract treated at 250 μg/mL (CTJ250) significantly suppressed lipid accumulation in the differentiated 3T3-L1 adipocytes. Likewise, CTJ250 significantly decreased the protein expression of peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α, and adipocyte lipid-binding protein, and regulated the mRNA expression of PPARγ, sterol regulatory element-binding protein-1c gene, fatty acid synthase, adipocyte lipid-binding protein, hormone-sensitive lipase, carnitine palmitoyl transferase 1, cluster of differentiation 36, and acetyl-CoA carboxylase in the differentiated 3T3-L1 adipocytes. Mice administered with CTJ showed dose-dependent decrease in body weight gain, starting from week 4 of the experiment. CTJ tea extract administered at 400 mg/kg body weight significantly decreased fat mass, food efficacy ratio, and levels of plasma triglyceride and total cholesterol.

CONCLUSION: CTJ attenuated weight gain in ob/ob mice and regulated the activity of the molecules involved in adipogenesis and lipolysis in 3T3-L1 adipocytes. CTJ is a potentially valuable herbal therapy for the prevention of obesity and/or obesity-related disorders.

Keywords: adipogenesis; Chungtaejeon; lipolysis; C57BL/6J-Lep ob/ob mice; weight loss


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1 Introduction

Obesity is a root cause of numerous metabolic diseases, including hyperglycemia, dyslipidemia, hypertension, atherosclerosis, and fatty liver disease. Obesity has become a serious global health problem in the 21st century, with an estimated 1.9 billion overweight adults worldwide. One theory is that obesity is caused when an imbalance between energy intake and energy expenditure causes excessive growth and expansion of adipose tissues, leading to the deposition of excessive fat, with morphological and functional changes in adipocytes. An increase in both the number and the mass of adipocytes is related to weight gain. Therefore, regulating adipogenesis and lipolysis may control weight gain. Adipogenesis is a differentiation process by which pre-adipocytes are converted to fully differentiated adipocytes and is mediated by a series of programmed changes in gene expression. Blocking the activities of the master regulator of adipogenesis, peroxisome proliferator-activated receptor γ (PPARγ), and its target genes, which include genes encoding fatty acid synthase (FAS), adipocyte lipid-binding protein (aP2), acetyl-CoA carboxylase (ACC), and cluster of differentiation 36 (CD36), could be a promising means of regulating body weight (BW). Lipolysis is a catabolic process leading to the breakdown of triglyceride (TG) and the release of free-fatty acids and glycerol, which also plays an important role in regulating lipid metabolism. The lipolysis pathways in adipocytes are firstly initiated by adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), both of which have the capacity to degrade TG, thus governing the lipolysis in adipose tissue. Adipose tissue lipolysis and adipogenesis have received much attention over recent years because of their important roles in regulating adipose mass. Yet, drugs available for the treatment and prevention of obesity are associated with undesirable side effects. Therefore, recent research has focused on developing healthy foods or drugs derived from botanical sources that can regulate both adipogenesis and lipolysis in 3T3-L1 adipocytes because 3T3-L1 adipocytes display phenotypic characteristics of multiple adipocyte lineages.

Tea is the most widely consumed beverage in the world. Promising experimental data on the beneficial effects of tea in various chronic diseases are available. Here, we investigated the effect of Chungtaejeon (CTJ) on body mass regulation using ob/ob mice. CTJ, a traditional Korean fermented tea that was first introduced in the Jianghyung area of the Jeonnam Province in Korea, is obtained by processing *Camellia sinesis*. This plant is reported to have potent antioxidant effects and important biological properties, such as antimutagenic, antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic properties. Herbal tea preparations have been used for many years in the management of diabetes and obesity, which are the primary risk factors for several diseases. This study was designed to delineate the beneficial effect of CTJ on BW regulation. We hypothesized that CTJ would strongly moderate BW gain in ob/ob mice and regulate the expression of genes associated with adipogenesis and lipolysis in 3T3-L1 adipocytes.

2 Materials and methods

2.1 Reagents

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), insulin, dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), oil-red O, 2-mercaptoethanol and anti-β-actin were purchased from Sigma-Aldrich (St Louis, MO, USA). Primary antibodies against PPARγ, CCAAT/enhancer-binding protein α (C/EBPα), and aP2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Nitrocellulose membrane for Western blotting was purchased from Bio-Rad (Richmond, CA, USA). Western blot chemiluminescent substrate and enzyme-linked immunosorbent assay kits were purchased from IMEGENEX (San Diego, CA, USA) and AsanPharm (Seoul, Korea), respectively. All solvents, chemicals, and reagents were of analytical grade and purchased from Sigma-Aldrich, unless otherwise specified.

2.2 Processing and extraction of CTJ

CTJ was processed as described in our previous study. Lyophilized tea (112 g) was extracted with 3.3 L of distilled water for 2 h. The extract was evaporated and freeze-dried to produce CTJ powder. The yield of the extract was about 12% of the starting material.

2.3 Cell culture and differentiation

3T3-L1 preadipocytes obtained from the American Type Culture Collection (Manassas, VA, USA) were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, 100 U/mL penicillin, 44 mmol/L NaHCO₃, and 1 mmol/L sodium pyruvate at 37 °C under a 5% CO₂ atmosphere. To induce differentiation, 3T3-L1 preadipocytes were cultured until confluence was reached (0 day), and the culture medium was replaced with a fresh induction medium containing 5 mg/mL insulin, 0.5 mmol/L IBMX, and 1 μmol/L dexamethasone in DMEM with 10% FBS for 2 d. The medium was then replaced with a differentiation medium containing 5 μg/mL insulin only and DMEM medium containing 10% FBS every 2 d for 8 d until the cells were harvested. To examine the effect of CTJ on adipogenesis, CTJ was dissolved in the differentiation medium.
2.4 Cell viability
3T3-L1 preadipocytes were seeded at a density of 4×10^3 cells/well in a 96-well plate. After 4 h, CTJ at different concentrations was added to each well and incubated for 24 h. MTT solution (2 mg/mL) was added to each 96-well plate and incubated for 4 h, and then the medium containing MTT solution was removed. The formazan crystals in the viable cells were solubilized with DMSO (150 μL), and absorbance was determined at 540 nm by a Microplate Reader (Immuno Mini NJ-2300, Japan).[13]

2.5 Oil red O staining
Intracellular lipid accumulation was measured using oil red O staining. The mature 3T3-L1 adipocytes were washed with PBS, fixed with 10% formalin (pH 7.4) for 30 min, and stained with oil red O solution for 1 h. The fat droplets in 3T3-L1 adipocytes were observed by phase-contrast microscopy.

2.6 Western blot analysis
3T3-L1 adipocytes, cultured in 6-well plates, were treated with CTJ for 8 d and harvested using protein extraction solution (PRO-PREP™). Cell debris was removed by centrifugation and the protein quantity in the lysate was determined using Bradford reagent (Bio-Rad Laboratories, Hercules, CA, USA). Cell lysates containing 30 μg of protein were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes, and the membranes were blocked with a solution of 0.1% Tween 20 in Tris-buffered saline containing 5% skim milk for 1 h at room temperature followed by overnight incubation with primary antibody at 4 °C. After overnight incubation with PPARγ, C/EBPα, and aP2, the membranes were incubated with anti-mouse horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Finally, protein bands were detected using the chemiluminescent substrate and VisionWorks™ LS (Analysis Software, Upland, CA, USA).

2.7 Animals and diets
C57BL/6J ob/ob mice were acclimatized for one week prior to experimentation. The animals were housed in an animal room controlled at (24 ± 1) °C and 55% ± 5% humidity with a 12-h light dark cycle and were provided standard normal pellet diet purchased from Central Lab Animal, Inc. (Seoul, Korea) and water ad libitum. Even though *Camellia sinensis* is edible, acute toxicity testing was done. As expected, no death was observed using a plant extract dose of up to 2 g/kg BW. After one week of acclimatization, the mice were randomly divided into three groups by avoiding any intergroup differences in BW. The first group (control) was given water only and the remaining two groups were given CTJ tea extract (dissolved in water) at the doses of 200 or 400 mg/kg BW once daily for 10 weeks by oral gavage (approximately 0.2 mL in volume). At the end of the experiment the mice were sacrificed by cervical dislocation and blood samples were collected from the inferior vena cava, centrifuged at 3 000 r/min for 15 min, and stored at −70 °C for TG and total cholesterol (TC) analyses. After collecting the blood, adipose tissues were removed, rinsed with a physiological saline solution, weighed, and immediately stored at −70 °C. The Animal Care Committee of the Graduate School of Natural Sciences, Mokpo National University (MNU-IACUC-2012-006) and all husbandry practices and animal care were in accordance with the guidelines of the Korean Council on Animal Care.

2.8 Body weight, adipose tissue weight, and food efficacy ratio
BW and the amount of food intake for each group were recorded every week. At the end of the experiment, adipose tissue (subcutaneous and visceral) was removed surgically, and weighed. Food efficacy ratio (FER) was calculated from BW gain in relation to the amount of food consumed.

2.9 Biochemical analyses
The blood was collected in a heparin-coated tube and centrifuged at 3 000 r/min for 20 min at 4 °C. Plasma TG and TC levels were measured spectrophotometrically using commercially available kits (AsanPharm, Seoul, Korea).

2.10 Reverse transcription-polymerase chain reaction
Total RNA was isolated from the tissues by using TRI REAGENT (Molecular Research Center, INC, USA). Isolated RNA was quantified by measuring the optical density (OD) at 260 and 280 nm using a Nanodrop 2000 spectrophotomer (Thermo Scientific, USA). Isolated RNA (50 ng) was added to Diastar™ 2X One Step reverse transcription-polymerase chain reaction (RT-PCR) Premix (final 30 μL volume) with each forward and downward primer. Temperature cycle for PCR reaction was 50 °C for 30 min, 95 °C for 15 min and 35 cycles of denaturation (95 °C for 20 s, annealing at 59 °C for 40 s, and extension at 72 °C for 1 min) followed by final denaturation at 72 °C for 5 min. The obtained PCR products were analyzed on Safe Red-stained agarose (1.5%) gel (iNtRON Biotechnology, Seoul, Korea) as described previously. The primers used in RT-PCR are shown in Table 1.

2.11 Statistical analysis
Values are expressed as mean ± standard error of mean. Statistical analyses were performed by one-way analysis of variance, followed by Duncan’s test and two-tailed *t*-test. In all cases, *P*-values < 0.05 were considered statistically significant.

3 Results
3.1 Effects of CTJ on cell viability, lipid accumulation, and expression of adipogenic factors in 3T3-L1 adipocytes
CTJ did not affect cell cytotoxicity up to 250 μg/mL.
Primers for reverse transcription-polymerase chain reaction

<table>
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<th>Description</th>
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<th>Anti-sense primer (5′→3′)</th>
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PPARγ: peroxisome proliferator-activated receptor γ; SREBP-1c: sterol regulatory element-binding protein-1c; FAS: fatty acid synthase; aP2: adipocyte lipid-binding protein; HSL: hormone-sensitive lipase; CPT-1: carnitine palmitoyl transferase-1; CD36: cluster of differentiation 36; ACC: acetyl Co-A carboxylase.

3.2 Effects of CTJ on mRNA expression of PPARγ, sterol regulatory element-binding protein-1c, FAS, aP2, HSL, carnitine palmitoyl transferase 1, CD36, and ACC in 3T3-L1 adipocytes

PPARγ and its target genes, such as sterol regulatory element-binding protein-1c (SREBP-1c), FAS, aP2, CD36, and ACC, were highly expressed in differentiated adipocytes, but were strongly inhibited by CTJ250 treatment (Figure 2). In addition, HSL and carnitine palmitoyl transferase 1 (CPT-1), which are rate-limiting enzymes of lipolysis, were effectively increased in CTJ250-treated adipocytes, suggesting that CTJ enhances lipolysis in adipocytes.

3.3 Effect of CTJ on body weight, total fat mass, and food efficacy ratio in obese mice

BW, total fat mass, and FER were significantly increased in control group mice when compared to CTJ-treated groups (Figures 3A–C). CTJ-treated groups tended to have decreased BW starting from week 4, with CTJ400 group displaying a significant 17% decrease in BW when compared to untreated ob/ob mice (Figure 3A). This group also displayed markedly decreased total fat mass and FER (Figures 3B and 3C). Visceral and subcutaneous fat did not differ in control and CTJ treatment groups (data not shown).

3.4 Effect of CTJ on plasma TC and TG levels in obese mice

Hypertriglyceridemia and hypercholesterolemia are important risk factors of obesity.[12] Presently, plasma TC and TG levels were significantly increased in the control group mice compared to CTJ-treated groups (Figure 4). The administration of CTJ at 400 mg/kg BW significantly decreased plasma TC and TG levels by 19% and 28%, respectively, compared to control.

4 Discussion

Obesity, thought to be caused by excessive fat accumulation and energy overload, is the root cause of several metabolic diseases.[1] However, the medicines available for the treatment and prevention of obesity and its metabolic disorders are not safe for long-term use.[6] Herbal medicines have been considered as an important part of therapy for weight control.[15] In our previous study, we did not find any difference in food consumption and BW among different groups of rats eating a high-fat diet.[10] Therefore, in this study we have used another mouse model of obesity—that is ob/ob mouse (Lep−/Lep− mouse)—in which a single-base spontaneous mutation of the ob gene prevents the secretion of bioactive leptin. The secretion of bioactive leptin is directly proportional to the amount of stored TG in fat tissues.[10] In this study, CTJ was found to prevent BW gain, and decrease total fat mass and FER in ob/ob mice. Similarly, CTJ strongly inhibited lipid accumulation in 3T3-L1 adipocytes via regulating the expression of different molecules involved in adipogenesis and lipolysis in 3T3-L1 adipocytes. PPARγ, the master regulator of adipogenesis, has an important role in the regulation of lipid metabolism.
Figure 1  Effects of Chungtaejeon on cell viability (A), lipid accumulation (B) and protein expression (C) in 3T3-L1 adipocytes
(A) To measure cell viability, 3T3-L1 adipocytes were treated with CTJ at 25, 50, 125, and 250-μg/mL doses. Data are representative of three independent experiments (mean and standard error of mean). (B) Expression of adipogenic factors in 3T3-L1 adipocytes, two-tailed t-test. **P < 0.01. Control: non-addition; CTJ: Chungtaejeon; PPARγ: peroxisome proliferator-activated receptor γ; C/EBPα: CCAAT/enhancer-binding protein α; aP2: adipocyte lipid-binding protein.

Figure 2  Effect of Chungtaejeon on mRNA expression in 3T3-L1 adipocytes
Data are representative of three independent experiments (mean and standard error of mean) and analyzed by two-tailed t-test. **P < 0.01. Control: non-addition; CTJ: Chungtaejeon; PPARγ: peroxisome proliferator-activated receptor γ; SREBP-1c: sterol regulatory element-binding protein-1c; FAS: fatty acid synthase; aP2: adipocyte lipid-binding protein; HSL: hormone-sensitive lipase; CPT-1: carnitine palmitoyl transferase1; CD36: cluster of differentiation 36; ACC: acetyl Co-A carboxylase.
in maintaining the size and number of adipocytes during adipogenesis. It also induces lipid accumulation in fibroblasts. Therefore, PPARγ is a key factor in controlling adipocyte differentiation and lipogenesis. Moreover, PPARγ regulates the expression of genes, such as aP2, FAS, CD36, and ACC, which are related to the regulation of fatty acid synthesis and TG accumulation.

BW is regulated by several physiological factors. Modulation of adipocyte function is directly related to BW regulation. Therefore, we speculated that the lower BW and FER in CTJ-treated mice might be due to the inhibition of the master regulators of adipogenesis, PPARγ and C/EBPα. They regulate adipocyte differentiation by modulating the expression of their target genes in a sequential manner, and act synergistically to promote terminal differentiation by activating the transcription of the genes for FAS, aP2, and fatty acid transporter CD36, which are involved in creating and maintaining the phenotype of adipocytes. In this study, the protein expressions of PPARγ, C/EBPα and aP2, and the mRNA expressions of CD36 and FAS were significantly decreased in CTJ-treated adipocytes. Furthermore, synthesis of fatty acids is controlled by SREBP-1c via regulating the expression of FAS and ACC. Herein, we speculated that CTJ could have two different mechanisms in attenuating BW in ob/ob mice. The first mechanism may be due to the inhibition of fatty acid synthesis via regulating FAS and ACC at the mRNA level. The other mechanism could be due to the regulatory effect of CTJ in sensing the nutrients on the central nervous system of the mice because, as mentioned earlier, ob/ob mice have a defect on the central regulation of food intake.

The balance of adipogenesis and lipolysis determines the TG accumulation in mature adipocytes. Increased levels of plasma TC and TG are important markers of increased fat in the body. CTJ decreased the levels of both plasma TC and TG.
and TG. These results suggest that CTJ may have inhibited adipogenesis genes and stimulated lipid catabolism in adipose tissue, which may help promote fatty acid utilization, thereby leading to fat burning. HSL, which is also known as TG lipase, is the rate-limiting step in cleaving fatty acids from the TG. The released fatty acids are then consumed for β-oxidation. CPT-1, an enzyme that is present at the cytosol side of the outer mitochondrial membrane, transfers the acyl group on to the carnitine group allowing the transportation of acetyl group into the mitochondria through the carnitine carrier which is an essential step in β-oxidation of long chain fatty acids.[22] Therefore, CPT-1 and HSL are considered as important targets for regulating lipid metabolism in obese subjects. In this study, CTJ-treated adipocytes showed increased CPT-1 and HSL mRNA expression, demonstrating its effect on lipid metabolism. CTJ is enriched in polyphenols, flavonoids, and several catechins including gallocatechin, epicatechin gallate, epigallocatechin gallate, and catechin gallate.[23] Epigallocatechin gallate strongly inhibits lipid accumulation and stimulates lipolysis by increasing HSL expression in 3T3-L1 adipocytes.[24] In addition, several studies support the anti-obesity effect of tea extract and their major components in different animal models.[25–26]

5 Conclusion

In conclusion, CTJ reduces lipid accumulation in 3T3-L1 adipocytes and attenuates BW gain in ob/ob mice. These effects may be mediated by the regulation of adipogenesis and lipolysis in adipocytes and adipose tissue. However, chemical profiling of the CTJ extract and a long-term in vivo studies are required to definitively understand the molecular mechanism of the anti-obesity effect of CTJ. Our findings indicate the potential value of CTJ for the prevention and treatment of obesity or its related diseases.

6 Acknowledgment

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7 Competing interests

The authors declare no conflict of interests. The authors alone are responsible for the writing and contents of the paper.

REFERENCES


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