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Germinated Waxy Black Rice Extract Inhibits Lipid Accumulation with Regulation of Multiple Gene Expression in 3T3-L1 Adipocytes

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Abstract The aim of this study was to investigate the anti-obesity effects of germinated waxy black rice (GWBR) extract in 3T3-L1 adipocytes. The inhibitory effect of GWBR extract against adipocyte differentiation was evaluated using Oil Red O staining and glycerol-3-phosphate dehydrogenase (GPDH) assay. GWBR extract inhibited adipocyte differentiation, but was not found to elicit any cytotoxicity. The mRNA levels of adipogenic transcriptional factors such as C/EBP-α and -β, PPAR-γ, and SREBP-1c, as well as adipogenic enzymes, including aP2, LPL, and FAS were significantly down-regulated by treatment with GWBR extract compared to untreated control cells. However, mRNA levels of lipolytic genes such as HSL and ATGL, β-oxidation related genes CPT1, and UCP2 involved in thermogenesis were significantly up-regulated by treatment with GWBR extract. These data suggest that GWBR extract may be a potential functional food, and may have pharmacological applications in both the prevention and treatment of obesity.

Keywords: anti-obesity, germinated waxy black rice, 3T3-L1 adipocyte, lipid accumulation

Introduction

Recently, many consumers have shown increased interest in improving personal health through an improved diet. Accordingly, several studies on "functional foods" have progressed in a competitive manner in order to fulfill this new demand (1). Rice is a dietary staple food, consumed on a daily basis by one-third of earth's population. Colored rice specifically has been shown to have a higher concentration of functional materials (i.e. protein, vitamins, minerals, phenolic compounds, and organic acids) than colorless rice. Black rice, one type of pigmented rice, contains fiber, minerals, phenolic acids, and flavonoid components, in addition to the basic nutrients found in other types of rice (2). Several studies have reported that black rice has the ability to reduce oxidative stress and decrease atherosclerosis, as well as protect against insulin resistance, alcoholic liver injury, and light-induced retinol damage (3-8). Furthermore, waxy black rice (also called glutinous or sticky black rice) contains anthocyanins at higher concentrations than in the non-waxy black rice variety (9). Germinated waxy black rice (GWBR) is created by "sprouting" the rice kernels, or soaking them in tap water until the length of the sprout is 1 mm. During the germination process, the texture and flavor of black rice may be enhanced, and digestibility and bioavailability have been shown to increase. Germinated rice generally has improved nutritional and physical quality compared to non-germinated rice (10). Therefore, research into the nutritional benefits of germinated rice may have implications for improved human health.

The development of obesity increases various metabolic disorders such as insulin resistance, type 2 diabetes mellitus, hyperlipidemia, and hypertension. An imbalance between energy intake and energy expenditure is a major cause of obesity (11-13). As the understanding of obesity has increased, many anti-obesity drugs (i.e., orlistat, sibutramine, dinitrophenol, and thyroid hormone) have been studied and marketed, but the prevalence of obesity continues to rise. These medications also have side effects, which in some cases are quite severe and limit their utility (14). Recent studies, using *in vitro* and *in vivo* assays, have identified several natural bioactive compounds that have anti-adipogenic effects (15,16). To determined inhibitory effect of GWBR extract against obesity, understanding of molecular mechanism related to adipogenesis in mature adipocytes was necessary. CCAAT/enhancer binding protein-α, β (C/EBP-α, β), peroxisome proliferator-activated receptor-γ (PPAR-γ), and sterol regulatory element binding protein-1c (SREBP-1c) are stimulated during adipocyte differentiation as major adipogenic transcriptional factors. Adipogenic enzymes, particularly adipocyte fatty acid-binding protein (aP2), lipoprotein lipase (LPL), and fatty acid synthase (FAS) are stimulated as well (17).

Conversely, lipolysis, β-oxidation, and thermogenesis are important mechanisms linked to reducing body fat, which are reduced in an adipogenic state (18). Hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), carnitine palmitoyl transferase 1 (CPT1), and uncoupling protein 2 (UCP2) are utilized accumulated fats. Both down-regulation of adipogenic genes and up-regulation of lipolytic genes are known to elicit suppression of lipid accumulation in mature adipocytes (19,20).

In present study, we investigated the effect GWBR has on proliferation of 3T3-L1 preadipocytes using MTT assay. The degree of lipid accumulation and glycerol-3-phosphate dehydrogenase (GPDH) activity were also determined. To understand the anti-adipogenic molecular mechanism of GWBR, the levels of adipogenic transcriptional factors (PPAR-γ, C/EBP-α, C/EBP-β, and SREBP-1c), adipogenic enzymes (aP2, LPL, and FAS), lipolytic enzymes (HSL and ATGL), CPT1 related to β-oxidation, and UCP2 involved in thermogenesis were also evaluated.

Materials and Methods

Reagents and materials Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Hyclone Co. (Logan, UT, USA). 3-isobutyl-1-methyl-xanthine (IBMX), dexamethasone (DEX), 3'-(4,5-dimethylthiazol-2-ly)-2,5-diphenyl tetrazolium bromide (MTT) and Oil Red O were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human insulin was purchased from Roche (Indianapolis, IN, USA).

Preparation of germinated waxy black rice (GWBR) extract GWBR was prepared with germinated waxy black rice collected from Hamyang-gun, Korea on October 2012. To induce germination, WBR was soaked for 2-3 days in water and subsequently dried at 60°C. The dried WBR and GWBR were ground and 50 g of the resultant material was used to prepare extracts using heat reflux extraction in methanol, ethanol, and water. The resultant extracts were filtered with filter paper, evaporated under partial vacuum conditions, and subsequently freeze-dried. The samples were stored at −20°C. Samples were added to a medium to obtain various final concentrations (0, 0.01, 0.1, 0.5, and 1.0 mg/mL). 0.1 mg/mL caffeine was used as a positive control.

Cell culture and differentiation The 3T3-L1 cells (#CL-173) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were seeded in cell culture dish 100×20 mm (Sarstedt, Newton, NC, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf serum (CS) and 1% penicillin/streptomycin in a humidified 5% CO_2 incubator at 37°C. The cells were treated on day 0 (2 days post-confluence) with a MDI medium composed of 10% FBS, 0.5 mM IBMX, 0.1 μM DEX, 1 μg/mL of insulin, and GWBR extracts. On day 2, the MDI medium was exchanged with an INS medium composed of 10% FBS, 1 μg/mL of insulin, and GWBR extracts. On day 4, cells were additional incubated with DMEM containing 10% FBS until day 8 for further experimentation.

Cell viability To investigate effects of GWBR on cytotoxicity in 3T3-L1 preadipocytes, the cell were treated with WBR, and GWBR methanol, ethanol, and hot water extracts. MTT assay was performed to measure cell viability. 3T3-L1 preadipocytes were seeded in a 96-well plate at a density of 1×10^4 cells, and cultured in DMEM containing 10% CS. After 24 h of incubation, the medium and extracts were reacted for 48 h. After the cells were rinsed with PBS, 0.5 mg/mL MTT solution was added to the cells and incubated in the dark at 37°C for 4 h. For quantitative analysis, the solution was removed and formed formazan was dissolved by dimethyl sulfoxide (DMSO). The optical density was measured using a microplate reader (Model 680; Bio-Rad Laboratories, Hercules, CA, USA) at 540 nm.

Oil Red O staining To examine the inhibitory effects of the various extracts against adipocyte differentiation, the cells were stained with Oil Red O reagent to quantify lipid droplets in mature adipocytes. For this assay, 3T3-L1 preadipocytes were seeded into a 6-well plate at a density of 2×10^5 cells/well. The differentiation procedure is described above. After adipocyte differentiation on day 8, the cells washed twice with phosphate buffered saline (PBS) and fixed with 10% formal in at room temperature for 1 h. The fixed cells were washed twice with distilled water (DW) and stained with Oil Red O solution at room temperature for 2 h. After the staining, the cells were rinsed twice with DW and excess water removed at 60°C for 5 min. Morphology of lipid droplets in 3T3-L1 cells were observed using light microscopy. For quantitative analysis, stained dye was dissolved with isopropyl alcohol, and the absorbance was measured using a microplate reader at 490 nm.

Glycerol-3-phosphate dehydrogenase (GPDH) assay To harvested mature adipocytes on day 8, cells were washed twice with PBS, and scraped with ice-cold GPDH buffer [100 μL of 50 mM Tris-Cl buffer (pH 7.5), 1 mM EDTA, and 1 mM β-mercaptoethanol]. After centrifuge the cells at 11,000x*g* for 5 min, the supernatant was mixed with a GPDH reaction buffer [100 mM triethanolamine/HCl buffer (pH 7.5), 2.5 mM EDTA, 0.12 mM NADH, 0.2 mM dihydroxyacetone phosphate]. Then, GPDH activity was measured by a spectrophotometric method at 340 nm (Perkin-Elmer, Fremont, CA, USA). One unit of enzyme activity corresponds to the oxidation of 1 nmol of NADH/min. The GPDH activity was expressed as units per milligram of protein, and as a percentage compared to untreated control cells. The protein concentration was measured with a Bradford protein assay kit (Bio-Rad Laboratories).

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) analysis To identify the inhibitory mechanism of adipocyte

Table 1. Gene-specific primers used for RT-PCR

Gene	Direction	Sequence
C/EBP-β	Forward	5-GGG GTT GTT GAT GTT TTT GG-3
	Reverse	5-CGA AAC GGA AAA GGT TCT CA-3
C/EBP-α	Forward	5-CGC AAG AGC CGA GAT AAA GC-3
	Reverse	5-AGA GGT CCA CAG AGC TGA TTC C-3
SREBP-1c	Forward	5-GGC ACT AAG TGC CCT CAA CCT-3
	Reverse	5-GCC ACA TAG ATC TCT GCC AGT GT-3
PPAR-γ	Forward	5-CGC TGA TGC ACT GCC TAT GA-3
	Reverse	5-AGA GGT CCA CAG AGC TGA TTC C-3
aP2	Forward	5-CAT GGC CAA GCC CAA CAT-3
	Reverse	5-CGC CCA GTT TGA AGG AAA TC-3
LPL	Forward	5-ACT TGT CAT CTC ATT CCT GG-3
	Reverse	5-TCT CAT ACA TTC CCG TTA CC-3
FAS	Forward	5-CCT GGA TAG CAT TCC GAA CCT-3
	Reverse	5-AGC ACA TCT CGA AGG CTA CAC A-3
HSL	Forward	5-ACT CAG ACC AGA AGG CAC TA-3
	Reverse	5-TAG TTC CAG GAA GGA GTT GA-3
ATGL	Forward	5-ATT TAT CCC GGT GTA CTG TG-3
	Reverse	5-GGG ACA CTG TGA TGG TAT TC-3
CPT1	Forward	5-GTG TTG GAG GTG ACA GAC TT-3
	Reverse	5-CAC TTT CTC TTT CCA CAA GG-3
UCP2	Forward	5-CAG AGC AGG AGG TTA CAG TC-3
	Reverse	5-TCA ACC CCT TCA TTA CAG AC-3
β-Actin	Forward	5-TGT CCA CCT TCC AGC AGA TGT-3
	Reverse	5-AGC TCA GTA ACA GTC CGC CTA GA-3

differentiation by GWBR hot water extract, the mRNA levels related to adipogenesis, lipogenesis, lipolysis, β-oxidation, and thermogenesis were measured. Total RNA extraction from 3T3-L1 adipocytes was performed using an easy-BLUE™ total RNA extraction reagent (iNtRON Biotechnology, Seongnam, Korea) according to the manufacturer's protocol. The extracted RNA was dissolved in diethyl pyrocarbonate-treated water, and the RNA concentration was measured using spectrophotometry at 260 and 280 nm. Template cDNA synthesis from the extracted RNA templates was performed using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). PCR was carried out pre-denaturing at 95°C for 5 min, denaturing at 95°C for 30 s, annealing at 52-63°C for 30 s, and extension at 72°C for 30 s (45 cycles). The primer sequence is shown in Table 1. Gel were stained after gel electrophoresis with ethidium bromide 0.5 μg/mL and visualized by semi-quantitative analysis (Gel Doc 2000 and the Quantity One program; Bio-Rad Laboratories). The mRNA levels were determined using ImageJ software (Research Services Branch, National Institutes of Mental Health, Bethesda, MD, USA).

Statistical analysis Data are presented as mean±standard deviation (SD). Statistical differences were determined by ANOVA and Turkey's test using SAS statistical software package (SAS Institute, Cary, NC, USA). Values were considered statistically significant when $p<0.05$.

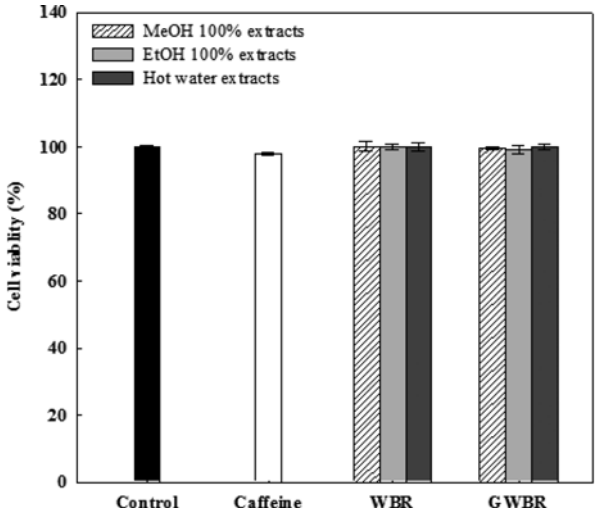

Fig. 1. Effect of WBR and GWBR extracts on cell viability in 3T3-L1 adipocytes. Cells were treated with WBR and GWBR extracts at 0.1 mg/mL for 24 h. Cell viability was measured by 3'-(4,5-dimethylthiazol-2-ly)-2,5-diphenyl tetrazolium bromide (MTT) assay. Results are presented as mean±SD. of at least three independent experiments. $p<0.05$ was considered a significant difference. WBR: Waxy black rice; GWBR: Germinated waxy black rice

Results and Discussion

Effects of WBR and GWBR on cell viability The effect of WBR and GWBR at 0.1 mg/mL on cell viability in 3T3-L1 preadipocytes was measured by MTT assay to confirm a lack of cytotoxicity. Caffeine and its metabolites have been shown to decrease lipid accumulation in 3T3-L1 adipocytes; therefore, caffeine 0.1 mg/mL was used as a positive control (21). As shown in Fig. 1, WBR, GWBR, and caffeine did not significantly affect cell viability at the same concentration.

Inhibitory effects of WBR and GWBR on adipocyte differentiation To investigate the inhibitory effects of WBR and GWBR on adipocyte differentiation, different forms of the extract were tested by Oil Red O staining at concentrations of 0.1 mg/mL in methanol, ethanol, or hot water. Among the extracts tested, GWBR hot water extract at 0.1 mg/mL exhibited the highest inhibitory effect against lipid accumulation, an increase of 41% compared to untreated control cells (Fig. 2A). Additionally, GWBR extracts inhibited lipid accumulation in 3T3-L1 cells significantly more than that by WBR extracts. These results suggest that the ability of WBR extract to inhibit lipid accumulation was improved by the germination process. Many studies found that the physical and biochemical changes of the germination process have various beneficial effects, including regulation of blood pressure and heart rate, decreasing blood glucose and lipid levels, suppression of fatty liver, inhibition of cancer cell proliferation, and protection against oxidative stress (22,23). The degree of lipid accumulation at various concentrations of the extracts is shown in Fig. 2B and 2C. The images in Fig. 2C illustrated the cells stained with Oil Red O solution. Morphological observation showed that GWBR hot water extract

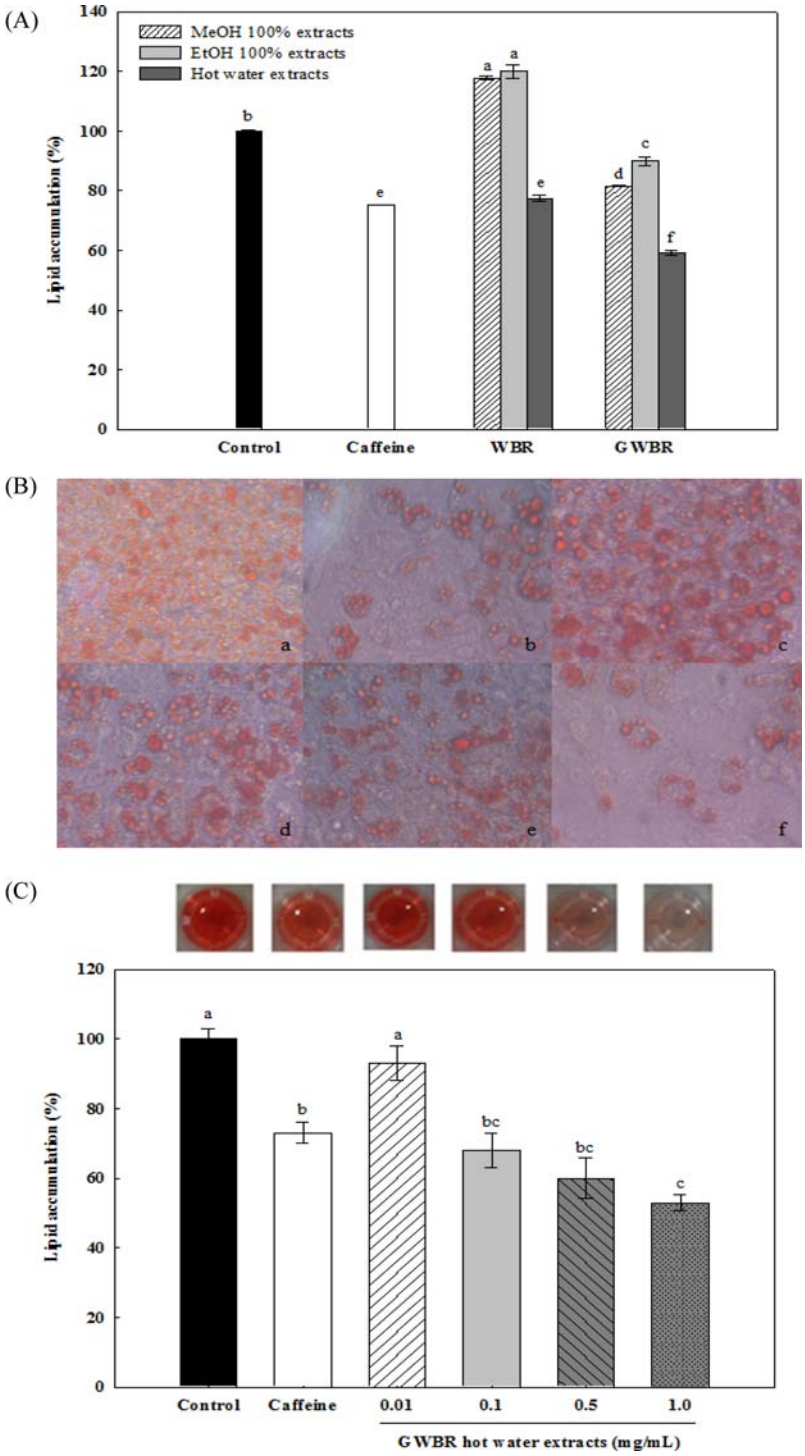

Fig. 2. Inhibitory effect of WBR and GWBR extracts on lipid accumulation in 3T3-L1 adipocytes. (A) Differentiated 3T3-L1 adipocytes were treated with WBR and GWBR extracts at 0.1 mg/mL, and intracellular lipid accumulation was measured by Oil Red O staining. (B) Differentiated 3T3-L1 adipocytes were treated with GWBR hot water extract at concentrations ranging from 0.01 to 1.0 mg/mL, and intracellular lipid accumulation was observed via microscope. a: control; b: caffeine 0.1 mg/mL, c: GWBR hot water extract 0.01 mg/mL; d: 0.1 mg/mL; e: 0.5 mg/mL; f: 1.0 mg/mL (C) The cells were measured by Oil Red O staining. Results are presented as mean±SD of at least three independent experiments. $p<0.05$ was considered a significant difference. WBR: Waxy black rice; GWBR: Germinated waxy black rice

dose-dependently reduced adipocyte differentiation and lipid accumulation (more red staining) when compared to the untreated control cells (Fig. 2B).

The inhibitory effect of GWBR hot water extract at concentrations of 0.01, 0.1, 0.5, and 1.0 mg/mL were significantly decreased by 7, 32, 40, and 47%, respectively, compared with untreated control cells.

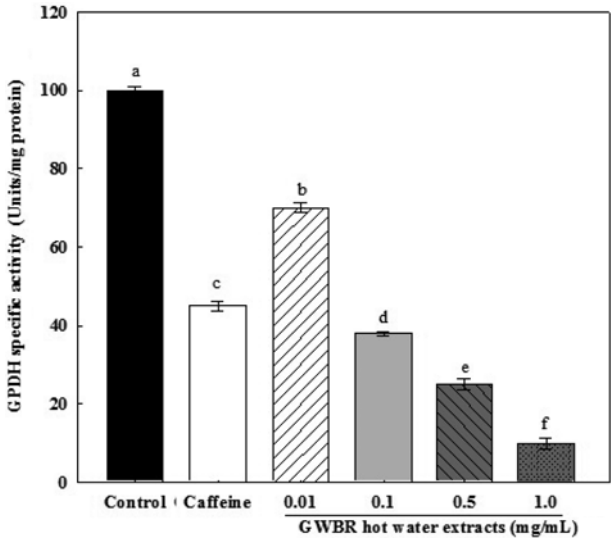

Fig. 3. Inhibitory effect of GWBR hot water extract against glycerol-3-phosphate dehydrogenase (GPDH) specific activity. Differentiated 3T3-L1 adipocytes were incubated with GWBR hot water extract (0.1 mg/mL) for 4 days. Cells were collected and measured for GPDH specific activity, as described in Materials and Methods, calculated as units/mg protein. The results are presented as means±SD of at least three independent experiments, each performed in triplicate (n=3). p<0.05 was considered a significant difference.

GWBR hot water extract also dose-dependently reduced lipid accumulation (Fig. 2C). Natural compounds such as minerals, phenolic compounds, and flavonoids were more easily extracted in water. Several studies have previously reported that hot water extracts of certain plants have inhibitory effects against lipid accumulation and related gene expression (24). Therefore, all subsequent experiments were performed using GWBR hot water extract.

Adipocyte differentiation and lipid accumulation are related to the development and perpetuation of obesity. Investigation of therapeutic strategies aimed at inhibition of adipocyte differentiation has been suggested as a promising approach in the prevention and treatment of obesity. Our results indicated that GWBR extract is a useful functional food material that may have anti-obesity effects.

Effects of GWBR on GPDH activity GPDH is an important cytosolic glycerophosphate dehydrogenase that catalyzes the conversion of glycerol to TG in adipocytes, which increases during adipocyte differentiation (25). The effect of GWBR extract on GPDH activity was investigated as an index of adipocyte differentiation. As shown in Fig. 3, GWBR extract significantly inhibited GPDH activity in a dose-dependent manner. Caffeine (positive control) also inhibited GPDH activity by 55%, and the GWBR hot water extract at concentrations of 0.01, 0.1, 0.5, and 1.0 mg/mL caused a dose-dependent decrease of 30, 62, 75, and 90%, respectively, compared with control cells. GPDH activity was more significantly suppressed by GWBR extract, compared to caffeine at the same concentration. GWBR treatment thus clearly suppressed GPDH levels in mature adipocytes. Several studies have reported that various natural bioactive compounds, including cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin, *p*-coumaric acid, ferulic acid, vanillic acid, and GABA, which are found in black rice, have beneficial effects in adipocytes and obese animals (5-9). The anti-obesity effect of natural plants and their compounds has been shown to be enhanced by the germination process (25). GWBR inhibits adipocyte differentiation through GPDH, via its' effect on TG synthesis.

Effect of GWBR extract on adipogenesis in mature 3T3-L1 adipocytes The effect of GWBR extract on the mRNA levels involved in adipocyte differentiation was examined by RT-PCR, in order to elucidate a possible underlying molecular mechanism. Adipogenesis is a tightly regulated process, which requires the coordination of expression and activation of several transcriptional factors. PPAR-γ, C/EBP-α, and SREBP-1c are the major transcriptional factors that promote adipocyte differentiation. During early stage of adipocyte differentiation, C/EBP-β is predominantly expressed. Expression of C/EBP-β is thought to mediate the expression of PPAR-γ and C/EBP-α during adipogenesis (26). SREBP-1c is highly expressed in adipose tissues, and its active form enhances lipogenic gene expression (25). Treatment with 1 mg/mL GWBR hot water extract exhibited the highest reduction in C/EBP-β, C/EBP-α, SREBP-1c, and PPAR-γ mRNA expression by 61, 80, 32, and 88%, respectively, compared to untreated control cells (Fig. 4A). These results reveal that the anti-adipogenic action of GWBR hot water extract was achieved in both early and terminal stages in adipocyte differentiation, including lipogenesis. PPAR-γ, C/EBP-α, and SREBP-1c regulate the expression of adipogenic enzymes, such as aP2, LPL, and FAS. These fatty acid anabolic genes are highly expressed during the late stage of differentiation (27). The expressions of aP2, LPL, and FAS mRNA in cells treated with 1 mg/mL of GWBR hot water extract were suppressed by 62, 48, and 51%, respectively, compared with control cells (Fig. 4B). The suppressive effect of GWBR extract against these three adipogenic enzymes contributes to decreased formation of lipid droplets. Consequently, these findings indicate that GWBR extract decreases fat storage via suppressing of adipogenic transcriptional factors and enzymes as a major regulator of adipocyte differentiation.

Effect of GWBR extract on lipolysis in mature 3T3-L1 adipocytes Lipolysis is one of the major mechanisms involved in reducing lipid accumulation. Both triacylglyceride hydrolyzation and free fatty acid emission are required for lipid catabolism during times of energy demand. Recently, many studies have demonstrated that both ATGL and HSL are rate-limiting enzymes in FFA metabolism. These lipases are responsible for predominantly TG hydrolysis in adipocytes (28). Previous research has reported that natural plant extracts appear to have anti-obesity effects by affecting lipolysis in adipocytes and in an obese mice model (29,30). The molecular mechanism of lipid catabolism affected by treatment with hot water extract from GWBR in mature adipocytes was investigated using RT-PCR. The mRNA

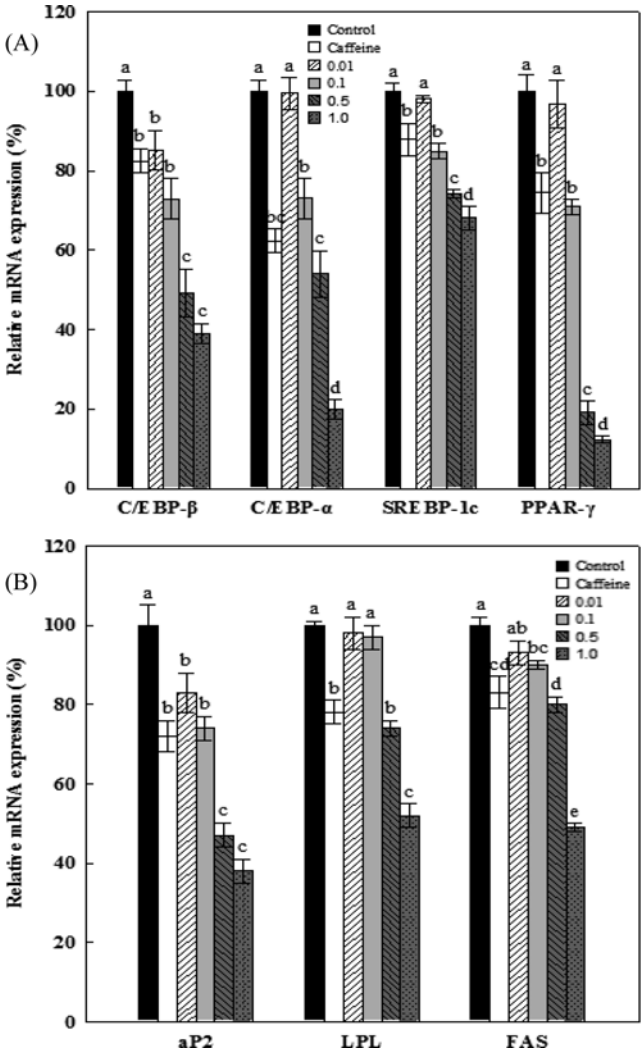

Fig. 4. Effect of GWBR hot water extract on mRNA expression in differentiation-related genes in 3T3-L1 adipocytes. mRNA expression of adipogenic transcriptional factors (A) and adipogenic enzymes (B), as examined by RT-PCR. Differentiated 3T3-L1 adipocytes were incubated with GWBR extract at concentrations ranging from 0.01 to 1.0 mg/mL for 4 days. Cell lysates were prepared and subjected to RT-PCR as described in Materials and Methods. Values are calculated as a percentage of differentiation-related gene mRNA expression versus β-actin as an internal control. The results are presented as means±SD of at least three independent experiments, each performed in triplicate (*n*=3).

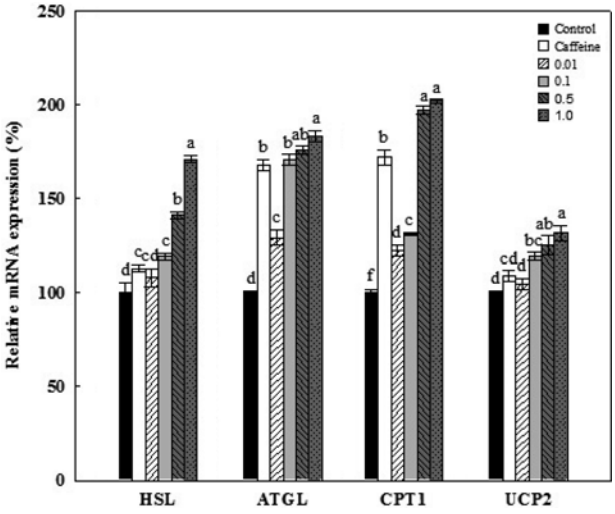

Fig. 5. Effect of GWBR hot water extract on mRNA expression involved in lipolysis, β-oxidation, and thermogenesis in 3T3-L1 adipocytes. Lipolysis, β-oxidation, and thermogenesis related to mRNA expression as examined by RT-PCR. Differentiated 3T3-L1 adipocytes were incubated with GWBR extract at concentrations ranging from 0.01 to 1.0 mg/mL for 4 days. Cell lysates were prepared and subjected to RT-PCR as described in Materials and Methods. Values are calculated as a percentage of lipolysis, β-oxidation, and thermogenesis related mRNA expression versus β-actin as an internal control. The results are presented as means±SD of at least three independent experiments, each performed in triplicate (*n*=3).

levels of lipolytic enzymes HSL and ATGL in cells treated with 1 mg/mL GWBR hot water extract increased notably by 71 and 83%, respectively, compared to untreated control cells (Fig. 5). The lipolysis-controlling effect of GWBR hot water extract is mediated by increased HSL and ATGL expression. These results reconfirm that GWBR extract has potent inhibitory effects against lipid accumulation via increased lipase activity.

Effect of GWBR extract on mRNA levels involved in β-oxidation and thermogenesis CPT1 mRNA expression related to β-oxidation and UCP2 mRNA expression involved in thermogenesis were significantly increased by 102 and 31%, respectively, in 3T3-L1 adipocyte cells treated with 1 mg/mL GWBR hot water extract, compared to untreated control cells (Fig. 5). CPT1 is rate-limiting enzyme in β-oxidation of long chain fatty acids. The enzyme catalyzes the esterification acyl group from coenzyme A to carnitine in order to transfer the group into the mitochondrial matrix. Both stored energy utilization and fatty acid oxidation were increased by stimulating CPT1 activity in an obese mice model (31). These results indicate that the anti-obesity effect of GWBR hot water extract was controlled by enhancing β-oxidation, as an approach to reducing amounts of fat stores. Recently, UCP2 known as regulate the energy metabolism or obesity in human and rodent models (32). Stimulating UCP2 expression with GWBR extract encourages transport of protons into the mitochondrial matrix to induce the release of energy as heat. These results indicate that GWBR hot water extract increased the mRNA expression of both CPT1 and UCP2 genes, improving lipolysis and thermogenesis in 3T3-L1 cells.

The inhibitory effects of GWBR extract against lipid accumulation were assessed in this *in vitro* study. In mature adipocytes, GWBR hot water extract inhibited lipid accumulation and reduced GPDH activity via down-regulation of adipogenesis-related genes (PPAR-γ, C/EBP-α, C/EBP-β, and SREBP-1c) and downstream genes (aP2, LPL, and FAS), and up-regulation of lipolysis-related genes (HSL and ATGL), β-oxidation-related gene CPT1, and thermogenesis-related gene UCP2.

These results might be partially mediated via regulation of expression multiple genes involved in adipogenesis, lipogenesis, lipolysis, β-oxidation, and thermogenesis in 3T3-L1 mature adipocytes. Consequently, GWBR hot water extract has a useful for effective agent in the treatment and prevention of obesity.

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Disclosure The authors declare no conflict of interest.

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