J Chin Med Assoc

ORIGINAL ARTICLE

Immature sword bean pods (*Canavalia gladiata*) inhibit adipogenesis in C3H10T1/2 cells and mice with high-fat diet–induced obesity

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Abstract

Background: Sword bean (SB; *Canavalia gladiata*) is a perennial vine used as a food and medicinal plant in Asia. SB is rich in nutrients, such as flavonoids and urease, and has various functions, including beneficial effects on dysentery, nausea, and hemorrhoids, as well as anti-inflammatory and antioxidant activity. Various plant parts are used; however, little is known about the physiological effects of SB pods (SBP). In this study, the anti-obesity effects of SBP extract were evaluated.

Methods: To investigate the anti-obesity effects of SBP extract, we confirmed the SBP extract downregulated lipogenesis-related genes and upregulated genes involved in lipolysis and brown adipocyte markers in differentiated C3H10T1/2 adipocytes in vitro. Next, we use a high-fat diet (HFD)–induced obesity mouse model to determine the anti-obesity effects of SBP extract.

Results: Treatment with SBP extract significantly reduced adipocytes. The extract decreased the HFD-induced increases in body weight and plasma triglyceride levels in mice after 8 weeks. mRNA and protein levels of the adipogenesis and lipogenesis-related factors CCAAT/enhancer binding protein-β, CCAAT/enhancer binding protein-α, peroxisome proliferator-activated receptor-γ (PPARγ), and their target genes *Ap2*, *SREBP-1c*, *FAS*, and *SCD-1* were reduced by SBP extract. In contrast, AMP-activated protein kinase and sirtuin1, involved in the thermogenic catabolism of fat, were activated by SBP extract in adipocytes and white adipose tissue, increasing the expression of peroxisome proliferator-activated receptor gamma coactivator-1α, peroxisome proliferator-activated receptor-α (PPARα), and uncoupling protein 1 and activating thermogenic activity.

Conclusion: SBP extract exerts an anti-obesity effect by inhibiting lipogenesis-related factors and activating fat-catabolizing factors; it is, therefore, a promising functional food and natural anti-obesity agent.

Keywords: Adipogenesis; Animals; Antioxidants; Canavalia; Lipogenesis

1. INTRODUCTION

Obesity is a noninfectious disease characterized by increases in the size and number of adipocytes with excessive body fat accumulation, resulting from an imbalance between energy intake and expenditure. Obesity is widespread and is a major risk factor for metabolic diseases, such as hyperlipidemia, hypertension, and type 2 diabetes.[1] According to a report by the World Health Organization, 30% of the global population is overweight or obese, and by 2025, one-third of the population is predicted to be obese. In Korea, the prevalence of obesity in adults has increased steadily from 29.7% in 2009 to 35.7% in 2018, with corresponding increases in the incidence of metabolic diseases. It is believed that additional management and treatment strategies, such as surgery and drugs, are needed to address the growing incidence of obesity and metabolic diseases.[2] However, current strategies have limitations, such as side effects, the need for long-term effort, and suitability for specific age groups. Therefore, there is a need to develop effective methods for the prevention and treatment of obesity.

Obesity, caused by fat accumulation, involves a series of processes in which preadipocytes differentiate into adipocytes by increasing the expression of adipocyte-specific genes.[3] Adipocyte differentiation is induced by adipogenic transcription factors, including CCAAT/enhancer binding protein-α, β (C/EBPα, β), and peroxisome proliferator-activated receptor-γ (PPARγ).[4] After differentiation, lipid synthesis regulates lipid metabolism by increasing the production of sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1).[5,6]

Some factors that function in lipid catabolism protect against obesity.[7] Among various pathways that break down fat, thermogenesis consumes energy by the production of heat via the oxidation of triglycerides (TGs).[8] Uncoupling protein 1 (UCP1) is involved in the thermogenic reaction and is present in a number of brown adipose tissues (BAT). Extensive research has focused on browning white adipose tissue (WAT), which oxidizes stored energy by converting energy-storing WAT into BAT with many

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Author Contributions: Dr. Hye-Jeong Hwang and Dr. Yu Jin Hwang contributed equally to this research.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2022) 85: 67-76.

Received July 5, 2021; accepted September 15, 2021.

doi: 10.1097/JCMA.0000000000000655.

mitochondria. Among the mechanisms by which WAT is converted to BAT, AMP-activated protein kinase (AMPK) activation mediates sirtuin1 (SIRT1) to increase the expression of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and activate mitochondrial biosynthesis. This increased expression of PGC-1α promotes UCP1 expression to break down TGs by heat generation.[9–12] This can be a useful defense mechanism against obesity by stimulating cellular energy expenditure and regulating adipocyte differentiation. Various natural ingredients, such as curcumin and resveratrol, have been shown to convert WAT into BAT via the regulation of the thermogenic pathway.[13]

Sword bean (SB; *Canavalia gladiata*) is a perennial vine plant originating in tropical Asia, Africa, and India. It has been used as a food and medicinal plant in Asia for thousands of years. Mature seeds are often roasted, ground, and consumed as a drink. SB is rich in phytochemicals, such as saponins, tannins, flavonoids, terpenoids, and steroids, and in nutrients, such as carbohydrates, proteins, vitamins, and minerals.[14] It also contains urease, hemagglutinin, canavanine, and canavalia gibberellin I and II. The seeds, pods, stems, and roots are used in folk remedies that are effective against dysentery, nausea, hemorrhoids, sinusitis, backaches, and obesity.[15] Furthermore, recent studies have shown that SBs possess physiological functions, including antioxidant, anti-inflammatory, hematopoietic expansion-improving, hepatoprotective, and antiangiogenic activities. Despite many studies of the bioactivities of SB, scientific analyses of the functionality of pods are limited, including analyses of anti-inflammatory and antioxidant activities. In this study, we investigated the efficacy of SB pod (SBP) extract against obesity in adipose cells and in mice fed a high-fat diet (HFD).

2. METHODS

2.1. Reagents

The Roswell Park Memorial Institute 1640 (RPMI 1640) and penicillin/streptomycin antibiotics (P/S) were purchased from Gibco (Gaithersburg, MD, USA). Fetal bovine serum (FBS) and anti-rabbit IgG horseradish peroxidase-conjugated antibodies were purchased from GenDEPOT (Barker, TX, USA). Dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), troglitazone, insulin, gelatin, and dimethyl sulfoxide (DMSO) Orlistat were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dodecyl sulfate-polyacrylamide gels for SDS-PAGE, polyvinylidene difluoride (PVDF) membranes, and a Chemi-doc image detector (Chemi-Doc XRS+ System) were purchased from Bio-Rad (Hercules, CA, USA). Antibodies against PPAR-γ, C/EBPα, C/EBPβ, FAS, CPT1α, UCP1, UCP2, peroxisome proliferator-activated receptor-α (PPARα), and AMPK were purchased from Abcam (Cambridge, MA, USA).

2.2. Sample extract preparation

SB and SBP were purchased domestically (Hwasun, Korea). Impurities were washed with water, and SB and SBP were minced, dried with hot air at 50 °C for 8 hours (Jeil Machine Co., Ltd., Icheon, Korea), pulverized with a grinder (IKA, M20, Staufen, Germany), and used to prepare the extract. Then, 30% ethanol was added to the pulverized SB and SBP and stirred at room temperature for 24 h. After the first extraction, the remaining solvent was added for the second extraction under the same conditions. Subsequently, the obtained extract was filtered through filter paper, and the filtrate was concentrated using a vacuum concentrator (R-100; Doo Young High Technology, Seoul, Korea) and lyophilized. SB and SBP extraction yields obtained after completion of lyophilization were 16.1% and 16.6%, respectively. The lyophilized extract was maintained at –20 °C until further use.

2.3. Cell culture and differentiation

Mouse-derived adipocyte mesenchymal cells C3H10T1/2 were purchased from the Korean Cell Line Bank (Seoul, Korea). C3H10T1/2 cells were maintained in RPMI 1640 with 10% FBS and 1% P/S in an incubator with 5% CO_2 at 37 °C. Five days after the cells were plated in 12-well plates, they were replaced with media containing MDI (1 μM dexamethasone, 0.5 mM IBMX, 5 μg/mL insulin, and 10 μM troglitazone; day 0). Three days after the medium change, C3H10T1/2 cells were differentiated with media containing insulin and troglitazone (day 3). When cell differentiation started on day 8, SB and SBP extracts were used to confirm adipogenesis and lipogenesis effects in adipocytes (Fig. 1).

2.4. Cell viability

C3H10T1/2 cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich). Cells (5×10^3 cells/well) were plated on 96-well plates and stabilized for 2 hours. SB and SBP extracts were added for 24 hours at various concentrations (0, 10, 20, 50, and 100 μg/mL) and incubated for 4 hours with 5 mg/mL MTT reagent. After removing the medium, formazan crystals were dissolved in DMSO and absorbance was measured at 540 nm (SpectraMax M5; Molecular Devices, LLC, Sunnyvale, CA, USA).

2.5. Lipid droplet staining

After 6 days of adipocyte differentiation, C3H10T1/2 cells were treated with SB and SBP extracts for 24 hours, washed with PBS, and fixed with 4% formaldehyde for 1 hour. After fixation, the cells were washed twice with 2-propanol and stained with Oil Red O (Sigma-Aldrich) reagent or Nile red (Invitrogen, Carlsbad, CA, USA) for 1 hour. The stained cells were observed under a light microscope (Leica Microsystems, Wetzlar, Germany) or a fluorescence microscope (Zeiss, Dublin, CA, USA).

2.6. RNA isolation and real-time RT-PCR analysis

Cells and WAT treated with SB and SBP extracts were washed with PBS, and RNA was isolated using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA). cDNA was synthesized using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocol. The synthesized cDNA was subjected to RT-PCR using the amfiSure qGreen Q-PCR Master Mix (genDEPOT) and Qiagen RotorGene Q real-time PCR machine. The target gene was amplified by denaturation at 95 °C for 15 s, annealing at 57 °C for 20 s, and extension at 72 °C for 30 s. For relative quantification, mRNA levels were normalized against levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Mouse primers were as follows: GAPDH, 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); C/EBPβ, 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); C/EBPα, 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); PPARγ, 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); Adipocyte fatty acid binding protein (Ap2), 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); SREBP1c, 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); FAS, 5′-GAG CCA AAA GGG TCA TCA TC-3′ (forward), 5′-TAA GCA GTT GGT GGT GCA GG-3′ (reverse); SCD-1, 5′-GTT CGT TAG CAC CTT CTT GCG-3′ (reverse); AMPK, 5′-CGA CCT GGA AGC GAA

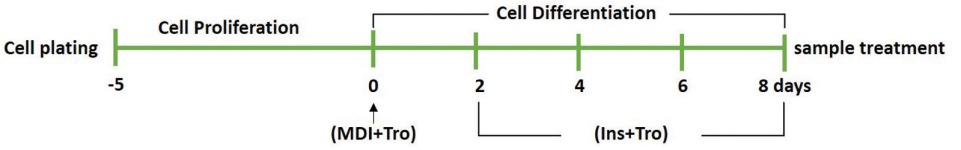

Fig. 1 Overview of the culture schedule during the differentiation of C3H10T1/2 cells. When C3H10T1/2 cells reached confluence (D – 5 to D + 0), the medium was changed to 3-isobutyl-1-methylxanthine, dexamethasone and insulin (MDI) and troglitazone (Tro; D + 0). Every 48 h, the medium was replaced with fresh medium containing insulin (Ins) and Tro until 8 d.

TGA TAA-3′ (forward), 5′-GTT GAA GGA CCC AGA CAA GTA G-3′ (reverse); SIRT1, 5′-GGA ACC TTT GCC TCA TCT ACA-3′ (forward), 5′-CAC CTA GCC TAT GAC ACA ACT C-3′ (reverse); PPARα, 5′-GGT TCC TGG TGC CGA TTT AT-3′ (forward), 5′-CAC AGA CTA GCA TCC CAC TTA AT-3′ (reverse); PGC-1α, 5′-CGG AAA TCA TAT CCA ACC AG-3′ (forward), 5′- TGA GGA CCG CTA GCA AGT TTG-3′ (reverse); UCP1, 5′-GGC AAA AAC AGA AGG ATT GC-3′ (forward), 5′-TAA GCC GGC TGA GAT CTT GT-3′ (reverse).

2.7. Western blot analysis

C3H10T1/2 cells were seeded in 6-well plates, differentiated, and treated with SB and SBP extracts. After incubation, the cells were harvested in lysis buffer on ice and centrifuged at 12 000 × g for 10 minutes at 4 °C to obtain the protein. WAT was also pulverized with lysis buffer to extract proteins following the same method used for cells. Protein quantification was performed using the BCA Protein Assay Reagent (GenDEPOT, Katy, TX, USA). Ten micrograms of protein were separated by 4% to 20% SDS-PAGE and transferred to PVDF membranes. The transferred membranes were blocked with 5% (w/v) skim milk for 1 hour, and the primary antibodies against C/EBPβ, PPAR-γ, C/EBPα, SREBP-1c, FAS, SCD-1, AMPKα, PPARα, and UCP1 were incubated overnight at 4 °C. The bands were visualized using enhanced chemiluminescence reagent (Thermo, Rockford, IL, USA), and images were captured using a Chemi-Doc image detector.

2.8. Animals and experimental groups

C57/BL6J mice (6 weeks old, male) were obtained from Samtako (Osan, Gyeonggido, Korea). Animals were maintained in a controlled environment at 25 ± 2 °C under a 12-hour dark/light cycle, with ad libitum access to water and food. After a week of acclimatization, the animals were randomly divided into seven groups (n = 6 per group) and normal or HFD provided for 8 weeks, and the composition for the diet is shown in Supplementary Table 1 http://links.lww.com/JCMA/A122: normal diet (NC) group, HFD group, orlistat with HFD group (Orlistat), and HFD with SB or SBP group. For mice in the SB and SBP groups, extracts were administered orally once a day at doses of 100 and 200 mg/kg body weight. Body weight and food intake were measured once per week. After 8 weeks, the mice were sacrificed and serum was collected. The liver and adipose tissues were promptly removed and stored at 80 °C until use. The study was approved by the Rural Development Administration (South Korea, NAS202009).

2.9. Lipid profiling of plasma

After animals were anesthetized, blood was collected and centrifuged at 3000 × g for 10 minutes. High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and TG levels in plasma obtained by centrifugation were measured using an ELISA kit (Abcam, Cambridge, MA, USA). Lipid contents in the plasma were measured and analyzed according to the manufacturer's instructions.

2.10. Histological analysis

Adipose tissues were examined for histopathological evaluation. The fixed tissues were subjected to general histological procedures, such as dehydration, paraffin embedding, and cutting. Hematoxylin and eosin staining was performed, and histopathological changes were evaluated by observation using an optical microscope (Leica).

2.11. Statistical analysis

Data are expressed as means ± SEM. Statistical analyses were performed using SPSS, version v25.0 (SPSS, Chicago, IL, USA). Data were analyzed by one-way analysis of variance. Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Effect of SBP extracts on viability in C3H10T1/2 cells

MTT assays were performed to evaluate the effect of SB and SBP extracts (10, 20, 50, 100, and 200 μg/mL) on C3H10T1/2 cell viability (Fig. 2). After treatment with SB and SBP extracts, cell viability was over 80% for all concentrations relative to the

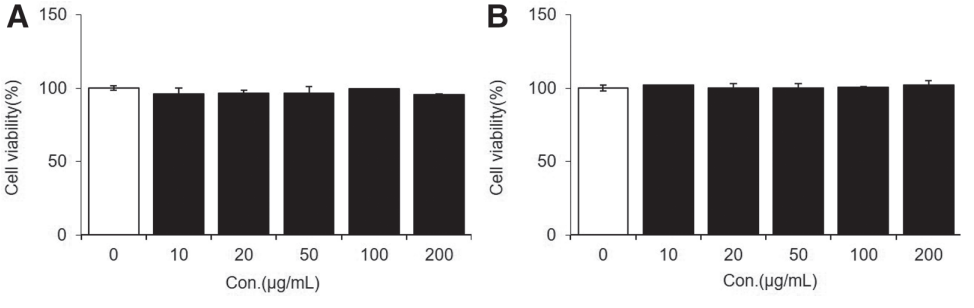

Fig. 2 Effects of sword bean (SB) and SB pod (SBP) extracts on C3H10T1/2 cell viability. (A) sword bean and (B) sword bean pod extract. Cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay after incubation with extracts for 24 h. Data are expressed as means±SEM. Con = Concentration.

control. When developing health functional food materials in the future, considering the safety of intake, we set 100 μg/mL as the maximum concentration and conducted future experiments. Therefore, concentrations of 10, 20, 50, and 100 μg/mL were used for subsequent analyses.

3.2. Effects of SBP extracts on lipid accumulation in C3H10T1/2 cells

To investigate the effects of SB and SBP extracts on lipid deposition in differentiated adipocytes, C3H10T1/2 fat cells were stained with Oil Red O and Nile Red. Staining intensity decreased in a concentration-dependent manner, confirming that the extract inhibited lipid accumulation in C3H10T1/2 cells (Fig. 3A, B).

3.3. Effects of SBP extracts on adipogenic, lipogenic, and thermogenic gene and protein expression in C3H10T1/2 cells

In adipocytes treated with SB and SBP extracts, adipogenic, lipogenic, and thermogenic gene expression levels were evaluated by real-time PCR and Western blotting (Fig. 4). The expression of adipogenic factors C/EBPβ, C/EBPα, and PPARγ and

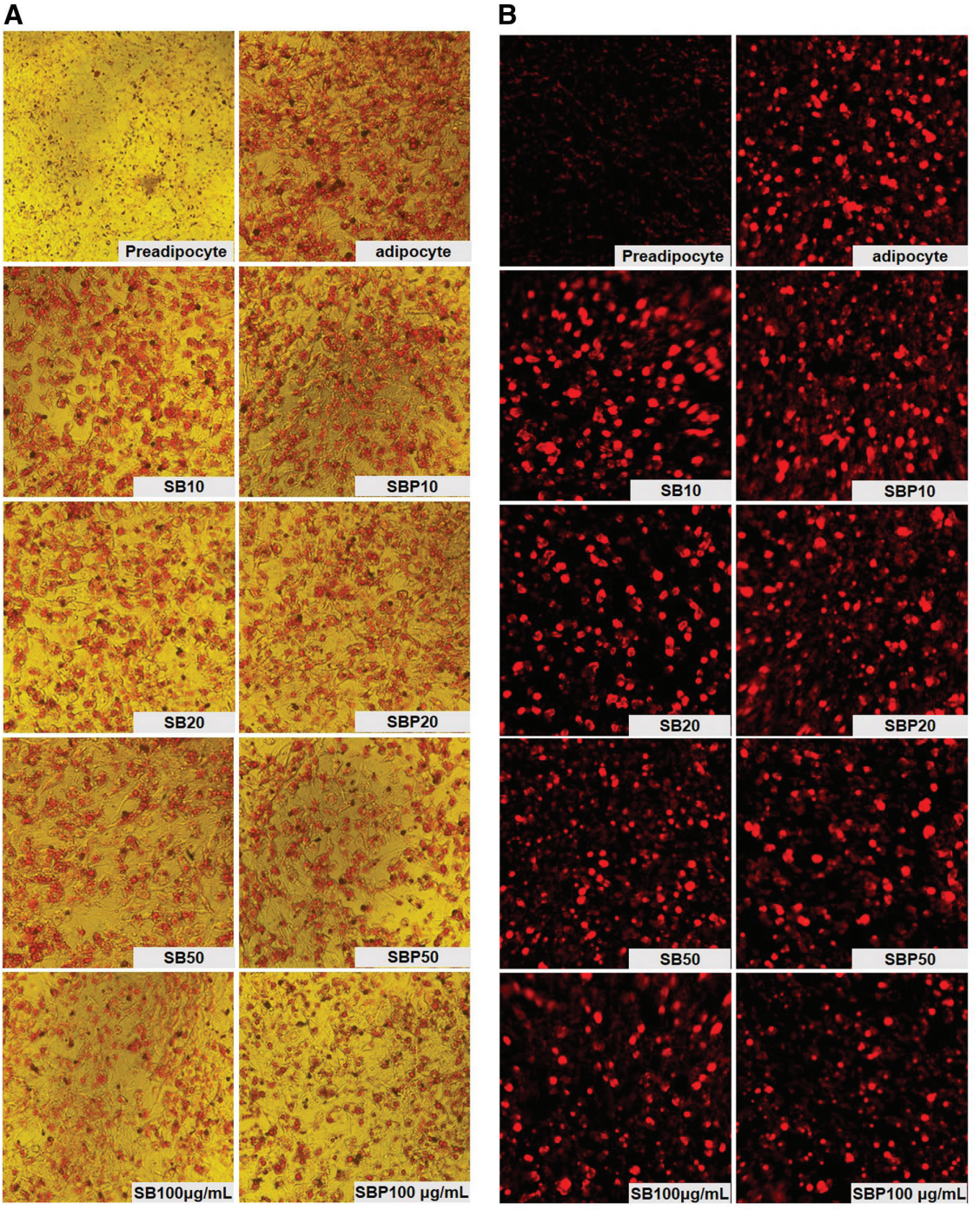

Fig. 3 Effects of sword bean (SB) and SB pod (SBP) extracts on lipid accumulation in differentiated C3H10T1/2 cells. After treatment of differentiated C3H10T1/2 cells with SB and SBP for 24 h, the cells were stained with (A) Oil Red O and (B) Nile red.

lipogenic factors Ap2, SREBP-1c, FAS, and SCD-1 was significantly reduced by SB and SBP extracts in adipocytes (Fig. 4A). In addition, the expression levels of major factors such as AMPK, SIRT1, PPARα, PGC-1α, and UCP1 involved in thermogenesis (ie, the mitochondrial heat generation mechanism) increased in a concentration-dependent manner by SB and SBP extracts (Fig. 4B). The results for adipogenic and lipogenic protein expression in the groups treated with SB and SBP were similar with the gene expression results (Fig. 4C–F). The SBP extract had a greater inhibitory effect on proteins related to fat synthesis than that of the SB extract.

3.4. Effects of SBP on mouse body and organ weight in the mouse model of obesity

The body weight of the HFD group was significantly higher than that of the NC group, and the weights of the groups fed orlistat, SB, and SBP extracts were significantly lower than that of the HFD group (Fig. 5A). In case of food intake, NC group showed

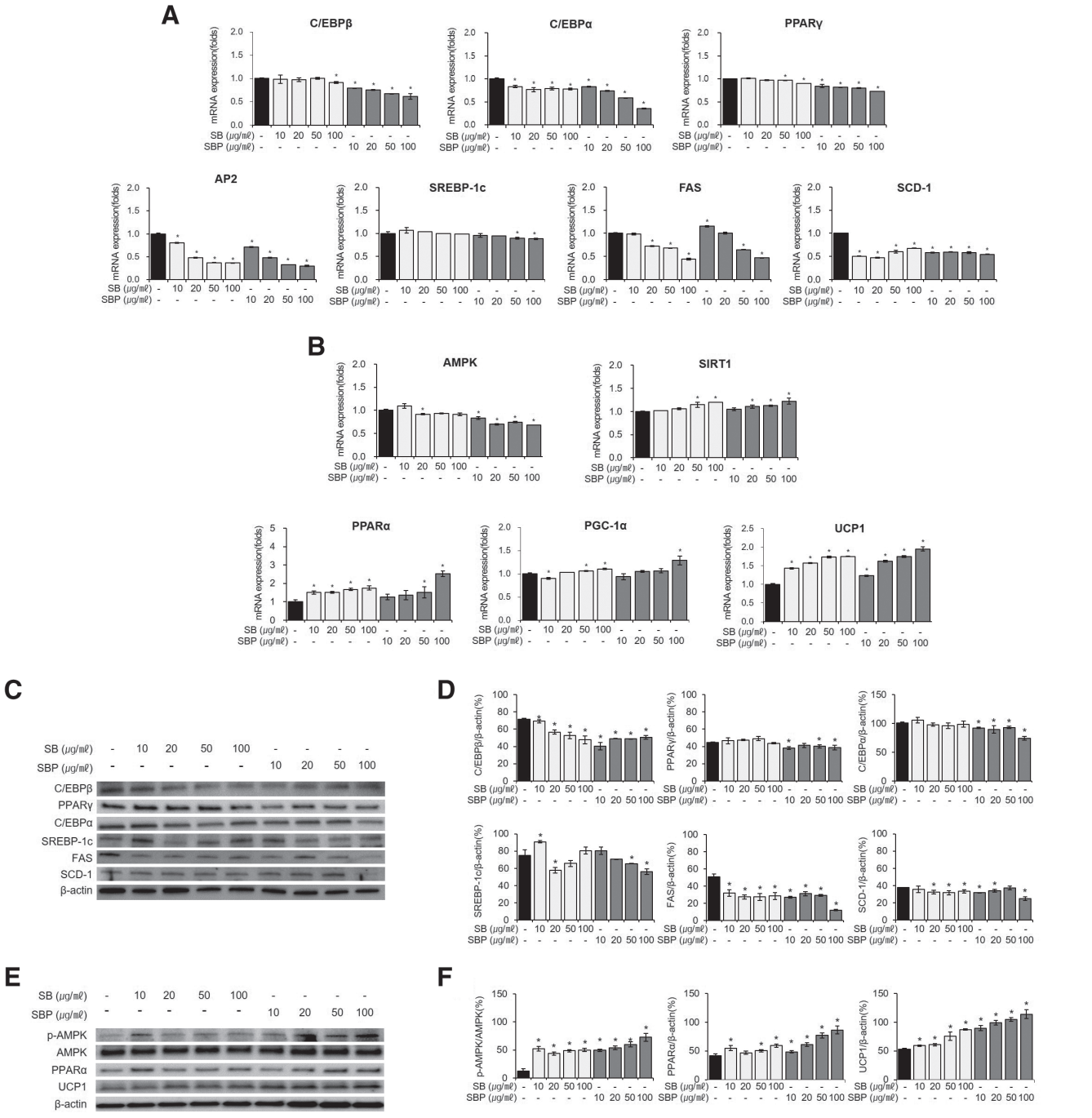

Fig. 4 Effects of sword bean (SB) and SB pod (SBP) extracts on the expression of adipogenic, lipogenic, and thermogenic factors in C3H10T1/2 cells. (A and B) mRNA expression (C and E) protein levels, and (D and F) densitometry. Measurement of gene and protein expression was measured by real-time reverse transcription polymerase chain reaction and western blot after 24 h of sample treatment in C3H10T1/2 cells. Data are expressed as means±SEM. *$p < 0.05$ compared with the untreated differentiation group.

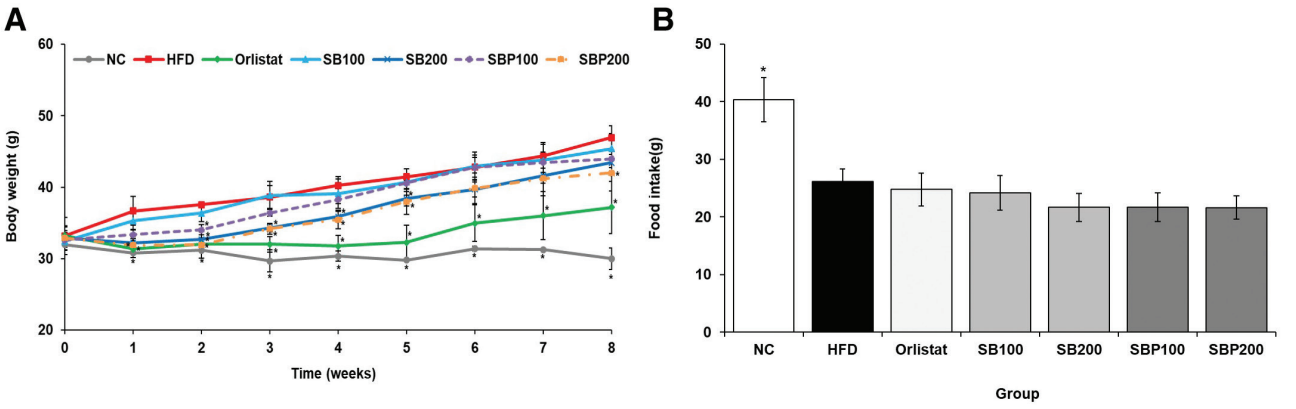

Fig. 5 Effects of sword bean (SB) and SB pod (SBP) extracts on body weight and food intake in mice with high-fat diet (HFD)–induced obesity. The (A) body weight and (B) food intake of mice fed a normal, HFD or sample with HFD was measured once a week. Results are presented as means ± SEM. NC = normal diet.

higher intake than HFD group. And there was no significant difference between the HFD groups. Therefore, it is considered that the difference in body weight between each group is due to sample intake, not food intake (Fig. 5B). In addition, the weights of tissues, such as the liver, spleen, and BAT, did not differ significantly between treatment groups and the HFD group. In case of WAT, it decreased significantly by orlistat and SBP treatment. Orlistat-treated group had no significant difference with NC group. And SBP-treated group had a decreased WAT weight in a dose-dependent manner (Table 1).

3.5. Effect of SBP on lipid levels in the plasma and histological of WAT in HFD-fed mice

Plasma lipid levels were higher in the HFD group than in the NC group; the TG levels were significantly decreased in the orlistat-, SB200-, and SBP100- or SBP200-treated groups than in the HFD group, and the TC levels were significantly lower only in the orlistat group than the HFD group. In addition, HDL-C was significantly higher in the SB and SBP groups than in the HFD group. The atherogenic index and cardiac risk factor were calculated to assess the risk of developing cardiovascular disease; the levels of both were significantly lower in the SB and SBP groups than in the HFD groups (Table 2). In addition, in an analysis of the effect of SBP treatment on the morphology of epididymal fat, adipocytes were larger in the HFD group than in the NC group, and the orlistat, SB, and SBP extract–treated groups showed a marked reduction in adipocyte size compared with that in the HFD group (Fig. 6). In particular, it was confirmed that the orlistat group as positive control and SBP200 treatment group showed a pattern very similar to that of the NC group.

Table 1

Tissue weights in mice with HFD-induced obesity

	Liver	WAT	Spleen	BAT
NC	1.269 ± 0.099	0.129 ± 0.068a	0.224 ± 0.068	0.048 ± 0.004a
HFD	1.647 ± 0.185	2.951 ± 0.119	0.129 ± 0.021	0.105 ± 0.007
Orlistat	1.165 ± 0.032	0.486 ± 0.219a	0.212 ± 0.056	0.074 ± 0.026
SB100	1.630 ± 0.146	2.634 ± 0.073	0.096 ± 0.006	0.136 ± 0.011
SB200	1.462 ± 0.094	2.505 ± 0.278	0.130 ± 0.017	0.157 ± 0.028
SBP100	1.670 ± 0.078	2.450 ± 0.131a	0.136 ± 0.038	0.129 ± 0.011
SBP200	1.347 ± 0.109	1.664 ± 0.252a	0.107 ± 0.012	0.106 ± 0.010

Data are expressed as means ± SEM.

BAT = brown adipose tissue; HFD = high-fat diet; NC = normal diet; SB = sword bean; SBP = sword bean pod; WAT = white adipose tissue.

[a]$p < 0.05$, compared with the HFD treatment group.

3.6. Effects of SBP extracts on the leptin and adiponectin contents in plasma

The plasma leptin concentration in the HFD group was significantly higher than that in the NC group. The levels of leptin in both the SB and SBP extract groups were lower than those in the HFD group (Fig. 7A). The plasma adiponectin concentrations were higher in the SB and SBP groups than in the HFD group (Fig. 7B). Adiponectin is an antagonist of adipogenesis and is effective in regulating lipid metabolism and increased expression of adiponectin decreases TG, LDL-C levels and increases HDL-C levels.[16] These results suggest that SB and SBP extracts improve obesity by more effectively regulating blood TG and HDL-C levels in obese mice as adiponectin levels are improved than in the orlistat group.

3.7. Effects of SBP extracts on adipogenic, lipogenic, and thermogenic gene and protein expression in WAT of mice with HFD-induced obesity

In the WAT of mice with diet-induced obesity treated with SB and SBP extracts, the expression levels of adipogenic (CEBPβ, CEBPα, PPARγ, and Ap2) and lipogenic (SREBP 1c, FAS, and SCD-1) factors were significantly higher in the HFD group than in the NC group. When mice were fed various concentrations of SB and SBP extracts, the expression levels were significantly decreased in a dose-dependent manner than those in the group fed the HFD. Levels were markedly lower in the SBP extract group than in the SB extract group. In particular, the SBP200-treated group showed a lower result than those in the group fed the obesity alleviation drug orlistat (Fig. 8A). In addition, we evaluated the effect of SB and SBP extracts on the major factors related to thermogenesis, which exert anti-obesity effects by releasing heat from the mitochondrial membrane and converting WAT into BAT to break down fat. Compared with levels in the HFD group, gene expression levels of AMPK, SIRT1, PGC1α, and UCP1 tended to be increased by treatment with SB and SBP extracts, and in particular, the SBP extract significantly increased the expression level of thermogenic factors than SB extract (Fig. 8B). In addition, the protein expression levels of adipogenesis and lipogenesis factors in the WAT of the HFD group were significantly higher than those in the NC group except PPARγ gene, and by the extract treatment, the mRNA expression levels of all genes were significantly decreased compared with the HFD group (Fig. 8C, D). The extract effectively improved obesity by oxidizing TGs via increases in the expression of factors involved in heat generation (Fig. 8E, F). These results suggest that the extracts can be utilized as a natural material for protecting

Table 2

Effects of SBP extracts on lipid profiles in mice with HFD-induced obesity

	Glucose	TG	TC	HDL cholesterol	LDL cholesterol	AI[a]	CRF[b]
NC	124.50 ± 8.68c	115.02 ± 29.14c	694.00 ± 124.4c	536.45 ± 28.30	144.10 ± 33.07c	0.307 ± 0.061c	1.307 ± 0.061c
HFD	280.80 ± 29.25	349.26 ± 27.13	1166.9 ± 17.30	626.88 ± 68.11	333.45 ± 29.31	1.033 ± 0.273	2.033 ± 0.273
Orlistat	191.33 ± 29.01	158.66 ± 5.14c	1001.5 ± 100.8c	452.25 ± 34.84	364.72 ± 50.01	1.242 ± 0.178	2.242 ± 0.178
SB100	246.00 ± 7.13	259.71 ± 22.51	1188.1 ± 14.41	996.73 ± 52.36c	397.58 ± 25.70	0.202 ± 0.063c	1.202 ± 0.063c
SB200	213.75 ± 10.59	222.01 ± 50.05c	1126.8 ± 57.04	1091.3 ± 53.10c	396.32 ± 24.15	0.096 ± 0.065c	1.096 ± 0.065c
SBP100	215.33 ± 16.84	240.45 ± 20.22c	1166.5 ± 23.65	945.45 ± 50.18c	409.35 ± 39.92	0.252 ± 0.070c	1.252 ± 0.070c
SBP200	188.40 ± 30.01	224.32 ± 15.49c	1163.1 ± 35.11	1060.4 ± 82.94c	383.42 ± 33.72	0.124 ± 0.087c	1.124 ± 0.087c

Data are expressed as means ± SEM.

AI = atherogenic index; CRF = cardiac risk factor; HDL = high-density lipoprotein; HFD = high-fat diet; LDL = low-density lipoprotein; NC = normal diet; SB = sword bean; SBP = sword bean pod; TC = total cholesterol; TG = triglyceride.

[a]AI = (total cholesterol – HDL cholesterol)/HDL cholesterol.

[b]CRF = total cholesterol/HDL cholesterol.

[c]$p < 0.05$ compared with the HFD treatment group.

against obesity, with a similar effect to that of orlistat, which is known to be effective in improving obesity.

4. DISCUSSION

We investigated the anti-obesity effect of the SBP extract on C3H10T1/2 adipocytes and high fat–induced obese mice. To confirm the anti-obesity effect, we examined the expression of genes and proteins related to adipogenesis, lipogenesis, and thermogenesis. The SBP extract significantly reduced fat cell accumulation by reducing the number and volume of lipid droplets (rich in differentiated adipocytes), as determined by staining with Oil Red O and Nile Red. According to Hsu et al, adipogenic transcription factors such as C/EBPα and PPARγ

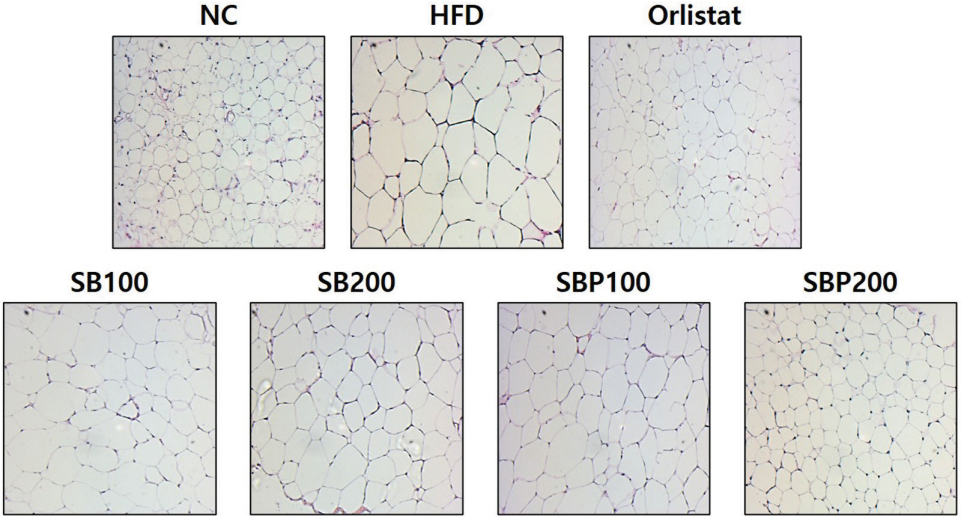

Fig. 6 Effects of sword bean (SB) and SB pod (SBP) extracts on histological properties of white adipose tissue (WAT) in mice fed a high-fat diet (HFD). Hematoxylin and eosin staining was performed on WAT of mice fed diet and extracts for 8 weeks. NC = normal diet.

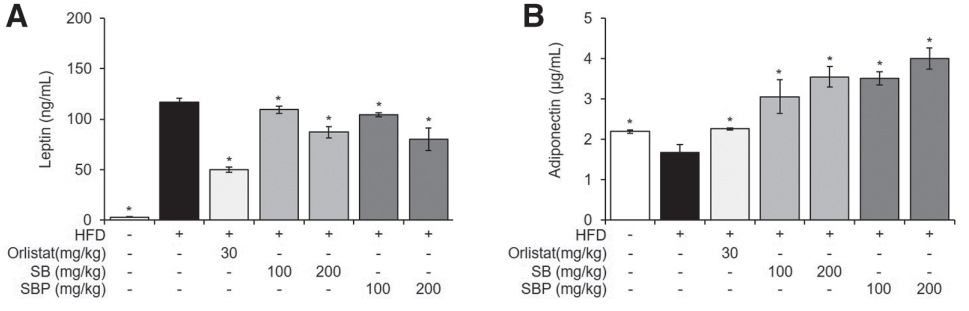

Fig. 7 Effects of sword bean (SB) and SB pod (SBP) extracts on the production of leptin and adiponectin in the plasma of mice with obesity. (A) Leptin and (B) adiponectin were measured using ELISA. Data are expressed as means±SEM. *$p < 0.05$ compared with the HFD treatment group. HFD = high-fat diet.

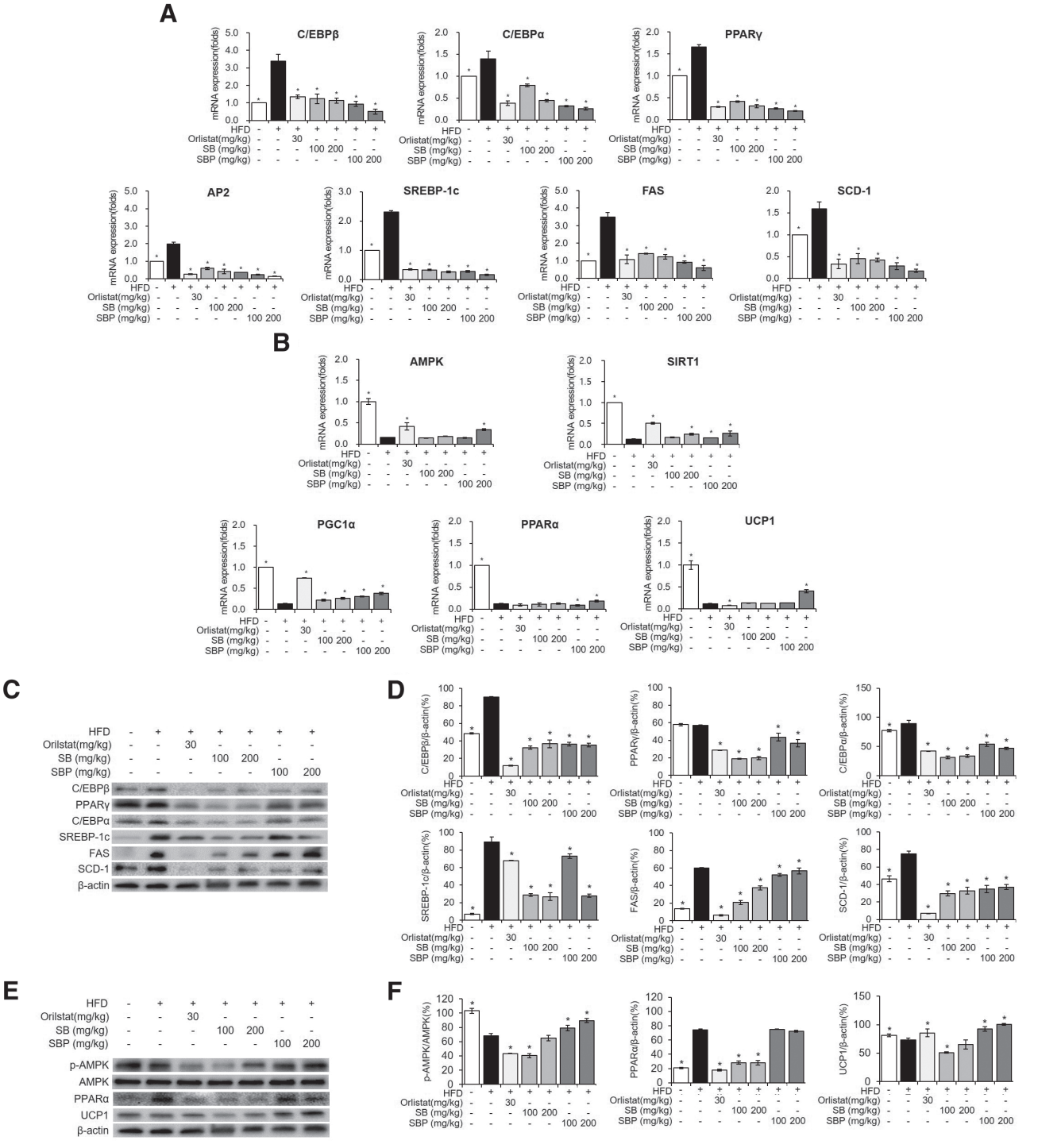

Fig. 8 Effects of sword bean (SB) and SB pod (SBP) extracts on the expression of adipogenic, lipogenic, and thermogenic factors in mice WAT. (A and B) mRNA expression (C and E) protein levels, and (D and F) densitometry. Measurement of gene and protein expression was done by real-time reverse transcription polymerase chain reaction and western blot after 24 h of sample treatment in WAT. Data are expressed as means±SEM. *$p < 0.05$ compared with the untreated differentiation group. AMPK = AMP-activated protein kinase; Ap2= adipocyte fatty acid binding protein; C/EBPα = CCAAT/enhancer binding protein-α; C/EBPβ = CCAAT/enhancer binding protein-β; FAS = fatty acid synthase; HFD = high-fat diet; PPARγ = peroxisome proliferator-activated receptor-γ; SCD-1 = stearoyl-CoA desaturase-1; SIRT1 = sirtuin1; SREBP-1c = sterol regulatory element-binding protein-1c; UCP1 = uncoupling protein 1.

were increased by C/EBPβ. Afterwards, the level of their target genes, SREBP-1c, FAS, Ap2, and SCD-1, was increased and TG was finally accumulated.[17] It is thought that our results of decreased adipocytes may have been due to the effects of SB and SBP extract administration on the expression of adipogenesis-related factors. In other words, the mRNA and protein

expression levels of the adipogenic transcriptioal factors and their target genes were decreased after treatment with SB and SBP extracts. The observed effects on gene expression were concentration dependent, and the effects were more pronounced for the SBP extract than the SB extract. SIRT1, AMPK, PGC1α, PPARα, and UCP1 are involved in the thermogenic pathway, which contributes to fat catabolism. SIRT1, which is activated by AMPK phosphorylation and a high NAD^+/NADH ratio, activates PGC1α and UCP1, thereby continuously promoting heat generation.[18–20] In our results, the expression levels of genes and proteins of thermogenic mediators, AMPK, SIRT1, PGC-1α, PPARα, and UCP1, were decreased in the HFD-treated group and increased by SBP200-treated group. Therefore, SBP extracts may be involved in fat catabolism by regulating the expression of thermogenic factors, thereby having anti-obesity effects. In addition, the body weight and plasma TG decreased in extract-fed mice and HDL-C levels increased, suggesting that the SB and SBP extracts could be effective materials for improving obesity.

Adipose tissue secretes adipokines, which regulate lipid metabolism, including leptin and adiponectin.[21,22] Leptin regulates appetite and energy metabolism and increases obesity; therefore, it is used as an indicator of body fat mass.[23] Conversely, adiponectin secretion is low in obesity, thus promoting inflammation and decreasing insulin sensitivity.[24] In our study, leptin levels were increased and adiponectin levels were decreased in the HFD group. In the case of adiponectin, it was confirmed that the production was greater than that of the orlistat-treated group, which is a positive control group. This is thought to be the effect of insulin regulating the secretion of adiponectin according to the report that the level of adiponectin is inversely proportional to insulin resistance.[25–27] Based on this theory, our study showed that insulin resistance level of the SB and SBP extract–treated groups was lower than that of the orlistat group, which is thought to increase the secretion of adiponectin (Supplementary Table 2 http://links.lww.com/JCMA/A122).

Adiponectin is a cytokine secreted from adipose tissue and is inversely proportional to body fat mass. In addition, adiponectin, LDL-C, TG, and TC exhibited an inverse relationship with HDL-C. Therefore, in obesity, decreased adiponectin secretion increases blood LDL-C, TG, and cholesterol levels and decreases the level of HDL-C, thereby causing diseases such as cardiovascular disease and hyperlipidemia.[28,29] Therefore, these results suggested that SB and SBP extracts are effective in improving hyperlipidemia, cardiovascular disease, and obesity by increasing the level of adiponectin in the blood, thereby regulating plasma lipid levels.

In addition, the orlistat group as a representative drug for improving obesity showed similar or better efficacy to the SB and SBP extract–treated groups. Improving obesity of orlistat was confirmed by regulating major biomarkers of adipogenic and thermogenic mechanisms. These results are similar to the previously reported results that orlistat significantly inhibited the expression of C/EBPα and PPARγ in WAT and also activated the thermogenic genes *AMPK*, *PRDM16*, and *UCP-1* to help weight loss.[30] Although orlistat is a representative drug effective for fat reduction, it is known to cause various side effects such as diarrhea, vomiting, headache, and nausea when taken for a long time. Therefore, it is expected that SB and SBP extracts, which exhibit similar efficacy to orlistat, can be used as an alternative to orlistat.

It is reported that the SBP extract contains a greater amount of gallic acid than the SB extract.

Gallic acid is known as a phenolic acid component that has a high anti-obesity effect, and it is speculated that this is why the SBP extract has a higher anti-obesity effect than the SB extract.[31–34]

Our results demonstrate that SB and SBP extracts inhibit fat production and increase the expression of thermogenic factors to prevent obesity via fat catabolism. In particular, the SBP extract showed a greater protective efficacy against obesity than that of SB and is expected to be a valuable raw material for product development aimed at controlling obesity.

ACKNOWLEDGMENTS

This study was supported by "Research Program for Agricultural Science & Technology Development (project number PJ01456201)," National Institute of Agricultural Sciences, Rural Development Administration, and Republic of Korea.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A122.

REFERENCES

1. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab* 2004;**89**:2595–600.
2. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract* 2014;**105**:141–50.
3. Goran MI. Energy metabolism and obesity. *Med Clin North Am* 2000;**84**:347–62.
4. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 1994;**79**:1147–56.
5. Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 2009;**458**:1056–60.
6. Lim CT, Kola B, Korbonits M. AMPK as a mediator of hormonal signalling. *J Mol Endocrinol* 2010;**44**:87–97.
7. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. *J Hepatol* 2017;**67**:862–73.
8. Jakus P. *Role of brown and white adipose tissue in cold-induced thermogenesis*. Hungary: University of Pécs; 2009.
9. Cao W, Medvedev AV, Daniel KW, Collins S. Beta-adrenergic activation of p38 MAP kinase in adipocytes: cAMP induction of the uncoupling protein 1 (UCP1) gene requires p38 map kinase. *J Biol Chem* 2001;**276**:27077–82.
10. Anderson RM, Barger JL, Edwards MG, Braun KH, O'Connor CE, Prolla TA, et al. Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. *Aging Cell* 2008;**7**:101–11.
11. Dong J, Zhang X, Zhang L, Bian HX, Xu N, Bao B, et al. Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKα1/SIRT1. *J Lipid Res* 2014;**55**:363–74.
12. Kamiya T, Nagamine R, Sameshima-Kamiya M, Tsubata M, Ikeguchi M, Takagaki K. The isoflavone-rich fraction of the crude extract of the Puerariae flower increases oxygen consumption and BAT UCP1 expression in high-fat diet-fed mice. *Glob J Health Sci* 2012;**4**:147–55.
13. Rabadan-Chávez G, Quevedo-Corona L, Garcia AM, Reyes-Maldonado E, Jaramillo-Flores ME. Cocoa powder, cocoa extract and epicatechin attenuate hypercaloric diet-induced obesity through enhanced β-oxidation and energy expenditure in white adipose tissue. *J Funct Food* 2016;**20**:54–67.
14. Kim OK, Nam DE, You YH, Jun WJ, Lee JM. Protective effect of *Canavalia gladiata* on gastric inflammation induced by alcohol treatment in rats. *J Korean Soc Food Sci Nutr* 2013;**42**:690-6.
15. Kumar CP, Reddy YN. Protective effect of *Canavalia gladiata* (sword bean) fruit extracts and its flavanoidal contents, against azathioprine-induced toxicity in hepatocytes of albino rats. *Toxicol Environ Chem* 2014;**96**:474-81.
16. Blüher M. Adipokines - removing road blocks to obesity and diabetes therapy. *Mol Metab* 2014;**3**:230–40.

17. Hsu CL, Lo WH, Yen GC. Gallic acid induces apoptosis in 3T3-L1 pre-adipocytes via a Fas- and mitochondrial-mediated pathway. *J Agric Food Chem* 2007;**55**:7359–65.
18. Cantó C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol* 2009;**20**:98–105.
19. Sakamoto T, Takahashi N, Goto T, Kawada T. Dietary factors evoke thermogenesis in adipose tissues. *Obes Res Clin Pract* 2014;**8**:e533–9.
20. Castrejón-Tellez V, Rodríguez-Pérez JM, Pérez-Torres I, Pérez-Hernández N, Cruz-Lagunas A, Guarner-Lans V, et al. The effect of resveratrol and Quercetin treatment on PPAR mediated uncoupling protein (UCP-) 1, 2, and 3 expression in visceral white adipose tissue from metabolic syndrome rats. *Int J Mol Sci* 2016;**17**:E1069.
21. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;**395**:763–70.
22. Kim JY, Shin M, Heo YR. Effects of stabilized rice bran on obesity and antioxidative enzyme activity in high fat di-et-induced obese C57BL/6 mice. *J Korean Soc Food Sci Nutr* 2014;**43**:1148-57.
23. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. *Diabetes* 1996;**45**:1455–62.
24. Gil JH, Lee JA, Kim JY, Hong YM. Leptin, adiponectin, interleukin-6 and tumor necrosis factor-α in obese adolescents. *Korea J Pediatrics* 2008;**51**:597-603.
25. Gomaa AA, EI-Sers DA, AI-Zokeim NI, Gomaa MA. Amelioration of experimental metabolic syndrome induced in rats by orlistat and Corchorus olitorius leaf extract; role of adipo/cytokines. *J Pharm Pharmacol* 2019;**2**:281-91.
26. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. *Obes Rev* 2005;**6**:13–21.
27. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006;**116**:1784–92.
28. Kim H, Lee SW, Lee HS, Shim KW, Cho CY, Yun JE, et al. Associations between lipid measures and metabolic syndrome, insulin resistance and adiponectin ratios in Korean men and women. *Circ J* 2010;**74**:931–7.
29. Bansal N, Charlton-Menys V, Pemberton P, McElduff P, Oldroyd J, Vyas A, et al. Adiponectin in umbilical cord blood is inversely related to low-density lipoprotein cholesterol but not ethnicity. *J Clin Endocrinol Metab* 2006;**91**:2244–9.
30. Choi J, Kim KJ, Koh EJ, Lee BY. Gelidium elegans regulates the AMPK-PRDM16-UCP-1 pathway and has a synergistic effect with orlistat on obesity-associated features in mice fed a high-fat diet. *Nutrients* 2017;**9**:E342.
31. Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab* 2002;**87**:2764–9.
32. Chan DC, Barrett PH, Ooi EM, Ji J, Chan DT, Watts GF. Very low density lipoprotein metabolism and plasma adiponectin as predictors of high-density lipoprotein apolipoprotein A-I kinetics in obese and non-obese men. *J Clin Endocrinol Metab* 2009;**94**:989–97.
33. Hwang KA, Heo W, Hwang HJ, Han BK, Song MC, Kim YJ. Anti-inflammatory effect of immature Sword Bean Pod (*Canavalia gladiata*) in Lipopolysaccharide-induced RAW264.7 cells. *J Med Food* 2020;**23**:1183–91.
34. Lee HS, Lee SJ, Ho JN, Cho HY. Preventive effect of fermented chestnut inner shell extract on obesity-induced hepatic steatosis. *Prev Nutr Food Sci* 2020;**25**:32–40.