SPANDIDOS PUBLICATIONS

Water-extracted plum (*Prunus salicina L. cv. Soldam*) attenuates adipogenesis in murine 3T3-L1 adipocyte cells through the PI3K/Akt signaling pathway

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Abstract. The objective of the present study was to evaluate the effects of water-extracted plum (WEP) on adipocyte differentiation, adipogenesis and inflammation in differentiated 3T3-L1 adipocyte cells. WEP was assessed for basic analyses, including high-performance liquid chromatography, total phenolic and flavonoid content and antioxidant activity [1,1-diphenyl-2-picrylhydrazyl (DPPH) assays] *in vitro*. Moreover, the cell viability was measured using an MTT assay. Adipogenesis and lipid accumulation in 3T3-L1 adipocytes was investigated using Oil Red O staining, and the expression of genes and proteins associated with adipogenesis and lipolysis were examined by reverse transcription polymerase chain reaction and western blotting. In addition, sulforaphane using a positive control was performed simultaneously. The WEP significantly suppressed adipocyte differentiation and lipid accumulation in differentiated adipocytes without cytotoxicity. WEP resulted in direct anti-obesity effects through the modulation of adenosine monophosphate-activated protein kinase, sterol regulatory element-binding protein 1c, cytidine-cytidine-adenosine-adenosine-thymidine/enhancer binding protein α and peroxisome proliferator-activated receptor γ. These regulations of molecular expressions were significantly activated via the phosphoinositide 3-kinase/Akt pathway. Moreover, these results provide potential anti-adipogenic effects of WEP and may have potential as a natural agent for the prevention and improvement of obesity.

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Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; SREBP1c, sterol regulatory element-binding protein 1c; PPARγ, peroxisome proliferator-activated receptor γ; PI3K, phosphoinositide 3-kinase/Akt; CCAAT/C/EBPα, cytidine-cytidine-adenosine-adenosine-thymidine/enhancer binding protein α

Key words: plum, anti-adipogenesis, adenosine monophosphate-activated protein kinase, sterol regulatory element-binding protein 1c, enhancer binding protein α, peroxisome proliferator-activated receptor γ, phosphoinositide 3-kinase/Akt

Introduction

Food intake behaviors, genes and the environment are important factors in causing people to be overweight and obese. The prevalence of obesity worldwide has rapidly increased over the past decades. In 2014, the National Health and Nutrition Survey in Korea reported that over a third of Korean adults (30.2%) were obese (1,2). Obesity-related conditions include heart disease, stroke, type 2 diabetes and certain types of cancer, which give rise to complex health issues, including hypertension, sleep apnoea, arthritis and decrease future life expectancy (3,4).

Prior studies have demonstrated that numerous dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* compared with vitamin E or C (5). In addition, increasing interest has been paid to dietary agents such as fruits and vegetables for the possibility of controlling obesity as lipolysis agents, since these dietary agents are known to have or enhance anti-adipogenic and/or lipolysis activity without causing cytotoxicity (6). Plum (*Prunus salicina L. cv. Soldam*) is an important fruit crop grown in Gimcheon, Gyungbuk, Korea. It is often used to help regulate the function of the digestive system and has been recently used as a supplement for irregularity treatment (7). This effect has been attributed to various compounds present in the fruits, such as dietary fiber, isatin and sorbitol (8). In addition, prunes and prune juice are often used to help regulate the functioning of the digestive system. Dried plums (or prunes) contain several antioxidants, including polyphenol, antocyannin and catechine amongst others (9,10). Moreover, plum extract has been characterized with the highest total phytonutrient content and exhibited the highest antioxidant activity compared with other fruits, including peach, pear and apple as shown by *in vitro* assays (11). Plum extract was subjected to high-performance liquid chromatography (HPLC) (9), and chlorogenic acid, quercetin and epicatechin were identified as main constituents. It has been well reported that phytochemicals as antioxidants have the ability to affect the lipid metabolism (12). However,

few studies have been conducted regarding the anti-adipogenic effects of aqua extracted plum, and so the molecular mechanisms of the effects in murin adipocyte 3T3-L1 cells remain unknown. Thus, the effects of water-extracted plum (WEP) on adipogenesis and the molecular signaling pathway in 3T3-L1 adipocytes were investigated in the present study.

Materials and methods

Reagents. High-glucose Dulbecco's modified Eagle's medium (DMEM), bovine calf serum (BCS), fetal bovine serum (FBS) and phosphate-buffered saline (PBS) were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Penicillin/streptomycin and 0.25% trypsin/EDTA were purchased from Gibco (Thermo Fisher Scientific, Inc.). 3-(4,5-dimethyl-thiazol)-2,5-diphenyltetrazolium bromide (MTT), insulin, dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), dimethyl sulfoxide (DMSO), rosiglitazone (RSG) and Oil red-O were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The other chemicals not specifically mentioned here were obtained from Sigma-Aldrich (Merck KGaA).

Preparation of plum. The *Prunus salicina L. cv. Soldam* were washed and pretreated in 60-70˚C for 10 min. The pretreated plum was pressed, concentrated on a waterbath under vacuum, freeze-dried and lyophilized (PVTFD 100R; Ilshin Biobase Co., Ltd., Korea) and the final yield of WEP was 15%.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, total phenolics and flavonoid contents. Free radical scavenging activities of solutions of the plum extracts were determined in accordance with the Blois method, which is based on the principle of scavenging radicals (13). Briefly, the reaction mixture (0.2 ml of WEP mixed with 3.8 ml of 0.4 mM DPPH solution) was incubated for 30 min at room temperature and the absorbance was recorded at 517 nm and then compared with that of ethanol as a negative control. The experiment was repeated three times. Butylated hydroxyanisole (BHA) was used as a standard control of radical scavenging activity. The results expressed electron-donating ability (EDA) according to the following formula: % of EDA=(Abs of control-Abs of Test)/Abs of control x 100. The total phenolics contents of the plum extract and the standard antioxidant materials were determined according to the Folin-Ciocalteu method (14). The results were calculated using the standard calibration curve of gallic acid and expressed as gallic acid equivalents (GAE, mg/g). In addition, the flavonoid content was determined using the $AlCl_3$ colorimetric method (15) and the total flavonoid content was expressed as mg of catechin equivalents (mg CE/kg WEP).

HPLC analysis. The antioxidant component of WEP, which is rich in polyphenols, was isolated using column chromatography and HPLC. HPLC was performed on the Agilent 1260 series (Agilent Technologies, Inc., Santa Clara, CA, USA), using a UV280 detector (model no. G4212B). Chromatographic separation was accomplished on an Eclipse XDB-C18 5 μm 4.6x150 mm column (Agilent Technologies, Inc.). A gradient solvent system consisting of 0.5% formic acid (solvent A) and acetonitrile (solvent B) was used at a flow rate of 1 ml/min. The standard stock solutions were prepared by dissolving standards in distilled water to set a final concentration of 200 ppm. Following ultracentrifugation and filtration through a syringe filter (0.45 μm; Pall Corporation, Port Washington, NY, USA), and 10 μl of sample was injected three times. The retention time of stock standards, chlorogenic acid, epicatechin and quercetin were ~9.683, 12.526 and 26.909 min, respectively.

Cell culture and toxicity. Murine 3T3-L1 preadipocytes were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured at 37˚C in 5% CO_2 in DMEM supplemented with 10% BCS for preadipocytes. In order to induce differentiation, two-day post-confluent preadipocytes (day 0) were cultured in differentiation medium (DMEM containing 10% FBS, 0.5 mM IBMX, 2 μM DEX and 2 μg/ml insulin) for 2 days and then cultured for a further 2 days in DMEM containing 10% FBS and 2 μg/ml insulin. The differentiated cells were maintained in post-differentiation medium (DMEM containing 1% PS and 10% FBS), which was replaced every two days. For the analysis of cell viability, the cells ($2x10^5$ cells/ml) were incubated with the indicated concentrations of WEP for 24 h. Moreover, cell viability was determined by the MTT assay. MTT solution (1 mg/ml) in each well was completely removed and 100 μl DMSO was added for dissolving formazan. The measurement of dissolved formazan was read using a microplate reader at 570 nm (Tecan Group Ltd., Männedorf, Switzerland).

Oil Red O staining. Accumulation of lipid droplets during adipogenesis was visualized by Oil Red O staining. Briefly, 3T3-L1 cells were washed twice with PBS and fixed with sodium cacodylate buffer for 3 h at 4˚C. Fixed cells were stained with Oil Red O solution (0.5% in 40% isopropanol) for 3 h at room temperature. Cells were then washed three times with 40% isopropanol and distilled water. In addition, images of the cells were captured using an inverted microscope (model no. AE31, Motic Insturments, Richmond, BC, Canada). For the quantification of lipid accumulation, cells were incubated with 1 ml of 100% isopropanol per well for 10 min. Following incubation, the absorbance was measured at 510 nm using a spectrophotometer (model no. LAMBDA 35; PerkinElmer, Inc., Waltham, MA, USA) and the experiments were repeated at least three times.

Reverse transcription-polymerase chain reaction (RT-PCR). Cellular RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. The cDNA was synthesized from 1 μg total RNA in a 20-μl reaction using OneStep RT-PCR PreMix (iNtRON Biotechnology, Inc., Kyunggi, Korea). PCR was performed in a Mastercycler (Eppendorf, Hamburg, Germany) and the following primers (Bioneer Corporation, Daejeon, Korea) were used for the RT-PCR analysis: Adenosine monophosphate-activated protein kinase (AMPK): Forward 5'-CCA GGTCATCAGTACACCAT-3' and reverse, 5'-CTGCCA AAGGATCCTGGTGA-3'; Sterol regulatory element-binding protein 1c (SREBP1c): forward, 5'-ATCCGCTTCTTACAG CACAG-3' and reverse, 5'-CCAATTAGAGCCATCTCTGC -3'; CCAAT/enhancer binding protein α (C/EBPα): forward, 5'-GCAACGCCGCCTTTGGCTTT-3' and reverse, 5'-AGT

GCGCGATCTGGAACTGC-3'; peroxisome proliferator-activated receptor γ (PPARγ) forward, 5'-ATTCTGGCCCACCAACTTCGG-3' and reverse, 5'-TGGAAGCCTGATGCTTTATCCCCA-3'; glyceraldehydes 3-phosphate dehydrogenase (GAPDH): forward, 5'-CGGAGTCAACGGATTTGGTCGTAT-3' and reverse, 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'. PCR amplification products were electrophoretically separated on a 1.5% agarose gel and visualized with ethidium bromide (Sigma-Aldrich; Merck KGaA) staining. All data were visualized using Biovis gel documentation software (version 6.0; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The band intensities were quantified by densitometry using L Process (version 2.01) and Multi Gauge software (version 2.02; FUJIFILM Medical Systems USA, Inc., Stamford, CT, USA).

Western blot analysis. Following treatment with/without WEP in the differentiated cells, the fully mature cells were washed with ice-cold PBS, collected and centrifuged at 500 x g for 5 min at 4˚C. The resulting cell pellets were resuspended in ice-cold lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 1 mM EGTA, phosphatase inhibitor cocktail, protease inhibitor, phenylmethanesulfonyl fluoride] and incubated on ice for 1 h. Following removal of the cell debris by centrifugation at 13,000 x g for 15 min at 4˚C, the total protein contents were quantified using the Bradford protein assay (cat no. 5000002; Bio-Rad Laboratories, Inc.). Aliquots of protein extracts (15-50 µg) were separated using 8 and 10% SDS-PAGE for 2 h at 60-110 V and electrophoretically transferred onto PVDF membranes (0.45 mm; EMD Millipore, Billerica, MA, USA) in a transfer buffer containing 25 mM Tris-HCl, 192 mM glycine and 20% methanol. Transferred membranes were blocked with 5% non-fat dry milk or 5% bovine serum albumin in Tris-buffered saline with 0.1% Tween-20 for 1 h at room temperature, and incubated with the following primary antibodies: AMPK (1:1,000; cat no. 9957), phospho-AMPK (1:1,000; cat no. 9957), PI3K, phosphor- phosphoinositide 3-kinase (1:1,000; cat no. 4255), phosphor-Akt (1:1,000; cat no. 2430s), Akt (1:1,000; cat no. 2920), (1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA), SREBP1c (1:1,000, cat no. sc-8984), PPARγ (1:1,000; cat no. sc-7196), C/EBPα (1:1,000; cat no. sc-9315) and β-actin antibody (1:10,000; cat no. sc-1616) purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) at 4˚C overnight. The membranes were further incubated for 1 h in appropriate HRP-conjugated secondary antibodies, including bovine anti-goat IgG-horseradish peroxidase-HRP (1:1,500; cat no. sc-2350), goat anti-rabbit IgG-HRP (1:1,500; cat no. sc-2004), goat anti-mouse IgG-HRP (1:1,500; cat no. sc-2005) purchased from Santa Cruz Biotechnology, Inc. The membranes were washed once again as described above for 5 min, six times each. The membranes were visualized through incubation with an Amersham ECL Western Blotting Detection Reagent (cat no. 2106; Amersham; GE Healthcare Life Sciences, Little Chalfont, UK) reagents. The band intensities that expressed protein were quantified by densitometry using L Process (version 2.01) and Multi Gauge software (version 2.02).

Statistical analysis. All data are presented as the mean ± standard deviation. Statistical analysis was performed using Student's t-test, with GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

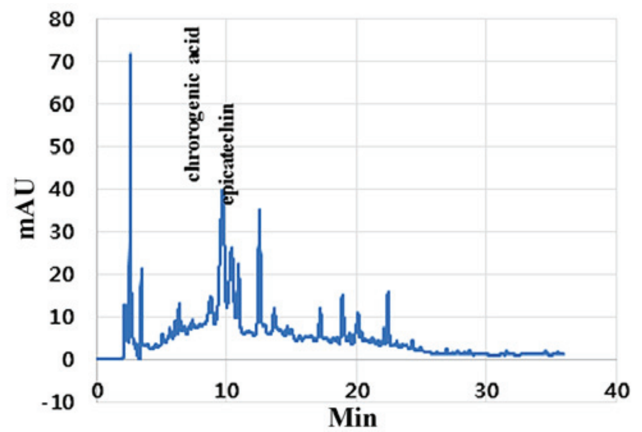

Figure 1. High-performance liquid chromatogram of phytochemicals obtained from stock standard, chlorogenic acid, epicathechin and quercetin used as positive controls.

Results

Contents of total phenolics and flavonoids, and activities of antioxidation in WEP. Antioxidant phytochemicals analyzed using HPLC analysis included 191.75 mg/100 g chlorogenic acid powder as hydroxycinnamic acid and 145.92 mg/100 g epicatechin powder in this WEP sample (Fig. 1). As shown in Fig. 2A, the total phenolics content expressed as GAE was 18.6 mg/g. In addition, the total flavonoid content expressed as mg of CE (mg CE/kg WEP) was 0.17 mg. As shown in Fig. 2B, the radical scavenging DPPH activities of the plum extracts were lower in 10 mg/ml WEP than those of BHA and quercetin, used as positive controls, but there was no significant difference in the 100 mg/ml WEP extract compared to those of controls, BHA and quercetin. These results indicate that 100 mg/ml WEP possesses prominent antioxidant properties and can be exploited as a natural drug to treat free-radical associated diseases.

Effects of WEP on the cytotoxicity and lipid accumulation in 3T3-L1 cells. The appropriate concentrations of WEP for the toxicity of 3T3-L1 adipocytes were determined by the MTT assay. WEP (up to 1 mg/ml) did not affect the viability and cytotoxicity of 3T3-L1 adipocytes (Fig. 3A). Therefore, 500 µg/ml WEP was treated for further study in the cells. The accumulated lipid droplets in differentiated 3T3-L1 adipocytes were visualized and quantified by Oil Red O staining. In order to compare the anti-adipogenesis effects sulforaphane was used as a positive control in the present study. As shown in Fig. 3B, on day 10 the mature 3T3-L1 adipocytes accumulated the intracellular lipid droplets. WEP inhibited lipid accumulation up to 1 mg/ml concentration without cytotoxicity compared to that of 5 mM RSG that was used as a positive control. Furthermore, the lipid accumulation increased by 50% compared with that of the control. The cells treated with 500 and 1,000 µg/ml WEP significantly decreased lipid accumulation by 35.5 and 50.4% (P<0.05), respectively, compared

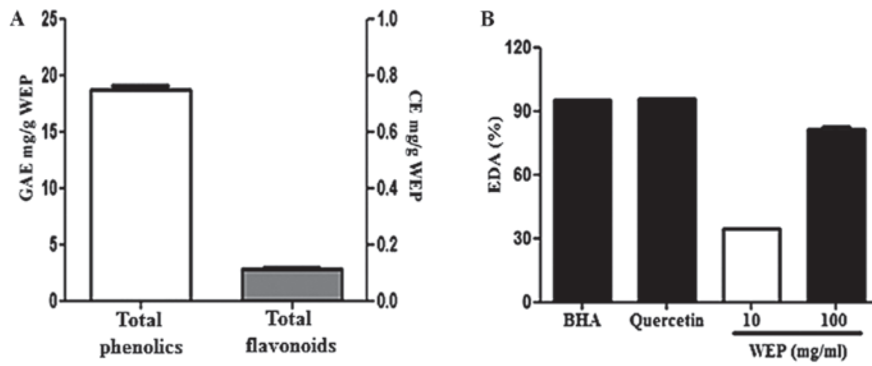

Figure 2. (A) Assay of total phenolic and flavonoid contents and (B) assays of DPPH radical scavenging activity. Total phenolics determined according to the Folin-Ciocalteu method are expressed as mg GAE/g WEP ± standard deviation (n=3). Total flavonoids determined using the $AlCl_3$ colorimetric method are expressed as mg CE/kg WEP ± standard deviation (n=3). Free radical scavenging activities determined in accordance with the Blois method are expressed as % of EDA. GAE, gallic acid equivalents for total phenolics; EDA, electron-donating ability; BHA, butylated hydroxyanisole for (+) control of radical scavenging activity; WEP, water-extracted plum; DPPH, 1,1-diphenyl-2-picrylhydrazyl; CE, cathechin equivalent for total flavonoids.

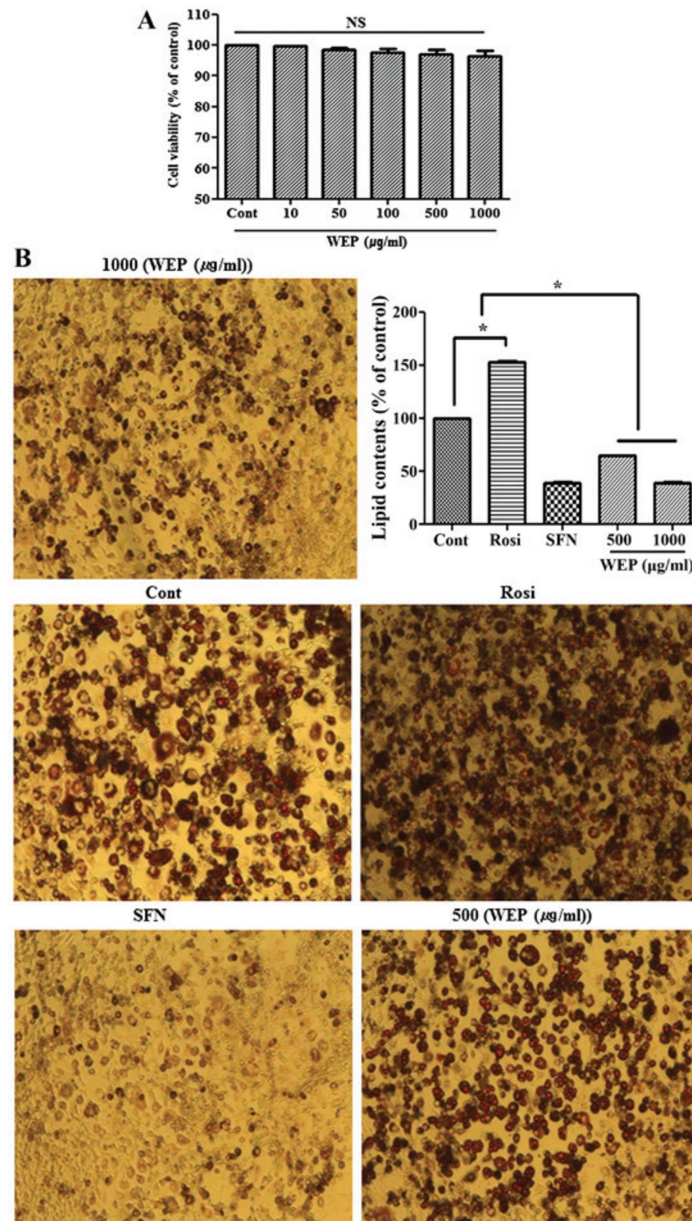

Figure 3. Effect of WEP on the cytotoxicity and lipogenesis in differentiated 3T3-L1 cells. (A) MTT assay for cell viability and cytotoxicity; (B) Oil Red O staining and lipid content in mouse adipocte 3T3-L1 cells treated with various doses of WEP. The data shown are representative of three independent experiments. *$P<0.05$ Cont vs. Rosi, SFN, WEP-500 and -1,000. NS, no significance; WEP, water-extracted plum; Cont, control; Rosi, rosigiltazone; SFN, sulforaphane.

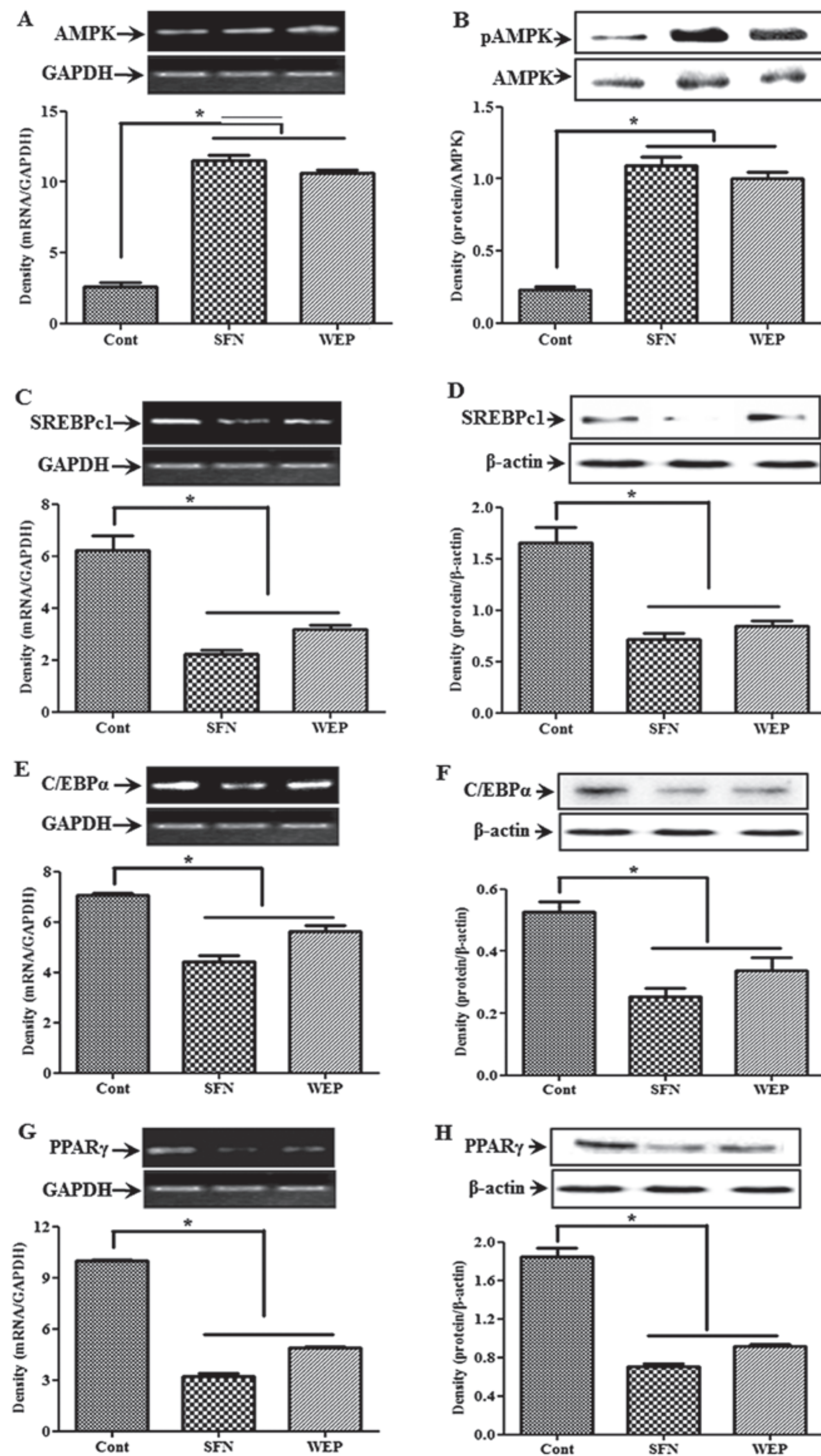

Figure 4. (A, C, E and G) Effects of WEP on the regulation of lipogenesis-associated genes in 3T3-L1 cells. Levels of mRNA detected using reverse transcription-polymerase chain reaction. (B, D, F and H) Protein expression by western blot analysis. mRNA and protein expression were normalized against the intracellular housekeeping genes, including GAPDH and β-actin, respectively. Each value is presented as the mean ± standard deviation. Data shown are representative of three independent experiments. *$P<0.05$ Cont vs. SFN, WEP. AMPK, adenosine monophosphate-activated protein kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SREBPc1, Sterol regulatory element binding transcript factor 1; SFN, sulforaphane; WEP, water-extracted plum; EBP, enhancer binding protein; PPARγ, peroxisome proliferator-activated receptor γ.

with that of the control. Moreover, there was no significant difference between the lipid accumulation between the positive control sulforaphane-treated and WEP-treated cells.

WEP regulated anti-adipogenesis related genes. As shown in Fig. 4A and B, the mRNA and protein expression of phosphorylated AMPK increased in WEP-treated cells compared with that of the control cells. Meanwhile, there was no difference between the treatments of WEP and sulforaphane, used as a positive control, respectively. This result indicates that WEP can promote the potential of anti-adipogenesis via activating AMPK in 3T3-L1 adipocytes. As shown in Fig. 4C and D, those

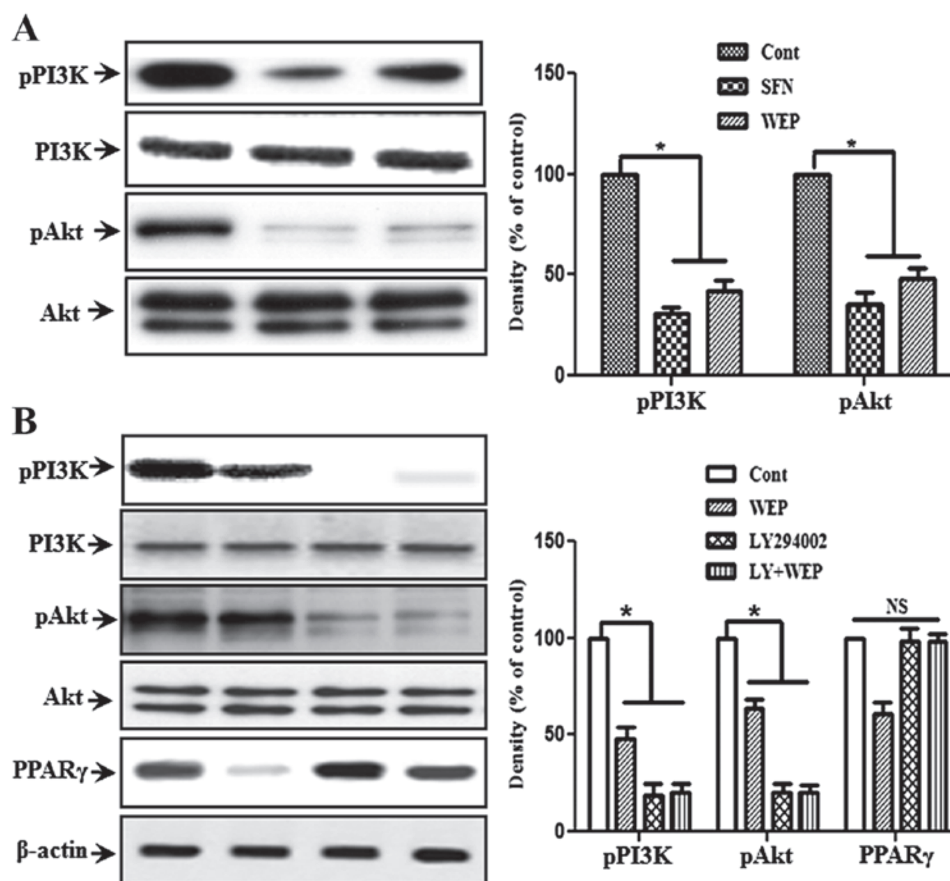

Figure 5. Effect of WEP induced PI3K/Akt on the inhibition of PPARγ expression with and without LY294002 as the PI3K/Akt inhibitor for conformation of the signaling pathway in 3T3-L1 adipocytes. Cells were pretreated for 2 h with LY294002 and then treated with WEP at the second day after beginning to differentiate. The total protein from differentiated control cells and LY294002-treated cells was extracted. The expression of pPI3K, PI3K, pAkt, Akt, PPARγ and actin was examined by western blot analysis. (A) Phosphorylated and total PI3K and Akt with WEP. (B) Phosphorylated and total PI3K, AKT and PPARγ protein levels with LY294002 and/or WEP. Band intensities were normalized relative to the internal controls (total PI3K, Akt and actin) and background, respectively. *P<0.05 vs. the untreated control. Each value indicates the mean ± standard deviation of the mean and is representative of the results obtained from three independent experiments. NS, no significance; Cont, control; WEP, water-extracted plum; PI3K, phosphoinositide 3-kinase; PPARγ, peroxisome proliferator-activated receptor γ; SFN, sulforaphane.

of SREBP1c were decreased in WEP-treated cells compared to those of the control, and no significant differences were observed between those of sulforaphane and WEP. Therefore, WEP in 3T3-L1 adipocytes can inhibit lipogenesis, particularly, sterol synthesis such as cholesterol. Next, the effects of WEP on C/EBPα and PPARγ were investigated in mature 3T3-L1 adipocytes. WEP and sulforaphane decreased those of C/EBPα and PPARγ compared to those of the control (Fig. 4E-H).

Effects of WEP on anti-adipogenesis via phosphorylation of the Akt pathway. As shown in Fig. 5A, WEP also decreased the expression of phosphorylation of the PI3K/Akt pathway, which acts upstream of PPARγ and C/EBPα in adipogenesis. In order to confirm these pathways, LY294002, an inhibitor of PI3K/Akt was treated in 3T3-L1 adipocytes (Fig. 5B). The expression of PPARγ induced during adipose differentiation was not inhibited. These results indicated that WEP inhibited lipid accumulation by downregulating the major transcription factors involved in the pathway of adipogenesis, including PPARγ via the regulation of the PI3K/Akt signaling pathway in 3T3-L1 adipocyte differentiation. This indicated the potential use of WEP as an anti-obesity agent.

Discussion

Accumulating evidence has indicated that polyphenol compounds in human diets are bioactive components of various fruit and vegetables (16). The present study investigates the role of WEP in the anti-adipogenesis metabolism and signaling pathway.

The present study aimed to provide comprehensive information on polyphenolic components and on the way through which they contribute to the antioxidant activities of WEP. Initially, the contents of antioxidant compounds were 18.6 mg/g total phenolics expressed as GAE (mg/g) and 0.17 mg total flavonoid expressed as mg of catechin equivalents (CE mg/WEP kg). In addition, the DPPH activity in 100 mg/ml WEP was not different compared to that of BHA and quercetin that were used as positive controls. Wang *et al* (17) reported that antioxidant effects of plant-based foods prevented oxidative injury and cell proliferation via several mechanisms. Therefore, these results indicated the sources of antioxidant activity in the health-related benefits and in particular lipogenesis-induced obesity.

Secondly, the present study focused on the expression of adipogenesis marker genes. AMPK, a potential therapeutic

target for the treatment of obesity and type 2 diabetes, indicates that AMPK activators are protective against and can rescue metabolic defects associated with obesity (18,19). AMPK activity modulates numerous aspects of the mammalian cell metabolism, and it also has diverse roles that extend from energy metabolism through to transcriptional control. In addition, AMPK functions modulate fatty acid oxidation, glycolysis, fatty acid and cholesterol synthesis (20,21). In order to investigate whether WEP regulates adipogenesis in differentiated 3T3-L1 adipocytes, the effects of WEP on the phosphorylation of AMPK were investigated. Consistently, WEP evidently increased the activity of the phosphorylation of AMPK in the cells compared with sulforaphane-treated cells that were used as a positive control. This result demonstrated that WEP has anti-obesity potency mediated by the AMPK pathway. Moreover, the effect of WEP on transcription factors of differentiation and lipogenesis in 3T3-L1 adipocytes was investigated. SREBP1c transcription factors regulate the expression of lipogenic enzymes, including ACC, fatty acid synthase and stearoyl-CoA desaturase (22,23). Thus, it is believed that phosphorylated AMPK in the cells treated with WEP triggers the suppression of lipogenesis transcription factors, including SREBP1c, C/EBPα and PPARγ expression. Moreover, effects of WEP on transcription factors of lipogenesis were similar to that of sulforaphane known to anti-adipogenesis as powerful antioxidant compounds in obesity and type 2 diabetes (12,24).

Altogether, these results indicate that WEP suppresses differentiation and adipogenesis associated genes, SREBP1c, C/EBPα and PPARγ expression by activating the AMPK phosphorylation in mature 3T3-L1 adipocytes. In addition, WEP may have similar properties to sulforaphane. Therefore, WEP including antioxidant phytochemicals, such as chlorogenic acid and epicathechin, has the potential to prevent obesity by modulating the lipid metabolism. Next, the cell signaling pathway needs to be investigated. The PI3K/Akt pathway is activated by growth factors and involved in various biological functions, including cell proliferation, survival and migration (25). Moreover, the pathway promotes GLUT4 expression in myocytes and adipocytes, and is important in mobilizing glucose throughout the body (26,27). Yu *et al* (28) suggested that the PI3K/Akt signaling pathway is essential for adipogenesis of human mesenchymal stem cells. In the present study phosphorylation of PI3K/Akt was suppressed by treatment of WEP in 3T3-L1 cells. Therefore, WEP may be a potential target for obesity associated with adipogenesis through phosphorylation of the PI3K/Akt pathway. In order to confirm that the PI3K/Akt pathway is important in the adipogenesis decreased by WEP, LY294002 was used, an inhibitor of PI3K. LY294002 with WEP did not suppress the activation of the PI3K/Akt pathway, and also PPARγ that is a master regulator in adipogenesis induced during the differentiation process was unblocked. Collectively, the results of the present study demonstrate that the PI3K/Akt signaling pathway is crucial for anti-adipogenesis in 3T3-L1 cells treated with WEP. Altogether, these results indicated the potential use of WEP as an anti-obesity agent through the PI3K/Akt signaling pathway. Consequently, the present study should provide useful information for identifying fruit (*Prunus salicina L. cv. Soldam*) that is rich in these protective components, and may have applications for the development of safe anti-obesity products with appropriate antioxidant properties.

Acknowledgements

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