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Effects of *Allium hookeri* Root Water Extracts on Inhibition of Adipogenesis and GLUT-4 Expression in 3T3-L1 Adipocytes

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Abstract Anti-adipogenic and antidiabetic activities of *Allium hookeri* root water extracts (ARW) were assessed. Oil Red O staining showed that treatment with ARW caused a dose-dependent reduction in lipid accumulation. ARW was also involved in adipocyte lipolysis via LPL activity, and in the concentration of glycerol in a culture medium. On the basis of the concentration of adipokines following ARW treatment, ARW appeared to inhibit expression of PPAR-γ, to reduce concentrations of leptin and resistin, to increase the concentration of adiponectin, and to inhibit lipid accumulation. ARW modulated adipokine expression associated with insulin resistance and sensitivity. 3T3-L1 adipocytes treated with ARW showed increased GLUT-4 expression with increased glucose uptake into adipocytes. ARW showed effectiveness for improvement of diabetic conditions.

Keywords: *Allium hookeri*, adipogenesis, GLUT-4, 3T3-L1

Introduction

Diabetes can be classified into Type 1 insulin dependent diabetes mellitus (IDDM), and Type 2 diabetes or non-insulin dependent diabetes mellitus, which accounts for at least 98% of diabetes cases in South Korea (1). Type 2 diabetes is often caused by dysfunction of insulin secreted from the pancreas, reduced insulin sensitivity of liver and muscle tissues, or insulin resistance, any of which can raise the blood glucose level, resulting in persistent hyper-glycemia and dysfunction of pancreatic beta-cells due to glucotoxicity (2). Obesity, a leading cause of Type 2 diabetes, is closely related to insulin resistance not only due to an increase in plasma cholesterol, plasma lipids, and plasma free fatty acid levels, but also due to destruction of beta-cells related to lipotoxicity leading to poor secretion of insulin and dysfunction of beta-cells (3,4).

Adipose tissues composed of adipocytes and small amounts of other types of cells may be involved in lipid metabolism for lipid secretion and storage, and in glucose metabolism for insulin-dependent glucose uptake, and also may control the endocrine system for hormone and cytokine secretion (5,6). However, dysfunction of adipose tissues, such as excessive differentiation of adipocytes, may increase the risk of obesity and diabetes (7). Lipid accumulation can cause insulin resistance via secretion of several types of adipokines, and failure of insulin secretion from pancreatic beta-cells to overcome the resistance may lead to impaired glucose tolerance, or Type 2 diabetes (8). Important types of adipokines related to obesity include leptin, which was first discovered in 1994, adiponectin, resistin, and the peroxisome proliferator activated receptor (PPAR)-γ, a nuclear receptor that is an important obesity-related indicator (7,8).

Research has been conducted on treatment of obesity and diabetes, including medication, diet therapy, and exercise therapy (9). While many patients with Type 2 diabetes use a combination of diet and exercise therapies to control blood sugar levels, a majority of patients use medication in addition to the combination, which alone is insufficient for control of blood sugar levels. However, since long-term medication has been found to have side effects and to cause tolerance, some researchers have recently tried to produce medicines from natural substances with fewer side effects, and much interest has developed in using a diversity of functional foods in development of anti-adipogenic products for improvement of diabetic conditions (10-12).

The perennial herb in the Liliaceae family *Allium hookeri*, known as a root leek, is used for foods and medicine and is sometimes called 3-taste namul due to 3 root tastes of sweet, bitter, and hot, and sometimes called ginseng namul due to a root taste like ginseng (13). Like *Allium fistulosum* L., *Allium cepa* L., *Allium sativum* L., and *Allium tuberosum*, *A. hookeri* is an accessible vegetable in the *Allium* genus (14) containing a high content of sulfur compounds with unique tastes and flavors that has been included in side dishes in Korean cuisine to increase appetite. These herbs exhibit physiological activities of anti-oxidant, anti-hypertensive, anti-cancer, antibacterial, and antidiabetic activities, and improvements in lipid metabolism (15-18).

While increasing interest in *A. hookeri* has led to research regarding physiological activities, few studies have been conducted regarding use in prevention and treatment of obesity and diabetes. The aim of this study was to use 3T3-L1 adipocytes for confirmation of anti-adipogenic and antidiabetic activities of water extracts of *A. hookeri* roots.

Materials and Methods

Materials Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin, trypsin/EDTA, bovine calf serum (BCS), fatal bovine serum (FBS), phosphate buffered saline (PBS), and PrestoBlue cell viability reagent were purchased from Invitrogen (Carlsbad, CA, USA). Insulin, isobutyl methylxanthine (IBMX), dexamethasone (DEX), Oil Red O, formalin solution, dimethyl sulfoxide (DMSO) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A protein assay kit and RIPA lysis buffer were purchased from Pierce (Rockford, IL, USA). Protease inhibitor cocktail was purchased from Roche (Mannheim, Germany). Concentrations of adipokine were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit purchased from R&D Systems (Minneapolis, MN, USA). Namely, a mouse resistin quantikine ELISA kit, a mouse leptin quantikine ELISA kit, and a mouse adiponectin quantikine ELISA kit. A lipoprotein lipase (LPL) ELISA kit was purchased from Mybiosource (San Diego, CA, USA) and a free glycerol assay kit and a glucose uptake assay kit were purchased from Abcam (Cambridge, MA, USA). Specific antibodies against PPAR-γ, GLUT-4, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). An enhanced chemiluminescence (ECL) detection kit were purchased from Bio-Rad (Hercules, CA, USA).

Preparation *A. hookeri* root water extracts (ARW) *A. hookeri* was collected from Imsil, Jeollabukdo, Korea in August of 2014. *A. hookeri* roots were immediately freeze-dried (FDA5508; Ilsinbiobase, Dongducheon, Korea) after removal of leaves. Dried roots were homogenized to a fine powder for 5 min using a mixer (HR-2860; Philips Family Electronic Equipment Co., Zhuhai, China) and powdered materials (20 g) were extracted with 1 L of water by maceration at room temperature (25°C) for 24 h. Extracts were filtered using Whatman filter paper No. 42 (150 mm) and stored at −20°C (DF8514; Ilsinbiobase).

Cell culture 3T3-L1 cells (Korean Cell Line Bank, Seoul, Korea) were cultured in DMEM medium containing 10%(v/v) BCS and 100 U/mL penicillin-streptomycin, followed by incubation under 8% CO_2 at 37°C (LCO-265AI; Lab Tech, Namyangju, Korea). Experiments were performed using cells from passages 3 through 10.

Cytotoxicity assay PrestoBlue cell viability reagent was used for measurement of cell viability. 3T3-L1 cells were cultured at a density of 1×10^4cells/well in a 96-well plates, followed by stabilization overnight under 8% CO_2 at 37°C (LCO-265AI; Lab Tech). Cells were then treated with ARW and incubated for 24, 48, and 72 h at 37°C (LCO-265AI; Lab Tech). After incubation, 10 μL of PrestoBlue cell viability reagent was added to each well, followed by incubation for 20 min at 37°C (LCO-265AI; Lab Tech). Cell viability was determined based on measurement of differences in absorbance at a wavelength of 570 nm (MRXe; Dynex Technologies, Chantilly, VA, USA).

Induction of 3T3-L1 cell differentiation Preadipocyte 3T3-L1 cells were grown in DMEM medium containing 10% (v/v) BCS until confluent. Two days after postconfluence (d 0), confluent cells were placed in a differentiation medium of MDI:DMEM medium containing 10% (v/v) FBS, 5 μg/mL insulin, 0.5 mM IBMX, and 1 μM DEX. The medium was subsequently replaced again with fresh MDI medium after 2 days and then every other day therafter. 8 days after induction in differentiation, accumulation of fat droplets had occurred in more than 90% of cells. From days −2 (postconfluence cells) to days 8, cells were exposed to ARW.

Oil Red-O staining Cells were washed with cold phosphate buffered saline (PBS) and fixed with 4%(v/v) formalin for 30 min on day 8. After Oil Red O staining, cells were photographed using a phase-contrast microscope (DMIR; Leica Microsystems, Wetzlar, Germany) at 100× magnification. Lipid droplets were dissolved in 125 μL DMSO and absorbance was measured at 540 nm (MRXe; Dynex Technologies).

Measurement of glycerol Glycerol levels were quantified using a free glycerol assay kit (colorimetric) according to manufacturer instructions on day 8. For measurement of glycerol levels in a culture medium, all cultured media were centrifuged at 1,200×*g* for 10 min at 4°C (VS-5000N; Vision Scientific, Daejeon, Korea), followed by storage at −20°C (F8514; Ilsinbiobase). Experiments were performed in triplicate.

ELISA LPL and adipokine (leptin, resistin, and adiponectin) levels were quantified using commercial ELISA kits following manufacturer instructions on day 8. For measurement of LPL and adipokine levels in a culture medium, all culture media were centrifuged at 1,200×*g* for 10 min at 4°C (VS-5000N; Vision Scientific), followed by storage at −20°C (F8514; Ilsinbiobase). Experiments were performed in triplicate.

Western blot analysis Cells were washed with cold PBS, then harvested using 0.25% (w/v) trypsin/EDTA on day 8. For preparation of whole-cell extracts, cells were lysed in RIPA cell lysis buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% (w/v) NP-40, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS) containing a 1% protease inhibitors (complete protease inhibitor cocktail) and 1 mM PMSF for 30 min on ice. Protein concentrations of cell lysates were determined using a protein assay reagent kit. Equal amounts of protein were subjected

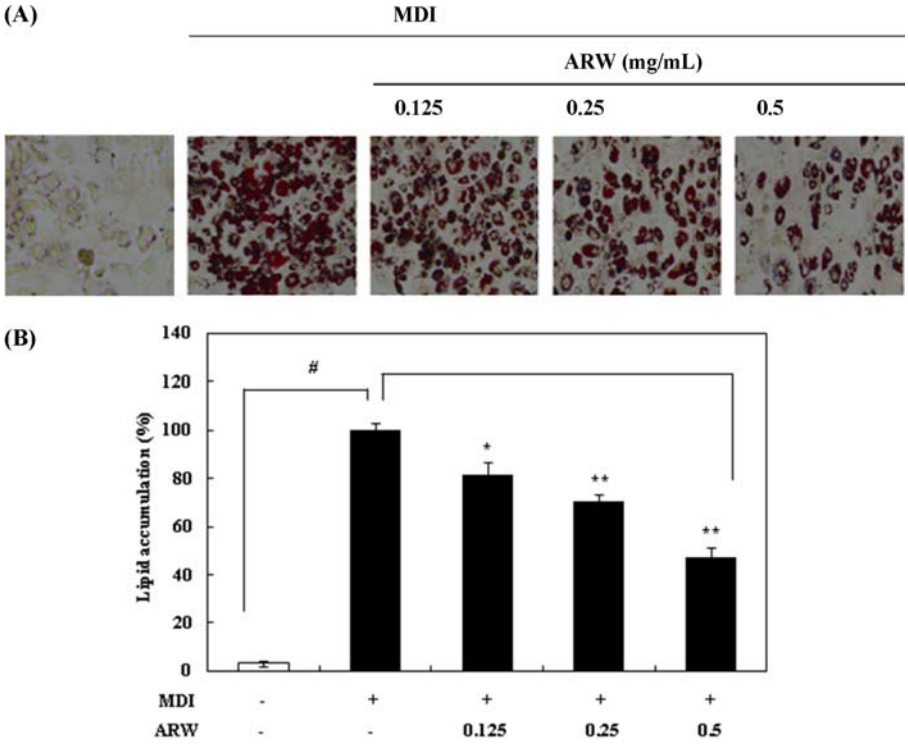

Fig. 1. Effects of ARW on lipid accumulation in 3T3-L1 cells. (A) 3T3-L1 preadipocytes were treated with different concentrations of ARW during differentiation. At day 8 of differentiation, 3T3-L1 cells were stained with Oil Red O and morphology changes in cells were observed under a microscope (100× magnification). (B) Subsequently, 125 μL of DMSO was used for elution of Oil Red O, and lipid accumulation was determined based on measurement of differences in absorbance. Data shown are means±SD values of duplicate determinations from 3 separate experiments. *p<0.05 and **p<0.01, vs. MDI alone-treated groups, $^{\#}p$<0.01, vs. none and MDI alone-treated groups. MDI: DMEM containing 10% FBS, 5 μg/mL of insulin, 0.5 mM IBMX and 1 μM DEX

to 12% (w/v) SDS-polyacrylamide gel electrophoresis (Mini Protein Tetra Cell; Bio-Rad), transferred onto a nitrocellulose membrane (Mini Trans-Blot Transfer Cell; Bio-Rad), which was washed once with a washing buffer (PBS, 0.05% (v/v) Tween 20), then blocked with a blocking buffer (PBS, 5% (w/v) skim milk, 0.05% (v/v) Tween 20) for 1 h. After blocking, membranes were incubated with primary antibodies overnight at 4. Mouse polyclonal antibodies were diluted to 1:1,000 (PPAR-γ; sc-7273, GLUT-4; sc-1608) (Santa Cruz), mouse polyclonal anti-β-actin was diluted to 1:2,000 (β-actin; sc-47778) (Santa Cruz) in blocking buffer. After incubation, membranes were washed twice with a washing buffer and subsequently incubated for 1 h at room temperature with goat anti-rabbit IgG HPR-conjugated secondary antibody (sc-2004; Santa Cruz) diluted to 1:5,000 using a blocking buffer. Membranes were washed twice with a washing buffer, and protein bands were detected using an ECL detection kit following manufacturer instructions.

Glucose uptake assay On day 8, fully differentiated cells were treated with ARW for 24 h. Glucose transport in 3T3-Ll adipocytes was assessed based on uptake of 2-deoxy-l-[3H]glucose, and quantified using an glucose uptake assay kit (colorimetric) following manufacturer instructions. Experiments were performed in triplicate.

Statistical methods Data were expressed as mean±standard deviation (SD), and statistical analysis for single comparisons was performed using Student's *t*-test. Each experiment was repeated at least in triplicate to yield comparable results. Values of p<0.05 and p<0.01 were considered significant.

Results and Discussion

Effects of ARW on 3T3-L1 cell viability ARW treatments at different concentrations of 0-1.0 mg/mL with 24 h incubation caused no cytotoxicity in cells. Control cells did experience cytotoxicity. Cell viability was also inhibited by 48 h incubation at a concentration of 0.5 mg/mL. Also, cell viability was decreased by 72 h incubation to 91±0.7% at 0.5 mg/mL and to 78±1.1% at 1.0 mg/mL (data not shown). ARW had no effect on cell viability, regardless of incubation time, at low concentrations. However, ARW inhibited adipocyte viability in proportion to incubation time and concentration at a high concentration of 0.5 mg/mL. Cells treated with ARW at different concentrations of 0-1.0 mg/mL and incubated for up to 72 h were observed microscopically. In the control group with no treatment, a 100% confluent cell density of 3T3-L1 pre-adipocytes led to the

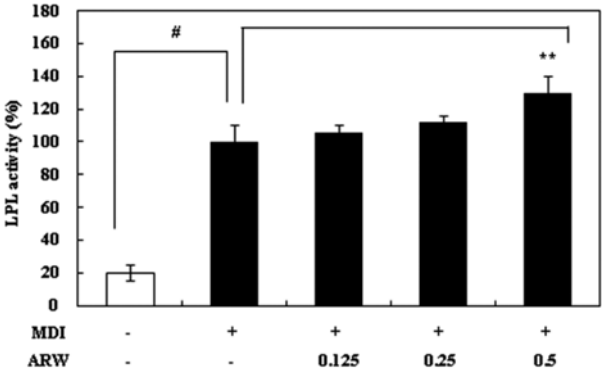

Fig. 2. Effect of ARW on the lipoprotein lipase (LPL) activity in 3T3-L1 cells. 3T3-L1 preadipocytes were treated with different concentrations of ARW during differentiation. At day 8 of differentiation, 3T3-L1 cells were harvested and LPL activation was tested using a lipoprotein lipase (LPL) activity assay kit. Data shown are means±SD values of duplicate determinations from 3 separate experiments. $^{**}p<0.01$, vs. MDI alone-treated groups, $^{\#}p<0.01$, vs. none and MDI alone-treated groups. MDI: DMEM containing 10% FBS, 5 μg/mL of insulin, 0.5 mM IBMX and 1 μM DEX

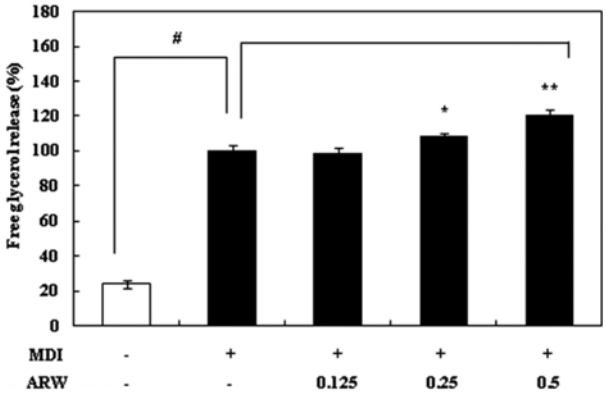

Fig. 3. Effect of ARW on free glycerol release in 3T3-L1 cells. 3T3-L1 preadipocytes were treated with different concentrations of ARW during differentiation. At day 8 of differentiation, cell supernatants were collected and free glycerol release was tested using a free glycerol assay kit. Data shown are means±SD values of duplicate determinations from 3 separate experiments. $^{*}p<0.05$, and $^{**}p<0.01$, vs. MDI alone-treated groups, $^{\#}p<0.01$, vs. none and MDI alone-treated groups. MDI: DMEM containing 10% FBS, 5 μg/mL of insulin, 0.5 mM IBMX and 1 μM DEX

stretched fibroblast form. In the experimental group treated with ARW, the fibroblast form was also observed at a low concentration of 0.25 mg/mL after 72 h. The same cell form was used in the experimental group with 0.5 mg/mL ARW, showing a lower cell density. Cells in the experimental group with 1.0 mg/mL ARW failed to maintain a stretched form and were suspended in culture medium. A concentration of 0.5 mg/mL was used, for which 80% of 3T3-L1 cells survived 72 h of incubation.

Effects of ARW on lipid accumulation in adipocytes An ORO staining reagent can bind to neutral lipids and cholesterol. Most lipid accumulation occurs due to lipid differentiation and the level of neutral lipid accumulation in adipocytes can be determined by the degree of redness of cells stained with a neutral fat (19). In this study, ORO staining was performed for assessment of ARW effects on lipid accumulation in 3T3-L1 adipocytes and the adipocyte form was observed (Fig. 1A), and amounts of intracellular lipids were quantified (Fig. 1B). Fat globule formation in adipocytes was inhibited in experimental group cells treated with ARW. In particular, experimental group cells with 0.5 mg/mL ARW showed inhibition of lipid accumulation based on a smaller number and size of fat globules compared with control group cells. When stained neutral fat in adipocytes was dissolved in DMSO and quantified, experimental group cells treated with ARW showed a dose-dependent reduction in the amount of fat, compared with differentiation group cells. Experimental group cells with 0.25 mg/mL ARW showed a 29% reduction and cells treated with 0.5 mg/mL ARW showed a 52% reduction in fat amounts. ORO staining revealed that ARW probably induced dose-independent 3T3-L1 adipocyte differentiation and reduced lipid accumulation.

Effects of ARW on adipocyte lipolysis LPL is a major enzyme secreted into cell membranes and extracellular and vascular endothelial cells for decomposition of neutral fat into fatty acids and glycerol (20). In this study, LPL activity was measured for determination of whether the effectiveness of ARW in inhibition of lipid accumulation was also associated with lipolysis (Fig. 2). Experimental group cells treated with ARW showed increased LPL activity. The experimental group treated with 0.25 mg/mL ARW showed a 12% increase and the group treated with 0.5 mg/mL ARW showed a 29% increase. The concentration of glycerol, a lypolytic product in the culture medium, increased by 8% in the experimental group with 0.25 mg/mL ARW and by 20% with 0.5 mg/mL ARW (Fig. 3). Thus, ARW not only inhibited lipid accumulation but also was involved in differentiation of generated lipids. Once increased LPL activity caused lipolysis, generated fatty acids could enter cells and be exhausted for energy metabolism in muscle cells, then remaining fatty acids be stored again as neutral lipids in adipocytes (21). However, higher concentrations of glycerol in culture media and poorer staining of ORO in experimental group cells treated with ARW confirmed an increase in neutral lipid differentiation due to increased LPL activity, and confirmed poor re-migration of lipolytic products, including fatty acids, into adipocytes, probably due to involvement of ARW in fatty acid resorption preventing accumulation of neutral lipids in adipocytes. Park *et al.* (22) reported that plants in the *Allium* genus, including leek, shallots, wild rocamboles, garlic, and onions, can induce adipogenesis and also adipocyte differentiation based on higher concentrations of glycerol. Thus, the effectiveness of ARW for inhibition of lipid accumulation and for activation of lipid differentiation was probably due to components similar to components of other plants in the *Allium* genus.

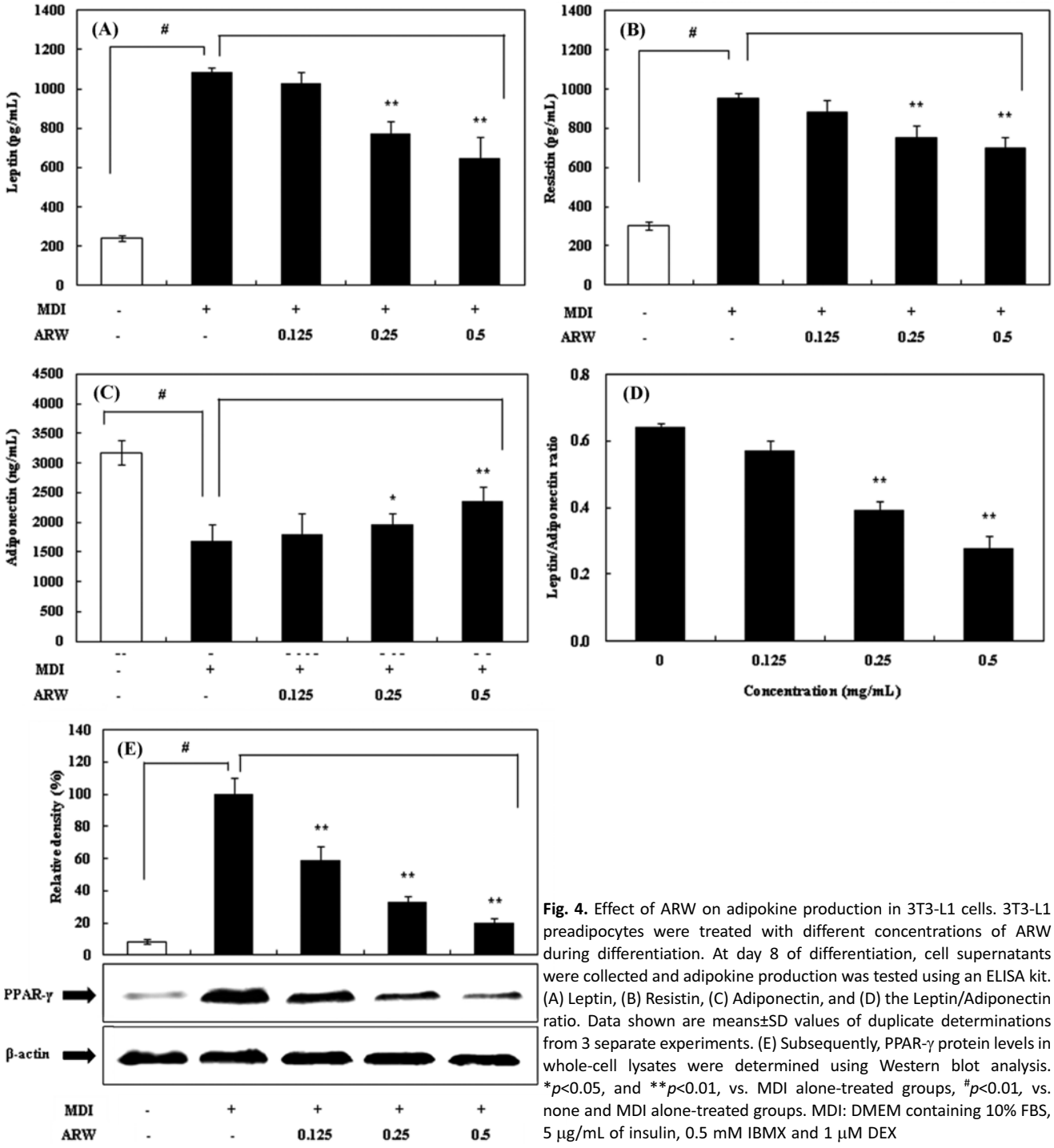

Fig. 4. Effect of ARW on adipokine production in 3T3-L1 cells. 3T3-L1 preadipocytes were treated with different concentrations of ARW during differentiation. At day 8 of differentiation, cell supernatants were collected and adipokine production was tested using an ELISA kit. (A) Leptin, (B) Resistin, (C) Adiponectin, and (D) the Leptin/Adiponectin ratio. Data shown are means±SD values of duplicate determinations from 3 separate experiments. (E) Subsequently, PPAR-γ protein levels in whole-cell lysates were determined using Western blot analysis. *p<0.05, and **p<0.01, vs. MDI alone-treated groups, $^{\#}p$<0.01, vs. none and MDI alone-treated groups. MDI: DMEM containing 10% FBS, 5 μg/mL of insulin, 0.5 mM IBMX and 1 μM DEX

Effects of ARW on concentrations of adipokines in adipocytes

Effects of ARW treatments on concentrations of adipokines in 3T3-L1 adipocytes are shown in Fig. 4. Leptin controls fat through food intake and energy variation, and then the activity of leptin is strongly correlated with body and fat weights. Over-accumulation of lipid, which may increase leptin production, is used as an obesity indicator. Lipid accumulation in adipocytes may promote production of leptin, which is then released into the blood to control energy consumption under normal conditions, and obesity is characterized by high blood leptin levels and leptin resistance. Leptin resistance is related either to obesity causing a reduction in reactivity of the central nervous system to offset the effects of leptin or to a reduction in transport capacity (23). The concentration of leptin in a culture medium was significantly (p<0.01) lower in the experimental group treated with ARW than in the control group. In particular, the leptin concentration decreased by 40% in experimental group cells treated with with 0.5 mg/mL ARW (Fig. 4A). High blood leptin levels failed to inhibit lipid accumulation in control cells with induced lipid differentiation, in

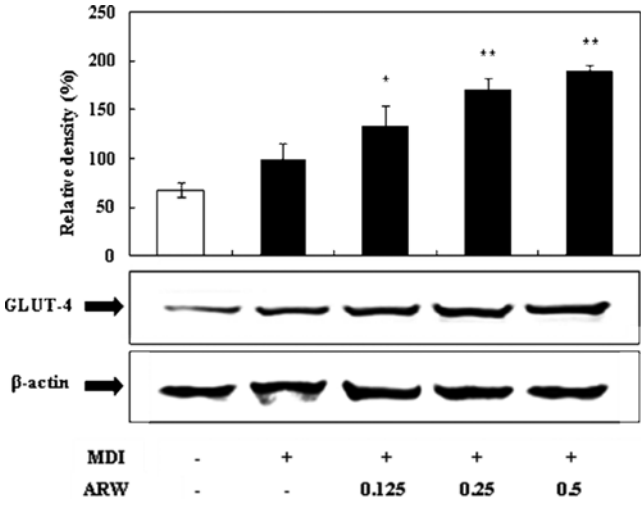

Fig. 5. Effect of ARW on GLUT-4 expression in 3T3-L1 cells. 3T3-L1 preadipocytes were treated with different concentrations of ARW during differentiation. At day 8 of differentiation, GLUT-4 protein levels in whole-cell lysates were determined using Western blot analysis. β-Actin was used as a loading control. *p<0.05, and **p<0.01, vs. MDI alone-treated groups. MDI: DMEM containing 10% FBS, 5 μg/mL of insulin, 0.5 mM IBMX and 1 μM DEX

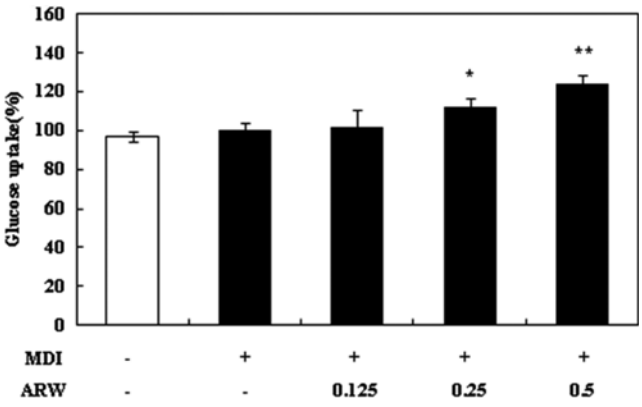

Fig. 6. Effect of ARW on glucose uptake in 3T3-L1 cells. Mature 3T3-L1 adipocytes were incubated with different concentrations of ARW for 24 h. 3T3-L1 cells were harvested and LPL activation was tested using a glucose uptake assay kit. Data shown are means±SD values of duplicate determinations from 3 separate experiments. *p<0.05, and **p<0.01, vs. MDI alone-treated groups. MDI: DMEM containing 10% FBS, 5 μg/mL of insulin, 0.5 mM IBMX and 1 μM DEX

agreement with a report that obesity itself may induce leptin resistance probably because ARW reduces lipid accumulation, thus, causing a smaller amount of leptin to be released from adipocytes and leading to reduced leptin resistance.

Release of resistin, which is known as a mediator between increase of adipose tissues and insulin resistance, may be increased for inhibition of lipid differentiation, like leptin, under conditions of obesity (24). The concentration of resistin decreased by 20% in experimental group cells with 0.25 mg/mL ARW and by 26% with 0.5 mg/mL ARW (Fig. 4B). Even increased resistin levels failed to inhibit lipid differentiation in control group cells for a reason similar to leptin resistance.

Adiponectin is expressed in adipose tissues, like leptin, but level of adiponectin tend to decrease under conditions of obesity, unlike leptin. Reduced adiponectin levels reportedly increase the risk of cardiovascular disease (25). ARW treatment resulted in a dose-dependent increase in the concentration of adiponectin (Fig. 4C), probably because ARW inhibited lipid accumulation and affected adiponectin secretion. ARW is also expected to be effective in prevention of cardiovascular disease.

The leptin-adiponectin ratio, a supplementary indicator of insulin resistance, was significantly (p<0.01) lower in experimental group cells treated with ARW than in control group cells (Fig. 4D). Based on associations of both resistin and adiponectin with diabetes on the basis of antagonism against insulin, ARW should also contribute to diabetes treatment (26). PPAR-γ, a representative modulator of adipocyte differentiation, is involved in glucose metabolism via promotion of adipocyte differentiation and lipogenesis and by increasing insulin sensitivity (27). Experimental group cells treated with ARW showed a decrease in the concentration of PPAR-γ (Fig. 4E). Thus, ARW appears to inhibit release of PPAR-γ, an important factor of adipocyte differentiation, to lower concentrations of leptin and resistin, to raise the concentration of adiponectin, and to inhibit lipid accumulation.

Among many studies of lipid accumulation inhibition activity of plants in the *Allium* genus, Choi and Kim (28) found that methanol extracts of *Allium tuberosum* inhibited ROS production in adipocytes, prevented expression of PPAR-γ for modulation of adipocyte differentiation, and inhibited accumulation of neutral lipids. Park *et al.* (29) reported that black garlic extracts could inhibit PPARγ, C/EBPα, and C/EBPβ expressions, reduce neutral fat generation, and inhibit differentiation into adipocytes. Ku *et al.* (30) reported that methanol extracts of *Allium victorialis* leaves lowered the concentration of leptin, raised the concentration of adiponectin, and mitigated obesity in a rat model of obesity induced using a high-fat diet. Of the major active components of plants in the *Allium* genus, sulfur compounds and saponin were effective for inhibition of lipid accumulation (31,32). Because *Allium hookeri* has a significantly higher content of sulfur compounds than onions or garlic and contains abundant saponin, its effectiveness for inhibition of lipid accumulation appears to be associated with sulfur compounds and saponin. However further research regarding effective components and mechanisms of ARW lipid accumulation inhibition activity in adipocytes will be necessary.

Effectiveness of ARW in improving diabetic conditions ARW controls expression of adipokines related to insulin resistance and sensitivity and, therefore, should be effective for improving diabetic conditions. Insulin-induced glucose uptake is caused by migration of GLUT-4 from the cytoplasm of muscle cells and adipocytes to cell membranes (33). In this study, effects of ARW on dependent glucose

transport of GLUT-4 in 3T3-L1 adipocytes were assessed. Experimental group cells treated with ARW showed a dose-dependent increase in GLUT-4 expression (Fig. 5). Also, glucose uptake was increased in ARW treated groups, by 12% in experimental group cells with 0.25 mg/mL ARW and by 24% with 0.5 mg/mL ARW (Fig. 6). 3T3-L1 adipocytes treated with ARW showed increased insulin sensitivity and GLUT-4 expression, with increased glucose uptake into adipocytes in proportion to GLUT-4 expression. Intracellular inflow of glucose due to GLUT-4 expression increased by ARW should increase glucose availability, lower the blood sugar level, and contribute to improvement of diabetic conditions. Kadan *et al.* (34) found that onions and garlic had antidiabetic effects due to increasing GLUT-4 expression in L6 muscle cells. Liu *et al.* (35) reported that garlic oil increased GLUT-4 expression and improved diabetic conditions in an animal model of diabetes. In this study, ARW showed effects similar to the increase in GLUT-4 expression by plants in the *Allium* genus. Therefore, the antidiabetic activity of ARW should be similar to the antidiabetic mechanism of plants in the *Allium* genus. However, further research regarding antidiabetic activities and mechanisms of ARW will be necessary.

In conclusion, ARW was effective for improving diabetes based on induction of GLUT-4 expression, leading to an increase of glucose uptake into adipocytes. However, ARW is probably involved in the process of adipogenesis with glucose entering adipocytes for inhibition of lipid accumulation in adipocytes.

Disclosure The authors declare no conflict of interest.

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