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Anti-obesity effects of *Laminaria japonica* fermentation on 3T3-L1 adipocytes are mediated by the inhibition of C/EBP-α/β and PPAR-γ

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Abstract: Obesity is global problem that contributes to disease, and is partly caused by fast-food, high-fat diets. Much attention has been focused on developing anti-obesity foods and chemical materials from natural sources. Seaweed has bioactive properties that influence immune activity and have anti-cancer and anti-obesity effects. *Laminaria japonica* is a widely consumed seaweed, and has been promoted as a health food in Korea. The bioactive properties of *L. japonica* include anti-cancer, anti-diabetic, and anti-inflammation effects. Most *Laminaria japonica* are distributed in a simple processing form such as drying, and their availability is very low. Therefore, various types of functional products can be developed if they can be applied to foods through functionalization using fermentation techniques. It is a structural problem that is the most problematic in seaweed processing. In this study, we used fermented *Laminaria japonica.* To increase physiological activity, fermentation treatment was performed to loosen the structure, thereby increasing the activity of the glycoprotein. First, we screened the anti-obesity potential of an *L. japonica* fermentation extract (LJF) using 3T3-L1 adipocyte cells. We determined cytotoxicity using an MTS assay and measured LJF for its ability to affect adipogenesis through glucose uptake, triglyceride levels, and Oil Red O staining. We confirmed that LJF inhibited adipocyte differentiation. CCAAT/enhancer-binding proteins α/β (C/EBP-α/β) and peroxisome proliferator-activated receptor-γ (PPAR-γ) are involved in the early and late stages of adipocyte differentiation. LJF significantly reduced the expression levels of C/EBP-α/β and PPAR-γ and decreased the concentration of adiponectin. Thus, our results suggest that LJF inhibits adipogenesis in 3T3-L1 cells, and may be valuable for its anti-obesity effects.

Key words: Obesity; C/EBP-α/β; PPAR-γ; 3T3-L1 adipocytes; *Laminaria japonica.*

Introduction

According to the World Health Organization (WHO), the terms obesity and overweight refer to abnormal excess fat accumulation that indicates a health risk. According to the Body Mass Index (BMI) standard proposed by the WHO, overweight is defined as a BMI of 23–24.9, and obesity is defined as over 25 (1). The obese population is increasing due to modernization and Westernized eating habits, the convenience of the living environment, decreased physical activity due to developments in transportation, and excessive caloric intake (2,3). Obesity contributes to a significantly increased incidence of diseases in adults, such as hypertension, diabetes, and hyperlipidemia (4,5). Obesity is itself a serious disease (6). The cause of the disease is unclear, but it is known that body fat accumulation is caused by unbalanced energy intake and consumption (7,8). Adipose tissue, composed of adipocytes and a small number of other cells, regulates lipid metabolism and functions to secrete and store lipids and in glucose metabolism, which regulates insulin-dependent glucose uptake, and in the endocrine system, which secretes hormones and cytokines (9). Thus, dysfunction of adipose tissue, such as excessive differentiation of adipocytes, increases the risk of diabetes and obesity.

In 3T3-L1 cells, the process of differentiation of preadipocytes into adipocytes is divided into early differentiation, involving C/EBP-β and δ, and late differentiation, involving C/EBP-α and PPAR-γ. When early differentiation is initiated by stimulation by mitogens and hormones, expression of C/EBP-β/δ is upregulated by various factors (10,11). C/EBP-β and δ cooperatively or independently regulate the expression of C/EBP-α and PPAR-γ (12-14). PPAR-γ and C/EBP-α are highly expressed in the late stage of differentiation as key transcription factors controlling adipogenesis, and they induce the expression of terminal markers of adipogenesis, including adiponectin and glucose transporter-4 (15-17). *Laminaria japonica*, a widely consumed seaweed, has traditionally been consumed in Asia and various bioactive effects have been reported. It is a brown alga and is an excellent source of minerals involved in physiological activities, such as iodine, potassium, sodium, calcium, and magnesium. Its alginic acid (70–80%), fucoidan, and laminarin contents are high (18,19). *Laminaria japonica* has been studied for various bioactive effects, such as anti-coagulant, anticancer, serum cholesterol lowering and excretion of harmful heavy metals in the body, immunity, and antioxidant activity (20-22). *Laminaria japonica* is widely used as a natural health material. In this study, we performed fermentation to increase the activity of *L. japonica* polysaccharides and proteins. Recently, it has been reported that fermentation can be used as prebiotics of seaweed extracts, and it has been reported that the fermentation process is effective for the production of new physiologically active substances and the increase

of useful components (23). The nutritional component of seaweeds is an indigestible polysaccharide whose part is difficult to digest in the body and has a relatively stable characteristic of acid or alkali, which makes it difficult to efficiently extract, and there is a high possibility that it may cause deterioration and loss of active substance. To overcome these drawbacks, studies have been actively conducted to extract useful components of seaweeds through fermentation (24). We investigated the effects of an *L. japonica* fermentation extract (LJF) in an artificially induced adipogenesis model using insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX) to determine its anti-obesity effects and its molecular mechanism of action in 3T3-L1 adipocyte cells. In particular, we observed the expression of adipogenic transcription factors, such as C/EBP-α/β/δ and PPAR-γ, which are involved in adipocyte differentiation.

Materials and Methods

Fermentation extract of L. japonica (LJF)

Laminaria japonica was collected from Wando, Korea in March, 2017. Healthy individuals were rinsed with water several times to remove mucus. The washed materials were incubated in fresh water overnight at room temperature for desalinization. 100g of cleaned *L. japonica* were fermented in fermentation devices (FER-50L; C&S Co., LTD., Daejeon, Korea) at 45°C for 4 h with 250 mL of water with 3% dry yeast and 30% commercial sugar relative to the weight of *L. japonica*. Fermented samples were cleaned with fresh water and freeze-dried using a freeze dryer (LFD-24L-DW; Lee Won Freezing, Busan, Korea).

Glycoprotein staining of *Laminaria japonica*

Fermented *Laminaria japonica* and raw state *Laminaria japonica* were subjected to 15% SDS-PAGE at 50 μg / mL for 2 h at 30 mA and glycoprotein staining confirmed the effect of fermentation on the increase of glycoprotein. That is, the fermented *Laminaria japonica* and raw state *Laminaria japonica* were subjected to SDS-PAGE and the glycoprotein staining was confirmed by the Gelcode® Glycoprotein Staining Kit (Pierce, USA). Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Composition of *Laminaria japonica*

In order to investigate the content of reducing sugar, ash, and water contained in *Laminaria japonica*, the biomass analytical procedure was modified to analyze the components. The content of reducing sugar was measured using sulfuric acid. The reducing sugar in the *Laminaria japonica* was measured at 580 nm using a spectrophotometer and standard sample was glucose. The ash content was calculated from the weight of ash remaining after 5 hours at 550 °C in a hot electric furnace. The water content was calculated from the weight of the dried sample after drying the *Laminaria japonica* in the dryer for one day and lipid content was measured by Soxhlet extraction with ether. Protein quantification was detected with absorbance at 562 nm using BCA assay kit (Pierce biothechnology, Rockford, IL, USA).

Cell culture, adipocyte differentiation, and treatment with LJF

3T3-L1 mouse fibroblast cells (American Type Culture Collection, Manassas, VA, USA) were maintained at 37°C in a 5% CO_2 humidified atmosphere. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Waltham, MA, USA) with 10% bovine calf serum (BCS; Gibco, Gaithersburg, MD, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin. When the 3T3-L1 preadipocyte cells reached 80% confluence, they were harvested, and the seed cells were allowed to grow for 4 d in a 6-well plate. When cells reached 100% confluence, they were maintained for another 48 h in this state to arrest cell division. Differentiation was initiated by DMEM medium containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) treated with MDI (0.5 mM IBMX, 1 μM dexamethasone, 10 μg/mL insulin) for 72 h. The medium was then replaced with DMEM supplemented with 10 μg/mL insulin and LJF, and changed once every 2 d.

Cell proliferation assays

3T3-L1 mouse fibroblast cell proliferation was measured using a CellTiter 96 aqueous non-radioactive cell proliferation assay (Promega, Madison, WI, USA), which is based on the cleavage of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium (MTS) into a formazan product which is soluble in the tissue culture medium. Cells were seeded onto 96-well plates at 1.5×10^4 cells/well and the medium was replaced with serum-free medium (SFM) after culture for 24 h. The medium was then replaced with SFM containing LJF (100 and 200 μg/mL) and incubated for 24 h. For the assay, MTS solution was added to each well and allowed to react for 30 min at 37°C. The absorbance at 490 nm was measured using a microplate ELISA reader (US/MQX 200; BIO-TEK instruments INC., Winwooski, VT, USA).

Glucose uptake assay

3T3-L1 preadipocytes were incubated with DMEM containing 10% BCS. Cell differentiation was induced by treatment with MDI in fresh DMEM containing 10% FBS. After differentiation, the medium was replaced with DMEM supplemented with 10 μg/mL insulin, LJF and LJ (raw state *Laminaria japonica*) (100 and 200 μg/mL) and changed once every 2 d. After collecting the cell culture medium, we confirmed glucose uptake using a kit according to the manufacturer's protocol (Asan Pharm. Co. Ltd., Gyeonggi, Korea). Enzyme solution was added to the culture medium and maintained at 37°C for 15 min in a 5% CO_2, humidified atmosphere. The absorbance at 500 nm was measured within 40 min.

Triglyceride (TG) component assay

3T3-L1 preadipocytes were incubated with DMEM containing 10% BCS. Cell differentiation was induced by MDI treatment in fresh DMEM containing 10% FBS. After differentiation, the medium was replaced with DMEM supplemented with 10 μg/mL insulin and LJF (100 and 200 μg/mL) and changed once every 2 d. The cell lysate was collected for TG assay. We performed the TG assay according to the kit protocol (Cleantech TG-S kit, Asan Pharm. Co. Ltd.). The enzyme solution

was added to the cell lysate and maintained at 37°C for 15 min in a 5% CO_2 humidified atmosphere. The absorbance at 550 nm was measured within 60 min.

Oil red O staining

3T3-L1 cells were washed carefully with phosphate-buffered saline (PBS) and fixed with 10% formalin for 5 min. The formalin was then refreshed and the cells were incubated for 1 h. After removal of formalin, 60% isopropanol was added to each well and dried. Oil Red O staining solution (60%) was then added to each well for 1 h. The wells were then washed three times with PBS and cell morphology and staining of lipid droplets were observed using a microscope.

Nuclear extraction

After differentiation and sample processing, 1 mL of fresh PBS per 20 cm^2 area was added and cells were scraped into a conical tube. Nuclear extraction was performed according to the abcam kit protocol (ab113474; abcam, Cambridge, MA, USA). Cells were centrifuged for 5 min at 1,000 rpm and the supernatant was discarded. One hundred microliters of pre-extraction buffer per 10^6 cells were added to the cell pellet and incubated on ice for 10 min. Samples were vortexed vigorously for 10 s and centrifuged for 1 min at 12,000 rpm. The cytoplasmic extract was removed from the nuclear pellet.

C/EBP α/β transcription factor assay

Using the pellet obtained in the nuclear extraction step, C/EBP α/β levels were measured following the abcam C/EBP α/β transcription factor assay kit (ab207199) protocol. Complete binding buffer was added and incubated for 1 h at RT with mild agitation. After 1 h, samples were washed three times with 1× wash buffer and primary antibody was added (1 h, RT). Samples were washed again three times with 1× wash buffer and secondary antibody was added. Finally, developing solution and stop solution were added. Absorbance was measured on a spectrophotometer at 450 nm within 5 min, with a reference wavelength of 665 nm.

PPAR γ transcription factor assay

Using the pellet obtained in the nuclear extraction step, PPAR γ levels were measured according to the abcam PPAR-γ transcription factor assay kit (ab133101) protocol. Active PPAR-γ was bound to the consensus sequence and PPAR-γ primary antibody was added. Goat anti-rabbit HRP was added and then developing solution was added. Absorbance was measured at 450 nm within 5 min of adding stop solution.

Reverse transcription polymerase chain reaction (RT-PCR)

3T3-L1 preadipocyte cells were seeded into 6-well plates at 2 × 10^4/well in 4 mL of BCS-DMEM. After cell cycle arrest, cell differentiation was induced by treatment with MDI in fresh DMEM containing 10% FBS. After differentiation, the medium was changed to insulin supplemented with LJF (100 and 200 μg/mL) for 48 h. RNA was purified from 3T3-L1 adipocytes using TRIzol reagent (Invitrogen Co., Carlsbad, CA, USA) and used as a template for cDNA synthesis using an oligo(dT) primer (Intron Co., Seongnam, Gyeonggi, Korea). The synthesized cDNA was mixed with 2× TOPsimple DyeMIX-nTaq (Enzynomics Inc., Daejeon, Korea) and primers in 0.1% diethylpyrocarbonate (DEPC)-treated water for polymerase chain reaction (PCR). Using a 1.5% agarose gel, the PCR products were separated and stained with RedSafe nucleic acid staining solution.

Statistical analysis

Results are expressed as means ± SD. SPSS software (ver. 10.0; SPSS, Inc., Chicago, IL, USA) was used. Comparisons were made using ANOVA and Duncan's multiple range test. The level of significance was set at $p < 0.05$.

Results

Increase of glycoprotein content as physiologically active substance

Fermentation of seaweed is a biochemical reaction that metabolizes organic substances in polymers and transforms them into relatively simple substances. It promotes nutrition of foods, improves protein quality and digestibility of fiber, and improves body absorption (25). In this study, the glycoproteins of fermented *Laminaria japonica* and raw state *Laminaria japonica* were identified at molecular weights below 10 kDa and many glycoproteins were detected more in the fermented *Laminaria japonica* group than raw state. This means that the fermentation treatment can induce an increase in the glycoprotein of *Laminaria japonica*, and it is considered that the increase of the glycoprotein can induce the increase of the physiological activity effect (Fig. 1).

Effect of fermentation treatment on the composition of *Laminaria japonica*

General component analysis was performed to quantify the changes identified in glycoprotein staining. The content of fat and ash was not significant and the increased water content was thought to be due to the large

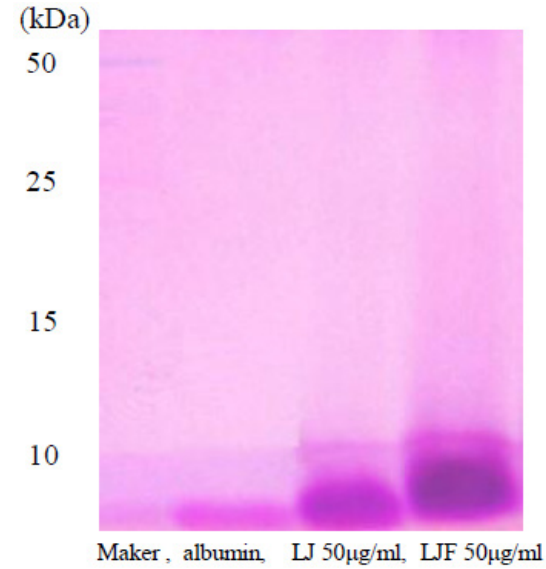

Figure 1. The glycoprotein staining of *Laminaria japonica*. The *Laminaria japonica* of raw state and fermentated *Laminaria japonica* were loaded using a SDS-PAGE. Loading volume is same concentraition (50 μg/ml).

Table 1. Composition of *Laminaria japonica.*

Biomass	Reducing sugar (%)	Protein (%)	Water (%)	Ash (%)	Lipid (%)	others
Laminaria Japonica (raw state)	29.7±0.7	6.3±0.4	4.67±0.2	14.96±0.13	0.58±0.08	43.79
Laminaria Japonica (fermentation)	41.5±0.8	13.3±0.2	11.06±0.03	12.10±0.06	1.55±0.01	20.76

amount of water administered during the fermentation process. As shown in the experiment of the glycoprotein, it was confirmed that the general composition analysis showed a structural change due to the fermentation process and the ratio of protein and reducing sugar in total nutrients was increased (raw state *Laminaria japonica* protein ratio: 6.3±0.4, reducing sugar ratio: 29.7±0.7, fermented *Laminaria japonica* protein ratio: 13.3±0.2, reducing sugar ratio: 41.5±0.8) (Table. 1).

Cell proliferation of LJF-treated 3T3-L1 preadipocytes

We observed cell toxicity of LJF in 3T3-L1 preadipocytes by MTS assay. Cells were seeded into 48-well plates at a density of 2×10^4 cells/well in DMEM and cultured for 1 d. The medium was replaced with fresh SFM containing LJF at concentrations of 100 and 200 μg/mL and the cells were incubated for 24 h. LJF did not result in toxicity in 3T3-L1 preadipocytes. No significant changes in cell proliferation were observed at the LJF concentrations used (Fig. 2).

Inhibitory effect of LJF on glucose uptake in 3T3-L1 adipocytes

Generally, glucose consumption increases during 3T3-L1 cell differentiation. Lipids are produced by consuming glucose when differentiation is induced with MDI treatment. In this study, after inducing differentiation with MDI, cells were treated with LJF and raw state *Laminaria japonica* at concentrations of 100 and 200 μg/mL in the insulin administration step, and glucose uptake was confirmed. As a result of treating the raw state *Laminaria japonica* and the fermented *Laminaria japonica* with the same concentration, the decrease in glucose consumption in the fermentation group was superior, and it was considered that the fermentation treatment could induce the increase of the anti-obesity activity, and the fermentation substance was treated in all

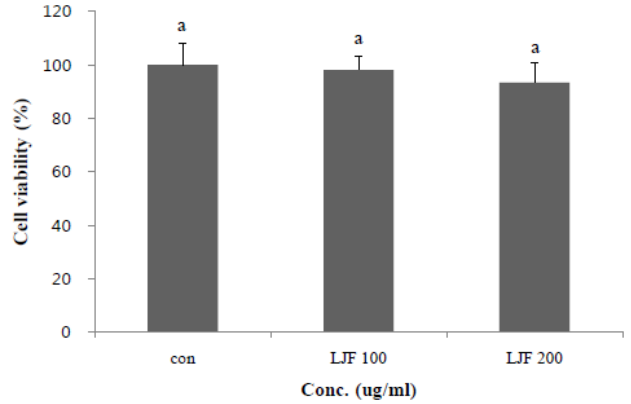

Figure 2. LJF affects the cell viability of 3T3-L1 preadipocyte cells. Cells were treated with LJF at various concentrations (100 and 200 ug/ml) for 24 h and viability was determined by MTS assay. At least 3 replicates were performed and Values represent means ± SD; $p < 0.05$ by ANOVA. Values indicated with different letters are significantly different according to Duncan's multiple range test.

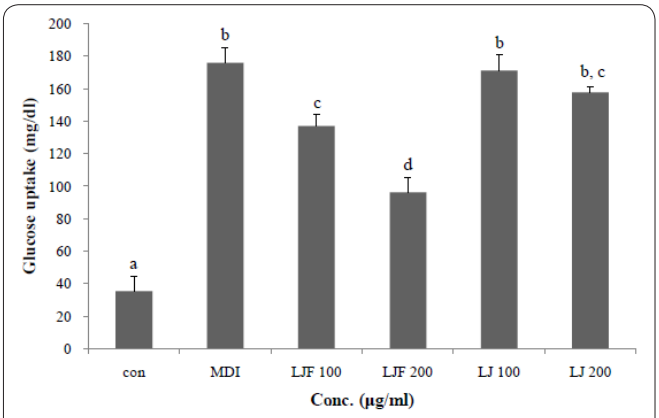

Figure 3. LJF and LJ inhibits glucose uptake of differentiated 3T3-L1 adipocyte cells. After induced differentiation by MDI, cells were treated with insulin, LJF and LJ at various concentrations (100 and 200 ug/ml) for 48 h. Cell culture DMEM-high glucose medium was assessed using the glucose uptake assay and an ELISA reader. Values represent means ± SD; $p < 0.05$ by ANOVA. Values indicated with different letters are significantly different according to Duncan's multiple range test. At least 3 replicates were performed and a, b, c, and d are significant.

the experiments. We found dose-dependent decreases in glucose uptake in 3T3-L1 adipocytes. The glucose uptake was 175.9 mg/dl, in the MDI group, and 137.1 and 96.2 mg/dl in the 100 and 200 μg/mL LJF groups, respectively. But, glucose uptake was 170.9 and 157.6 mg/dl in raw state LJ groups. LJF reduces glucose uptake and inhibits differentiation of 3T3-L1 adipocytes (Fig. 3). In the initial screening, we observed various concentrations of LJF as 50, 100, 200, and 400 μg/mL. As a result, the glucose consumption in 50 μg/mL group was 161 mg/dl, which was not significant when compared to the MDI group. Also, Considering that the concentration was doubled in the 200 group, in the 400 group, the activity was not as high as about 85 mg/dl. Therefore, we decided the concentration to be 100 and 200 μg/mL and proceeded to experiment.

Inhibitory effect of LJF on TG levels in 3T3-L1 adipocytes

To evaluate the effects LJF on TG levels in differentiating 3T3-L1 cells, cells were treated with LJF at concentrations of 100 and 200 μg/mL. Glucose consumption involved in the production of many lipids and TG accumulation is proportional to glucose uptake. TG content in 3T3-L1 adipocytes treated with LJF at 100 and 200 μg/mL was significantly decreased in a dose-dependent manner. TG level was 123.9 mg/dl in the MDI group, and 90.9 and 72.3 mg/dl in the 100 and 200 μg/mL LJF groups, respectively (Fig. 4). LJF reduces glucose uptake and inhibits differentiation of 3T3-L1 adipocytes and TG production was inhibited by the decrease in glucose consumption.

Inhibition of lipid accumulation by LJF in 3T3-L1 adipocytes

In the glucose uptake and TG assays, we confirmed

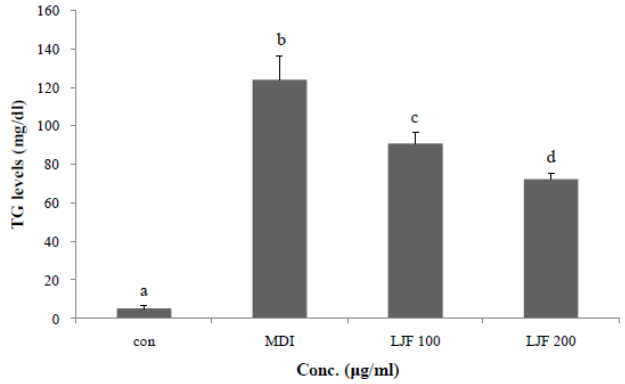

Figure 4. LJF inhibits TG accumulation of differentiated 3T3-L1 adipocyte cells. After induced differentiation by MDI, cells were treated with insulin and LJF at various concentrations (100 and 200 ug/ml) for 48 h. Cell pellet was assessed using the TG level and an ELISA reader. Values represent means ± SD; $p < 0.05$ by ANOVA. Values indicated with different letters are significantly different according to Duncan's multiple range test. At least 3 replicates were performed and a, b, c, and d are significant.

dose-dependent decreases in glucose consumption and TG content by LJF treatment (100 and 200 μg/mL). This was visually confirmed through Oil Red O staining, which is used for histological visualization of neutral fat or lipid cells. The size, density, and number of lipids were highest in the MDI group and decreased in a dose-dependent manner in the LJF groups (Fig. 5).

Inhibitory effect of LJF on the expression of adipogenic genes (C/EBP-α/β and PPAR-γ) during 3T3-L1 differentiation process

In differentiated 3T3-L1 adipocytes, post-translational regulation of C/EBP gene activity may be an important transcription factor in adipogenesis. Overexpression of C/EBP-α and C/EBP-β were essential in differentiating cells (26,27) and C/EBP-α is required in the late phase of differentiation and upregulates the lipid-related genes required for adipogenic synthesis, while C/EBP-β is involved the early phase of adipocyte differentiation (28). PPAR- γ is expressed in adipose tissue and is a regulator of adipogenesis, playing an important role in 3T3-L1 cell differentiation. PPAR- γ and C/EBPs are master regulators of adipogenesis and interact to increase the expression of terminal makers, such as

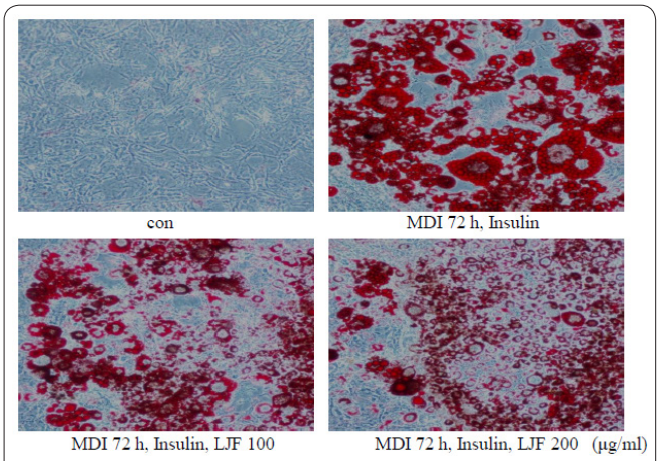

Figure 5. LJF inhibits effect of lipid droplet in differentiated 3T3-L1 adipocyte cells. Cells were treated with insulin and LJF (100 and 200 ug/ml) for 48 h and Lipid droplets were stained by oil red O staining.

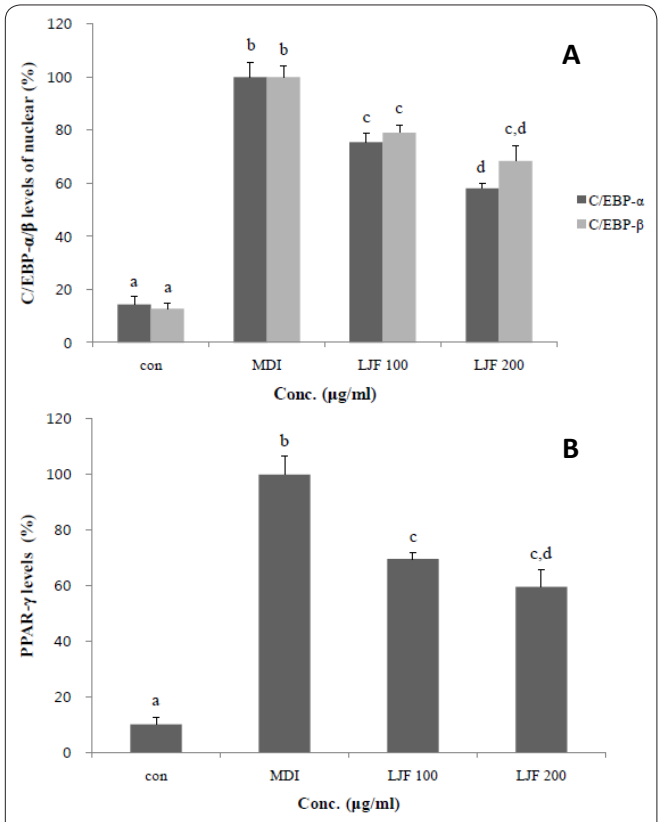

Figure 6. Inhibition of nuclear C/EBP-α/β and PPAR-γ levels in 3T3-L1 adipocyte cells. Cells were treated with insulin and LJF (100 and 200 ug/ml) for 48 h and nuclear transcription factor were extracted. (A) Expression levels of nuclear C/EBP-α/β. (B) Expression levels of nuclear PPAR-γ. Values represent means ± SD; $p < 0.05$ by ANOVA. Values indicated with different letters are significantly different according to Duncan's multiple range test. At least 3 replicates were performed and a, b, c, and d are significant.

glucose transporter-4 (GLUT-4) and fatty acid synthase (FAS) (29,30).

In this study, we observed adipogenesis-related gene expression by using nuclear extracts and reverse transcription polymerase chain reaction (RT)-PCR. The expression levels of PPAR- γ and C/EBP-α/β were markedly increased in 3T3-L1 adipocyte cells treated with the differentiation inducer. The expression levels of these genes in the LJF-treated group were significantly reduced at the nuclear protein and mRNA levels.

LJF-treated groups (100 and 200 μg/mL) showed a decrease in nuclear C/EBP-α levels by ~25 and 42%, compared with the MDI group. C/EBP-β was reduced by about 21 and 32% in the LJF-treated groups compared to the MDI group (Fig. 6A). Treatment with LJF decreased the nuclear PPAR-γ level in a dose-dependent manner compared with the MDI group (Fig. 6B) and downregulated mRNA levels (C/EBP-α/β, PPAR-γ, GLUT-4, and FAS) (Fig. 7).

Discussion

Adipocytes play a very important role in energy homeostasis and metabolism (31). Excess fat accumulation in adipocytes is known to be a causative agent of chronic diseases including diabetes, hypertension, cardiovascular disease, and cancer (32). Therefore, studies on the inhibition of the accumulation and decomposition of intracellular fat have attracted much attention,

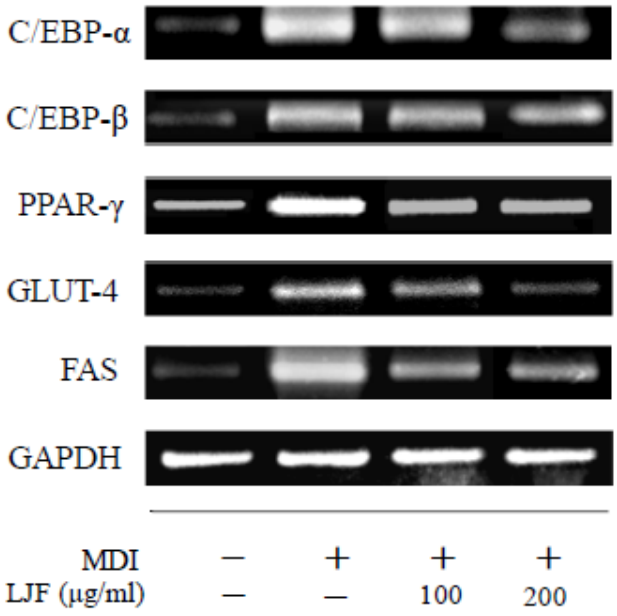

Figure 7. Inhibition of mRNA levels (C/EBP-α/β, PPAR-γ, FAS and GLUT-4) in 3T3-L1 adipocyte cells. cDNAs were subjected to RT-PCR.

and research on functional anti-obesity materials has been actively conducted worldwide.

Laminaria japonica contains many physiologically active polysaccharides, such as alginate, fucoidan, and laminaran, and is widely used as a functional material and health food. Alginic acid is a slippery component of *L. japonica* and forms about 20% of the total content. It has an excellent ability to lower cholesterol synthesis and inhibit increases in blood pressure, and has anti-cancer and heavy metal detoxification activities (33). In addition, it not only helps colon function, but also inhibits the absorption of fat in diabetic rats and mice (34). It can eliminate free radicals and was reported to be effective in counteracting aging and diseases in adults (35). Adipogenesis involves several regulatory factors, among which C/EBP-β is a typical transcription factor which regulates C/EBP-α and PPAR-γ. C/EBP-α and PPAR-γ are highly expressed in the late phase of differentiation as key transcription factors which induce the expression of terminal markers of adipogenesis, including adiponectin, FAS, and GLUT-4. GLUT-4 is involved in the active transport of glucose and FAS is a related gene in adipogenesis, and their expression levels are increased by C/EBPs. Differentiated cells have typical morphological characteristics, such as lipid droplet generation and increased cell size, and the expression of specific genes is induced. In this study, we investigated the effect of LJF on the expression levels of adipogenic transcription factors.

LJF (100 and 200 μg/mL) was not cytotoxic and it decreased glucose uptake and TG accumulation. Treatment with LJF decreased the amount of lipid droplets in a dose-dependent manner compared with the MDI group, as revealed by Oil Red O staining. We observed the inhibitory effects of LJF on the differentiation of 3T3-L1 adipocytes by examining the expression levels of nuclear C/EBP-α/β and PPAR-γ. We also confirmed the mRNA levels of their corresponding genes and those of sub-factors (FAS, GLUT-4) using RT-PCR.

The expression levels of C/EBP-α/β, PPAR-γ, FAS, and GLUT-4 were decreased in the LJF-treated groups compared to the MDI group. These results indicate that LJF reduces lipid droplet production by inhibiting the expression of C/EBP-α/β and PPAR-γ, which are adipogenic transcription factors, in 3T3-L1 adipose precursor cells. This has a promising anti-obesity effect that may inhibit differentiation into adipocytes. Through the fermentation process, the amount of reducing sugar increased and the amount of glycoprotein detected increased and it is considered that *Laminaria japonica* glycoprotein enhances the anti-obesity effect.

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Author Contributions
The first author, KIM, experimented and wrote a paper The correspondent author JANG, checks the experiment and is the research director.

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