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Optimized *Cirsium setidens* Nakai Fermented by *Lentinula edodes* Attenuates Lipid Accumulation by Regulating Fatty Acid Oxidation-mediated Lipolysis in 3T3-L1 Cells and High Calorie Diet-induced Obese Zebrafish

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Abstract *Cirsium setidens* Nakai is an edible herb. Previously we found that fermented *Cirsium setidens* Nakai (FCSN) has a large amount of major bioactive compound compared to *Cirsium setidens* Nakai. In this study, we aimed to examine the anti-obesity effect of FCSN using 3T3-L1 cells *in vitro* and high calorie diet-induced obese (HDIO) zebrafish model *in vivo*. Our results demonstrated that FCSN significantly inhibited intracellular lipid accumulation in 3T3-L1 cells. FCSN was shown to reduce the expressions of crucial adipocyte differentiation markers, including PPARγ and aP2. FCSN also decreased the production of ROS due to the up-regulated expressions of SOD1, SOD2, GPx, and catalase. Furthermore, we observed that FCSN also altered the levels of energy metabolism and β-oxidation-associated genes such as AMPK, ACC, and CPT-1. In addition, ATGL, a key lipolysis enzyme, was stimulated while the differentiation of 3T3-L1 was suppressed by FCSN. Strikingly, we found that FCSN dramatically increased both the energy metabolism and β-oxidation associated genes and subsequently prevented the increase of body fat accumulation in high calorie diet-induced obese zebrafish. Taken together, this is the first study that demonstrates that FCSN has the beneficial activity to suppress adipogenesis in 3T3-L1 cells and ameliorate an obese-associated health condition *in vivo*.

Keywords: *fermented Cirsium setidens Nakai, 3T3-L1, adipogenesis, zebrafish, β-oxidation, high fat diet*

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1. Introduction

Obesity is the most popular diet-associated chronic disease worldwide [1,2]. A large number of studies have suggested that a calorie rich diet tends to promote obesity in humans, which may diminish the life span due to the incidence of chronic diseases including cardiovascular disease (CVD), diabetes, and cognitive impairment [3,4]. On the other hand, diets abundant in fruits, vegetables, and edible plants have been shown to decrease the risk of CVD, diabetes, cognitive impairment, and obesity [5,6,7,8]. Bioactive compounds, such as flavonoids and phenolic pigments in fruits, vegetables, and edible plants [9,10], act as a positive modulator of signal transduction pathways elicited by ligand-binding domain in cells and tissues [11,12,13]. In addition, certain types of bioactive compound-enrich extracts play an upstream regulator of energy expenditure and β-oxidation in adipocyte cells and obese animal models [14,15,16]. Therefore, it has become a great interest in finding natural edible plants for managing obesity.

Cirsium setidens Nakai is a species of *Cirsium* from the East Asia region and has been used as an emergency alternative crop until the late 18th in South Korea [17]. And, in recent years, it has been re-centered as the focus of considerable public attention due to its number of beneficial biological activities [18,19,20]. Besides the effects *Cirsium setidens* Nakai has on anti-oxidant, anti-inflammation, anti-cancer, and anti-adipogenesis [18,21,22,23], it has been supported that *Cirsium setidens* Nakai contains potential as a natural edible plant against obesity. Moreover through this study, we showed that the fermentation improved the quantity of bioactive compound in *Cirsium setidens* Nakai [24], which might stimulate the protective mechanisms against abnormalities in obesity. However, the detailed mechanism of how

fermented *Cirsium setidens* Nakai (FCSN) influences adipocyte cells and obese animal as experimental models still remains unclear. Therefore, the aim of present study was to determine whether FCSN has beneficial effects on adipogenesis and lipid accumulation *in vitro* and *in vivo*.

2. Materials and Methods

2.1. Materials

FCSN was prepared through bioconversion by *Lentinula edodes* using submerged liquid fermentation system. For the optimization of inoculation amount and incubation time of the mycelium, the growth curve was fitted at 3 points according to cell mass. And, then enzymatic treatment of the secondary bioconversion process is performed. To remove the cell wall of cultured mycelium, 0.1~2% cellulase, hemi-cellulase, pectinase, β-glucanase, and β-glucosidase were treated at 50~60°C for 1~3 h. FCSN was extracted with 80% ethanol. Extracts were filtered and evaporated under vacuum, dried at -70°C. FCSN was standardized by HPLC (Waters 2695, Waters Co., Milford, MA, USA) [24].

2.2. Materials

3T3-L1 pre-adipocyte (CL-173) was purchased from ATCC (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM), bovine calf serum (BCS), fetal bovine serum (FBS), penicillin-streptomycin (P/S), insulin and trypsin-EDTA were from Gibco (Gaithersburg, MD, USA). Dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), Oil Red O, and isopropanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Specific antibodies for aP2, AMPK, p-AMPK, p-ACC, G6PDH, and β-actin were purchased from Cell Signaling Technology (Danvers, MA, USA). PPARγ, SOD1, SOD2, catalase, and CPT-1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. Cell Culture and Differentiation

3T3-L1 pre-adipocytes were cultured in DMEM with 10% BCS, 3.7 g/L sodium bicarbonate, and 1% P/S. Induction of adipocyte differentiation, the cells were treated 10% FBS and hormone cocktail (MDI: 0.5 mM IBMX, 1.0 μM DEX, and 1 μg/mL insulin). The cells were maintained at 37°C in 5% CO_2 incubator and medium with 10% FBS and 1 μg/mL of insulin was changed every two days.

2.4. Cell Viability Assay

3T3-L1 pre-adipocyte (5×10^3 cells/well) were incubated with DMEM with 10% BCS media for overnight in 96-well plates. 3T3-L1 cells were treated with FCSN (0, 25, 50, and 100 μg/mL) for 6 days. The growth was detected using XTT assay. XTT reagent and PMS were added to the 96 well plates and incubated at 37°C for 4 h. After 4 h, the absorbance was measured at 490 and 660 nm. The growth medium alone was used as a blank. The specific absorbance of the sample is expressed as the followed: Specific absorbance=$A_{450\ nm}$(sample)−$A_{450\ nm}$(blank) − $A_{690\ nm}$(sample).

2.5. Oil Red O Staining

Differentiated 3T3-L1 cells were fixed with 10% formaldehyde at 4°C for 1 h. After washing with 60% isopropanol, the fixed cells were stained with 0.4% Oil Red O in 60:40 (v/v) isopropanol/H_2O at room temperature for 30 min, and then rinsed three times with water. Lipid accumulation was observed by the inverted light Olympus CKX41 microscope (Olympus, Tokyo, Japan). The Oil Red O dye was eluted by 100% isopropanol, and the absorbance at 490 nm was determined by microplate reader (SpectraMax i3; Molecular Devices, Sunnyvale, CA, USA)

2.6. Nitrobluetetrazolium (NBT) Assay

3T3-L1 pre-adipocytes were grown to confluence and induced to differentiate into adipocytes, as described. Production of ROS was detected by NBT assay. Nitrobluetetrazolium (NBT) is reduced by ROS to a dark-blue, insoluble form of NBT called formazan. On day 6 post-induction, the cells were incubated for 90 min in PBS containing 0.2% NBT. Formazan was dissolved in 100% acetic acid and the absorbance was determined at 570 nm.

2.7. Animal Husbandry

Adult zebrafishes (wild-type *Danio rerio*) were initially obtained from Chungnam National University (South Korea). Embryos and larvae were obtained by mating naturally and raised embryo in the water containing sea salts (Sigma, St. Louis, MO, USA) of 60 μg/mL until 5 days post-fertilization (5 dpf). Then, larvae were maintained in 100 mm plate, at a density of around 20 larvae per 100 mL, and fed hardboiled egg yolk as a high calorie diet once a day in the presence or absence of FCSN for 12-15 days (17-20 dpf).

2.8. Nile Red Staining, Fluorescence Imaging

The stock solution (1.25 mg/mL) of nile red (Invitrogen N-1142) was prepared in acetone, and protected from the light at -20°C before using. For staining of fish, stock solution was diluted to 50 ng/mL in egg water, and incubated at 28°C in the dark for 5-10 min. Fishes were washed with distilled water three times and anesthetized with a few drops of tricain (Sigma, St. Louis, MO, USA) stock solution (4 mg/mL, pH 7.0). Zebrafish larvae used for imaging analysis were starved for 24 h to empty digestive tract before Nile red staining. Fishes were mounted in 3% methylcellulose and images were taken with Leica Z16 APO macroscope (Leica, Wetzlar, Germany) with the attached DFC320 digital camera (Leica, Wetzlar, Germany) and subsequently processed with Leica LAS software.

2.9. Total RNA Isolation, Reverse Transcription Polymerase Chain Reaction (RT-PCR), and Quantitative RT-PCR Assay

Total RNAs were extracted using TRIzol® reagent (Invitrogen) following the manufacturer's instructions. Subsequently, total RNAs were converted to cDNA with Maxime RT PreMix kit (iNtRON Biotechnology, South Korea). Then, quantitative RT-PCR or conventional RT-PCR was carried out with SYBR® or Dr. Taq-HOT Master Mix (MGmed, South Korea), respectively. Target gene amplification was performed using specific oligonucleotide primers in a normal PCR system and the primer sequences were as the followed: iNOS, forward (5'-CCC TTC CGA AGT TTC TGG CAG CAG C-3') and reverse (5'-GGC TGT CAG AGC CTC GTG GCT TTG G-3'); COX-2, forward (5'-CAC TAC ATC CTG ACC CAC TT-3') and reverse (5' – ATG CTC CTG CTT GAG TAT GT- 3'); GAPDH, forward (5'- CAA GGT CAT CCA TGA CAA CTT TG-3') and reverse (5'-GGC CAT CCA CAG TCT TCT GG-3'). GAPDH was selected as a control of housekeeping genes, as its expression was not significantly different between samples. The expression of target genes was normalized to that of the control gene. RT-PCR products were analyzed on 1% agarose gel and bands were visualized using ethidium bromide. The expression levels were quantified by scanning with the gel documentation and analysis system (Image J, NIH, Maryland, USA).

2.10. Western Blot Analysis

Cell were washed with PBS and harvested by lysis buffer containing protease inhibitors and phosphatase inhibitor cocktail II and III. Protein samples were separated by SDS-PAGE and transferred onto PVDF membrane. The membranes were blocked 5% skim milk in Tris-buffered saline and 0.1% Tween-20 for 1 h and incubated with primary antibodies (1:1000) diluted in 1% bovine serum albumin (BSA) in TBST at 4°C overnight. After three washes with TBST, Secondary antibodies were conjugated with horseradish peroxidase (1:5000) in TBST containing 5% skim milk at room temperature for 1 h. The bands were visualized by enhanced chemiluminescence, and proteins were detected with Chemidoc image software (Bio-Rad, Hercules, CA, USA).

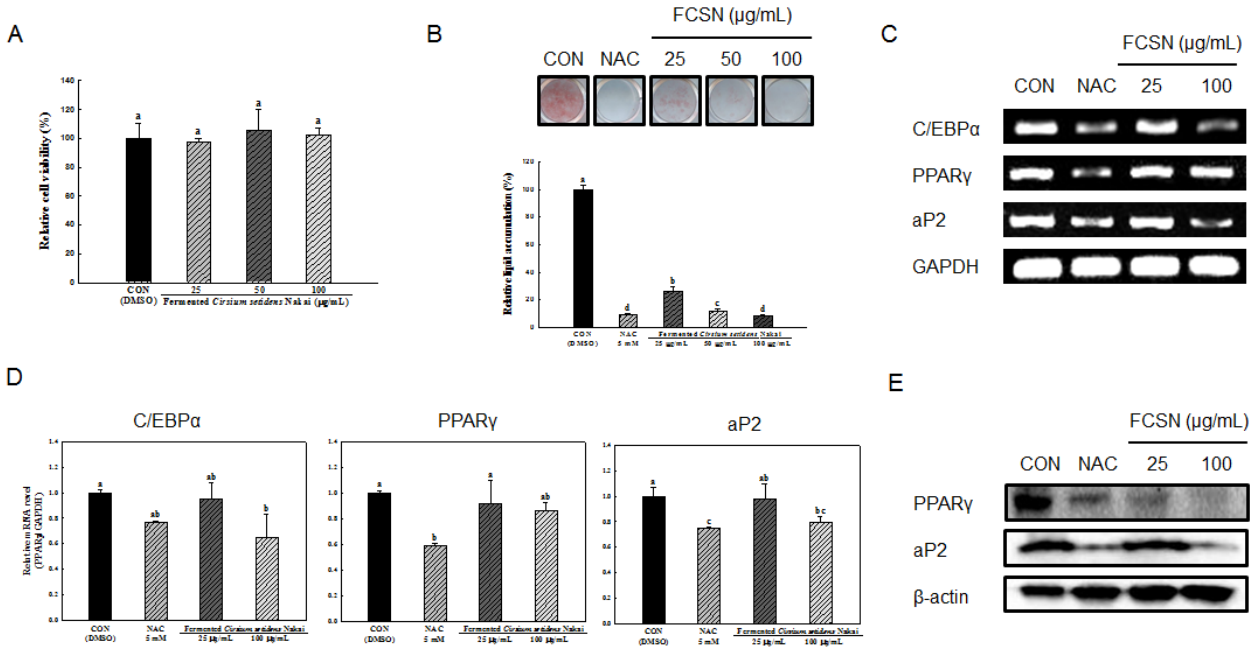
2.11. Statistical Analysis

All values are presented as mean standard deviation values. All data were obtained at least triplicate experiments independently. Differences among multiple groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SAS 9.0 software (SAS Institute, NC, USA). A difference of *p-value* < 0.05 was considered significantly.

3. Results

3.1. Analysis of Optimized FCSN

FCSN was standardized to use a validation method [24]. During the bioconversion processing, pectolinarin converted to pectolinarigenin. The contents of pectolinarin and pectolinarigenin in FCSN were 2.23 mg/g and 28.91 mg/g, respectively. The total phenol contents and the total flavonoid contents were 38.11 mg GAE/g and 71.44 mg GE/g, respectively.



(A) 3T3-L1 cells were plated into 96-well plates at a 1×10^4 cells/well density in growth medium, and incubated with FCSN (0, 25, 50, and 10 μg/mL) for 24 h. (B) Lipid accumulation was measured by Oil Red O staining in 3T3-L1 cells differentiated with the presence or absence of 25, 50, and 100 μg/mL FCSN during 6 days and determined at 490 nm. (C, D). Cell lysates differentiated during 6 days were subjected to RT-PCR for analyzed for C/EBPα, PPARγ, and aP2 mRNA expression. (E) Cell lysates differentiated during 6 days were subjected to western blot for analyzed for PPARγ and aP2 protein expression. Values with different letters are significantly different, $p < 0.05$.

Figure 1. Attenuation of FCSN on adipogenic differentiation through the regulation of adipogenic factors in 3T3-L1

3.2. Effect of FCSN on Cell Viability and Adipocyte Differentiation in 3T3-L1 Cells

Cell viability was firstly evaluated using XTT assay to choose the concentration of FCSN for further experiments. As shown in Figure 1A, 100 μg/mL of FCSN in 3T3-L1 cells was the non-toxic level to the cells. Thus the concentrations of 25, 50, and 100 μg/mL of FCSN were chosen for the further investigation.

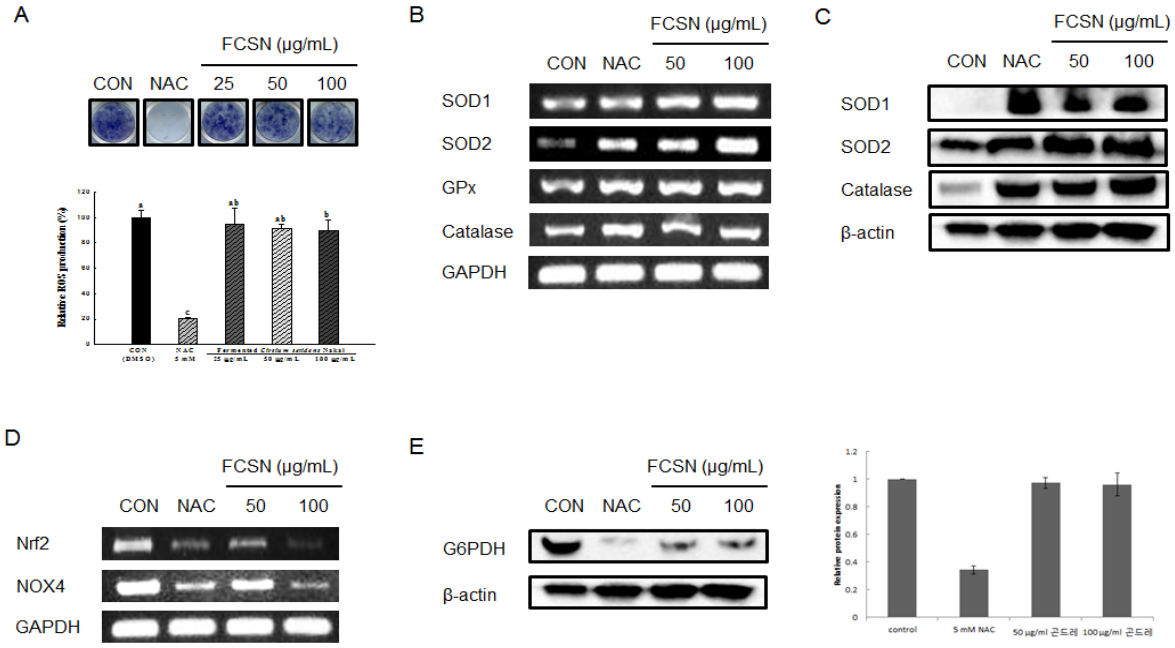
To evaluate the effect of FCSN, 3T3-L1 cells were cultured under differentiation media as described in materials and methods. Oil Red O (ORO) staining was performed to assess the effect of FCSN on intracellular lipid accumulation in 3T3-L1 cells. ORO staining showed that FCSN dramatically decreased intracellular lipid accumulation compared to 3T3-L1 cells under differentiation condition without FCSN as shown in Figure 1B.

Next, we performed RT-PCR and western blot analysis whether FCSN regulated the adipocyte differentiation by crucial markers including C/EBPα, PPARγ, and aP2. As shown in Figure 1C and 1D, FCSN significantly decreased the mRNA expressions of C/EBPα, PPARγ, and aP2 in 3T3-L1 cells. Consistently, both protein expressions of PPARγ and aP2 were suppressed in 3T3-L1 cells in the presence of FCSN, compared to corresponding control (Figure 1E). In addition, we used N-acetyl cysteine (NAC) as a positive control which is a well-known inhibitor for adipocyte differentiation through blocking to generate ROS during adipogenesis in 3T3-L1 [25]. We observed that FCSN showed a similar tendency of ORO staining, RT-PCR, and western blot analysis in 3T3-L1 treated with NAC. These data potentially indicated that FCSN can suppress not only adipocyte differentiation markers but also ROS-associated pathway in 3T3-L1 cells.

3.3. Effect of FCSN on Oxidative-Associated Pathway in 3T3-L1 Cells

To investigate whether FCSN regulated the production of ROS during adipogenesis, we then assessed the nitric oxide (NO) accumulation by NBT assay. As shown in Figure 2A, a large amount of NO production was observed in 3T3-L1 cells, compared to NAC treated group. The level of NO in the presence of FCSN was significantly decreased compared to the control. So, to know how FCSN involved in the production of NO, we analyzed the changes of the expression levels of oxidative stress-associated pathway involvements including SOD1, SOD2, GPx, and catalase by RT-PCR and western blot analysis. As shown in Figure 2B and Figure 2C, we found that 3T3-L1 cells with FCSN dramatically showed the stimulated expressions of SOD1, SOD2, GPx, and catalase, compared to the control. Moreover, western blot data revealed that oxidative stress-associated proteins with 100 μg/mL of FCSN induced even higher expression than one treated with NAC in 3T3-L1 cells.

On the other hand, this oxidative stress pathway is regulated by upstream modulator such as Nrf2 and NOX4 [26,27]. Thus, we evaluated the expressions of Nrf2 and NOX4 to confirm whether FCSN affected oxidative stress pathway through modulating Nrf2 and NOX4. As shown in Figure 2D, our result showed that both expressions of Nrf2 and NOX4 mRNA were strongly inhibited in the presence of FCSN, in a dose dependent manner. We also found that 3T3-L1 cells with FCSN consistently produced the suppressed level of glucose-6-phosphate dehydrogenase (G6PDH) which is another downstream target of endogenous Nrf2 during antioxidant response (Figure 2E) [28]. Recently, Nrf2-knockout is associated with an increase in energy expenditure *in vivo* [29]. Thus, we inquired further examination in the energy metabolism pathway involvements in 3T3-L1 cells.



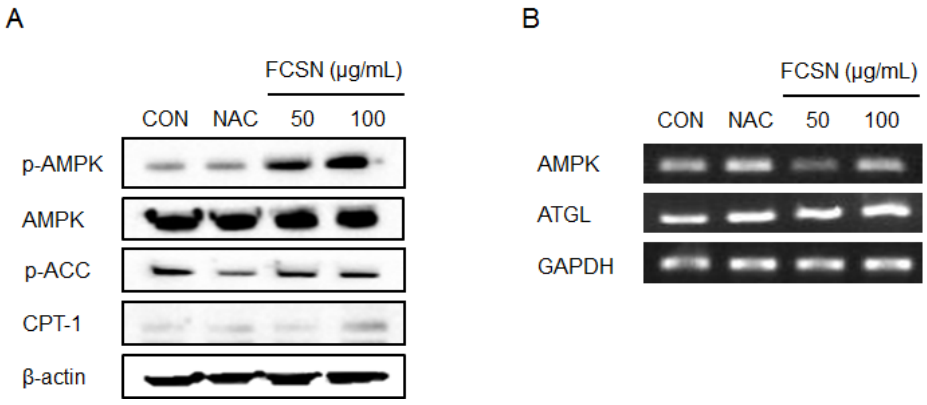
(A) ROS production was subjected to NBT assay in cells differentiated during 6 days for the indicated tables and determined at 570 nm. (B) Cell lysates differentiated during 6 days were subjected to RT-PCR for analyzed for SOD1, SOD2, GPx, and catalase mRNA expression. (C) Cell lysates differentiated during 6 days were subjected to Western blot for analyzed for SOD1, SOD2, and catalase protein expression. (D) RT-PCR analysis for Nrf2 and NOX4 mRNA expression. (E) Western blot analysis for G6PDH. Values with different letters are significantly different, $p < 0.05$.

Figure 2. Modulation of FCSN on the production of ROS and the expression of oxidative stress-associated enzymes during adipogenesis

3.4. Effects of FCSN on Energy Metabolism and β-Oxidation in 3T3-L1 Cells

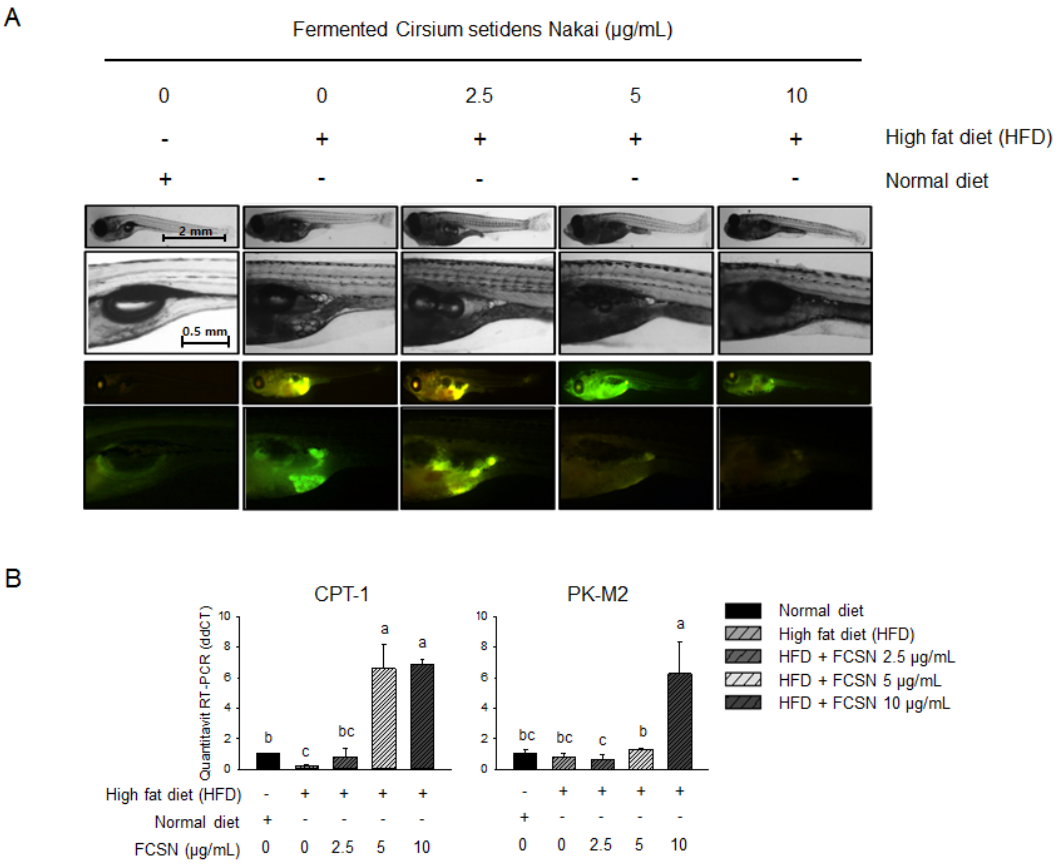
To determine the effect of FCSN on the energy metabolism, we validated the changes of the expressions of AMP-activated protein kinase (AMPK), acetyl CoA carboxylase 1 (ACC) and carnitine palmitoyltransferase I (CPT-1) by immunoblotting. As shown in Figure 3A, both levels of phosphorylation of AMPK and inhibition of its downstream target ACC were enhanced in 3T3-L1 cells in various FCSN concentrations. When AMPK inhibits the phosphorylation of ACC resulted in decreasing intracellular lipid synthesis, it activates β-oxidation [30].

Western blot analysis revealed that FCSN consistently ameliorated the expression of CPT-1 protein, which controls mitochondrial β-oxidation compared to corresponding control. Moreover, the expression of adipose triglyceride lipase activation (ATGL) was increased in 3T3-L1 cells in the presence of FCSN. This indicates that FCSN also stimulated lipolysis activity in 3T3-L1 cells (Figure 3B). The afore-stated evidence suggested that the beneficial effect of FCSN on adipogenesis could be due to the altered energy metabolism and β-oxidation and, subsequently, the inhibited adipocyte differentiation of 3T3-L1 cells.



(A) Western blot analysis for p-AMPK, AMPK, p-ACC, and β–actin. (B) RT-PCR analysis for AMPK, ATGL, and GAPDH.

Figure 3. Effect of FCSN on energy metabolism and β–oxidation-associated genes in 3T3-L1 cells



(A) Lipid accumulation was evaluated by Nile Red staining in zebrafishes grown with HDIO in the presence or absence of 2.5, 5, and 10 μg/mL FCSN during 20 dpf and visualized under a fluorescence microscope. (B) Quantitative RT-PCR analysis for CPT-1 and PK-M2. Values with different letters are significantly different, $p < 0.05$.

Figure 4. Effect of FCSN on the lipid accumulation and the expression of β–oxidation-associated genes in high calorie diet-induced zebrafish

3.5. Effects of FCSN on Energy Metabolism and β-Oxidation in High Calorie Diet-Induced Obese Zebrafish Model

To finalize the analysis of the effect of FCSN on energy metabolism and β-oxidation *in vivo*, we used high calorie diet-induced obese (HDIO) zebrafish. As shown in Figure 4A, the analysis of the fluorescent staining showed that the body fat of HDIO zebrafish was highly accumulated, compared to normal diet zebrafish. In contrast, HDIO zebrafish in the presence of FCSN strongly lost its body fat mass in a dose dependent manner. Consistent with finding *in vitro*, quantitative RT-PCR analysis indicated that the expression of CPT1 was significantly increased when HDIO zebrafish was fed 10 μg/mL of FCSN (Figure 4B). Moreover, we found that 10 μg/mL of FCSN supplemented HDIO zebrafish significantly induced the increased expression of pyruvate kinase M2 (PK-M2), which decrease in adipose tissue in obese animal model [31], compared to HDIO zebrafish. Here, we provided a part of evidence that FCSN may lead to suppress body fat accumulation through stimulating both energy metabolism and β-oxidation in HDIO zebrafish.

4. Discussion

In this study, we showed that FCSN led the suppressed adipogenesis of pre-adipocyte in 3T3-L1 via the inhibition of adipocyte differentiation markers and the stimulation of genes involved in energy metabolism in 3T3-L1 adipocytes. In addition, we also demonstrated that FCSN contributed to prevent the accumulation of lipid droplet as well as an increase the expression of fatty acid oxidation-associated genes in HDIO zebrafish.

High calorie diet causes a typical abnormality of energy metabolism and promotes an increase of the excessive energy intake [32]. It results in an increase of adipose tissue mass and, then, subsequently alters the body condition into obesity. The majority of adipose tissue in organisms is adipocyte [33]. A number of factors including C/EBPα, PPARγ, and aP2 are important for the development of adipocyte [34]. The beneficial effect of dietary extracts derived from fruits or vegetables have been showed on controlling C/EBPα, PPARγ, and aP2 *in vitro* and *in vivo* [35,36,37]. Inafuku and colleagues [38] provided a clue that *Cirsium Brevicaule* A. GRAY leaf led to suppress C/EBPα, PPARγ, and aP2, resulted in a decrease lipid accumulation in 3T3-L1 and HDIO mice model.

Cirsium setidens Nakai is an edible herbal plant in South Korea. It is believed that *Cirsium setidens* Nakai plays a crucial role in its anti-obesity activity [23]. Given that *Cirsium setidens* Nakai has a potential to prevent lipid drop accumulation, *Cirsium setidens* Nakai appears as a good candidate for anti-obesity food ingredient. However, the detailed mechanism is poorly understood. Our previous study revealed that fermentation increased main bioactive component of *Cirsium setidens* Nakai such as pectolinarigenin. In the present study, we examined the effect of FCSN on the expressions of C/EBPα, PPARγ, and aP2 in 3T3-L1 cells. We observed that 3T3-L1 treated with FCSN produced the significantly reduced protein levels of C/EBPα, PPARγ, and aP2 compared to the control as indicated by less amount of lipid droplet. These data indicated that FCSN contributed to inhibit adipogenesis and lipid accumulation during the differentiation of 3T3-L1.

Oxidative stress is involved in promoting the initiation of adipogenesis in adipose tissue and is caused by excessive production of ROS [39]. Endogenous enzymes such as SOD and catalase can convert super oxide into hydrogen peroxide to prevent adipogenesis by the excessive amount of ROS. In addition, GPx regulates glutathione homeostasis through the conversion of hydrogen peroxide into alcohols and water [40,41], resulted in a decrease of the adipocyte differentiation [42]. FCSN remarkably suppressed the production of ROS through the stimulation of the anti-oxidant enzymes including SOD1, SOD2, catalase, and GPx in 3T3-L1. Moreover, both Nrf2 and NOX4 act as pro-oxidant enzymes which produce ROS via the regulation of NADPH [43,44]. The reduced expressions in mRNA levels of Nrf2, NOX4 and its downstream target G6PDH were also in consistent with our findings.

The AMPK signaling pathway is a nutrient sensor and a crucial energy balance mediator through modulating fatty acid β-oxidation transcription factors [45]. In adipocytes, several factors such as active exercise and nutrients activate AMPK and, then, inhibit the phosphorylation of ACC and its activity. Sequentially, decreased ACC phosphorylation, induces the reduced CPT-1 activity. This indicates that the activated AMPK can stimulate fatty acid β-oxidation by CPT-1 [46].

Lipid droplet is decomposed into fatty and glycerol via β-oxidation. Lipolysis is also involved in the process of resolving glycerol from fatty acids by ATGL in lipid droplet such as triglyceride [47]. In this study, we found that FCSN promoted an increase in the expression of AMPK, resulted in a decrease of ACC phosphorylation in 3T3-L1, compared to the control. Furthermore, the expression of CPT-1 and ATGL was also induced by FCSN treatment. Taken together, our data suggested that anti-adipogenic effect of FCSN was mediated by the down-regulation of transcription factors such as C/EBPα, PPARγ, and aP2 during adipogenesis and the up-regulation of genes involved in β-oxidation and lipolysis. This is also partially agree with previous report, Noh *et al*., described that *Cirsium setidens* Nakai reduced the hepatic lipid accumulation in HDIO mice [48].

Our findings suggested that FCSN decreased the lipid accumulation through modulated β-oxidation-associated pathway. These results prompted us to evaluate whether FCSN can suppress lipid accumulation in HDIO zebrafish. To examine whether FCSN contributed to decrease body fat accumulation or not, we chose well-established HDIO zebrafish model. As we expected, HDIO zebrafish showed the increased body fat mass as indicated fluorescence intensity, while FCSN led the significantly eliminated level of body fat accumulation in a dose dependent manner. In addition, FCSN at 10 μg/mL significantly suppressed the expression of CPT-1 and PK-M2 in HDIO zebrafish, compared to corresponding control. These suggest that FCSN strongly stimulated both β-oxidation and lipolysis-associated pathway, and subsequently prevented lipid accumulation in HDIO zebrafish.

Our results supported that FCSN has the potential to inhibit lipid accumulation and to increase β-oxidation-mediated lipolysis both in 3T3-L1 cells and HDIO zebrafish. To our best knowledge, this is the first evidence that FCSN ameliorates the molecular events in lipogenesis *in vitro* and *in vivo*. We suggested that the underlying mechanisms of anti-lipogenic function of FCSN might be able to induce the β-oxidation-associated pathway. Therefore, we suggest that FCSN can hold the potential to beneficially repress the body fat mass in HDIO populations.

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Conflict of Interest

The authors declare no conflict of interest.

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