

# Suppressive Effects on Lipid Accumulation and Expression of Interleukin-1 $\beta$ -Mediated Inducible Nitric Oxide Synthase in 3T3-L1 Preadipocytes by a Standardized Commercial Noni Fruit Juice

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## Noni Fruit Juice의 3T3-L1 지방전구세포 분화 억제 및 인터루킨-1 $\beta$ 유도 Inducible Nitric Oxide Synthase 염증유전자 발현 감소 효과

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**Objectives:** Noni fruit juice (NFJ) is liquor extracted from *Morinda citrifolia* (noni) fruit and has been used as an herbal remedy in many countries. However, the NFJ's anti-adipogenic and anti-inflammatory effects on adipocytes are poorly understood. The purpose of this study was to explore the commercially standardized NFJ effects on lipid accumulation throughout 3T3-L1 preadipocytes differentiation and interleukin-1 $\beta$  (IL-1 $\beta$ )-mediated inducible nitric oxide synthase (iNOS) expression in 3T3-L1 preadipocytes.

**Methods:** Cellular lipid accumulation and triglyceride (TG) content in differentiating 3T3-L1 preadipocytes were assessed subsequently via the Oil Red O staining and AdipoRed assay. MTS assay was used to examine NFJ cytotoxicity in (differentiating) 3T3-L1 preadipocytes. Immunoblotting and reverse transcriptase polymerase chain reaction analysis were used to measure the expression levels of target protein and mRNA in (differentiating) 3T3-L1 preadipocytes, respectively.

**Results:** NFJ treatment at 150  $\mu$ L/mL led to a substantial reduction of fat accumulation and TG content during 3T3-L1 adipogenesis with no discernable impact on the cell viability. Of note, while NFJ treatment (150  $\mu$ L/mL) largely inhibited the CCAAT/enhancer-binding protein- $\alpha$  (C/EBP- $\alpha$ ) and peroxisome proliferator-activated receptor- $\beta$  (PPAR- $\beta$ ) protein expressions, it did not influence PPAR- $\gamma$  in differentiating 3T3-L1 preadipocytes. Of interest, treatment with IL-1 $\beta$  at 20 ng/mL for 4 hours elicited in firm induction of iNOS mRNA expression in 3T3-L1 preadipocytes. However, NFJ treatment at 100 or 200  $\mu$ L/mL greatly attenuated the IL-1 $\beta$ -induced iNOS mRNA expression in 3T3-L1 preadipocytes.

**Conclusions:** NFJ has anti-adipogenic and anti-inflammatory effects on (differentiating) 3T3-L1 preadipocytes which are in part intervened via control of the expression of C/EBP- $\alpha$ , PPAR- $\beta$ , and iNOS.

**Key Words:** Noni, C-EBPalpha, PPARbeta, Nitric oxide synthase type II, 3T3-L1 cells

## Introduction

Obesity prevalence has risen as global epidemic issue

over past years<sup>1,2</sup>). Preadipocyte differentiation, also called adipogenesis, is a multistep pathway of preadipocyte expansion and adipocyte maturation orchestrated by a variety

of transcription factors, enzymes, and signaling proteins<sup>3,4</sup>). However, inordinate preadipocyte differentiation leads to hypertrophic adipocytes and abnormal expansion of the adipose tissue (AT) and eventually the development of obesity, a disease with metabolic dysregulation<sup>5,6</sup>). Hence, any material that interferes with excessive or abnormal fat accumulation in preadipocyte differentiation have probability to act as an implied anti-obesity treatment.

It has been shown that numerous transcription factors play key roles in induction of adipogenesis<sup>7-9</sup>). Upon the exposure of different inducers to preadipocytes, the cell differentiation initiates with the temporary expression of members of the CCAAT/enhancer-binding proteins (C/EBPs) family, including C/EBP- $\beta$  and CEBP- $\delta$ , which leads to further induction and activation of C/EBP- $\alpha$  and peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ )<sup>10</sup>). In addition, PPAR- $\beta$ , another isoform of the family of PPARs, participates in the initial stage of preadipocyte differentiation process<sup>11</sup>).

There are strong evidences shown that chronic inflammation is tightly associated with obesity which further intensifies the AT dysfunction<sup>12,13</sup>). Inducible nitric oxide synthase (iNOS) is a key inflammatory mediator in many cell types and tissues<sup>14</sup>). Notably, iNOS over-expression is reported to play an essential role in the pathogenesis of obesity<sup>15</sup>). Thus, inhibition (inhibitor) of iNOS expression in fat cells could be a promising target to combat or treat obese inflammation.

Presently, study involving in anti-obesity material(s) invention from natural products with its safety to the human health has been risen. The noni fruit juice (NFJ, fruit juice of *Morinda citrifolia* L.) has been widely used as an alternative medicine with abundant bioactive compounds<sup>16,17</sup>). It also has been reported that the NFJ possesses anti-inflammatory, anti-bacterial, and antioxidant properties<sup>18-20</sup>). Moreover, diverse studies have markedly elucidated that NFJ has prospective anti-obesity and anti-cancer effects<sup>21-24</sup>). However, up to date, the NFJ's anti-adipogenic and anti-inflammatory effects on fat cells are poorly understood.

The purpose of this study was to explore the effect of a standardized commercial NFJ on fat accumulation in differentiating 3T3-L1 preadipocytes and expression of interleukin-

1 $\beta$  (IL-1 $\beta$ )-mediated iNOS, an inflammatory enzyme, in 3T3-L1 preadipocytes. Overall, our present work exemplified, for the first time that NFJ has anti-adipogenic and anti-inflammatory effects on (differentiating) 3T3-L1 preadipocytes and these effects are in part mediated by inhibiting the expression of C/EBP- $\alpha$ , PPAR- $\beta$ , and iNOS.

## Materials and Methods

### 1. Materials

NFJ, the fruit juice of *M. citrifolia* L., was purchased from Tahitian Gold Co., Inc. and stored at 4 °C refrigerator until use. Primary antibodies for anti-C/EBP- $\alpha$ , PPAR- $\gamma$  and PPAR- $\beta$  were acquired from Santa Cruz Biotechnology. IL-1 $\beta$  was bought from R&D Systems. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent was from Promega. Oil Red O working solution was obtained from Sigma.

### 2. 3T3-L1 preadipocytes differentiation

3T3-L1 murine preadipocytes (ATCC) were maintained in Dulbecco's Modified Eagles' Medium (DMEM) (Welgene) with the addition of 10% heat-inactivated fetal calf serum (Gibco) and 1% penicillin/streptomycin (Welgene) at 37 °C under a humidified atmosphere of 5% CO<sub>2</sub>. To induce 3T3-L1 cells differentiation, the medium was changed to DMEM containing 10% fetal bovine serum (FBS; Welgene), 0.5 mM of IBMX (M) (Sigma), 0.5  $\mu$ M of dexamethasone (D) (Sigma), and 5  $\mu$ g/mL of insulin (I) (Sigma) either with or without NFJ. After 2 days, the first differentiation medium was changed to DMEM supplemented with 10% FBS and 5  $\mu$ g/mL insulin added with or without NFJ at the desired doses for further 3 days. Then, the cells were maintained in DMEM with the addition of 10% FBS in the presence or absence of NFJ for additional 3 days.

### 3. Oil Red O staining

At the end of differentiation process (day 8), after control or NFJ-treated 3T3-L1 cells were washed with phosphate-buffered saline (PBS), the cells were fixed with formaldehyde

(10%) for 2 hours. Eventually, after cells were washed with 60% isopropanol and dried, Oil Red O working solution was put in the fixed cells for 1 hour and then washed with distilled water. Then, lipid droplets (LDs) were visualized under light microscopy (Nikon; TS100).

#### 4. Cell viability assay

On day 8, differentiated 3T3-L1 cells-treated with or without varying concentrations of NFJ and seeded in a 96-well plate overnight. At which point, the cells were incubated with MTS (20  $\mu$ L/well) for 1.5 hours at 37 °C. The absorbance was then measured at 595 nm using a micro-plate reader.

#### 5. AdipoRed assay

To assess the intracellular triglyceride (TG) deposition, AdipoRed Assay Reagent kit was utilized and it was done following the manufacturer's instruction (Lonza). After 10-minute incubation, the plates were put in a Victor<sup>3</sup> (Perkin Elmer), and fluorescence was conducted with 485 nm excitation and 572 nm emission wavelength.

#### 6. Whole-cell lysates preparation

3T3-L1 cells were washed with PBS and lysed in a modified RIPA buffer (50 mM Tris-Cl [pH 7.4], 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.25% sodium deoxycholate, 1% Triton X-100, 1% Nonidet P-40, 1 mM ethylene-diamine-tetraacetic acid, 1 mM EGTA, proteinase inhibitor cocktail [1 $\times$ ]) at the selected time point. Then, the cells were scraped to collect the whole-cell lysates, followed by centrifuge the lysed cell suspension at 14,000 revolutions per minute for 15 minutes at 4 °C. The supernatant was saved, and protein concentrations were estimated by Pierce BCA Protein Assay Kit (Thermo Scientific).

#### 7. Western blot analysis

A total of 50  $\mu$ g amount of protein was separated from each other based on their size by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) gel electrophoresis. Next, the protein was transferred from the gel to the nitro-

cellulose membranes (Millipore). Then, the membranes were rinsed briefly with Tris-buffered saline (10 mM Tris, 150 mM NaCl) supplemented with 0.05% (v/v) Tween 20 (TBST) and blocked with blocking buffer (TBST containing 5% [w/v] non-fat dried milk). The membranes were incubated overnight with primary antibodies solution against the target protein for C/EBP- $\alpha$  (1:1,000), PPAR- $\gamma$  (1:1,000), PPAR- $\beta$  (1:1,000), or  $\beta$ -actin (1:10,000) at 4 °C. The membranes were rinsed with TBST and then incubated with horseradish peroxidase-conjugated secondary antibodies for 2 hours. The membranes were then rinsed with TBST. ECL reagents was applied to develop the blot (Advansta). Equal loading of proteins was confirmed by  $\beta$ -actin antibody.

#### 8. Reverse transcriptase polymerase chain reaction (PCR) analysis

The total RNA from control or Noni-treated 3T3-L1 cells was extracted using an RNAiso Plus (TaKaRa) at the selected time point. Random hexadeoxynucleotide primer and reverse transcriptase were subjected for reverse transcribed the total RNA (3  $\mu$ g). The newly synthesized cDNA was amplified by PCR with primers of iNOS and actin with sequences as iNOS sense; 5'-GACAAGCTGCATGTGACATC-3'; iNOS anti-sense; 5'-GCTGGTAGGTTCTGTTGTT-3'; Actin sense; 5'-TCATGAAGTGTGACGTTGACATCCGT-3'; Actin anti-sense; 5'-CCTAGAAGCATTTGCGGTGCACGATG-3'. The following PCR conditions were applied: iNOS, 25 cycles of denaturation at 94 ° for 30 seconds, annealing at 52 ° for 30 seconds, and extension at 72 ° for 30 seconds; Actin, 18 cycles of denaturation at 94 ° for 30 seconds, annealing at 57 ° for 30 seconds, and extension at 72 ° for 30 seconds. Actin were used as a loading control to normalize iNOS expression.

#### 9. Statistical analysis

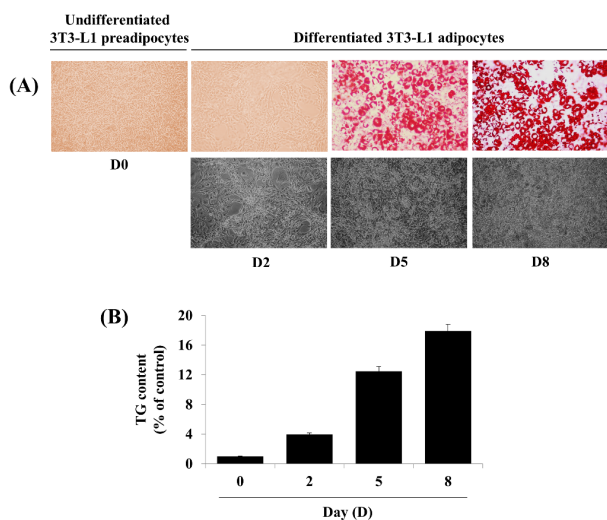
The IBM SPSS Statistics 25 software was utilized for statistical analysis (IBM Corp.). MTS analysis was defined in triplicate and three times replication. The data were demonstrated as mean $\pm$ standard error. One-way ANOVA was used to assess the significance difference. The level of significance

was indicated as a P-value of <0.05 in each experiment.

## Results

### 1. Confirmation of 3T3-L1 preadipocyte differentiation in response to the exposure of different inducers

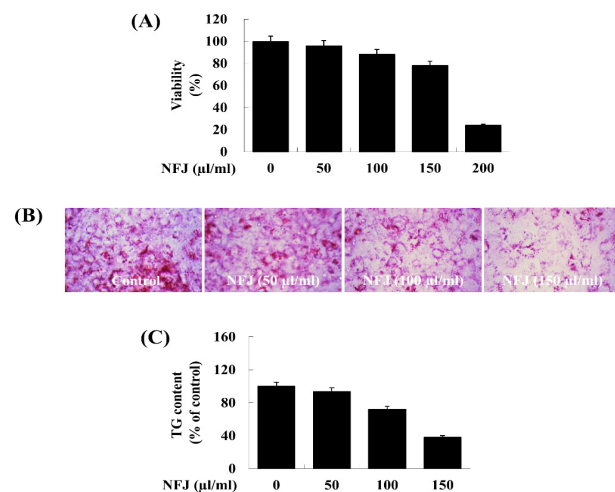
Initially, to induce 3T3-L1 preadipocytes differentiation, we used an induction medium containing MDI, insulin, and FBS for 8 days. On day 8 (D8), cellular lipid accumulation in the conditioned cells was confirmed by using Oil Red O staining. As shown in Fig. 1A (upper panels), there was high lipid (LDs) accumulation on D8 of 3T3-L1 cells differentiation, collated with undifferentiated cells (on D0). The phase-contrast images were further illustrated a high fat accumulation on D8 of adipogenesis in 3T3-L1 cells (Fig. 1A lower panels). As shown in Fig. 1B, there was also a time-dependent increase in the amount of TG in 3T3-L1 preadipocyte differentiation.



**Fig. 1.** Effects of different inducers on lipid accumulation in differentiating 3T3-L1 preadipocytes. (A) To induce adipocyte differentiation, 3T3-L1 preadipocytes were grown in induction medium containing MDI, insulin, and fetal bovine serum (FBS) for 8 days. On day 8 (D8), the intracellular lipid droplets were evaluated by Oil Red O staining (upper panels in A). Phase-contrast cell images of the differentiation D8 were also taken (lower panels in A) ( $\times 100$ ). (B) 3T3-L1 preadipocytes were induced to differentiate with an induction medium containing MDI, insulin, and FBS for 8 days. On day 8 (D8), intracellular triglyceride (TG) stores was assessed by AdipoRed assay.

### 2. NFJ treatment dose-dependently interferes with cellular fat accumulation in 3T3-L1 preadipocyte differentiation

During the differentiation process, cells were treated with or without NFJ at different concentrations (50, 100, 150, and 200  $\mu\text{L}/\text{mL}$ ) for 8 days. Under these experimental conditions, results of MTS assay, as shown in Fig. 2A, showed that treatment with NFJ at 50, 100, or 150  $\mu\text{L}/\text{mL}$  was not cytotoxic to these cells, whereas that with NFJ at 200  $\mu\text{L}/\text{mL}$  was highly cytotoxic to the cells. Hence, NFJ at 200  $\mu\text{L}/\text{mL}$  was excluded for further works. Of note, as shown in Fig. 2B, data of Oil Red O staining demonstrated that NFJ treatment vastly suppressed fat accumulation throughout 3T3-L1 adipogenesis in a dose-dependent manner. In addition, AdipoRed assay was next performed to study whether NFJ treatment also lowers TG stores on D8 of 3T3-L1 cells adipogenesis. Interestingly, as shown in Fig. 2C, treatment with NFJ led to a concentration-dependent reduction of TG

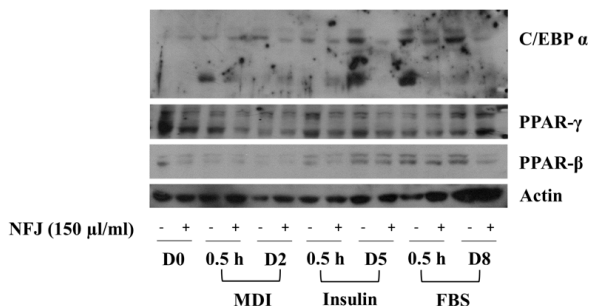


**Fig. 2.** Effects of noni fruit juice (NFJ) at different concentrations on lipid accumulation, triglyceride (TG) content, and cell viability in differentiating 3T3-L1 preadipocytes. (A, B) To induce adipocyte differentiation, 3T3-L1 preadipocytes were grown in induction medium containing MDI, insulin, and fetal bovine serum for 8 days with or without of NFJ at the selected concentrations. On day 8 (D8), cell viability was calculated using MTS assay (A) and the intracellular lipid accumulation was estimated by Oil Red O staining (B) ( $\times 100$ ). Values are mean  $\pm$  standard error (SE) of data from three independent experiments with triplicate. (C) Measurement of TG content in 3T3-L1 adipocytes (D8) that were grown in the absence or presence of NFJ at the designated concentrations by AdipoRed assay. Each experiment was conducted independently in triplication. Results are mean  $\pm$  SE of three independent experiments.

content on D8 of differentiation in 3T3-L1 cells, collated with the mock-treated cells. Thus, in consequence of strong reductive effects on fat accumulation and TG content without significant cytotoxicity, 150  $\mu\text{L}/\text{mL}$  of NFJ concentration was selected for further studies.

### 3. NFJ treatment at 150 $\mu\text{L}/\text{mL}$ significantly inhibits the protein expression of C/EBP- $\alpha$ and PPAR- $\beta$ in 3T3-L1 preadipocyte differentiation

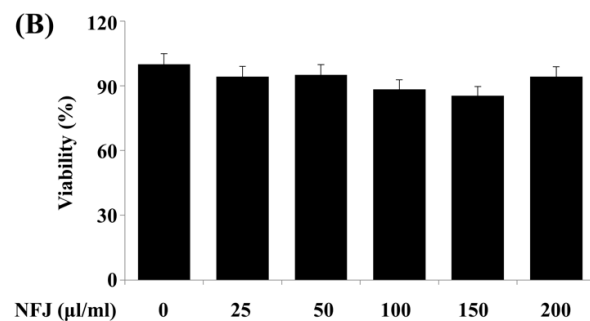
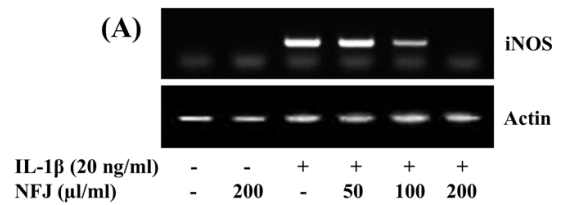
Next, to discover molecular mechanisms underlying the NFJ's lipid-reducing effects, 3T3-L1 cells were grown in the differentiation induction media either with or without presence NFJ at 150  $\mu\text{L}/\text{mL}$  for 8 days and further subjected for protein expression levels measurement of known associated adipogenic transcription factors in control or NFJ-treated 3T3-L1 cells by using Immunoblotting analysis. Distinctly, as shown in Fig. 3, while treatment with NFJ (150  $\mu\text{L}/\text{mL}$ ) had no effect on protein expression levels of PPAR- $\gamma$  throughout the adipogenesis in 3T3-L1 cells, it highly reduced C/EBP- $\alpha$  protein expression levels. Treatment with NFJ also greatly reduced the protein expression levels of PPAR- $\beta$  during differentiation D5 and D8 of 3T3-L1 adipogenesis. Total expression levels of  $\beta$ -actin proteins remained unchanged under these studies.



**Fig. 3.** Effects of noni fruit juice (NFJ) at 150  $\mu\text{L}/\text{mL}$  on the expression of CCAAT/enhancer-binding protein- $\alpha$  (C/EBP- $\alpha$ ), peroxisome proliferator-activated receptor (PPAR)- $\beta$ , and PPAR- $\gamma$  in differentiating 3T3-L1 preadipocytes. To induce adipocyte differentiation, 3T3-L1 preadipocytes were grown in induction medium containing MDI, insulin, and fetal bovine serum (FBS) for 8 days with or without of NFJ at 150  $\mu\text{L}/\text{mL}$ , and harvested at day 2 (D2), D5, and D8, subsequently. Whole-cell lysates at the selected time point were collected and analyzed by Western blot analysis with relevant antibodies.

### 4. NFJ treatment at 100 or 150 $\mu\text{L}/\text{mL}$ vastly suppresses the pro-inflammatory cytokine IL-1 $\beta$ -induced iNOS expression in 3T3-L1 preadipocytes

To see any anti-inflammatory effect, we next investigated whether IL-1 $\beta$  at 20 ng/mL induces expression of iNOS in 3T3-L1 preadipocytes and treatment with NFJ at different concentrations could interfere with it. As shown in Fig. 4A, IL-1 $\beta$  treatment for 4 hours maximally induced the iNOS mRNA expression in 3T3-L1 preadipocytes. Of note, NFJ treatment at 100 or 150  $\mu\text{L}/\text{mL}$  greatly suppressed the cytokine-induced the mRNA expression of iNOS in 3T3-L1 preadipocytes. Under these experimental conditions, results of MTS assay revealed no cytotoxic effects on 3T3-L1 preadipocytes by NFJ at dosed tested (Fig. 4B).



**Fig. 4.** Effects of noni fruit juice (NFJ) on the interleukin (IL)-1 $\beta$ -induced inducible nitric oxide synthase (iNOS) expression in 3T3-L1 preadipocytes. (A) 3T3-L1 cells were incubated without or with IL-1 $\beta$  in the absence or presence of NFJ at the designated doses for 4 hours. Total cellular RNA from the conditioned cells was extracted and analyzed by reverse transcriptase polymerase chain reaction for measurement of the mRNA expression levels of iNOS or actin. (B) 3T3-L1 cells were incubated without or with IL-1 $\beta$  in the absence or presence of NFJ at the designated doses for 4 hours, followed measurement of cell viability by a MTS assay. Values are mean $\pm$ standard error of results from three independent experiments with triplicate.

## Discussion

White adipose tissue (WAT) regulates energy homeostasis storage through adipogenesis<sup>3)</sup>. However, excessive fat accumulation has been reported to stimulate inflammatory cascades in WAT, leading to aggravate AT impairment<sup>25,26)</sup>. Thus, any inhibitor of excessive preadipocyte differentiation and obesity inflammation may hold promising effect as an anti-obesity agent.

Aforementioned, NFJ is a herbal remedy that has been known over decades to improve and treat various symptoms, including inflammation, lipid-lowering, and anti-bacterial<sup>27-29)</sup>. Due to its bitter flavor, many companies commercially developed flavored NFJ to make it better-taste product<sup>30)</sup>. However, presently, the anti-obesity effect and mode of action of NFJ in fat cells are still poorly understood. Here, I provide evidence suggesting for the first time, that NFJ at 150  $\mu\text{L}/\text{mL}$  has strong anti-adipogenic effect on differentiating 3T3-L1 cells, which is mediated through the modulation of C/EBP- $\alpha$  and PPAR- $\beta$  expression levels.

It has been previously demonstrated that aqueous leaf extract of Noni reduces abdominal fat and triacylglycerols in Wistar rats with metabolic syndrome, exhibiting its lipid-lowering effect<sup>29)</sup>. Other study also revealed that NFJ has anti-diabetic and anti-lipidemic effects<sup>31)</sup>. In agreement with it, treatment with NFJ at 150  $\mu\text{L}/\text{mL}$  herein vastly inhibits intracellular fat accumulation and TG stores with no cytotoxicity on D8 of differentiation in 3T3-L1 cells, as confirmed through Oil Red O staining, AdipoRed assay, and cell viability analysis. These results elucidate that NFJ at 150  $\mu\text{L}/\text{mL}$  has strong lipid-lowering effects throughout 3T3-L1 adipogenesis.

Previous studies have implicated that 3T3-L1 preadipocytes into adipocytes differentiation process mainly associated with the expression and activation of multiple adipogenic transcription factors, such as C/EBP- $\alpha$ , C/EBP- $\beta$ , and PPARs<sup>32,33)</sup>. Previous studies have further elucidated that C/EBP- $\beta$  and C/EBP- $\delta$  play a role at initiation stage of adipogenesis, leading to the activation of C/EBP- $\alpha$  and PPAR- $\gamma$ , which are the key regulators of middle and terminal stages of pre-

adipocyte differentiation<sup>34-36)</sup>. However, up to date, the NFJ regulation of C/EBP- $\alpha$ , PPAR- $\beta$ , and PPAR- $\gamma$  in adipocytes differentiation are unknown. Of note, the present study shows the ability of NFJ at 150  $\mu\text{L}/\text{mL}$  to selectively down-regulate C/EBP- $\alpha$  and PPAR- $\beta$  protein expression levels throughout 3T3-L1 cells on D5 and D8. These results thus point out that the NFJ's lipid-reducing effect during 3T3-L1 cells adipogenesis is not through controlling of the expression of PPAR- $\gamma$  but via the reduction of C/EBP- $\alpha$  and PPAR- $\beta$  expression levels.

To my best knowledge, this is the first study to exhibit the NFJ's lipid-lowering mechanism through down-regulation of C/EBP- $\alpha$  and PPAR- $\beta$  in fat cell adipogenesis. Of note, previous in vivo and in vitro studies also have shown that kaempferol, a flavonoid isolated from the fruit and leaves of *M. citrifolia*, inhibits the expressions several pro-inflammatory cytokines including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-6 in 3T3-L1 adipocytes and WAT of high fat diet-obese mice, which is mediated through downregulation of adipogenesis-related genes, such as C/EBP- $\alpha$ , FAS, and others<sup>37)</sup>. Of interest, previous in vivo study shown that high-fat dietary hamsters supplemented with NFJ had low levels of liver iNOS, cyclooxygenase-2, TNF- $\alpha$ , and IL-1 $\beta$  expressions compared to those without NFJ<sup>38)</sup>. However, until now, NFJ regulation of IL-1 $\beta$ -induced expression of iNOS in 3T3-L1 preadipocytes has not yet reported. In the present study, of interest, NFJ is able to largely interfere with the IL-1 $\beta$ -induced iNOS mRNA expression in 3T3-L1 cells in a dose-dependent manner. These results thus advocate the NFJ's anti-inflammatory effect on preadipocytes, which may further contribute to its anti-obesity effect.

In addition, noni is usually found in several tropical climate regions of the USA, such as Hawaii, to Brazil, reaching Tahitian, Malaysia, and Fiji Islands. Notably, there is compelling evidence showing that noni and its products, including NFJ used herein, possess a clear Chinese medicine property, further laying a theoretical foundation for the compatibility of noni and its products with traditional Chinese medicines, which can enrich traditional Chinese medicine resources and promote the development of a new and in-

novative product composed of Chinese medicine and noni or its products<sup>39,40</sup>). This notion may be supported by many known biological and beneficial effects of noni and its products that can tonify the kidney and liver, strengthen tendons and bone, and improve many other human illnesses<sup>39</sup>. Given that the disease-modifying effects of Korean medicine have a high similarity with those of Chinese medicine, it is further likely that there would be a theoretical foundation for the compatibility of Korean medicine and noni or its products, which can boost the disease-modifying effects of Korean medicine, enrich Korean medicine resources, and challenge the development of a new and innovative product composed of Korean medicine and noni or its products. Hence, by knowing this concept and the NFJ's anti-adipogenic and anti-inflammatory effects herein, it is worthy to state the meaning or significance of the present study in advocating that exotic natural products like NFJ herein may/can be used as an adjuvant therapy or constituent with Korean medicine in boosting or elevating the anti-obesity effect of Korean medicine, although there remains to test or evaluate the efficacy of combined treatments (Korean medicine with NFJ) in proper fat cell culture and obese animal models.

## Conclusion

The present study demonstrates firstly that NFJ has strong anti-adipogenic and anti-inflammatory effects in (differentiating) 3T3-L1 preadipocytes, that are partly mediated through the reduction of C/EBP- $\alpha$ , PPAR- $\beta$ , and iNOS expression. This study suggests NFJ as an alternative substance to prevent or treat obesity by modulating fat accumulation, TG synthesis, and inflammation in fat cells.

## Conflict of interest

No potential conflict of interest relevant to this article was reported.

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