J Vet Sci. 2021 Jul;22(4):e55
https://doi.org/10.4142/jvs.2021.22.e55
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Original Article

Molecular and Cellular Biology

Check for updates

Restoration of the adipogenic gene expression by naringenin and naringin in 3T3-L1 adipocytes

Lakshi A. Dayarathne, Sachithra S. Ranaweera, Premkumar Natraj, Priyanka Rajan, Young Jae Lee, Chang-Hoon Han *

College of Veterinary Medicine, Jeju National University, Jeju 63243, Korea

OPEN ACCESS

Received: Apr 13, 2021
Revised: May 29, 2021
Accepted: Jun 20, 2021
Published online: Jul 1, 2021

***Corresponding author:**
Chang-Hoon Han
College of Veterinary Medicine, Jeju National University, 102 Jejudaehang-ro, Jeju 63243, Korea.
E-mail: chhan@jejunu.ac.kr

ORCID iDs
Lakshi A. Dayarathne
https://orcid.org/0000-0001-5679-4952
Sachithra S. Ranaweera
https://orcid.org/0000-0001-9589-3240
Premkumar Natraj
https://orcid.org/0000-0003-2615-3809
Priyanka Rajan
https://orcid.org/0000-0002-0638-5808
Young Jae Lee
https://orcid.org/0000-0001-7892-5546
Chang-Hoon Han
https://orcid.org/0000-0002-0763-9590

Funding
This work was supported by Korean Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through

ABSTRACT

Background: Naringenin and its glycoside naringin are well known citrus flavonoids with several therapeutic benefits. Although the anti-adipogenic effects of naringenin and naringin have been reported previously, the detailed mechanism underlying their anti-adipogenesis effects is poorly understood.
Objectives: This study examined the anti-adipogenic effects of naringenin and naringin by determining differential gene expression patterns in these flavonoids-treated 3T3-L1 adipocytes.
Methods: Lipid accumulation and triglyceride (TG) content were determined by Oil red O staining and TG assay. Glucose uptake was measured using a 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-d-glucose fluorescent d-glucose analog. The phosphorylation levels of AMP-activated protein kinase (AMPK) and acetyl Co-A carboxylase (ACC) were observed via Western blot analysis. Differential gene expressions in 3T3-L1 adipocytes were evaluated via RNA sequencing analysis.
Results: Naringenin and naringin inhibited both lipid accumulation and TG content, increased phosphorylation levels of both AMPK and ACC and decreased the expression level of 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) in 3T3-L1 adipocytes. RNA sequencing analysis revealed that 32 up-regulated (> 2-fold) and 17 down-regulated (< 0.6-fold) genes related to lipid metabolism, including *Acaca*, *Fasn*, *Scd1*, *Mogat1*, *Dgat*, *Lipin1*, *Cpt1a*, and *Lepr*, were normalized to the control level in naringenin-treated adipocytes. In addition, 25 up-regulated (> 2-fold) and 25 down-regulated (< 0.6-fold) genes related to lipid metabolism, including *Acaca*, *Fasn*, *Fabp5*, *Scd1*, *Srebf1*, *Hmgcs1*, *Cpt1c*, *Lepr*, and *Lrp1*, were normalized to the control level by naringin.
Conclusions: The results indicate that naringenin and naringin have anti-adipogenic potentials that are achieved by normalizing the expression levels of lipid metabolism-related genes that were perturbed in differentiated 3T3-L1 cells.

Keywords: Naringenin; naringin; adipogenesis; gene expression; RNA sequencing

INTRODUCTION

Obesity is a serious consequence of prolonged disruption of energy homeostasis caused by an imbalance of energy intake and expenditure [1]. In that imbalance, surplus energy

Innovational Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (grant No. 11901303).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Han CH, Dayarathne LA; Data curation: Ranaweera SS; Funding acquisition: Han CH; Investigation: Dayarathne LA, Han CH; Resources: Han CH; Software: Natraj P, Rajan P; Supervision: Han CH, Lee YJ; Visualization: Dayarathne LA, Han CH; Writing - original draft: Dayarathne LA; Writing - review & editing: Natraj P, Rajan P.

is stored as a lipid, specifically triglyceride (TG), in white adipose tissues [2]. Obesity is a major contributor to the global burden of chronic diseases and complications, including cardiovascular diseases, diabetes, and cancers [3].

AMP-activated protein kinase (AMPK) is an energy sensor that regulates glucose and lipid metabolism to modulate energy homeostasis in the body. The activation of AMPK inhibits preadipocyte differentiation and suppresses transcription factors, including peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT/enhancer-binding protein alpha (C/EBPα), and sterol regulatory element-binding protein 1 (SREBP-1c), which are necessary for adipogenesis [4]. In addition, the activation of AMPK inactivates key metabolic enzymes, including acetyl Co-A carboxylase (ACC) and 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (HMGCR), involved in fatty acid and cholesterol synthesis [5]. Therefore, AMPK is considered a target in the treatment of obesity and associated metabolic diseases.

Previous studies investigated anti-adipogenic agents as potential therapeutics for preventing obesity and associated disorders [6]. Recently, the use of natural bioactive compounds as alternative methods for treating obesity and related diseases is increasing because of their low risks of side effects compared to those of synthetic drugs [4].

Flavonoids are plant-derived compounds with multiple therapeutic effects, including anti-hyperglycemic and anti-hyperlipidemic activities [7]. Flavonoids mediate these biological effects via cell signaling pathways that are based on molecular interactions with numerous enzymes [8]. For naringin or its aglycon naringenin, which are citrus-derived flavonoids, diverse biological activities of therapeutic interests have been described, including anti-diabetic and anti-dyslipidemic effects [9]. However, the detailed mechanism associated with the actions of naringenin and naringin on anti-adipogenesis has not yet been fully described.

In this context, the present study hypothesized that naringenin and naringin exert anti-adipogenic potential by normalizing the activities of key enzymes related to lipid metabolism and restoring the expression of genes related to lipid metabolism to a normal level. To that end, the effects of naringenin and naringin on the phosphorylation levels of AMPK, ACC, and the expression levels of HMGCR were observed. In particular, RNA sequencing analysis was performed to examine the anti-adipogenic effects of naringenin and naringin, in which they normalized the expressions of lipid metabolism-related genes that had been perturbed in differentiated 3T3-L1 adipocytes.

MATERIALS AND METHODS

Induction of adipocyte differentiation and incubation with test compounds

The 3T3-L1 fibroblasts (KCLB 42835) obtained from the Korean Cell Line Bank (KCLB, Korea) were cultured Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (1% PS) (Gibco, USA) in a humidified atmosphere containing 5% CO_2 at 37°C.

The 3T3-L1 cells were cultured in 96-well plates (1×10^5 cells/well), and after reaching post-confluence, cells were induced for differentiation for 48 h by adding differentiation initiation media (DIM) containing DMEM, 1% PS, 10% fetal bovine serum (FBS; Gibco), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, USA), 0.5 μM dexamethasone (Sigma),

2 μM rosiglitazone (Sigma) and 10 μg/mL insulin (Sigma). Subsequently, the DIM was changed to a normal medium (DMEM containing 1% PS and 10% FBS) containing 10 μg/mL insulin. Media were changed to normal growth media after 48 h and every 2 days thereafter until differentiation to mature adipocytes. To examine the effects of test samples on lipid accumulation, cells were cultured in DIM with or without the following test samples: 5 μM of simvastatin (Sigma) and different concentrations of naringenin and naringin (Sigma) diluted in DMSO until differentiation to mature adipocytes. At day 8, completely differentiated adipocytes were assessed for lipid accumulation through Oil red O staining and Western blot analysis.

Monitoring cellular viability: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The 3T3-L1 cells were seeded in 96-well plates at the cell density of 1×10^5 cells/well and cultured overnight using routine culture media. After reaching confluence, the cells were treated with different concentrations (0–100 μM) of the naringenin and naringin flavonoids for 48 h. Cell culture media was removed and fresh media containing 10% Ez-Cytox (DogenBio, Korea) was added into each well, according to the manufacturer's instructions. Plates were incubated for 3 h at 37°C and 5% CO_2. Cell viability, as indicated by formazan production, was measured with an enzyme-linked immunosorbent assay microplate reader (TECAN, Austria) at 450 nm wavelength.

Oil red O staining

Post-confluence preadipocytes were differentiated into adipocytes as described above. According to a previously described method, on day 8 after adipocyte differentiation, cells were stained with Oil red O [10] with slight modifications. The fully differentiated adipocytes were fixed in 10% (v/v) formaldehyde (Biosesang, Korea) for 1 h at room temperature. Next, the cells were rinsed twice with phosphate-buffered saline (PBS; Gibco) and stained with 0.5% Oil red O solution (Sigma) (60% of Oil red O stock solution and 40% distilled water) for 1 h in the dark. The cells were washed with distilled water to remove the unbound dye, and images were captured using an IncuCyte ZOOM (Essen BioScience, USA) at 20× magnification. Quantitative analysis was performed using IncuCyte ZOOM processing software.

Measurement of TG content

Cellular TG contents were quantified using a commercially available colorimetric TG assay kit (BioAssay Systems, USA). Differentiated 3T3-L1 cells at day 8 were washed with PBS and harvested to cell lysis buffer containing 5% Triton X100 (Bio-Rad Laboratories, USA). Lysed cells were homogenized and centrifuged at 3,000 × *g* for 5 min. The TG content of the diluted supernatants was analyzed according to the manufacturer's instructions. The protein concentration of each sample was measured using the Bio-Rad DC protein assay (Bio-Rad Laboratories). TG contents were normalized to the respective protein concentration as detected by using bovine serum albumin (Sigma) as the calibration standard.

2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-d-glucose (2-NBDG) glucose uptake assay

Glucose uptake assays were performed on fully differentiated 3T3-L1 adipocytes. Briefly, preadipocytes were cultured in 96-well plates and induced to differentiate using the DIM protocol described above. Adipocytes were serum-starved for 12 h and then incubated with samples for 24 h. The cells were incubated with 40 μM 2-NBDG (Carlsbad, USA) for 30 min at 37°C. Cells were then washed 3 times with ice-cold PBS, and fluorescent images were obtained using an IncuCyte ZOOM fluorescence microscope (Essen BioScience). IncuCyte

ZOOM fluorescence processing software (Essen BioScience) was used to analyze the total fluorescence intensities of each well.

Western blot analysis

Cells were washed with PBS and lysed with ice-cold RIPA buffer containing protease inhibitor mixture. The whole-cell lysates were centrifuged at 12,000 rpm for 10 min. The supernatant was separated, and the amount of protein determined by Bradford assay (Bio-Rad Laboratories). Equal amounts of protein was mixed with 20% loading buffer, separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis, and subjected to Western blot with AMPK (Cell Signaling Technology, USA), ACC (Cell Signaling Technology), p-AMPK (Cell Signaling Technology), and p-ACC (Cell Signaling Technology), and β-actin (Thermo Fisher, USA) antibodies. A chemiluminescence bioimaging instrument (NeoScience, Korea) was used to detect the proteins of interest. Densitometry analysis was performed using the ImageJ analysis software.

RNA sequencing analysis

RNA sequencing analysis was performed as previously described [11]. Briefly, total RNA was isolated from 3T3-L1 adipocytes using an Easy-blue RNA extraction kit (iNtRON Biotechnology, Korea). RNA quality was assessed by Agilent 2100 bioanalyzer using the RNA 6000 Nano Chip (Agilent Technologies, Netherlands). Based on the manufacturer's instructions, libraries for RNAs were constructed using Ouantseq 3′mRNA-Seq Library Prep Kit (Lexogen, Austria). High-throughput sequencing was performed as single-end 75 sequencings using the NextSeq 500 (Illumina, USA). QuantSeq 3′mRNA-Seq reads were aligned using Bowtie2 version 2.1.0 [12]. Differentially expressed genes were determined based on counts from unique and multiple alignments using EdgeR within R version 3.2.2 and Bioconductor version 3.0 [13]. The RT (read count) data were processed based on the quantile normalization method using the Genowiz version 4.0.5.6 (Ocimum Biosolutions, India). Gene classification was performed using the Medline database (National Center for Biotechnology Information, USA).

Statistical analysis

Values were expressed as means ± SE of 3 independent experiments. Data were statistically analyzed with the aid of IBM SPSS Statistics (Ver.17.0; IBM, USA). The statistical differences among groups were assessed using a 1-way analysis of variance followed by Tukey's test. The $p < 0.05$, $p < 0.005$, and $p < 0.0005$ indicate statistically significant differences from the differentiated cell control group.

RESULTS

Cell viability of 3T3-L1 cells

The MTT assay was performed to assess the effect of naringenin and naringin on 3T3-L1 cell viability (**Fig. 1D and E**). Significant cellular toxicity was not observed for up to 25 μM concentrations of both naringenin and naringin in 3T3-L1 cells. Accordingly, the following experiments were conducted using non-toxic concentrations (10 and 20 μM) of the 2 flavonoids.

Lipid accumulation and TG content

The effects of naringenin and naringin on lipid accumulation and TG content in 3T3-L1 adipocytes were observed by Oil red O staining and TG assay (**Fig. 2**). Differentiated

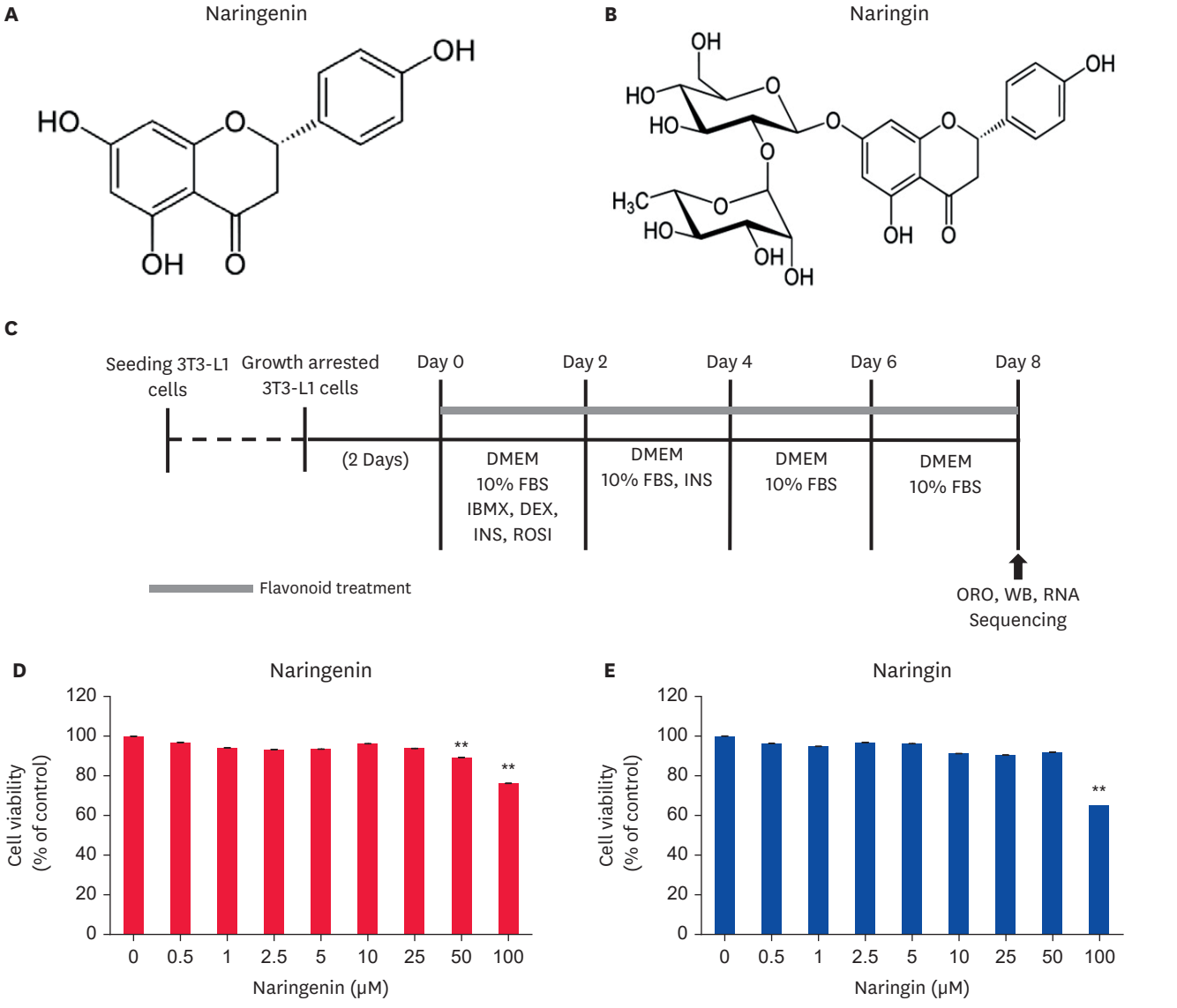

Fig. 1. Effect of naringenin and naringin on 3T3-L1 cell viability. Chemical structure of naringenin (A) and naringin (B). Differentiation procedure and assay schedule (C). Effect of naringenin (D) and naringin (E) on 3T3-L1 cell viability. The 3T3-L1 cells were cultured at a density of 1×10^5 in 96-well plates; after reaching confluence, cells were treated with 0–50 μM of the flavonoids for 48 h, and cell viability was measured by MTT assay (C). Values represent mean ± SE.
DMEM, Dulbecco's modified Eagle's medium; IBMX, 3-isobutyl-1-methylxanthine; DEX, dexamethasone; INS, insulin; ROSI, rosiglitazone; FBS, fetal bovine serum; ORO, Oil red O; WB, Western blot; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
**$p < 0.005$ vs. the control.

adipocytes significantly increased lipid accumulation and TG content compared to those of undifferentiated adipocytes (**Fig. 2B and C**). In contrast, treatment with simvastatin (positive control) and the higher concentrations (20 μM) of naringenin and naringin significantly inhibited ($p < 0.0005$) both lipid accumulation and TG content (**Fig. 2B and C**). The treatment of naringin shows more significant effectiveness ($p < 0.0005$) in inhibiting lipid accumulation and TG content of 3T3-L1 adipocytes than naringenin even at the low concentration (10 μM). Therefore, the results suggest that both naringenin and naringin suppress both lipid accumulation and TG content in differentiated 3T3-L1 adipocytes.

https://doi.org/10.4142/jvs.2021.22.e55

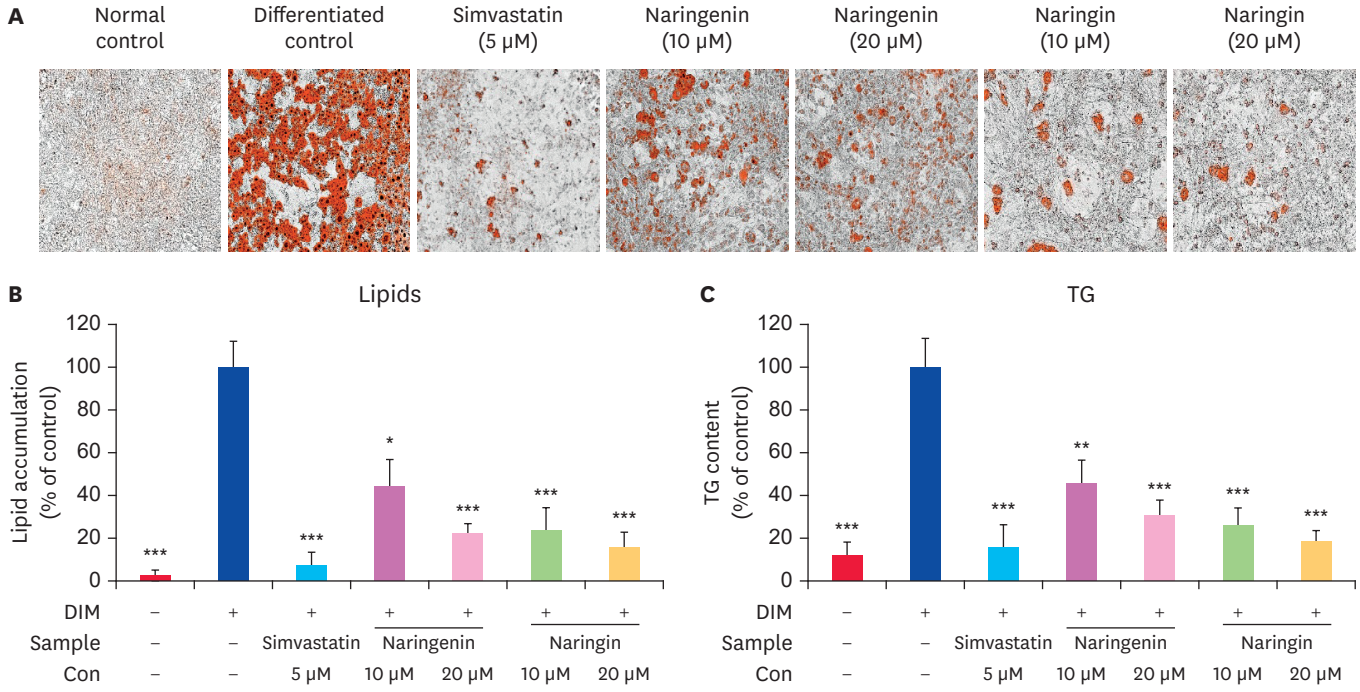

Fig. 2. Effect of naringenin and naringin on intracellular lipid accumulation. Cells were differentiated and treated with samples as described in material and methods. Lipid accumulation was measured by Oil red O staining on day 8. Representative images were captured using the IncuCyte ZOOM at 20× magnification (A). Quantitative analysis of lipid accumulation in 3T3-L1 adipocyte calculated by IncuCyte ZOOM fluorescence processing software (B). Effect of naringenin and naringin on TG accumulation in 3T3-L1 adipocytes. Total intracellular TG concentration was determined using commercial assay kits (C). Values represent mean ± SE.
DIM, differentiation initiation media; TG, triglyceride; Con, concentration.
$^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.0005$ vs. the differentiated control.

Glucose uptake in 3T3-L1 adipocytes

The glucose uptake effects of naringenin and naringin in 3T3-L1 adipocytes were assessed using the 2-NBDG glucose uptake assay (**Fig. 3**). Differentiated 3T3-L1 adipocytes showed slightly increased glucose uptake when compared with undifferentiated adipocytes. However, simvastatin treatment significantly increased ($p < 0.005$) glucose uptake compared to that of the differentiated control. In particular, both naringenin and naringin significantly increased ($p < 0.005$) glucose uptake compared to the differentiated control when used at the 20 μM concentration (**Fig. 3A and B**). Naringin at the low concentration (10 μM) also produced a significant increase in glucose uptake ($p < 0.005$); however, that was not observed with naringenin treatment at the low concentration. These results indicate that both naringenin and naringin stimulate cellular glucose uptake in differentiated 3T3-L1 adipocytes.

Phosphorylation levels of AMPK pathway molecules

Western blot analysis was conducted to observe the effects of naringenin and naringin on the regulation of key enzymes in the AMPK signaling pathway (**Fig. 4**). The phosphorylation levels of both AMPK (Thr172) and ACC (Ser79) in 3T3-L1 adipocytes were significantly decreased ($p < 0.05$) in differentiated adipocytes compared to undifferentiated adipocytes. In contrast, the phosphorylation levels of both AMPK (Thr172) and ACC (Ser79) were significantly increased by the treatment of simvastatin, naringenin, and naringin in differentiated 3T3-L1 adipocytes (**Fig. 4A and B**). The effects of naringin on the phosphorylation levels of AMPK (Thr172) and ACC (Ser79) were greater ($p < 0.0005$) than those of naringenin and were similar to those of simvastatin. In addition, compared to the differentiated control treatment, naringenin and naringin treatment significantly reduced

https://doi.org/10.4142/jvs.2021.22.e55

Journal of Veterinary Science

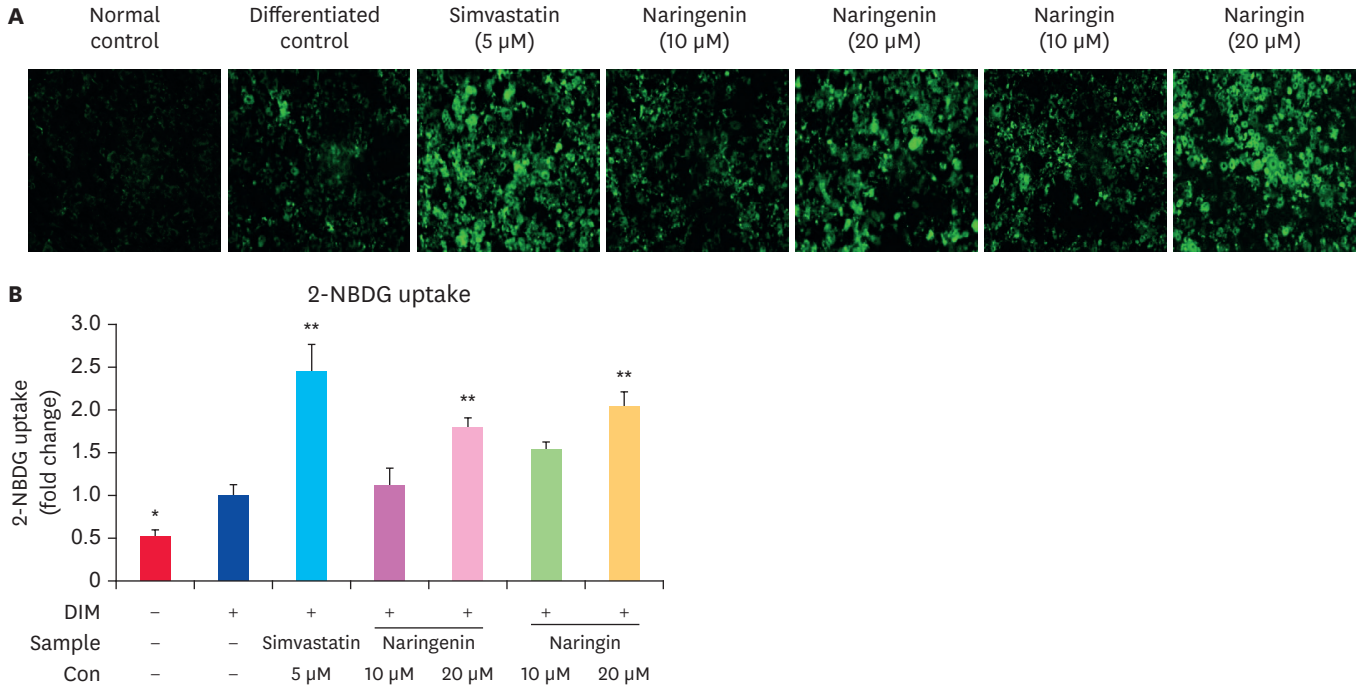

Fig. 3. Effect of naringenin and naringin on glucose uptake. Glucose uptake was assessed by a fluorescent probe, 2-NBDG. Differentiated 3T3-L1 cells were incubated with Flavonoids for 24 h, then incubated with 2-NBDG (40 uM) for 30 min. Cells were washed with PBS 3 times, and images were obtained using an IncuCyte ZOOM fluorescence microscope at 20× magnification (A). Total fluorescence intensity was calculated using IncuCyte ZOOM fluorescence processing software (B). Values represent mean ± SE.
DIM, differentiation initiation media; 2-NBDG, 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-d-glucose; PBS, phosphate-buffered saline; Con, concentration.
*p < 0.05 and **p <0.005 vs. the differentiated control.

(p < 0.0005) the protein expression of HMGCR (**Fig. 4C**). The results suggest that both naringenin and naringin activate the AMPK signaling pathway in 3T3-L1 adipocytes.

Differential gene expression

RNA sequencing analysis was performed to observe the effects of naringenin and naringin on the expression levels of genes related to lipid metabolism in 3T3-L1 adipocytes. Based on the results of gene ontology analysis, a large proportion of the genes related to lipid metabolism was either up- or down-regulated in naringin and naringenin-treated 3T3-L1 adipocytes (**Fig. 5**). Thirty-two up-regulated (> 2-fold) genes, including *Scd1*, *Scd3*, *Elovl3*, *Ppara*, *Lipin 1*, and *Cebpa*, and 17 down-regulated (< 0.6-fold) genes, including *Cpt1a*, *Lrp1*, *Lrp5*, *Adh7*, and *Lepr* were normalized to the control level in naringenin-treated adipocytes (**Tables 1** and **2**). In addition, 25 up-regulated (> 2-fold) genes, including *Scd1*, *Apoa4*, *Ppara*, *Cebpa*, *Fabp5*, and *G6pc* and 25 down-regulated (< 0.6-fold) genes, including *Pitpnc1*, *Spns2*, *Cpt1c*, *Lepr*, and *Pigv* were normalized to the control level in naringin-treated adipocytes (**Tables 3** and **4**).

Search Tool for the Retrieval of Interacting Genes/Proteins analysis was performed to identify protein-protein interactions (PPIs) among the normalized genes induced by naringenin and naringin, and the results were visualized as a set of nodes and edges (**Fig. 6A and B**). The proteins related to lipid metabolism, including *Acaca*, *Fasn*, *Scd1*, *Mogat1*, *Dgat*, and *Lipin1*, were closely localized and interacted directly with each other within the PPI network of naringenin. In addition, the normalized down-regulated genes, including *Cpt1a*, *Lepr*, and *Lrp1*, were closely localized in the functional hub in the PPI network of naringenin. In the naringin PPI network, the normalized up-regulated genes, including *Acaca*, *Fasn*, *Fabp5*, *Scd1*, *Srebf1*, and

https://doi.org/10.4142/jvs.2021.22.e55

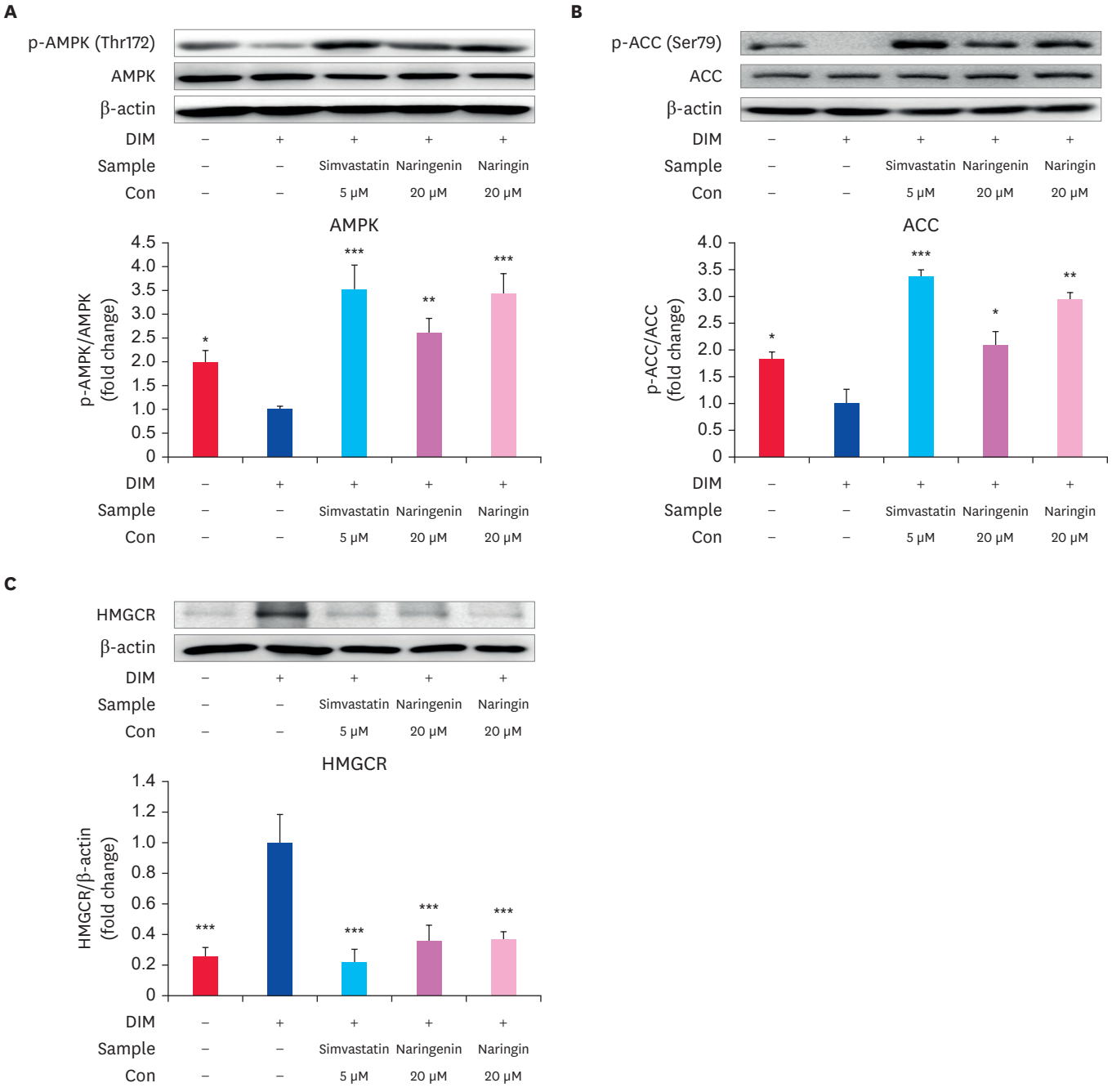

Fig. 4. Effects of naringenin and naringin on the expression of AMPK pathway molecules. The 3T3-L1 preadipocytes were differentiated in the absence or presence of flavonoids for 8 days. The p-AMPK and p-ACC and the expression of HMGCR were then determined by Western blot analysis. Immunoblots of p-AMPK (A), p-ACC (B), and HMGCR (C). Relative protein levels were quantified using densitometry analysis. Values represent mean ± SE.
p-AMPK, phosphorylation of AMP-activated protein kinase; p-ACC, phosphorylation of acetyl Co-A carboxylase; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; DIM, differentiation initiation media; Con, concentration.
$^{*}p < 0.05$, $^{**}p < 0.005$ and $^{***}p < 0.0005$ vs. the differentiated control.

Hmgcs1, were closely localized and formed a functional cluster. In addition, normalized down-regulated genes, including *Cpt1c*, *Lrp1*, and *Lepr*, were located in the functional hub of the naringin PPI network. Overall, RNA sequencing analysis reveals that naringenin and naringin normalize the expression levels of lipid metabolism-related genes that were perturbed in differentiated 3T3-L1 adipocytes.

https://doi.org/10.4142/jvs.2021.22.e55

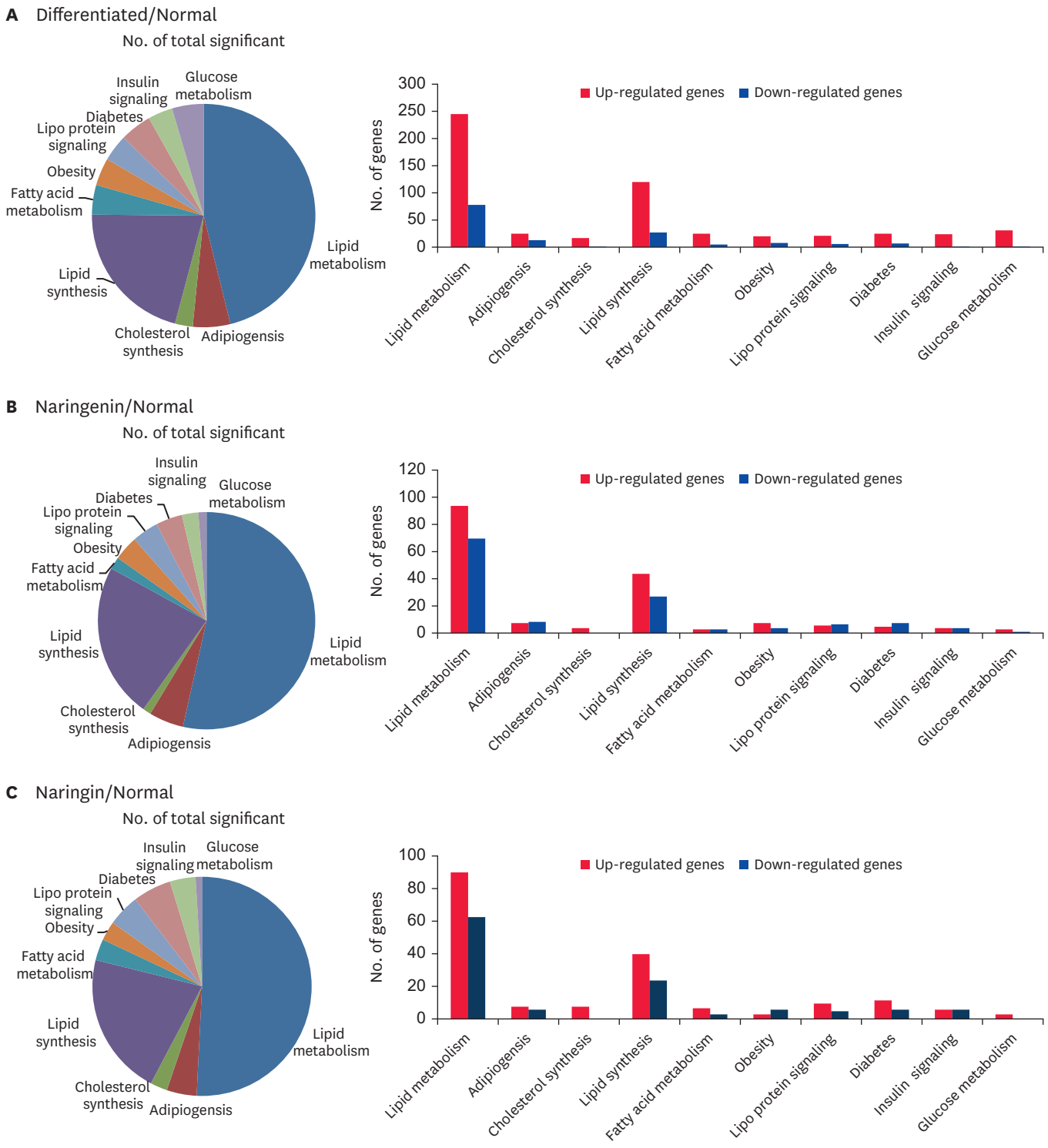

Fig. 5. Effects of naringenin and naringin on differential gene expression. Gene ontology analysis of 3T3-L1 adipocytes compared to normal cells (A). Naringenin-treated 3T3-L1 adipocytes compared to normal cells (B). Naringin-treated 3T3-L1 adipocytes compared to normal cells (C). The pie chart indicates functional categorization of the differentially expressed genes in 3T3-L1 adipocytes, and the bar graph represents the number of genes that were up- and down-regulated.

https://doi.org/10.4142/jvs.2021.22.e55

Table 1. Up-regulated genes normalized by naringenin in 3T3-L1 adipocytes

Gene symbol	Differentiated/normal	Simvastatin/normal	Naringenin/normal	Gene name
Scd3	188.817	87.202	1.031	Stearoyl-coenzyme A desaturase 3
Elovl3	110.594	74.751	1.002	Elongation of very long chain fatty acids like 3
Scd1	95.233	86.417	1.160	Stearoyl-coenzyme A desaturase 1
Ppara	77.170	39.919	1.578	Peroxisome proliferator-activated receptor alpha
Lpin1	25.118	21.260	1.283	Lipin 1
Apoa4	21.349	7.713	1.001	Apolipoprotein A-IV
Mogat1	19.342	16.389	1.001	Monoacylglycerol O-acyltransferase 1
Cebpa	18.250	12.830	1.182	CCAAT/enhancer-binding protein, alpha
Cyp2f2	15.210	14.921	1.081	Cytochrome P450, family 2, subfamily f, polypeptide 2
Dgat1	10.944	9.938	1.444	Diacylglycerol O-acyltransferase 1
Pla2g5	7.756	5.796	1.001	Phospholipase A2, group V
Acsf2	6.340	4.254	1.071	Acyl-coa synthetase family member 2
Cyp27a1	5.879	1.000	1.000	Cytochrome P450, family 27, subfamily a, polypeptide 1
G6pc	5.879	1.000	1.000	Glucose-6-phosphatase, catalytic
Pparg	5.716	5.128	1.383	Peroxisome proliferator-activated receptor gamma
Dbi	5.654	7.595	1.082	Diazepam binding inhibitor
Adipor2	5.577	4.734	1.244	Adiponectin receptor 2
Fasn	5.001	3.137	1.267	Fatty acid synthase
Awat1	4.885	3.898	1.000	Acyl-coa wax alcohol acyltransferase 1
Hacd2	4.571	4.082	0.945	3-hydroxyacyl-coa dehydratase 2
Acox1	4.282	4.007	1.042	Acyl-Coenzyme A oxidase 1, palmitoyl
Hmgcs1	3.370	3.956	1.214	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
Pik3r6	2.950	1.000	1.000	Phosphoinositide-3-kinase, regulatory subunit 6
Cyp3a57	2.950	1.000	1.000	Cytochrome P450, family 3, subfamily a, polypeptide 57
Mogat2	2.950	1.000	1.000	Monoacylglycerol O-acyltransferase 2
Apoa2	2.944	1.000	1.000	Apolipoprotein A-II
Scp2	2.776	2.641	0.818	Sterol carrier protein 2, liver
Oxsm	2.656	2.235	0.936	3-oxoacyl-ACP synthase, mitochondrial
Fads2	2.242	1.908	1.197	Fatty acid desaturase 2
Cebpb	2.161	1.279	0.964	CCAAT/enhancer-binding protein, beta
Ppargc1a	2.101	2.743	1.129	Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
Galc	2.037	1.562	1.056	Galactosylceramidase

Table 2. Down-regulated genes normalized by naringenin in 3T3-L1 adipocytes

Gene symbol	Differentiated/normal	Simvastatin/normal	Naringenin/normal	Gene name
Akr1c14	0.151	0.296	0.792	Aldo-keto reductase family 1, member C14
Gata2	0.163	0.199	1.007	GATA binding protein 2
Npy1r	0.290	0.489	1.072	Neuropeptide Y receptor Y1
Mtmr11	0.291	0.381	0.765	Myotubularin related protein 11
Cpt1a	0.302	0.257	0.789	Carnitine palmitoyltransferase 1a
Adh7	0.316	0.635	1.050	Alcohol dehydrogenase 7 (class IV)
Pld1	0.333	0.462	0.949	Phospholipase D1
Hacd4	0.355	0.211	1.150	3-hydroxyacyl-coa dehydratase 4
Lrp1	0.379	0.387	0.797	Low density lipoprotein receptor-related protein 1
Asah1	0.383	0.467	0.850	N-acylsphingosine amidohydrolase 1
Lepr	0.394	0.984	1.224	Leptin receptor
Lrp5	0.407	0.348	0.918	Low density lipoprotein receptor-related protein 5
Lmna	0.412	0.347	0.726	Lamin A
E2f1	0.440	0.517	1.045	E2F transcription factor 1
Ccnd1	0.454	0.267	0.718	Cyclin D1
Gla	0.474	0.714	1.083	Galactosidase, alpha
Cyb5r3	0.498	0.726	1.009	Cytochrome b5 reductase 3

DISCUSSION

The present study demonstrated the anti-adipogenic effects of naringenin and naringin on differentiated 3T3-L1 adipocytes. The naringenin and naringin treatments suppressed both

https://doi.org/10.4142/jvs.2021.22.e55

Table 3. Up-regulated genes normalized by naringin in 3T3-L1 adipocytes

Gene symbol	Differentiated/normal	Simvastatin/normal	Naringin/normal	Gene name
Scd1	95.233	86.417	1.327	Stearoyl-Coenzyme A desaturase 1
Ppara	77.170	39.919	1.670	Peroxisome proliferator-activated receptor alpha
Apoa4	21.349	7.713	1.043	Apolipoprotein A-IV
Cebpa	18.250	12.830	1.161	CCAAT/enhancer-binding protein alpha
Fabp5	15.580	12.573	1.001	Fatty acid binding protein 5, epidermal
Lpl	7.613	7.816	1.152	Lipoprotein lipase
G6pc	5.879	1.000	1.012	Glucose-6-phosphatase, catalytic
Pparg	5.716	5.128	1.579	Peroxisome proliferator-activated receptor gamma
Adipor2	5.577	4.734	1.174	Adiponectin receptor 2
Fasn	5.001	3.137	1.289	Fatty acid synthase
Bmp4	3.927	1.000	1.008	Bone morphogenetic protein 4
Ghrl	3.901	3.893	1.018	Ghrelin
Hmgcs1	3.370	3.956	1.326	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
Cyp3a57	2.950	1.000	1.005	Cytochrome P450, family 3, subfamily a, polypeptide 57
Mogat2	2.950	1.000	1.005	Monoacylglycerol O-acyltransferase 2
Srebf1	2.907	2.027	1.271	Sterol regulatory element-binding transcription factor 1
Lias	2.898	2.812	1.050	Lipoic acid synthetase
Eci3	2.828	1.684	1.234	Enoyl-Coenzyme A delta isomerase 3
Socs2	2.756	2.257	1.058	Suppressor of cytokine signaling 2
G6pdx	2.737	3.247	1.264	Glucose-6-phosphate dehydrogenase X-linked
Oxsm	2.656	2.235	1.020	3-oxoacyl-ACP synthase, mitochondrial
Fads2	2.242	1.908	0.974	Fatty acid desaturase 3
Cebpb	2.161	1.279	1.055	CCAAT/enhancer-binding protein (C/EBP), beta
Ppargc1a	2.101	2.743	0.903	Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
Galc	2.037	1.562	1.256	Galactosylceramidase

Table 4. Down-regulated genes normalized by naringin in 3T3-L1 adipocytes

Gene symbol	Differentiated/normal	Simvastatin/normal	Naringin/normal	Gene name
Akr1c14	0.151	0.296	0.993	Aldo-keto reductase family 1, member C14
Pitpnc1	0.164	0.204	0.899	Phosphatidylinositol transfer protein, cytoplasmic 1
Spns2	0.280	0.232	0.775	Spinster homolog 2
Gba	0.288	0.379	0.707	Glucosidase, beta, acid
Npy1r	0.290	0.489	1.069	Neuropeptide Y receptor Y1
Mtmr11	0.291	0.381	0.956	Myotubularin related protein 11
Cpt1c	0.302	0.257	1.012	Carnitine palmitoyltransferase 1c
Adh7	0.316	0.635	1.001	Alcohol dehydrogenase 7 (class IV)
Pld1	0.333	0.462	0.972	Phospholipase D1
Hacd4	0.355	0.211	1.025	3-hydroxyacyl-coa dehydratase 4
Pigv	0.360	0.735	0.802	Phosphatidylinositol glycan anchor biosynthesis, class V
Hexa	0.375	0.491	0.870	Hexosaminidase A
Lrp1	0.379	0.387	0.861	Low density lipoprotein receptor-related protein 1
Lepr	0.394	0.984	1.056	Leptin receptor
Fgf10	0.403	0.615	0.998	Fibroblast growth factor 10
Lrp5	0.407	0.348	1.096	Low density lipoprotein receptor-related protein 5
Lmna	0.412	0.347	0.784	Lamin A
Scpep1	0.438	0.533	0.772	Serine carboxypeptidase 1
E2f1	0.440	0.517	1.112	E2F transcription factor 1
Lima1	0.457	0.334	1.008	LIM domain and actin binding 1
Fgf7	0.463	0.480	1.167	Fibroblast growth factor 7
Gla	0.474	0.714	1.101	Galactosidase, alpha
Pigt	0.491	0.587	1.124	Phosphatidylinositol glycan anchor biosynthesis, class T
Socs5	0.494	0.584	0.921	Suppressor of cytokine signaling 5
Tmem150a	0.497	0.640	0.782	Transmembrane protein 150A

lipid accumulation and TG content and increased glucose uptake in 3T3-L1 adipocytes. Both naringenin and naringin increased the phosphorylation levels of AMPK and ACC and inhibited the expression level of HMGCR, which are the key enzymes in lipogenesis and

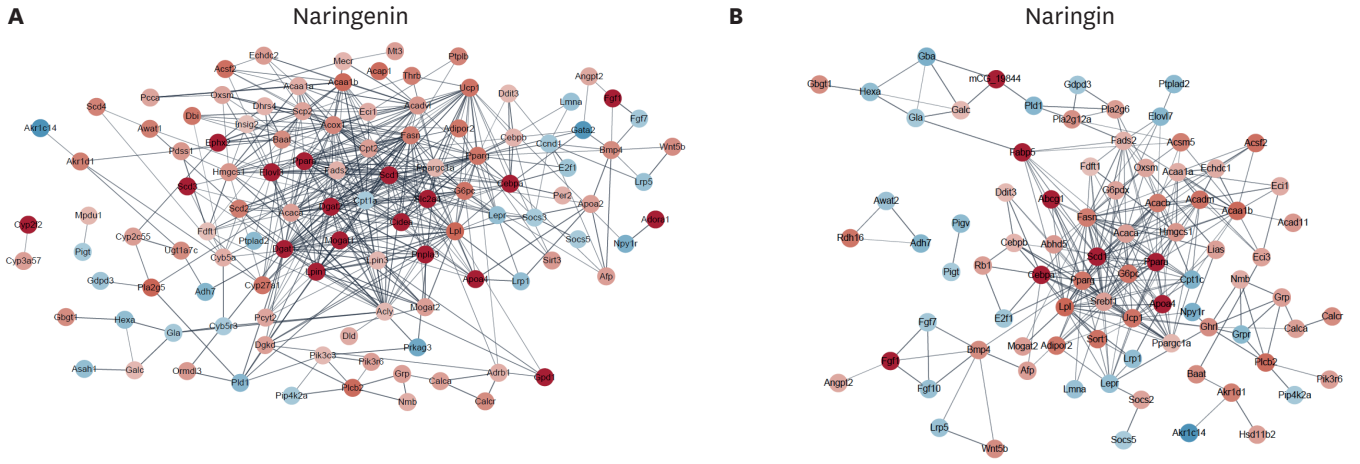

Fig. 6. PPI networks of normalized genes related to lipid metabolism in naringenin and naringin treated 3T3-L1 adipocytes. PPI network of naringenin (A). PPI network of naringin (B). Red circles represent up-regulated genes, and blue circles represent down-regulated genes in 3T3-L1 adipocytes. PPI, protein-protein-interaction.

cholesterol synthesis. RNA sequencing results revealed that naringenin treatment normalizes the expression of genes involved in lipid metabolism, including *Acaca*, *Fasn*, *Scd1*, *Mogat1*, *Dgat*, *Lipin1*, *Cpt1a*, and *Lepr*. In addition, the treatment of naringin normalizes the expression of genes related to lipid metabolism, including *Acaca*, *Fasn*, *Fabp5*, *Scd1*, *Srebf1*, *Hmgcs1*, *Cpt1c*, *Lepr*, and *Lrp1* in 3T3-L1 adipocytes.

The present study shows that naringenin and naringin treatments reduce intracellular lipid accumulation and TG content in 3T3-L1 adipocytes, as was observed previously [6,14]. Naringenin reduces diet-induced weight gain and improves glucose and lipid metabolism in animal models [15]. In addition, naringin-rich pomelo (*Citrus grandis* (L.) Osbeck) peel extract has been shown to reduce body weight, TG, and total cholesterol level in obese rats [16]. Therefore, the present study of differentiated 3T3-L1 adipocytes confirms that both naringenin and naringin have significant roles in inhibiting lipid accumulation and TG synthesis.

Furthermore, the present study in differentiated 3T3-L1 adipocytes confirmed that both naringenin and naringin significantly increase glucose uptake in 3T3-L1 adipocytes. Many flavonoids are known to increase glucose uptake via the activation of AMPK, which enhances glucose transporter type 4 translocation in 3T3-L1 adipocytes [17,18]. Naringenin and naringin enhance glucose utilization through increased glycolysis, which ultimately reduces the utilization of lipids [19,20]. Therefore, the previous and present findings suggest that naringenin and naringin produce anti-adipogenic effects by increasing glucose uptake, which might enhance glucose metabolism in differentiated 3T3-L1 adipocytes.

In the present study, the naringenin and naringin treatments increased the phosphorylation levels of both AMPK and ACC and decreased the expression level of HMGCR. Activation of AMPK suppresses lipid synthesis [21] through downregulation of key adipogenic factors, including PPARγ, C/EBPα, and SREBP-1c [4]. Phosphorylation of ACC inhibits fatty acid synthesis by blocking the conversion of acetyl-CoA to malonyl-CoA [22]. Naringenin is known to reduce the levels of plasma cholesterol and hepatic TG that accompany the decreased expression of HMGCR in animal models [23]. In addition, naringin is known to activate AMPK [20] and suppress HMGCR expression in type 2 diabetic mice [24]. The

https://doi.org/10.4142/jvs.2021.22.e55

previous and present findings suggest that naringenin and naringin treatments regulate lipid metabolism by activating the AMPK pathway in 3T3-L1 adipocytes, which potentially contributes to a reduction of adipogenesis.

RNA sequencing results revealed that both naringenin and naringin treatments normalized the expressions of genes involved in lipid metabolism in 3T3-L1 adipocytes. The treatment of naringenin normalized the expressions of up-regulated genes, including *Acaca*, *Fasn*, *Scd1*, *Mogat1*, *Dgat*, and *Lipin1*. The *Acaca* encoding enzyme catalyzes the carboxylation of acetyl-CoA to malonyl-CoA [25], while *Fasn* catalyzes the conversion of malonyl-CoA into palmitate [26]. *Scd1* is a rate-limiting enzyme in the synthesis of mono-unsaturated fatty acids [27], and *Mogat* catalyzes the synthesis of diacylglycerol, the precursor of triacylglycerol [28]. *Dgat* catalyzes the covalent addition of a fatty acyl chain to diacylglycerol [29] and reduces TG levels in *Dgat* knockout mice [30]. *Lipin1* is a key regulator of TG metabolism and lipoprotein synthesis [31]. In this regard, normalization of genes related to lipid metabolism, including *Acaca*, *Fasn*, *Scd1*, *Mogat1*, *Lipin1*, and *Dgat*, by naringenin contributes to reducing adipogenesis in 3T3-L1 adipocytes.

Naringenin treatment normalized the expressions of down-regulated genes, including *Cpt1a*, and *Lepr*. *Cpt1* catalyzes fatty acid oxidation by converting acyl-CoAs into acylcarnitines [32], and *Cpt1* knockout mice are reported to be susceptible to high-fat-diet- induced obesity [33]. *Lepr* regulates energy expenditure and reduces lipid accumulation in the body [34]. A previous study showed attenuation of obesity via *Lepr* gene therapy [35]. Naringenin has been reported to stimulate the mRNA expression of *Cpt1* [36], an observation supported by the present findings. Therefore, the previous and present findings suggest that naringenin reduces adipogenesis by normalizing lipid metabolism-related genes in 3T3-L1 adipocytes.

Naringin treatment normalized the expressions of up-regulated genes, including *Acaca*, *Fasn*, *Scd1*, *Fabp5*, *Srebf1*, and *Hmgcs1*. As described previously, the expression of *Acaca* catalyzes the first committed step in fatty acid synthesis [25]. *Fasn* is the central enzyme involved in *de novo* lipogenesis [26], and *Scd1* is the rate-limiting enzyme in the synthesis of mono-unsaturated fatty acids [37]. In addition, *Fabp5* is involved in lipid trafficking and intracellular fatty acid storage [38]. *Srebf1* regulates SREEBP1a and SREBP1c that are involved in fatty acid and cholesterol synthesis [39]. *Hmgcs1* is involved in cholesterol synthesis, converting acetyl-CoA and acetoacetyl-CoA into HMG-CoA [40]. In this regard, normalization of genes related to lipid metabolism, including *Acaca*, *Fasn*, *Fabp5*, *Scd1*, *Srebf1*, and *Hmgcs1*, by naringin treatment contributes to reducing adipogenesis in 3T3-L1 adipocytes.

Naringin treatment normalized the expressions of down-regulated genes, including *Cpt1c*, *Lepr*, and *Lrp1*. *Cpt1* is involved in fatty acid oxidation [32], and *Lepr* regulates energy expenditure, which reduces lipid accumulation [34]. *Lrp1* is a key factor in maintaining lipid homeostasis, insulin sensitivity, and glucose homeostasis [41]. Overall, the results suggest that naringin restores adipogenesis to the normal level by normalizing the expression of *Cpt1c*, *Lepr*, and *Lrp1* genes in 3T3-L1 adipocytes.

In the present study, both naringenin and naringin showed significant anti-adipogenic effects, with naringin having a higher potency than naringenin in mitigating obesity. Naringenin and naringin are major flavonoids present in grapefruit and are abundant in many other citrus fruits [42]. Naringin is a 7 O-glycoside in which 2 rhamnose units are attached to its aglycon portion, naringenin, at the 7-carbon position [43]. The glycoside

forms of flavonoids are the most abundant polyphenols in plants [44]. Glycosides are known to be more biologically active than the respective aglycone, since the bound sugar moiety of O-glycosides is known to influence their bioavailability [45]. Attaching a glycosidic moiety increases hydrophilicity, which influences the pharmacokinetic properties of the respective compounds [46]. Solubility has a major role in the therapeutic efficacy of flavonoids. Low solubility of flavonoid aglycones in water, coupled with its short residence time in the intestine, results in their low absorption. The low solubility of the flavonoids in water often presents a problem in medicinal applications [47]. Further, O-glycosylation can enhance some biological benefits, including anti-adipogenic activity, as reported previously [48]. However, as the glycoside linkages are less stable than most glucuronide linkages and might not withstand the acidic environment in the stomach; moreover, glycosidase from intestinal bacteria may cleave sugar residues, which will generate the aglycone naringenin [49,50]. Therefore, the absorption of naringin inside the body remains to be clarified further [50]. However, being a 7 O-glycoside, naringin shows higher anti-adipogenic potential than its aglycone naringenin in 3T3-L1 adipocytes.

Overall results of the present study indicate that both naringenin and naringin treatments reduce lipid accumulation and TG content in 3T3-L1 adipocytes by increasing the phosphorylation of both AMPK and ACC while reducing the expression of HMGCR. In particular, both naringenin and naringin normalize the expression of genes involved in lipid metabolism in 3T3-L1 adipocytes (**Fig. 7**). Further studies are needed to delineate the potential role of these genes as therapeutic targets in adipogenesis. The overall results suggest that naringenin and naringin treatments have potent anti-adipogenic effects related to normalizing the expression of lipid metabolism-related genes that are perturbed in differentiated 3T3-L1 adipocytes.

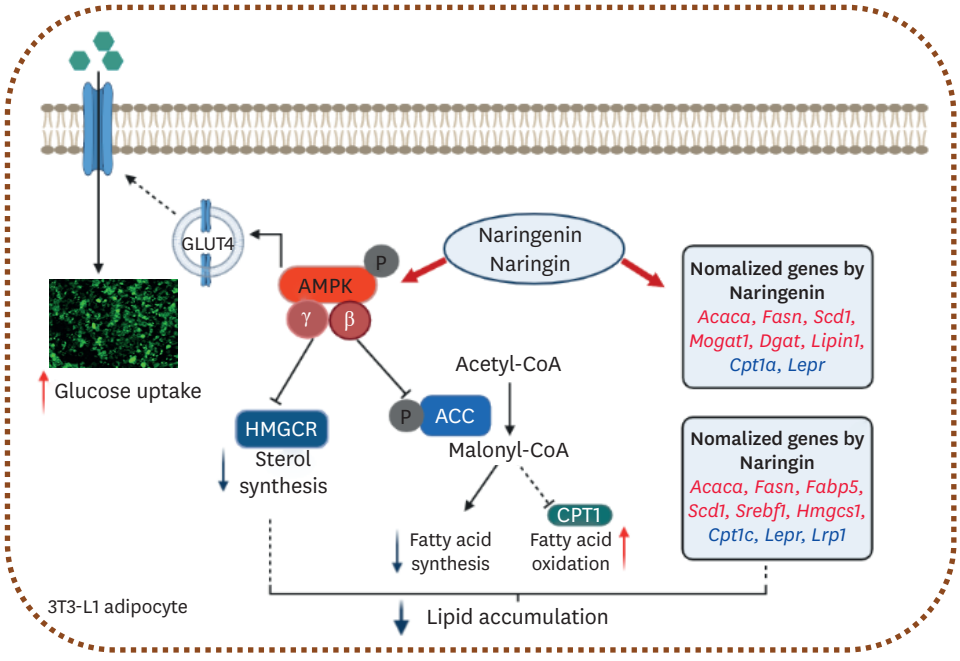

Fig. 7. Proposed model of the anti-adipogenic effects of naringenin and naringin on 3T3-L1 adipocytes. GLUT4, glucose transporter type 4; AMPK, AMP-activated protein kinase; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; ACC, acetyl Co-A carboxylase; CPT1, carnitine palmitoyltransferase I.

https://doi.org/10.4142/jvs.2021.22.e55

REFERENCES

1. Sun NN, Wu TY, Chau CF. Natural dietary and herbal products in anti-obesity treatment. Molecules. 2016;21(10):1351.
PUBMED | CROSSREF
2. Park J, Kim HL, Jung Y, Ahn KS, Kwak HJ, Um JY. Bitter orange (Citrus aurantium Linné) improves obesity by regulating adipogenesis and thermogenesis through AMPK activation. Nutrients. 2019;11(9):1988.
PUBMED | CROSSREF
3. Xu L, Li Y, Dai Y, Peng J. Natural products for the treatment of type 2 diabetes mellitus: Pharmacology and mechanisms. Pharmacol Res. 2018;130:451-465.
PUBMED | CROSSREF
4. Kang SW, Kang SI, Shin HS, Yoon SA, Kim JH, Ko HC, et al. Sasa quelpaertensis Nakai extract and its constituent p-coumaric acid inhibit adipogenesis in 3T3-L1 cells through activation of the AMPK pathway. Food Chem Toxicol. 2013;59:380-385.
PUBMED | CROSSREF
5. Hardie DG, Pan DA. Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. Biochem Soc Trans. 2002;30(Pt 6):1064-1070.
PUBMED | CROSSREF
6. Richard AJ, Amini-Vaughan Z, Ribnicky DM, Stephens JM. Naringenin inhibits adipogenesis and reduces insulin sensitivity and adiponectin expression in adipocytes. Evid Based Complement Alternat Med. 2013;2013:549750.
PUBMED | CROSSREF
7. Mahmoud AM, Hernández Bautista RJ, Sandhu MA, Hussein OE. Beneficial effects of citrus flavonoids on cardiovascular and metabolic health. Oxid Med Cell Longev. 2019;2019:5484138.
PUBMED | CROSSREF
8. Claussnitzer M, Skurk T, Hauner H, Daniel H, Rist MJ. Effect of flavonoids on basal and insulin-stimulated 2-deoxyglucose uptake in adipocytes. Mol Nutr Food Res. 2011;55 Suppl 1:S26-S34.
PUBMED | CROSSREF
9. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr. 2014;5(4):404-417.
PUBMED | CROSSREF
10. Rizzatti V, Boschi F, Pedrotti M, Zoico E, Sbarbati A, Zamboni M. Lipid droplets characterization in adipocyte differentiated 3T3-L1 cells: size and optical density distribution. Eur J Histochem. 2013;57(3):e24.
PUBMED | CROSSREF
11. Ranaweera SS, Dissanayake CY, Natraj P, Lee YJ, Han CH. Anti-inflammatory effect of sulforaphane on LPS-stimulated RAW 264.7 cells and ob/ob mice. J Vet Sci. 2020;21(6):e91.
PUBMED | CROSSREF
12. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357-359.
PUBMED | CROSSREF
13. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.
PUBMED | CROSSREF
14. Guo X, Liu J, Cai S, Wang O, Ji B. Synergistic interactions of apigenin, naringin, quercetin and emodin on inhibition of 3T3-L1 preadipocyte differentiation and pancreas lipase activity. Obes Res Clin Pract. 2016;10(3):327-339.
PUBMED | CROSSREF
15. Goldwasser J, Cohen PY, Yang E, Balaguer P, Yarmush ML, Nahmias Y. Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPARalpha, PPARgamma and LXRalpha. PLoS One. 2010;5(8):e12399.
PUBMED | CROSSREF
16. Lien DN, Quynh NT, Phuc DV, Phong VC, Huong PT. Effect of pomelo (citrus grandis (l). osbeck) peel extract on lipid-carbohydrate metabolic enzymes and blood lipid, glucose parameters in experimental obese and diabetic mice. VNU J Sci Nat Sci Technol. 2010;26(4):224-232.
17. Ha T, Trung TN, Phuong TT, Yim N, Chen QC, Bae K. The selected flavonol glycoside derived from Sophorae Flos improves glucose uptake and inhibits adipocyte differentiation via activation AMPK in 3T3-L1 cells. Bioorg Med Chem Lett. 2010;20(20):6076-6081.
PUBMED | CROSSREF

18. Fang XK, Gao J, Zhu DN. Kaempferol and quercetin isolated from Euonymus alatus improve glucose uptake of 3T3-L1 cells without adipogenesis activity. Life Sci. 2008;82(11-12):615-622.
PUBMED | CROSSREF

19. Rajappa R, Sireesh D, Salai MB, Ramkumar KM, Sarvajayakesavulu S, Madhunapantula SV. Treatment with naringenin elevates the activity of transcription factor Nrf2 to protect pancreatic β-cells from streptozotocin-induced diabetes *in vitro* and *in vivo*. Front Pharmacol. 2019;9:1562.
PUBMED | CROSSREF

20. Constantin RP, Constantin RP, Bracht A, Yamamoto NS, Ishii-Iwamoto EL, Constantin J. Molecular mechanisms of citrus flavanones on hepatic gluconeogenesis. Fitoterapia. 2014;92:148-162.
PUBMED | CROSSREF

21. Pu P, Gao DM, Mohamed S, Chen J, Zhang J, Zhou XY, et al. Naringin ameliorates metabolic syndrome by activating AMP-activated protein kinase in mice fed a high-fat diet. Arch Biochem Biophys. 2012;518(1):61-70.
PUBMED | CROSSREF

22. Ceddia RB. The role of AMP-activated protein kinase in regulating white adipose tissue metabolism. Mol Cell Endocrinol. 2013;366(2):194-203.
PUBMED | CROSSREF

23. Lee SH, Park YB, Bae KH, Bok SH, Kwon YK, Lee ES, et al. Cholesterol-lowering activity of naringenin via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A:cholesterol acyltransferase in rats. Ann Nutr Metab. 1999;43(3):173-180.
PUBMED | CROSSREF

24. Jung UJ, Lee MK, Park YB, Kang MA, Choi MS. Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. Int J Biochem Cell Biol. 2006;38(7):1134-1145.
PUBMED | CROSSREF

25. Matsumoto H, Sasaki K, Bessho T, Kobayashi E, Abe T, Sasazaki S, et al. The SNPs in the ACACA gene are effective on fatty acid composition in Holstein milk. Mol Biol Rep. 2012;39(9):8637-8644.
PUBMED | CROSSREF

26. Hillgartner FB, Salati LM, Goodridge AG. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. Physiol Rev. 1995;75(1):47-76.
PUBMED | CROSSREF

27. Jiang Z, Michal JJ, Tobey DJ, Daniels TF, Rule DC, Macneil MD. Significant associations of stearoyl-CoA desaturase (SCD1) gene with fat deposition and composition in skeletal muscle. Int J Biol Sci. 2008;4(6):345-351.
PUBMED | CROSSREF

28. Hall AM, Kou K, Chen Z, Pietka TA, Kumar M, Korenblat KM, et al. Evidence for regulated monoacylglycerol acyltransferase expression and activity in human liver. J Lipid Res. 2012;53(5):990-999.
PUBMED | CROSSREF

29. Harris CA, Haas JT, Streeper RS, Stone SJ, Kumari M, Yang K, et al. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. J Lipid Res. 2011;52(4):657-667.
PUBMED | CROSSREF

30. Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, et al. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nat Genet. 2000;25(1):87-90.
PUBMED | CROSSREF

31. Péterfy M, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. Nat Genet. 2001;27(1):121-124.
PUBMED | CROSSREF

32. Bonnefont JP, Djouadi F, Prip-Buus C, Gobin S, Munnich A, Bastin J. Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. Mol Aspects Med. 2004;25(5-6):495-520.
PUBMED | CROSSREF

33. Gao XF, Chen W, Kong XP, Xu AM, Wang ZG, Sweeney G, et al. Enhanced susceptibility of Cpt1c knockout mice to glucose intolerance induced by a high-fat diet involves elevated hepatic gluconeogenesis and decreased skeletal muscle glucose uptake. Diabetologia. 2009;52(5):912-920.
PUBMED | CROSSREF

34. Bjørbaek C. Central leptin receptor action and resistance in obesity. J Investig Med. 2009;57(7):789-794.
PUBMED | CROSSREF

35. Morton GJ, Niswender KD, Rhodes CJ, Myers MG Jr, Blevins JE, Baskin DG, et al. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (fa(k)/fa(k)) rats. Endocrinology. 2003;144(5):2016-2024.
PUBMED | CROSSREF

36. Rebello CJ, Greenway FL, Lau FH, Lin Y, Stephens JM, Johnson WD, et al. Naringenin promotes thermogenic gene expression in human white adipose tissue. Obesity (Silver Spring). 2019;27(1):103-111.
PUBMED | CROSSREF

37. Mauvoisin D, Mounier C. Hormonal and nutritional regulation of SCD1 gene expression. Biochimie. 2011;93(1):78-86.
PUBMED | CROSSREF

38. Guaita-Esteruelas S, Gumà J, Masana L, Borràs J. The peritumoural adipose tissue microenvironment and cancer. The roles of fatty acid binding protein 4 and fatty acid binding protein 5. Mol Cell Endocrinol. 2018;462(Pt B):107-118.
PUBMED | CROSSREF

39. Eberlé D, Clément K, Meyre D, Sahbatou M, Vaxillaire M, Le Gall A, et al. SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. Diabetes. 2004;53(8):2153-2157.
PUBMED | CROSSREF

40. Quintana AM, Hernandez JA, Gonzalez CG. Functional analysis of the zebrafish ortholog of HMGCS1 reveals independent functions for cholesterol and isoprenoids in craniofacial development. PLoS One. 2017;12(7):e0180856.
PUBMED | CROSSREF

41. Actis Dato V, Chiabrando GA. The role of low-density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. Int J Mol Sci. 2018;19(6):1780.
PUBMED | CROSSREF

42. Hsiu SL, Huang TY, Hou YC, Chin DH, Chao PDL. Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits. Life Sci. 2002;70(13):1481-1489.
PUBMED | CROSSREF

43. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013;2013:162750.
PUBMED | CROSSREF

44. Veitch NC, Grayer RJ. Flavonoids and their glycosides, including anthocyanins. Nat Prod Rep. 2011;28(10):1626-1695.
PUBMED | CROSSREF

45. Morand C, Manach C, Crespy V, Remesy C. Respective bioavailability of quercetin aglycone and its glycosides in a rat model. Biofactors. 2000;12(1-4):169-174.
PUBMED | CROSSREF

46. Křen V. Glycoside vs. aglycon: the role of glycosidic residue in biological activity. In: Fraser-Reid BO, Tatsuta K, Thiem J, editors. *Glycoscience*. Berlin: Springer; 2008, 2589-2644.

47. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002;96(2-3):67-202.
PUBMED | CROSSREF

48. Xiao J. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? Crit Rev Food Sci Nutr. 2017;57(9):1874-1905.
PUBMED | CROSSREF

49. Ribeiro IA, Ribeiro MHL. Naringin and naringenin determination and control in grapefruit juice by a validated HPLC method. Food Control. 2008;19(4):432-438.
CROSSREF

50. Ameer B, Weintraub RA, Johnson JV, Yost RA, Rouseff RL. Flavanone absorption after naringin, hesperidin, and citrus administration. Clin Pharmacol Ther. 1996;60(1):34-40.
PUBMED | CROSSREF