

ORIGINAL ARTICLE

# Sunflower (*Helianthus annuus*) seed extract suppresses the lipogenesis pathway and stimulates the lipolysis pathway in high-fat diet-induced obese mice

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## Popular scientific summary

- Dietary supplementation with extract from sunflower seed inhibited adipogenesis and lipogenesis in high fat diet-induced obese mice.
- Dietary supplementation with extract from sunflower stimulated the hydrolysis of triacylglycerols and thermogenesis in high fat diet-induced obese mice.

## Abstract

**Background:** Obesity, abnormal fat accumulation in the adipose tissue, has become a serious global public health problem as it increases an individual's risk of developing various diseases.

**Objective:** This study sought to determine whether the extract from sunflower seed (SUNCA) prevents the development of obesity in high-fat diet (HFD)-induced obese mice.

**Design:** C57BL/6J mice were fed with AIN93G normal diet (Normal diet), 60% HFD, HFD containing Catechin 100 mg/kg body weight (b.w.) (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks.

**Results:** Body weight gain, food efficiency rate, adipose tissue weight, adipose tissue mass, size of adipocytes, and serum levels of triglyceride, total cholesterol, very low-density lipoprotein/low-density lipoprotein (VLDL/LDL)-cholesterol, aspartate aminotransferase, and alanine aminotransferase were significantly decreased by SUNCA supplementation in HFD-fed mice. Furthermore, SUNCA supplementation decreased the expression of proteins related to the adipogenesis and lipogenesis pathways and increased the expression of proteins related to the lipolysis and thermogenesis pathways in the adipose tissues of HFD-induced obese mice.

**Conclusions:** Altogether, SUNCA might prevent obesity by suppressing the adipogenesis/lipogenesis pathway and stimulating the lipolysis/thermogenesis pathway in HFD-induced obese mice.

Keywords: *obesity; sunflower seed; lipolysis; adipogenesis; lipogenesis*

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**O**besity is a medical condition associated with abnormal fat accumulation in the adipose tissue. Obesity has become a serious global public health problem as it increases an individual's risk

of developing various diseases, including type 2 diabetes, dyslipidemia, cardiovascular disease, hypertension, non-alcoholic fatty liver diseases, and certain cancers (1–3). Obesity is characterized by excessive expansion

of the adipose tissue owing to an imbalance in energy intake and expenditure. This expansion of adipose tissue is caused by the accumulation of triglycerides in adipocytes via the stimulation of the adipogenesis and *de novo* lipogenesis pathways (4, 5).

Adipogenesis, the process of differentiation from preadipocytes to adipocytes, is a key process for determining the number of adipocytes regulated by adipogenic transcription factors (6). Adipogenic transcription factors also stimulate several lipogenic enzymes, thereby inducing the conversion of acetyl-CoA into fatty acids and the accumulation of triglyceride in adipocyte via lipogenesis pathway activation (6–8). Nutrient excess, including high-fat diet (HFD), high-sucrose diet, and western diet, stimulates adipose tissue expansion through adipogenesis and lipogenesis (8, 9). During the development of obesity, lipolysis, the process of triglyceride breakdown, is inhibited by the inhibition of lipase activation and  $\beta$ -oxidation of fatty acids in adipose tissue. These conditions accelerate the development of obesity (10, 11). Therefore, the administration of substances that inhibit adipogenesis and lipogenesis and stimulate lipolysis is required to prevent and treat obesity. Recently, several studies have proposed alternative therapies, including herbal remedies, for inhibiting the development of obesity through the suppression of adipogenesis and lipogenesis or the stimulation of lipolysis (12, 13).

In this study, we investigated the potential anti-obesity effects of sunflower (*Helianthus annuus* L.) seed extracts (SUNCA) on HFD-induced obesity. Sunflower belongs to the Asteraceae family and possesses various biological activities, such as antioxidant, antimicrobial, anti-lipidemia, and anti-inflammatory effects (14, 15). The main polyphenol in the sunflower seed extract is the chlorogenic acid (CGA) (16); thus, we used CGA for the standardization of

SUNCA. Previously, Leverrier *et al.* revealed that the consumption of 500 mg/day sunflower extract for 12 weeks reduced body weight, fat mass, and serum lipid (17). In the present study, we determined the effects of standardized SUNCA on adipogenesis, lipogenesis, and lipolysis in HFD-induced obese mice to elucidate the mechanism of its anti-obesity effect.

## Materials and methods

### Standardization of SUNCA

The powdered *Helianthus annuus* L. seeds were extracted with 70% ethanol and concentrated *in vacuo*. The product was dried in a spray dryer to produce dried extracts (SUNCA). SUNCA was procured from the department of phytochemistry, Vidya Herbs Pvt. Ltd. (Bengaluru), analyzed using high-performance liquid chromatography (HPLC), and standardized using 200 mg/g CGA (Fig. 1).

### Animals

C57BL/6J mice (4-week-old, male) were purchased from Saeron Bio (Uiwang, Korea) and housed in cages under controlled conditions ( $22 \pm 2^\circ\text{C}$ , 55% humidity, and 12:12 h light-dark cycle). Mice were allowed to adapt for 1 week. Thereafter, they were fed with the AIN93G normal diet (Normal diet), 60% HFD, HFD containing Catechin 100 mg/kg body weight (b.w.) (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Mice were killed, and their tissues and blood (by orbital venipuncture) were collected. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Kyung Hee University (KHGASP-20-271).

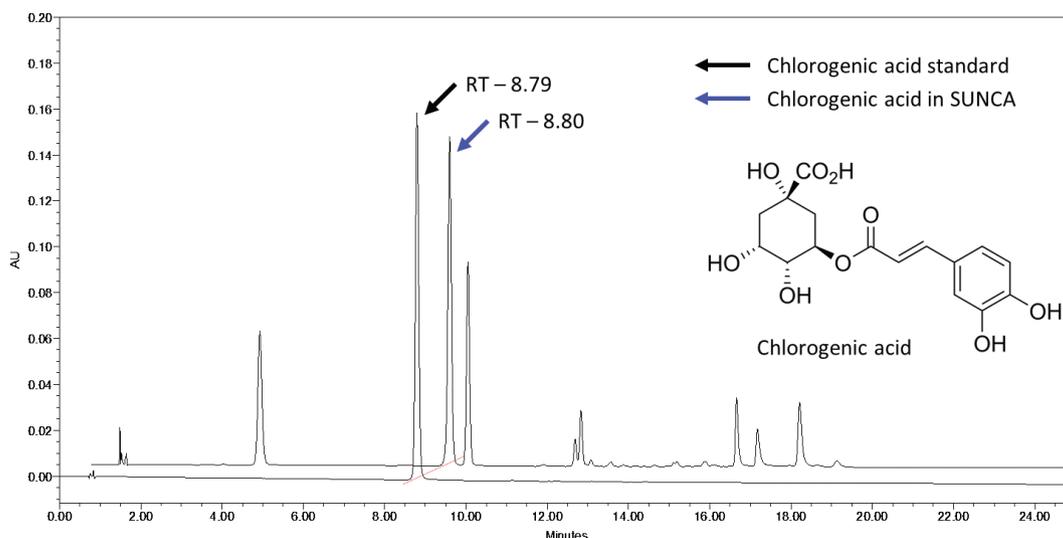


Fig. 1. High-performance liquid chromatography analysis of the levels of chlorogenic acid (CGA) in SUNCA.

### *Serum and feces biochemical analysis*

Triglyceride (Triglyceride Quantification Kit, Biomax, Seoul, Korea), total cholesterol (Total Cholesterol Assay Kit, Biomax), low-density lipoprotein (LDL)/high-density lipoprotein (HDL)-cholesterol (Biovision), aspartate aminotransferase (AST; Aspartate Aminotransferase Activity Colorimetric Assay Kit, Biovision), and alanine aminotransferase (ALT; Alanine Aminotransferase Activity Colorimetric Assay Kit, Biovision) were measured in the serum or feces according to the respective manufacturers' instruction.

### *Micro-CT*

Mice were examined whole *via* abdominal tomography using a micro-CT equipment (VIVA CT 80, Scano Medical AG, Switzerland).

### *Hematoxylin and eosin staining*

Epididymal adipose tissues from mice were fixed with 10% neutral buffered formaldehyde solution and embedded in paraffin. The paraffin blocks were sliced into 5  $\mu$ m sections, stained with hematoxylin and eosin (H&E), and observed using an optical microscope.

### *Western Blot*

Epididymal adipose tissues and brown adipose tissues (BAT) were lysed using CellLytic MT cell lysis reagent (Sigma, St. Louis, MO, USA). Briefly, equal amount (100  $\mu$ g/lane) of protein (100 was separated using 10% MiniPROTEAN<sup>®</sup>TGX<sup>™</sup>Precast Protein Gel (Bio-Rad Laboratories)) was transferred electrophoretically onto membranes using the Trans-Blot<sup>®</sup> Turbo<sup>™</sup> Transfer system (Bio-Rad). Membranes were blocked with 5% skimmed milk in TBST for 1 h and then incubated for 12 h at 4°C with antibodies against p-MAPK MAPK(Erk1/2) (Cell signaling, #9101), MAPK (Erk1/2) (Cell signaling, #9102), p-CREB (Cell signaling, #9198S), CREB (Cell signaling, #9197S), SREBP1(Abcam, ab3259), PPAR- $\gamma$  (Cell signaling, #2443), C/EBP $\alpha$  (Cell signaling, #2295S), p-ACL (Cell signaling, #4331), ACL (Cell signaling, #13390), p-ACC (Cell signaling, #3676), ACC (Cell signaling, #11818S), FAS (Cell signaling, #3189), LPL (Abcam, ab21356), PKA (Cell signaling, #4782), p-HSL (Cell signaling, #4139S), HSL (Cell signaling, #4107S), ATGL (Cell signaling, #2138), Leptin (Abcam, ab16227), p-AMPK (Cell signaling, #2531), AMPK (Cell signaling, #2532), UCPI (Cell signaling, #14670), CPT1A (LSBio, C482415), and  $\beta$ -actin (Cell signaling, #3700). All antibodies were diluted 1:1,000. After incubation with the primary antibody, the membranes were incubated with a secondary antibody (anti-rabbit IgG HRP-linked antibody, 1:5,000, Cell signaling, Beverly, MA, USA) for 1 h at room temperature. The protein bands were detected using EzWestLumi Plus (ATTO, Tokyo, Japan) and developed using Ez-Capture II (ATTO, Tokyo, Japan). The

bands were quantified using the CS Analyzer 3.0 (ATTO), and  $\beta$ -actin was used as a loading control to normalize the levels of protein.

### *Statistical analysis*

All data are expressed as mean  $\pm$  standard deviation (SD). Significant differences were determined using one-way analysis of variance (ANOVA) and Duncan's multiple range test (SPSS PASW Statistic v.23.0, SPSS Inc., Chicago, IL, USA). Statistical significance was determined at  $P < 0.05$ .

## **Results**

### *Effects of SUNCA on the body and organ weights of HFD-induced obese mice*

HFD-fed mice (Control) had a marked increase in body weight gain, food efficiency rate (FER), and relative tissue weights of white adipose tissue (WAT) and BAT compared with the mice fed with the normal control diet. Compared to the HFD-fed mice (Control), HFD containing SUNCA-fed mice displayed a significant decrease in body weight gain, FER, and relative tissue weights of WAT and BAT ( $P < 0.05$ ). However, dietary supplementation with SUNCA did not affect the ratio of WAT/total adipose tissue and BAT/total adipose tissue in HFD-induced obese mice (Table 1). These results indicate that dietary supplementation with SUNCA can prevent the development of obesity.

### *Effects of SUNCA on serum and feces lipid profiles and serum AST and ALT levels in HFD-induced obese mice*

The serum levels of triglyceride, total cholesterol, very low-density lipoprotein/low-density lipoprotein (VLDL/LDL)-cholesterol, AST, and ALT were significantly increased in the Control group compared with those in the Normal Control group; however, HDL-cholesterol was significantly decreased. Dietary supplementation with catechin and SUNCA significantly decreased the levels of triglyceride, total cholesterol, VLDL/LDL-cholesterol, AST, and ALT, and significantly increased the level of HDL-cholesterol in the HFD-induced obese mice. Interestingly, dietary supplementation with catechin and SUNCA significantly increased the levels of triglyceride and total cholesterol in feces ( $P < 0.05$ ) (Table 2).

### *Effects of SUNCA on adipose mass and size of adipocytes in HFD-induced obese mice*

Based on the results of the tomography analysis using microCT, adipose tissue mass significantly increased in HFD-fed mice (Control) compared with that in the normal diet-fed mice (Normal Control). However, dietary supplementation with catechin and SUNCA significantly decreased the adipose tissue mass compared to treatment

**Table 1.** Effects of SUNCA on the body and organ weights of high-fat diet-induced obese mice

Measurements	Normal diet	HFD	Catechin	SUNCA 25	SUNCA 50	SUNCA 100
Weight gain (g)	14.30 ± 2.17 <sup>c</sup>	28.50 ± 3.92 <sup>a</sup>	22.66 ± 4.93 <sup>b</sup>	22.30 ± 4.20 <sup>b</sup>	20.50 ± 6.24 <sup>b</sup>	18.42 ± 5.71 <sup>bc</sup>
Food consumption (g/day/mouse)	2.91 ± 0.32 <sup>a</sup>	2.48 ± 0.23 <sup>c</sup>	2.23 ± 0.25 <sup>d</sup>	2.68 ± 0.16 <sup>bc</sup>	2.52 ± 0.22 <sup>bc</sup>	2.73 ± 0.33 <sup>ab</sup>
Food efficiency ratio	4.38 ± 0.83 <sup>d</sup>	9.97 ± 1.37 <sup>a</sup>	8.59 ± 1.87 <sup>ab</sup>	7.25 ± 1.37 <sup>bc</sup>	6.90 ± 2.10 <sup>bc</sup>	5.81 ± 1.80 <sup>cd</sup>
Relative tissue weights (percent body weight, %)						
Liver	4.56 ± 0.39 <sup>ns</sup>	4.25 ± 0.50	4.01 ± 0.32	4.35 ± 0.29	4.26 ± 0.66	4.38 ± 0.68
Subcutaneous fat	2.42 ± 0.28 <sup>d</sup>	6.62 ± 0.63 <sup>a</sup>	5.65 ± 0.80 <sup>bc</sup>	6.34 ± 0.79 <sup>ab</sup>	5.46 ± 1.16 <sup>bc</sup>	4.76 ± 0.70 <sup>c</sup>
Visceral fat	0.52 ± 0.24 <sup>e</sup>	2.92 ± 0.45 <sup>a</sup>	1.99 ± 0.22 <sup>c</sup>	2.39 ± 0.43 <sup>b</sup>	1.83 ± 0.34 <sup>c</sup>	1.42 ± 0.22 <sup>d</sup>
Epididymis fat	1.99 ± 0.29 <sup>d</sup>	5.26 ± 0.96 <sup>a</sup>	3.79 ± 0.29 <sup>c</sup>	4.54 ± 0.53 <sup>b</sup>	4.29 ± 0.72 <sup>bc</sup>	3.56 ± 0.32 <sup>c</sup>
Brown adipose tissue	0.28 ± 0.05 <sup>c</sup>	0.49 ± 0.11 <sup>a</sup>	0.42 ± 0.12 <sup>ab</sup>	0.44 ± 0.03 <sup>a</sup>	0.43 ± 0.08 <sup>a</sup>	0.33 ± 0.08 <sup>bc</sup>
White adipose tissue/total adipose tissue (%)	94.63 ± 00.84 <sup>b</sup>	96.76 ± 00.97 <sup>a</sup>	96.40 ± 01.01 <sup>a</sup>	96.74 ± 00.23 <sup>a</sup>	96.36 ± 00.42 <sup>a</sup>	96.73 ± 00.73 <sup>a</sup>
Brown adipose tissue/total adipose tissue (%)	5.37 ± 0.84 <sup>a</sup>	3.37 ± 0.97 <sup>b</sup>	3.60 ± 1.01 <sup>b</sup>	3.26 ± 0.23 <sup>b</sup>	3.64 ± 0.42 <sup>b</sup>	3.27 ± 0.73 <sup>b</sup>

C57BL/6J mice fed with the AIN93G normal diet (Normal diet), high-fat diet (diet with 60 kcal% fat, HFD), HFD containing Catechin 100 mg/kg b.w. (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Values are presented as mean ± standard deviation ( $n = 8$ ). Different letters (a > b > c > d > e) indicate significant difference among treatments. Multiple comparisons of means were performed using the Duncan's multiple range test at the 0.05 significance level.

**Table 2.** Effects of SUNCA on serum and feces lipid profiles and serum AST and ALT in high-fat diet-induced obese mice

Measurements	Normal diet	HFD	Catechin	SUNCA 25	SUNCA 50	SUNCA 100
<b>Serum</b>						
Triglyceride (μg/μL)	19.08 ± 1.55 <sup>d</sup>	35.80 ± 2.64 <sup>a</sup>	27.58 ± 2.84 <sup>b</sup>	33.36 ± 2.11 <sup>a</sup>	27.31 ± 2.50 <sup>b</sup>	22.93 ± 2.91 <sup>c</sup>
Free fatty acid (mM)	0.223 ± 0.028 <sup>d</sup>	0.658 ± 0.093 <sup>a</sup>	0.307 ± 0.097 <sup>cd</sup>	0.516 ± 0.075 <sup>b</sup>	0.364 ± 0.031 <sup>c</sup>	0.264 ± 0.041 <sup>cd</sup>
Total cholesterol (μg/μL)	1.16 ± 0.14 <sup>d</sup>	3.82 ± 0.46 <sup>a</sup>	3.03 ± 0.34 <sup>b</sup>	3.69 ± 0.31 <sup>a</sup>	2.98 ± 0.25 <sup>b</sup>	2.61 ± 0.23 <sup>c</sup>
VLDL/LDL-cholesterol (μg/μL)	0.53 ± 0.04 <sup>e</sup>	2.89 ± 0.26 <sup>a</sup>	1.55 ± 0.16 <sup>d</sup>	2.08 ± 0.22 <sup>b</sup>	1.85 ± 0.15 <sup>c</sup>	1.74 ± 0.14 <sup>cd</sup>
HDL-cholesterol (μg/μL)	0.41 ± 0.03 <sup>d</sup>	0.94 ± 0.09 <sup>b</sup>	0.79 ± 0.06 <sup>c</sup>	0.72 ± 0.08 <sup>c</sup>	0.98 ± 0.10 <sup>b</sup>	1.12 ± 0.07 <sup>a</sup>
AST (mU/mL)	27.03 ± 3.83 <sup>c</sup>	37.92 ± 5.78 <sup>a</sup>	33.28 ± 1.53 <sup>b</sup>	36.41 ± 2.66 <sup>ab</sup>	33.15 ± 1.20 <sup>b</sup>	32.22 ± 2.63 <sup>b</sup>
ALT (mU/mL)	7.95 ± 1.32 <sup>b</sup>	11.41 ± 2.61 <sup>a</sup>	8.92 ± 1.61 <sup>b</sup>	9.46 ± 1.47 <sup>ab</sup>	8.41 ± 0.99 <sup>b</sup>	8.13 ± 1.58 <sup>b</sup>
<b>Feces</b>						
Triglyceride (μg/μL)	0.90 ± 0.10 <sup>e</sup>	1.87 ± 0.12 <sup>d</sup>	2.44 ± 0.22 <sup>b</sup>	2.10 ± 0.17 <sup>c</sup>	2.59 ± 0.20 <sup>ab</sup>	2.67 ± 0.21 <sup>a</sup>
Total cholesterol (μg/μL)	1.23 ± 0.14 <sup>e</sup>	1.78 ± 0.20 <sup>d</sup>	2.33 ± 0.23 <sup>b</sup>	2.05 ± 0.22 <sup>c</sup>	2.60 ± 0.28 <sup>a</sup>	2.66 ± 0.24 <sup>a</sup>

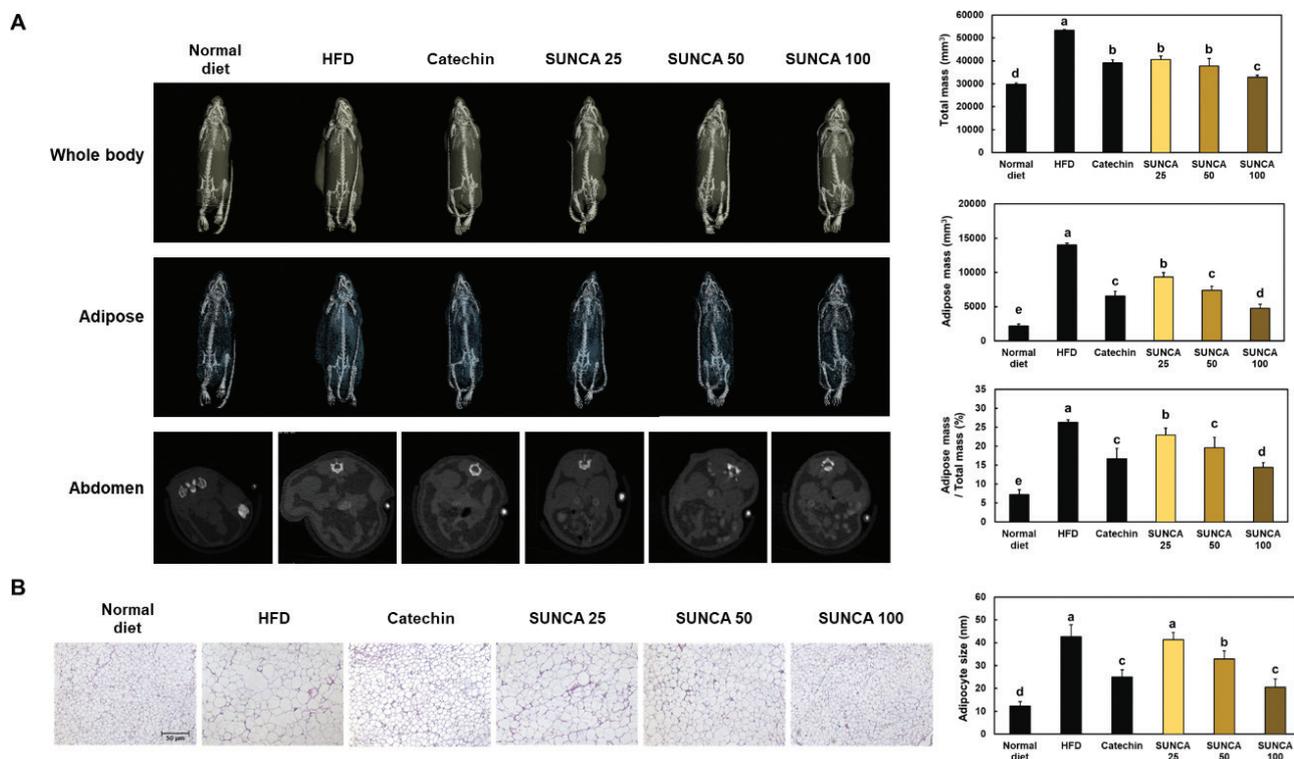
C57BL/6J mice fed with the AIN93G normal diet (Normal diet), high-fat diet (diet with 60 kcal% fat, HFD), HFD containing Catechin 100 mg/kg b.w. (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Values are presented as mean ± standard deviation ( $n = 8$ ). Different letters (a > b > c > d > e) indicate significant difference among treatments. Multiple comparisons of means were performed using the Duncan's multiple range test at the 0.05 significance level.

with the Control ( $P < 0.05$ ) (Fig. 2A). Furthermore, lipid size in the catechin and SUNCA groups decreased significantly compared with that in the Control group ( $P < 0.05$ ) (Fig. 2B). Therefore, dietary supplementation of SUNCA can decrease body weight gain by decreasing adipose tissue mass and the size of adipocytes.

#### Effect of low SUNCA on the adipogenesis and lipogenesis pathways in WAT from HFD-induced obese mice

To determine the effect of SUNCA on lipid metabolism in adipogenesis, lipogenesis, and lipolysis, western

blotting was carried out to detect specific protein molecules. Activated mitogen-activated protein kinase (MAPK; Erk1/2) and cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) via phosphorylation stimulate adipogenic transcription factors, including sterol regulatory element binding protein 1 (SREBP1), peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), and CCAAT/enhancer binding proteins (C/EBPs). Herein, the expression levels of p-MAPK, p-CREB, SREBP1, PPAR $\gamma$ , and C/EBP $\alpha$  were significantly increased by HFD compared to treatment with the



**Fig. 2.** Effects of SUNCA on adipose tissue mass (A) and size of adipocytes (B) in high-fat diet-obese mice. C57BL/6J mice fed with the AIN93G normal diet (Normal diet), high-fat diet (diet with 60 kcal% fat, HFD), HFD containing Catechin 100 mg/kg b.w. (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Values are presented as mean  $\pm$  standard deviation ( $n = 8$ ). Different letters (a > b > c > d > e) indicate significant difference among treatments. Multiple comparisons of means were performed using the Duncan's multiple range test at the 0.05 significance level.

Normal Control and significantly decreased by dietary supplementation with catechin and SUNCA compared to treatment with the Control. Moreover, the expression levels of *de novo* lipogenesis-related lipogenic enzymes, including ATP-citrate lyase (ACL) dephosphorylation, acetyl-CoA carboxylase (ACC) dephosphorylation, fatty acid synthase (FAS), and lipoprotein lipase (LPL), were significantly decreased by dietary supplementation with catechin and SUNCA compared to treatment with the Control ( $P < 0.05$ ) (Fig. 3).

#### Effect of low SUNCA on the lipolysis pathway, adiponectin, and leptin in WAT from HFD-induced obese mice

According to Fig. 3, HFD inhibited the expression of proteins in the lipolysis pathway, including cAMP/protein kinase A (PKA)/hormone-sensitive lipase (HSL) phosphorylation/adipose triglyceride lipase (ATGL) in WAT in mice. In addition, HFD supplementation caused an increase in the protein expression of leptin compared with that induced by normal diet supplementation. However, the expression levels of cAMP/PKA/HSL/ATGL in the lipolysis pathway were significantly decreased by dietary supplementation with catechin and SUNCA compared to treatment with the Control.

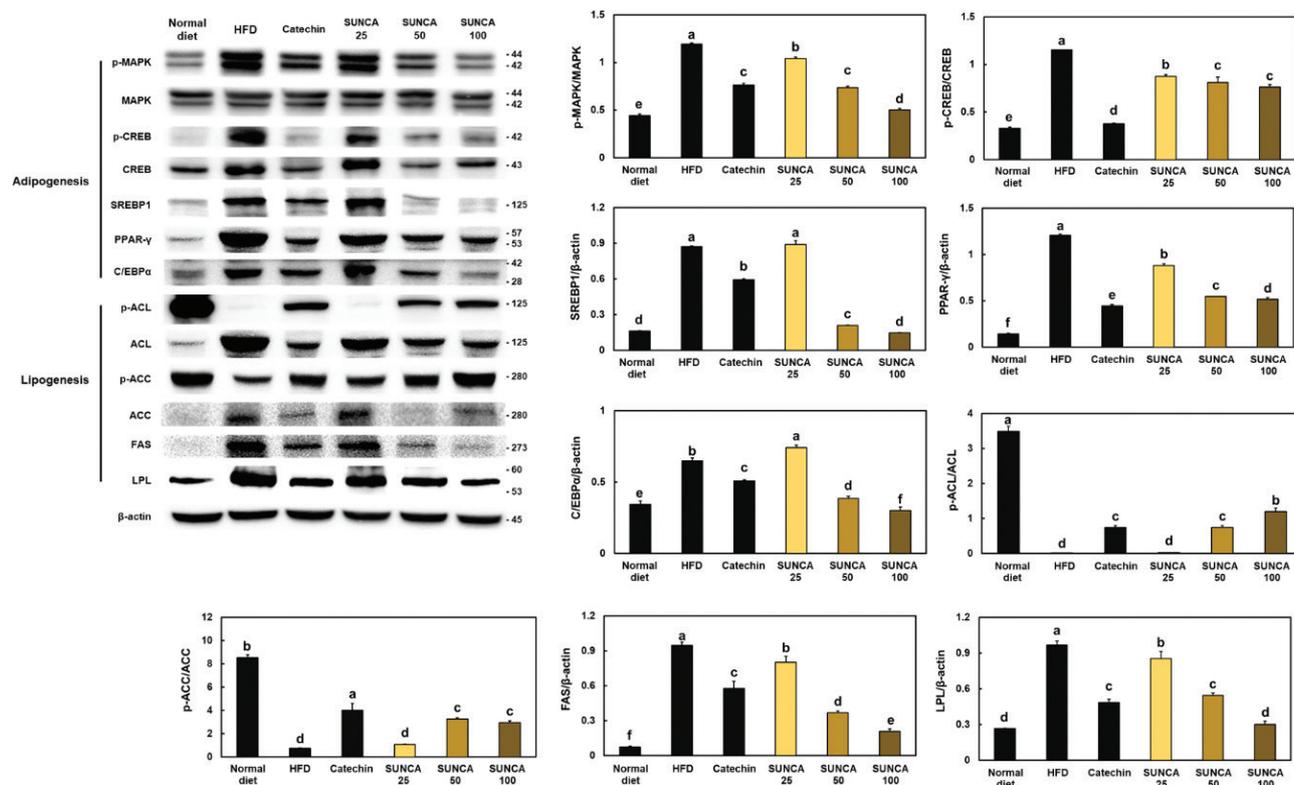
Moreover, the catechin and SUNCA supplementation groups had a significant decrease in the protein expression of leptin compared with the Control group ( $P < 0.05$ ) (Fig. 4).

#### Effect of low SUNCA on the thermogenesis pathway in BAT from HFD-induced obese mice

The expression levels of proteins in the thermogenesis pathway, including AMP-activated protein kinase (AMPK)/carnitine palmitoyltransferase 1 (CPT1)/uncoupling protein-1 (UCP-1) in the BAT from HFD-induced obese mice, were significantly decreased compared to levels in the normal diet-fed mice. However, catechin and SUNCA supplementation in HFD-induced obese mice increased the expression levels of AMPK/CPT1/UCP1 in this pathway compared to levels found in Control mice ( $P < 0.05$ ) (Fig. 5).

## Discussion

Sunflower seeds are used commercially as a baking ingredient, snack, and for oil production. Owing to the notable medicinal and nutritional benefits of sunflower seeds, their application in the food industry is increasing. Sunflower seed contains approximately 20% protein, 40–50%



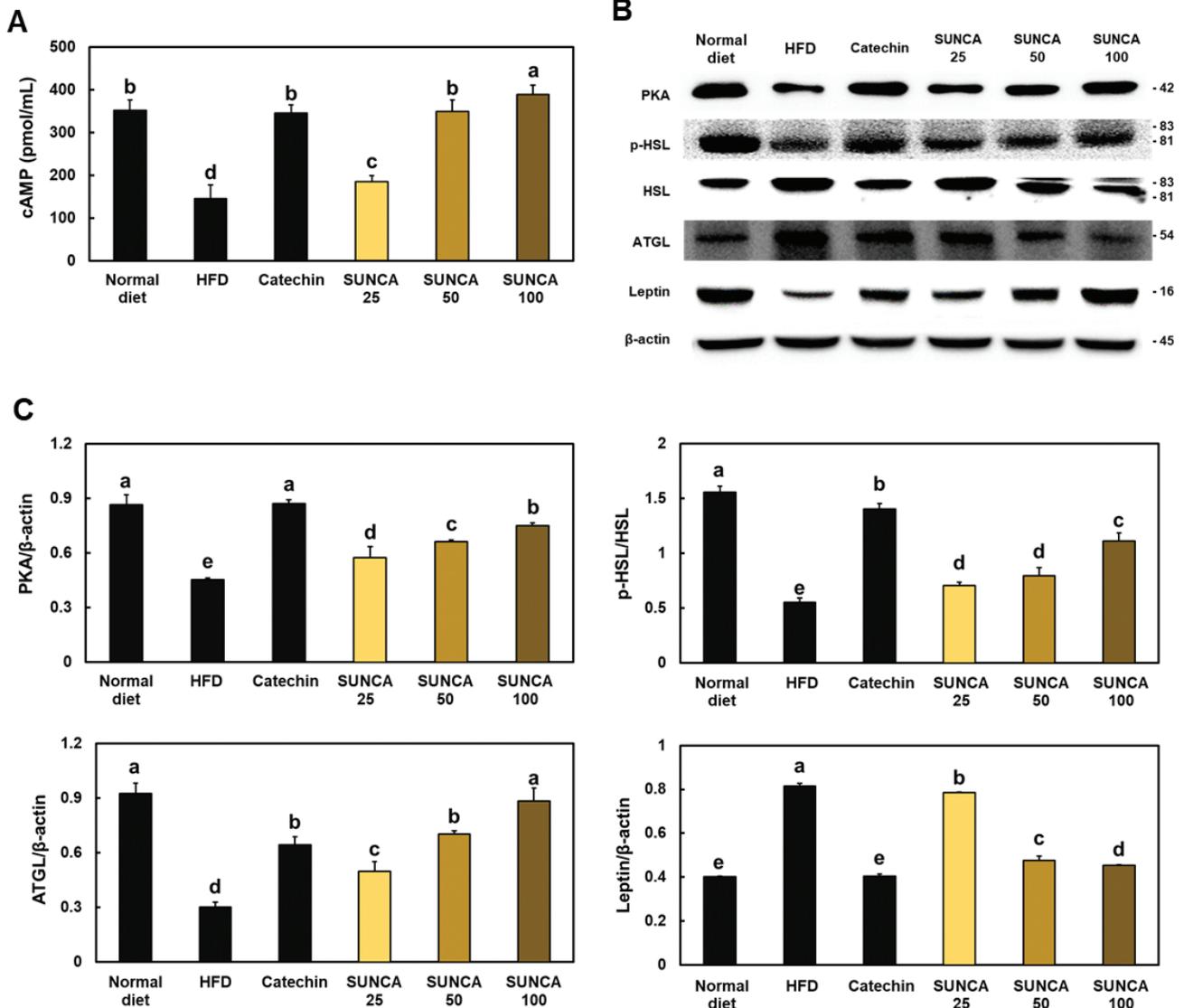
**Fig. 3.** Effect of low SUNCA on the adipogenesis and lipogenesis pathways in white adipose tissue from HFD-induced obese mice. C57BL/6J mice fed with the AIN93G normal diet (Normal diet), high-fat diet (diet with 60 kcal% fat, HFD), HFD containing Catechin 100 mg/kg b.w. (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Values are presented as mean  $\pm$  standard deviation ( $n = 8$ ). Different letters (a > b > c > d > e) indicate significant difference among treatments. Multiple comparisons of means were performed using the Duncan's multiple range test at the 0.05 significance level.

oil (55–70% linoleic acid and 20–25% oleic acid), and 20% carbohydrate. Furthermore, several compounds including phenolic acids and flavonoids, which have pharmaceutical activities, are found in the seed (14, 18, 19). We sought to determine whether SUNCA standardized to CGA has an anti-obesity effect in HFD-induced obese mice, which would ultimately increase the potential utilization of sunflower seeds. As HFD-induced obese mice are pathophysiologically similar to obese humans, they are well known as an appropriate animal model for evaluating anti-obesity effects (19). Green tea catechins are widely known for their anti-obesity effects through the modulation of energy metabolism and reducing appetite. Catechins increase norepinephrine by inhibiting the catechol O-methyltransferase, which causes an increase in energy expenditure and fat oxidation; thus, we used catechin as a positive control (21, 22). In the present study, we showed that HFD caused significant increases in body weight gain, FER, adipose tissue weight, adipose tissue mass, size of adipocytes, and serum levels of triglyceride, total cholesterol, VLDL/LDL-cholesterol, AST, and ALT

compared with the normal diet, indicating the normal development of obesity, lipidemia, and liver damage.

A significant decrease in body weight gain, FER, adipose tissue weight, adipose tissue mass, size of adipocytes, and serum levels of triglyceride, total cholesterol, VLDL/LDL-cholesterol, AST, and ALT was observed when HFD-fed mice were supplemented with SUNCA. To identify additional biochemical mechanisms that prevent obesity due to the SUNCA supplementation, we determined the effect of SUNCA on lipid metabolism, including the adipogenesis, lipogenesis, lipolysis, and thermogenesis pathways in adipose tissues.

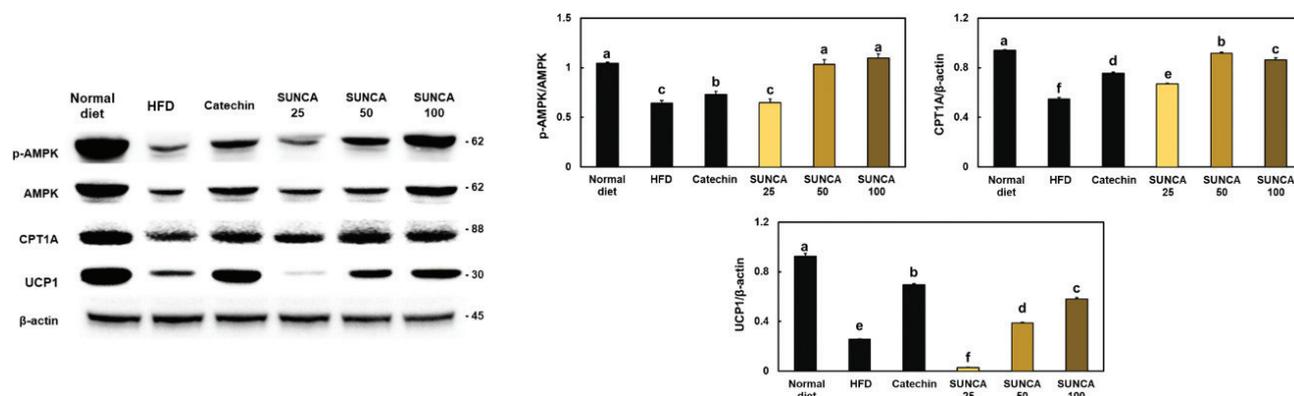
Adipogenesis is initiated by the phosphorylation of MAPK and CREB, which stimulate the transcription factors, SREBP1, PPAR $\gamma$ , and C/EBP $\alpha$  in adipocytes (23, 24). After differentiation of preadipocytes into mature adipocytes, the cells can act as lipid-storing adipocytes. When LPL is activated and hydrolyzes triglyceride lipoproteins in blood, fatty acids are internalized by adipocytes. Fatty acids from blood and new fatty acids synthesized via the *de novo* lipogenesis pathway are stored as triglycerides.



**Fig. 4.** Effect of low SUNCA on the cAMP level (A) and protein expression of PKA, HSL, p-HSL, ATGL, and leptin (B, band image; C, quantification of bands) in white adipose tissue from HFD-induced obese mice. C57BL/6J mice fed with the AIN93G normal diet (Normal diet), high-fat diet (diet with 60 kcal% fat, HFD), HFD containing Catechin 100 mg/kg b.w. (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Values are presented as mean  $\pm$  standard deviation ( $n = 8$ ). Different letters (a > b > c > d > e) indicate significant difference among treatments. Multiple comparisons of means were performed using the Duncan's multiple range test at the 0.05 significance level.

New fatty acid synthesis owing to acetyl-CoA via the dephosphorylation of ACL and ACC, and activation of FAS plays a key role in fat accumulation (8, 25). Interestingly, we showed that SUNCA supplementation induced a significant decrease in the expression of proteins related to the adipogenesis and lipogenesis pathways in WATs from HFD-induced obese mice. Such findings suggest that dietary supplementation with SUNCA can reduce adipose tissue weight, adipose tissue mass, and size of adipocytes by inhibiting adipogenesis and *de novo* lipogenesis.

The present study revealed that supplementation with SUNCA in HFD-induced obese mice increased the level of cAMP, the secondary messenger in intracellular signal transduction, in the WATs. cAMP activates PKA by releasing catalytic subunits, which can initiate the catabolism of triglycerides via PKA-mediated stimulation of HSL and ATGL (26). Moreover, supplementation with SUNCA was found to activate the AMPK/CPT1/UCP1 pathway in BAT. The key function of BAT is to participate in thermogenesis for the conversion of



**Fig. 5.** Effect of low SUNCA on the expression of proteins in the thermogenesis pathway in brown adipose tissue from HFD-induced obese mice. C57BL/6J mice fed with the AIN93G normal diet (Normal diet), high-fat diet (diet with 60 kcal% fat, HFD), HFD containing Catechin 100 mg/kg b.w. (Catechin), HFD containing SUNCA 25 mg/kg b.w. (SUNCA 25), HFD containing SUNCA 50 mg/kg b.w. (SUNCA 50), or HFD containing SUNCA 100 mg/kg b.w. (SUNCA 100) for 15 weeks. Values are presented as mean  $\pm$  standard deviation ( $n = 8$ ). Different letters ( $a > b > c > d > e > f$ ) indicate significant difference among treatments. Multiple comparisons of means were performed using the Duncan's multiple range test at the 0.05 significance level.

energy to heat, which is stimulated by AMPK, a regulator of energy metabolism (26, 27). Thus, we suggest that SUNCA in the diet potentially exerts anti-obesity effects by stimulating the hydrolysis of triacylglycerols and thermogenesis.

Jiang et al. (28) revealed that leptin plays a critical role in lipid metabolism by suppressing the expression of lipogenic enzymes. Leptin, a hormone secreted by adipose tissue, acts as a central regulator of systemic energy homeostasis by suppressing food intake. Abnormal fat accumulation in adipose tissue leads to overproduction of leptin, leading to leptin resistance and failure to control appetite. In this study, supplementation with SUNCA was found to reduce both lipogenic enzymes and leptin. Therefore, our results suggest that supplementation with SUNCA could reduce the secretion of leptin and prevent leptin resistance by suppressing adipocyte hypertrophy.

In our study, we measured main polyphenol CGA amount in SUNCA. In the study of Cho *et al.*, they showed that CGA supplementation lowered body weight and visceral fat mass in high-fat fed mice and suggested the effect of CGA in body weight reduction and regulation of lipid metabolism (29). Additional studies on the nutritional composition and potential active compounds from SUNCA, including CGA, and on the clinical effects are required to verify the effective levels of SUNCA in the future.

In conclusion, our findings indicate that SUNCA supplementation significantly suppressed the adipogenesis/lipogenesis pathways and stimulated the lipolysis/thermogenesis pathways in HFD-induced obese mice. Therefore, SUNCA has a potential function in suppressing the development of obesity.

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