

Inhibitory Effect of Olive Leaf Extract on Obesity in High-fat Diet-induced Mice

YUN-CHAN JUNG^{1*}, HYUN WOO KIM^{1*}, BOK KEE MIN², JAE YOUNG CHO², HYO JEONG SON²,
JA YOUNG LEE², JI-YOUNG KIM², SAE-BOM KWON², QIANG LI¹ and HEE-WOO LEE¹

¹*Institute of Research and Development, Chaon Corp., Seongnam, Republic of Korea;*

²*Nova K Health Corp., Seoul, Republic of Korea*

Abstract. *Background/Aim:* The rapid increase in the number of people who are overweight or obese, which increases the risk of diseases and health problems, is becoming an important issue. Herein, we investigated whether olive leaf extract (OLE) has potent anti-obesity effects in high-fat induced mouse models. *Materials and Methods:* C57BL/6 mice were randomized into normal control, high-fat diet (HFD), HFD with OLE, and HFD with garcinia groups and administered experimental diets for 12 weeks. Body weight and food intake were measured once per week and obesity-related biomarkers were evaluated in the serum and adipose tissue. *Results:* OLE significantly suppressed weight gain, food efficiency ratio, visceral fat accumulation, and serum lipid composition in HFD-induced mice. Furthermore, the expression of adipogenesis- and thermogenesis-related molecules was decreased in the OLE-treated group. *Conclusion:* OLE prevents obesity development by regulating the expression of molecules involved in adipogenesis and thermogenesis.

Obesity is a complex disorder involving an excessive amount of body fat resulting from the accumulation of white adipose tissue (1). The number of people who are overweight or obese is rapidly increasing, creating an important health issue by increasing the risk of diseases and health problems such as

cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer, osteoarthritis, hypertension, hyperlipidemia, and depression (2-4). Obesity typically results from a combination of causes and contributing factors, including genetics, family lifestyle, inactivity, unhealthy diet, age, pregnancy, social determinants, and medical problems such as Prader-Willi syndrome, Cushing's syndrome, and other conditions. It is characterized by an increase in adipocyte cell size and number, leading to an abnormal increase in fat mass and excessive fat accumulation in various organs. Adipogenesis is the process by which adipocytes differentiate from the mesenchymal lineage and is regulated by the Wnt/ β -catenin signaling pathway (1, 5). This differentiation is controlled by the activities of several important transcription factors, such as peroxisome proliferator-activated receptor- γ (PPAR- γ) and members of the CCAAT/enhancer-binding protein family (6-9). These factors play important roles in adipogenesis and lipogenesis, and studies of their signaling pathways have improved the understanding of adipogenesis mechanisms. Additionally, obesity results from a positive energy balance that occurs when energy intake exceeds expenditure. Thermogenesis increases energy expenditure in mitochondria-rich brown adipocytes in brown fat tissue and is triggered by intracellular mechanisms in response to exercise, diet, or cold exposure (10-14). Regulators of thermogenesis, such as peroxisome proliferator-activated receptor γ coactivator-1alpha (PGC-1 α), interferon regulatory factor 4, and PR-domain containing 16 regulate the expression of genes encoding uncoupling protein 1 (UCP1) (15-17), which depolarizes the inner mitochondrial membrane to cause proton transfer and heat dissipation. Therefore, screening for therapeutic agents that control the expression of key regulators of adipogenesis and thermogenesis is important for preventing obesity.

Olive leaf extract (OLE) is widely consumed in the human diet and has various health benefits. OLE contains some polyphenols in common with olive fruit and oil and has been shown to prevent hypertension, atherosclerosis, cancer, diabetes, and cardiovascular diseases (18-23). In this study,

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*These Authors contributed equally to this study.

Correspondence to: Qiang Li, Institute of Research and Development, Chaon Corp., 230 Pangyoyeok-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13493, Republic of Korea. Tel/Fax: +82 316984780/+82 316984784, e-mail: liqiang8589@snu.ac.kr; Hee-Woo Lee, Institute of Research and Development, Chaon Corp., 230 Pangyoyeok-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 13493, Republic of Korea. Tel/Fax: +82 316984780/+82 316984784, e-mail: dinks90@chaon.kr

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we investigated the *in vivo* preventive effects of OLE on obesity in high-fat diet-fed mice by evaluating the expression of molecules involved in adipogenesis and thermogenesis.

Materials and Methods

Materials. OLE was provided by Nova K health (Seoul, Korea). The compound was stored at 4°C and suspended in sterile phosphate-buffer saline (Invitrogen, Carlsbad, CA, USA). Garcinia extract was provided by Nova K health and administered to mice in the positive control group.

Animals. Five-week-old male C57BL/6 mice weighing 18-21 g were purchased from Orient Bio (Gyeonggi-do, Korea). All mice were housed in a specific pathogen-free standard room under controlled temperature (21.5-22.3°C), humidity (47-53.1%), and light cycle (12:12 h light-dark) conditions; the mice had access to rodent chow and tap water *ad libitum* for 1 week.

Experimental design. The mice were randomized into the following groups (n=9 in each group): normal control group (NC), high-fat diet (HFD) group, HFD with OLE (150 mg/kg) administered group, and HFD with garcinia (200 mg/kg) administered group. Mice were fed the experimental diets for 12 weeks. The body weight and food intake of mice were measured once per week. The mice were sacrificed by cardiac puncture under anesthetization and their blood and adipose tissues were collected for further analysis. The serum was isolated from the blood samples by centrifugation at 200 × g for 10 min and immediately stored at -80°C. The adipose tissue (epididymal fat, perirenal, retroperitoneal fat, and mesenteric fat) from each mouse was harvested and weighted. All experimental procedures involving animals were approved by the institutional Animal Care and Use Committee of CHA University (IACUC180078), and the experiments were performed according to the guidelines for animal experiments.

Histological examination. The epididymal fat was rinsed and fixed in 10% neutral-buffered formalin, embedded in paraffin, and cut into 4-µm sections that were stained with hematoxylin and eosin and observed under a microscope at 100× magnification. To measure adipocyte diameter, 10 random fields per group were evaluated with Image J software (NIH, Bethesda, MD, USA).

Biochemical analysis. Serum concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) cholesterol, free fatty acid (FFA), and glucose were evaluated with commercial assay kits and using the automatic analyzer TBA-30FR Accute (Toshiba, Tokyo, Japan). The levels of LDL+VLDL cholesterol were calculated by subtracting the HDL cholesterol levels from the total cholesterol levels. A small amount of blood was collected from the tail vein, and leptin and adiponectin levels were evaluated at 4, 8, and 12 weeks using the Mouse/Rat Leptin ELISA Kit (Morinaga, Yokohama, Japan) and Adiponectin ELISA kit (FineTest, Wuhan, China), respectively.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from the homogenized adipose tissue using an Easy-BLUE Total RNA Extraction kit (Intron Biotechnology, Seongnam, Republic of Korea) according to the manufacturer's instructions. cDNA was synthesized from 1 µg of

Table I. Primer sequences for RT-PCR amplification of target genes.

Gene		Primer sets
PPAR γ	F	5'-TTCGGAATCAGCTCTGTGGA-3'
	R	5'-CCATTGGGTCAGCTCTTGTG-3'
C/EBP- α	F	5'-AAGGCCAAGAAGTCGGTGA-3'
	R	5'-CCATAGTGGAAAGCCTGATGC-3'
UCPI	F	5'-GGTTTTGCACCACACTCCTG-3'
	R	5'-ACATGGACATCGCACAGCTT-3'
PGC-1 α	F	5'-TAAATCTGCGGGATGATGGA-3'
	R	5'-GTTTCGTTTCGACCTGCGTAA-3'
GAPDH	F	5'-AGAACATCATCCCTGCATCC-3'
	R	5'-TCCACCACCCTGTTGCTGTA-3'

F: Forward primer; R: reverse primer.

total RNA with CellScript All-in-One cDNA Master Mix (CellSafe, Yongin, Republic of Korea), and the samples were analyzed in triplicate with HiPi PCR Premix (Elpisbio, Daejeon, Republic of Korea). The expression of target genes was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences used in this study are listed in Table I.

Statistical analysis. Data are shown as the mean±standard error of the mean (SEM). Differences between groups were compared by one-way or two-way analysis of variance followed by Bonferroni post hoc test with GraphPad Prism v.6.01 software (GraphPad, Inc., La Jolla, CA, USA). $p < 0.05$ was considered as statistically significant.

Results

Effect of OLE on weight gain, food intake, and food efficiency ratio (FER) in HFD-fed mice. After 12 weeks of feeding of the different diets, the HFD group showed a significantly increased final body weight and cumulative weight gain compared to the NC group (Figure 1). A decrease in body weight and weight gain was observed in the OLE group (Figure 1B, C). Food intake levels of the HFD, OLE, and garcinia groups were approximately equal, while the FER was significantly attenuated by OLE and garcinia treatment (Figure 1D, E). Lower body weight and FER were observed in the OLE group compared to the garcinia group.

Effect of OLE on fat accumulation in HFD diet-fed mice. In both the OLE and garcinia groups, the epididymal and retroperitoneal tissue weights were significantly decreased compared to those in the HFD group, with similar changes observed in the total visceral fat weight (Figure 2A). Moreover, histological sections from the epididymal adipose tissue of the HFD group revealed a greater adipocyte diameter compared to that in the NC group; this change in adipocyte size was significantly reversed by OLE and garcinia administration (Figure 2B).

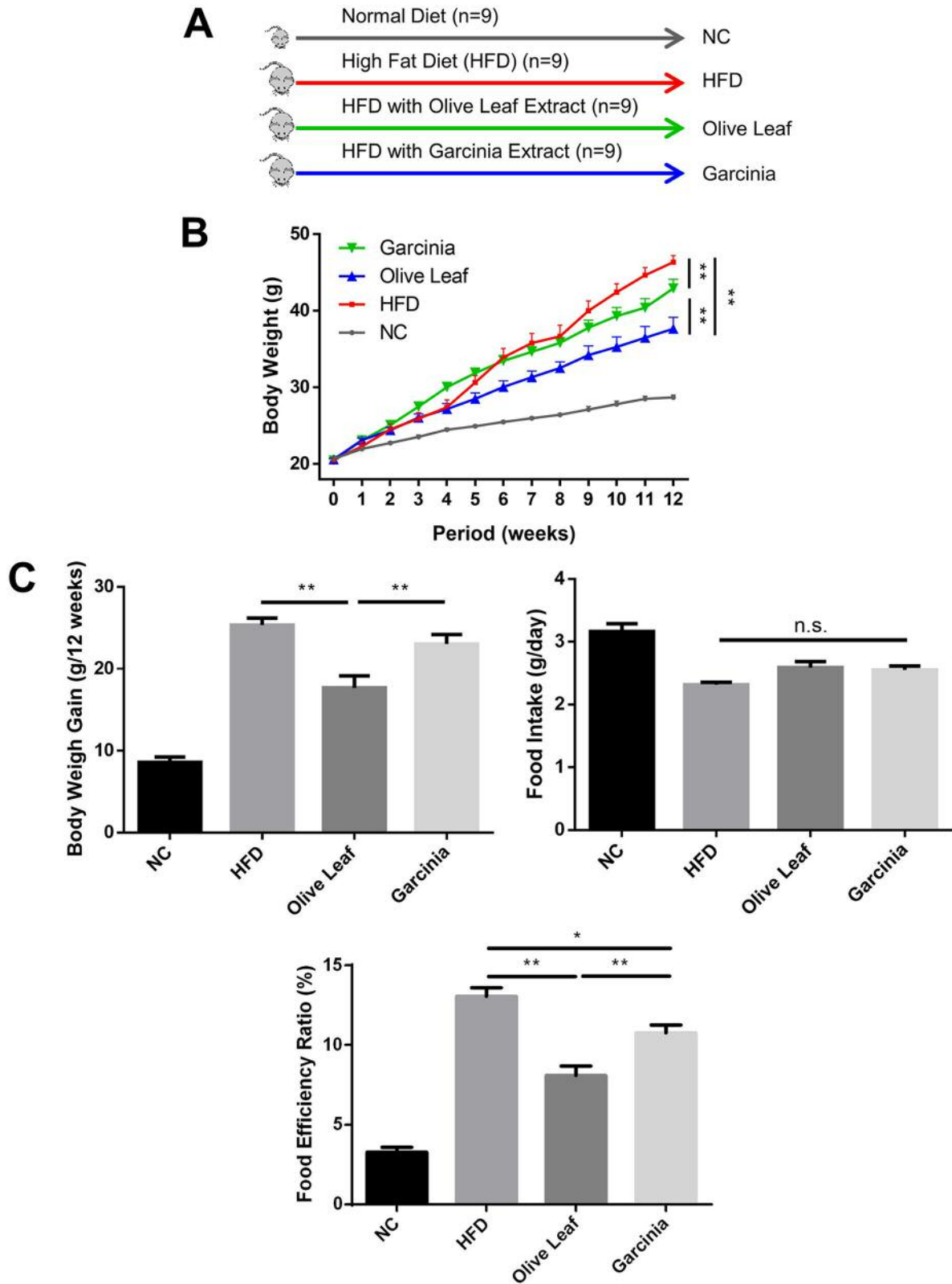


Figure 1. Effects of olive leaf extract on body weight gain, food intake, and food efficiency ratio (FER) of mice fed high-fat diet (HFD). (A) Experimental groups. (B) Body weight change over a 12-week period. (C-E) Body weight gain, food intake, and FER at 12 weeks. Results are presented as the mean±SEM (n=9) (*p<0.05, **p<0.01, N.S.: Not significant, FER=body weight gain for experimental period/food intake for experimental period).

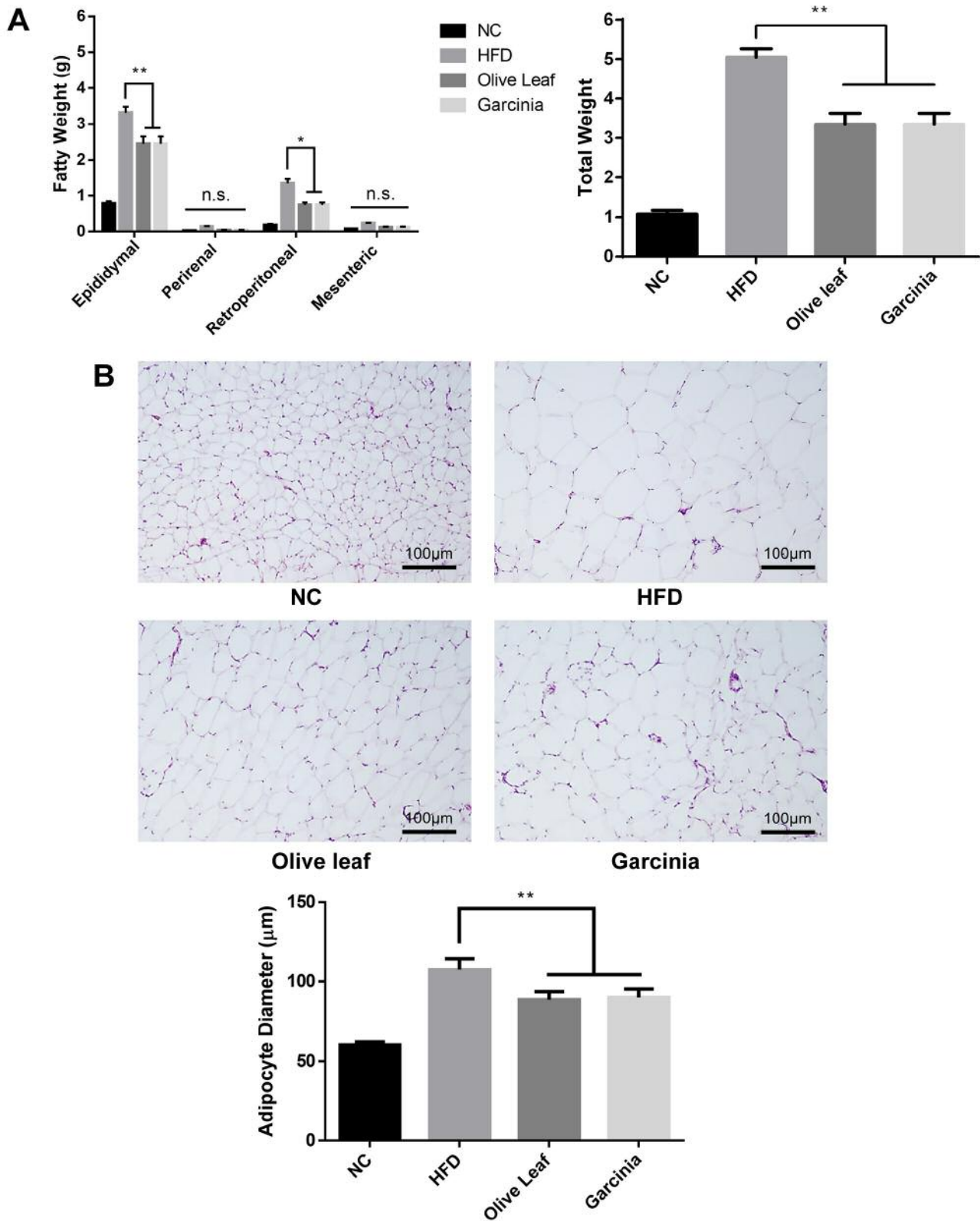


Figure 2. Effect of olive leaf extract on adipose tissue in HFD-induced mice. (A) Fatty weight of epididymal, perirenal, retroperitoneal, mesenteric, and total weight in each experimental group. (B) Histological analysis of the epididymal adipose tissue from each group was conducted to quantify the size of adipocytes. Results are presented as the mean±SEM (n=9) (*p<0.05, **p<0.01, N.S.: Not significant).

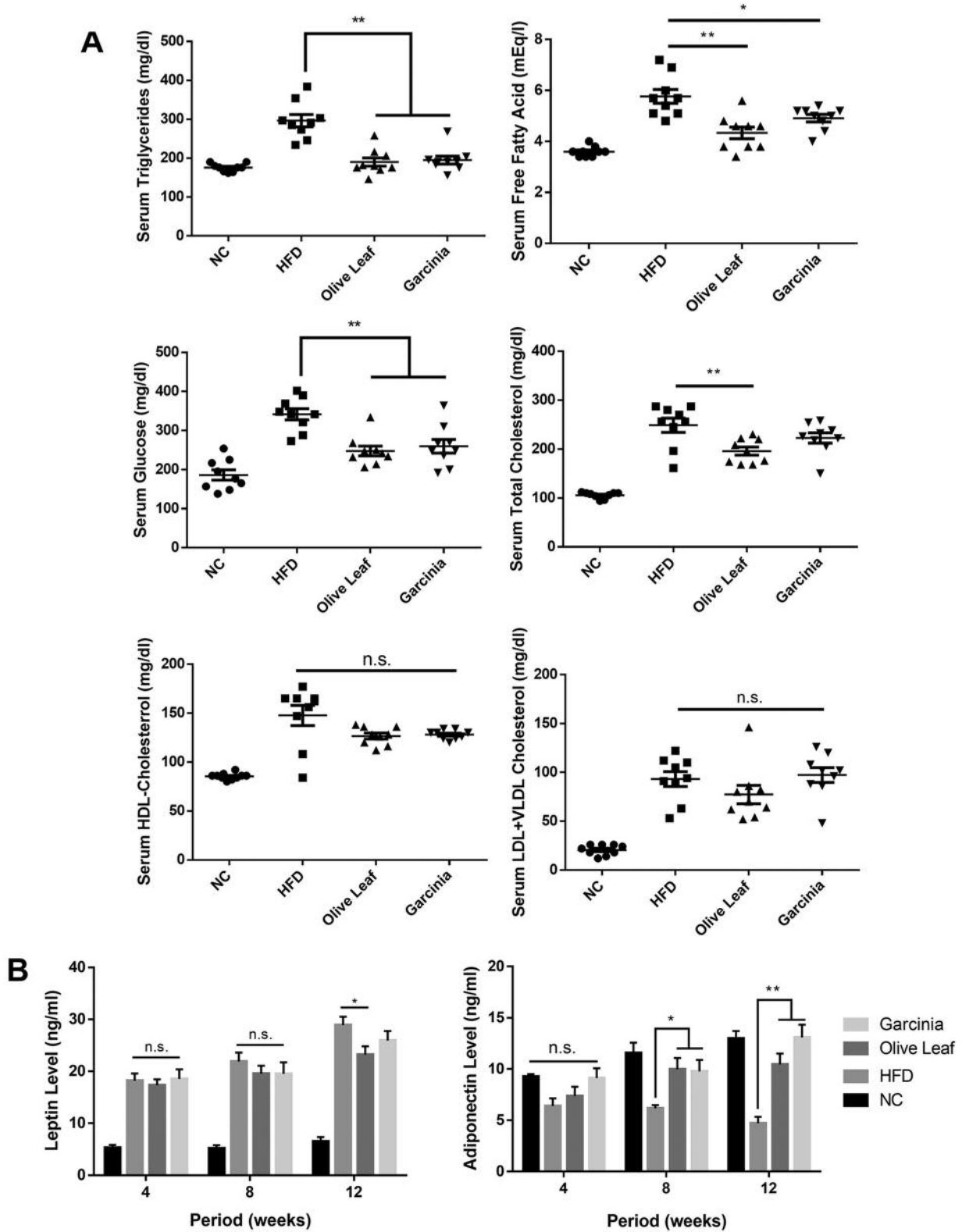


Figure 3. Effects of olive leaf extract on the levels of serum biochemicals and cytokines in obese mice fed high-fat diet. (A) Serum levels of triglycerides, free fatty acid, glucose, total cholesterol, HDL-cholesterol, LDL+VLDL-cholesterol were measured. (B) Leptin and adiponectin levels were analyzed by ELISA. Results are presented as the mean±SEM (n=9) (*p<0.05, **p<0.01, N.S.: Not significant).

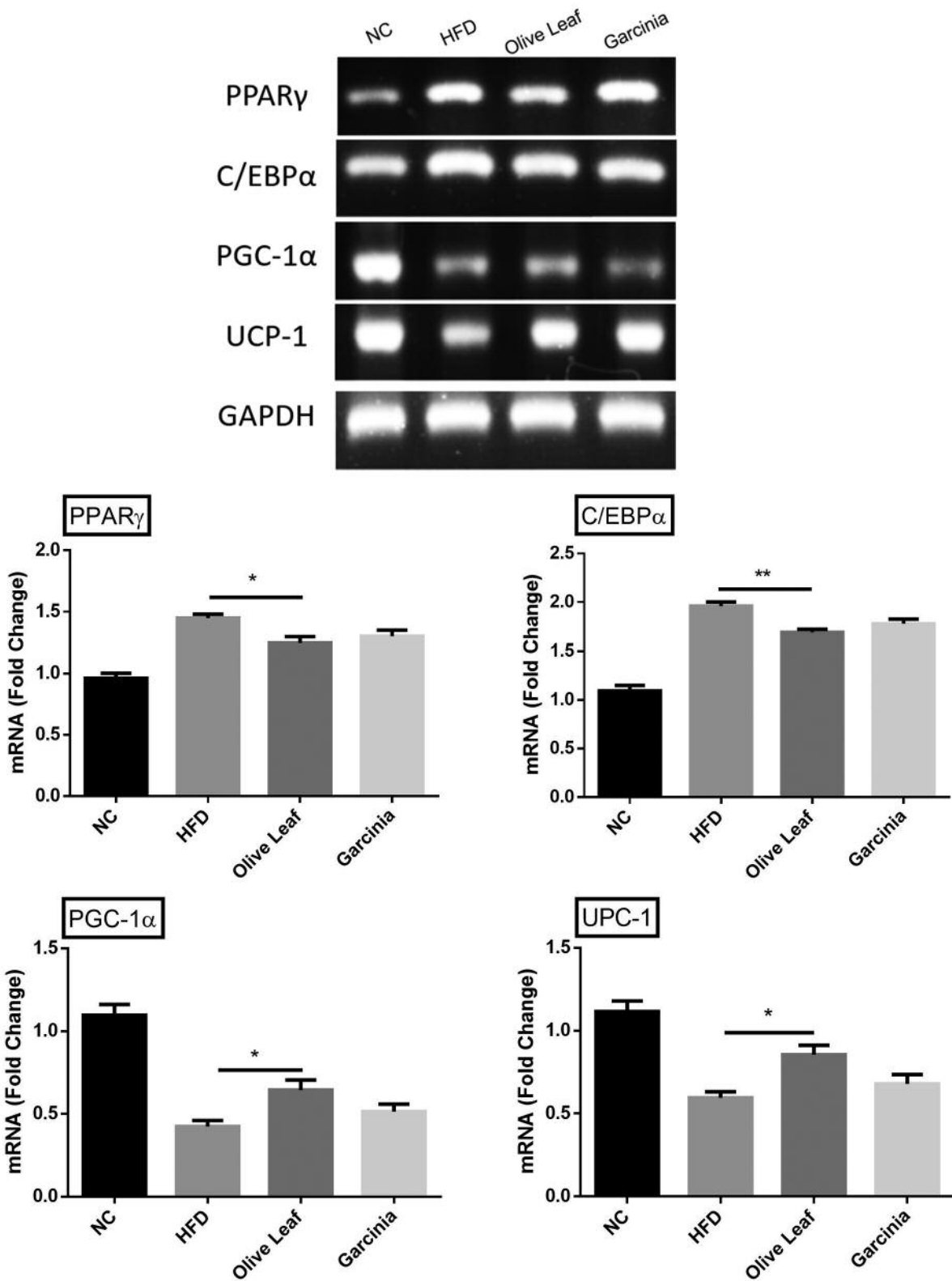


Figure 4. RT-PCR analysis of gene expression in the epididymal adipose tissue. The expression levels of adipogenesis-related (PPAR, C/EBP) and thermogenesis-related (PGC-1, UPC-1) mRNAs were evaluated by RT-PCR and normalized to that of GAPDH. Results are presented as the mean \pm SEM (n=9) (*p<0.05, **p<0.01, N.S.: Not significant).

Effect of OLE on serum biochemical and cytokines in HFD-fed mice. In serum biochemical and cytokine analysis, the levels of TG, FFA, and glucose were significantly ameliorated in both the OLE and garcinia groups compared to those in the HFD group (Figure 3A). Furthermore, the OLE group showed significantly ameliorated TC serum levels, while administration of OLE or garcinia had no significant effect on the serum levels of HDL and low-density lipoprotein (LDV) + very low-density lipoprotein (VLDL). Additionally, OLE administration resulted in significantly lower concentrations of leptin at 12 weeks and both the OLE and garcinia groups showed significantly upregulated adiponectin levels from 8 to 12 weeks (Figure 3B).

Effect of OLE on adipogenesis and thermogenesis gene expression in adipose tissue. Expression analysis of genes related to adipogenesis and thermogenesis in the epididymal fat of the OLE and garcinia groups showed that the mRNA expression levels of PPAR- γ and C/EBP α were significantly downregulated compared to those in the HFD group. OLE treatment significantly upregulated PGC-1 α and UPC-1 mRNA levels (Figure 4).

Discussion

This study was conducted to investigate whether OLE has anti-obesity effects in high-fat diet-induced mice. The results indicated that OLE prevented obesity by down-regulating adipogenesis and up-regulating thermogenesis in adipose tissue.

The incidence of obesity has increased because of the lack of exercise and poor eating habits of people; therefore, methods for managing obesity have gained attention (24). Additionally, studies have focused on the development of therapeutic agents from natural products that have few side effects. Olive leaf is a natural substance with therapeutic properties, and its extract contains several bioactive compounds including oleuropein, luteolin, hydroxytyrosol, apigenin, caffeic acid, and rutin (25). Recent studies demonstrated that olive leaf constituents improve management of obesity by decreasing the accumulation of intracellular lipid in the 3T3-L1 pre-adipocyte cell line (26-32). According to the results of these studies, OLE compound has anti-obesity effects. The present study showed that cumulative weight gain and FER were significantly decreased in HFD-fed mice after OLE treatment (Figure 1). Furthermore, the total visceral fatty weight was reduced by OLE administration (Figure 2).

Adipocytes in fat tissue secrete numerous hormones and cytokines to regulate lipid, glucose, and energy metabolism, thus maintaining the energy balance (33). However, adipocyte accumulation in adipose tissue may cause obesity and related complications. Recent studies demonstrated that long-term HFD increases in serum TG, FFA, glucose, TC, HDL, and

LDL decrease cholesterol levels, leading to an imbalance in serum lipid composition (24, 34, 35). This imbalance is considered as the main risk factor for several diseases. OLE effectively decreased TG, FFA, glucose, and TC levels in the serum, while HDL and LDL-cholesterol levels were not significantly changed compared to those in the HFD group. Leptin and adiponectin are secreted mainly from adipose tissue to control energy homeostasis (36). Leptin in the plasma and adipose tissue are related to energy stores; leptin levels are increased in obesity (37) and the serum levels of leptin are proportional to fat mass in various obesity rodent models (3, 38). Additionally, adiponectin exerts potent effects on the nervous system to regulate energy expenditure (37, 39, 40). Serum levels of adiponectin decrease with obesity and are positively associated with insulin sensitivity; a previous study showed that weight loss elevates plasma adiponectin levels (41, 42). In this study, we found that leptin levels were significantly reduced in the OLE group, while the opposite results were observed for adiponectin.

Adipogenesis is the process of a pre-adipocyte becoming a mature adipocyte; both PPAR γ and C/EBP α are key transcription factors involved in adipocyte differentiation (7, 43-45). Recent studies evaluated thermogenesis in obesity mouse models by measuring PGC-1 α and UPC-1 levels (46-48). The present study confirmed that OLE treatment significantly inhibited the expression of PPAR γ and C/EBP α and significantly elevated PGC-1 α and UPC-1 levels. Taken together, these results suggest that the anti-obesity effects of OLE occur through the control of adipogenesis and thermogenesis in vesical adipose tissue. Recently, hepatic lipid metabolism was shown to influence obesity and may be a therapeutic target (49). Therefore, further studies are needed to evaluate the effects of OLE on liver metabolism and predict the efficacy of OLE in obesity patients.

Conclusion

In conclusion, OLE significantly ameliorated increases in body weight, visceral fat, and serum lipid levels in HFD-diet mice. Furthermore, OLE showed anti-obesity effects by regulating the expression of molecules involved in adipogenesis and thermogenesis in the adipose tissue. Taken together, our results suggest that OLE is useful for preventing or treating obesity.

Conflicts of Interest

There are no conflicts of interest to declare regarding this study.

Authors' Contributions

Y CJ, HWK: conception and design, collection and/or assembly of data, data analysis and interpretation; BKM, JYC, HJS, JYL, JYK,

SBW: collection and/or assembly of data; QL, HWL: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript.

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