

The Extract of *Humulus japonicus* Inhibits Lipogenesis and Promotes Lipolysis via PKA/p38 Signaling

Jaw Long Sun^a Young Jin Kim^b Wonjun Cho^a Sung Su Park^a
A.M. Abd El-Aty^{c,d} Enas H. Mobarak^e Tae Woo Jung^a Ji Hoon Jeong^{a,f}

^aDepartment of Pharmacology, College of Medicine, Chung-Ang University, Seoul, Republic of Korea;

^bDepartment of Surgery, College of Medicine, Chung-Ang University, Seoul, Republic of Korea; ^cDepartment of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; ^dDepartment of Medical Pharmacology, Medical Faculty, Ataturk University, Erzurum, Turkey; ^eDepartment of Restorative Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia; ^fDepartment of Global Innovative Drugs, Graduate School of Chung-Ang University, Seoul, Republic of Korea

Keywords

Humulus japonicus · Obesity · Adipocyte · Protein kinase A · p38 MAPK · Lipolysis

Abstract

Introduction: Previous research has shown that an aqueous extract of *Humulus japonicus* (EH) can ameliorate hypertension, nonalcoholic fatty liver disease, and oxidative stress in adipocytes by activating the thermogenic pathway. However, the effects of an ethanol (30%) extract of EH on obesity are unknown.

Methods: Various protein expression levels in fully differentiated 3T3-L1 adipocytes were assessed by Western blotting. Lipid deposition in 3T3-L1 adipocytes was examined by oil red O staining. The MTT assay was used to evaluate adipocyte viability. Caspase 3 activity and glycerol release were determined using commercial assay kits. **Results:** In this study, we discovered that EH treatment inhibited lipogenesis and promoted lipolysis in both differentiated 3T3-L1 adipocytes and adipose tissue of mice fed a high-fat diet. EH treatment also increased phosphorylated protein kinase A (PKA) levels while reducing p38 phosphorylation. When H89, a PKA inhibitor, was used, the effects of EH on

lipogenic lipid accumulation and lipolysis in 3T3-L1 adipocytes were eliminated. Treatment with luteolin 7-O- β -D-glucoside (LU), the major active compound in EH, also suppressed lipid deposition and p38 phosphorylation but enhanced lipolysis in 3T3-L1 adipocytes. These changes were abrogated by H89. **Conclusion:** These findings indicate that EH containing LU reduces lipogenesis and stimulates lipolysis via the PKA/p38 signaling pathway, leading to an improvement in obesity in mice. Therefore, our study suggested that EH could be a promising therapeutic agent for treating obesity.

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Introduction

As society advances, the prevalence of obesity is rapidly increasing due to a sedentary lifestyle and excessive calorie intake [1, 2]. This surge in obesity has led to an

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increase in metabolic diseases, such as insulin resistance, diabetes, nonalcoholic fatty liver disease (NAFLD), and cardiovascular disease [3]. Obesity is a significant social health problem because it not only has an aesthetic impact but also triggers the onset of various metabolic diseases [4]. Although steady aerobic exercise is the most effective way to control obesity [5], it can be challenging for severely obese individuals or those who cannot exercise effectively due to environmental factors. Therefore, the discovery of candidate substances that are less toxic to the human body and efficiently improve obesity is crucial not only for treating obesity but also for preventing metabolic diseases.

Humulus japonicus (EH) is a traditional food and/or medicinal herb that has been historically used to treat a range of ailments, such as diarrhea, hypertension, pneumonia, and leprosy [6]. In Korea, the leaves of EH have been utilized for treating pulmonary tuberculosis, tuberculosis, cervical lymphadenitis, and hypertension [7]. Recent studies have shown that the extract of EH has beneficial effects on metabolic disorders. Notably, the extract has been found to possess antioxidative and anti-inflammatory properties [8, 9]. In addition, research conducted by Jung et al. [8] indicated that an aqueous extract of EH enhances thermogenesis, reduces oxidative stress, and promotes lipolysis in adipocytes, thereby reducing lipid accumulation. Furthermore, Chung et al. [10] demonstrated that an aqueous extract of EH ameliorates hyperlipidemia and hepatic steatosis in obese mice. Peng et al. [11] and Cho et al. [12] previously identified vitexin, which has an antiobesity property [11], and luteolin 7-O- β -d-glucoside (LU) as a major ingredient in the ethanol (30%) extract of EH and demonstrated the suppressive effects of LU on hepatic steatosis [12].

To the best of our knowledge, the impact of EH ethanol extract on adipocyte lipid metabolism has not been examined, although Jung et al. reported the effects of EH water extract on oxidative stress and thermogenesis in adipocytes [8]. This study aimed to examine the effects of EH on obesity through in vitro and in vivo experiments. Additionally, we sought to investigate the molecular mechanisms underlying the impact of EH on cultured adipocytes.

Materials and Methods

EH Preparation

In this study, an ethanol extract of EH leaves was kindly provided by Hanpoong Nature Pharm (Iksan, South Korea). The EH leaves were extracted as follows: 1.2 kg of EH (obtained from

Woori Pharm, Gimpo, Republic of Korea) was mixed with 10 volumes of 30% (v/v) ethanol and heated at 85°C for 3 h. The extract was then filtered through a 5- μ m filtering system, and the residue was re-extracted under the same conditions. The resulting extract was concentrated under reduced pressure at less than 60°C and then dried using a vacuum system to yield 103 g of extract (8.58% yield). The botanical identification of EH was performed by Dr. Yuan Lu Sun at Solvit P&F (Goyang, Republic of Korea).

Animal Experiments

The animal study was approved by the Institutional Animal Review Board at Chung-Ang University (Seoul, Korea; approval No. A2022012) and conducted in accordance with the Laboratory Animal Care and Use Guide (NIH publication, 8th edition, 2011). Male C57BL/6J (B6) mice aged 7 weeks were divided into different groups ($n = 5$) and fed a normal diet (ND) (Cat. No. 710027; Dyets, Bethlehem, PA, USA), a high-fat diet (HFD) (60% fat and 20% carbohydrate) (Cat. No. D12492; Research Diets, New Brunswick, NJ, USA), and/or EH at doses of 0–300 mg/kg via oral administration every other day for 8 weeks. EH was dissolved in distilled water (DW). Equivalent volumes of DW were orally administered to both the ND and HFD groups, similar to the treatment group. Throughout the in vivo studies, the weight of the mice was monitored weekly, and food intake was determined as the average of four measurements taken once per week.

Adipocyte Culture/Differentiation and Reagents

3T3-L1 mouse preadipocytes (ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% bovine calf serum (Invitrogen), 100 units/mL penicillin, and 100 μ g/mL streptomycin (Invitrogen) at 37°C in a humidified atmosphere containing 5% CO₂. After reaching confluence on day 2, the cells were differentiated by adding medium supplemented with 10% fetal bovine serum (Invitrogen), 1 μ M insulin, 0.5 mM IBMX, and 0.5 μ g/mL dexamethasone for 2 days, followed by another 3 days in medium containing 1 μ M insulin. EH suspended in phosphate-buffered saline at concentrations ranging from 0 to 100 μ g/mL was used to treat 3T3-L1 adipocytes for 24 h. The negative control was phosphate-buffered saline or dimethyl sulfoxide (DMSO), while LU (Sigma) and H89 (Sigma) dissolved in DMSO were used in the experiments. Lipopolysaccharide (LPS) (Sigma) was dissolved in DW.

Western Blot Analysis and Antibodies

To obtain protein samples for the experiment, cultured cells and mouse adipose tissue were collected and treated with lysis buffer (PRO-PREP; Intron Biotechnology, Seoul, Republic of Korea) for 60 min at 4°C. The extracted protein samples were between 35 and 40 μ g and subjected to SDS-PAGE on gels ranging from 7 to 12%, followed by transfer to nitrocellulose membranes (Amersham Biosciences, Westborough, MA, USA). Then, primary antibodies specific to the target protein were used to probe the membranes, followed by incubation with secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology, USA). Enhanced chemiluminescence kits (Amersham Biosciences) were used to detect signals. The following antibodies were used for the experiments: anti-SCD1 (1:1,500), anti-SREBP1 (1:1,500), anti-phospho-PKA (1:1,000), anti-PKA (1:2,000), anti-phospho-

p38 (1:1,000), anti-p38 (1:2,000), and anti- β -actin (1:2,500) from Santa Cruz Biotechnology and anti-phospho hormone-sensitive lipase (HSL) (1:1,000) and anti-HSL (1:2,000) from Cell Signaling Technology (Danvers, MA, USA).

Oil Red O Staining

To evaluate cellular lipid accumulation, we employed oil red O staining. The cells were fixed in 10% formalin for 1 h, followed by staining with preprepared oil red O solution (Sigma) for an hour at 37°C. The stained lipids were quantified by adding isopropanol to each sample and gently agitating them for 10 min at room temperature. The quantity of extracted samples (100 μ L) was then analyzed using a spectrophotometer at 510 nm.

Hematoxylin and Eosin Staining and Adipocyte Size Measurement in Visceral Adipose Tissue

As a routine protocol, we performed hematoxylin and eosin staining and analyzed the cross-sectional areas of the visceral adipose tissue to measure the size of adipocytes. The analysis was carried out using ImageJ software provided by the NIH (Bethesda, MD, USA).

Glycerol Quantitation

To examine the glycerol release levels in mouse serum and 3T3-L1 adipocyte culture media, we used a colorimetric lipolysis assay kit (Abcam, Cambridge, MA, USA).

Apoptosis Assay

We used two assays to evaluate apoptosis: the MTT assay and caspase 3 activity assay [13]. To perform the MTT assay, the experimental 3T3-L1 adipocyte cultures were incubated in 96-well plates with MTT solution (Sigma) for 2 h at 37°C. The red formazan generated inside the cells was then dissolved in DMSO (Sigma) for 10 min at room temperature with gentle agitation. Afterward, the optical density was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) at 550 nm. For the caspase 3 activity assay, the researchers utilized a colorimetric caspase 3 activity assay kit (Abcam).

TNF α Release Assay

Following the experimental treatments, the supernatants of the culture media from 3T3-L1 adipocytes were collected and stored at -70°C for further analysis. To quantify the levels of TNF α in the collected culture media, commercially available enzyme-linked immunosorbent assay kits (R&D Systems) were used, and the manufacturer's instructions were followed.

Statistical Analyses

The statistical analysis was conducted using GraphPad Prism version 6 software (San Diego, CA, USA), and the results are presented as the mean $\pm$ standard deviation (SD) relative to the highest values. The experiments were repeated independently three to five times, and statistical significance was determined by performing one-way repeated analysis of variance (ANOVA) followed by Tukey's post hoc test. The protein expression levels were analyzed with ImageJ software (NIH, Bethesda, MD, USA).

Results

EH Administration Alleviates Obesity in Mice by Modulating Lipogenesis and Lipolysis

As obesity is characterized by the enlargement of adipose tissue due to an increase in adipocyte size [14], we investigated whether EH administration affected adipocyte size in visceral fat tissue. Our results showed that mice fed an HFD for 8 weeks had increased body weight (Fig. 1a) without changes in caloric intake or adipocyte size in visceral adipose tissue (Fig. 1b), along with increased expression of lipogenic proteins, such as SREBP1 (processed form) and SCD1 (Fig. 1c). However, EH administration reversed these changes (Fig. 1). Additionally, HFD-fed mice exhibited increased phosphorylation of HSL in adipose tissue and increased serum glycerol levels. EH administration further increased the effects of HFD on lipolysis (Fig. 1d). These results indicate that EH has the potential as a therapeutic agent for obesity-related metabolic disorders.

EH Treatment Suppresses Lipogenic Lipogenesis and Enhances Lipolysis in Cultured Adipocytes

To optimize the cell treatment conditions, we conducted preliminary in vitro experiments to assess the effect of EH on adipocyte viability. Treatment of 3T3-L1 adipocytes with EH at concentrations of 0–50 μ g/mL for 24 h did not affect cell viability. However, at a concentration of 100 μ g/mL, EH significantly ($p < 0.05$) decreased cell viability (Fig. 2a). Therefore, we administered EH at concentrations of 0–50 μ g/mL to investigate its effect on lipid metabolism in adipocytes. We observed a dose-dependent reduction in lipid accumulation in differentiated 3T3-L1 adipocytes treated with EH (Fig. 2b), along with a decrease in the expression of lipogenic proteins, such as SREBP1 (processed form) and SCD1 (Fig. 2c). Moreover, treatment with EH increased phosphorylated HSL expression and glycerol release in 3T3-L1 adipocytes (Fig. 2d). The lipolysis evaluation was conducted without the preadipocyte group, as the effects of EH on mature adipocytes needed to be assessed (Fig. 2d).

Protein Kinase A/p38 Signaling Contributes to the Effects of EH on Lipogenic Lipid Deposition and Lipolysis in Adipocytes

Previous studies have shown that protein kinase A (PKA) can decrease the formation of lipids while increasing their breakdown in adipocytes [15]. Additionally, p38 mitogen-activated protein kinase (MAPK) has

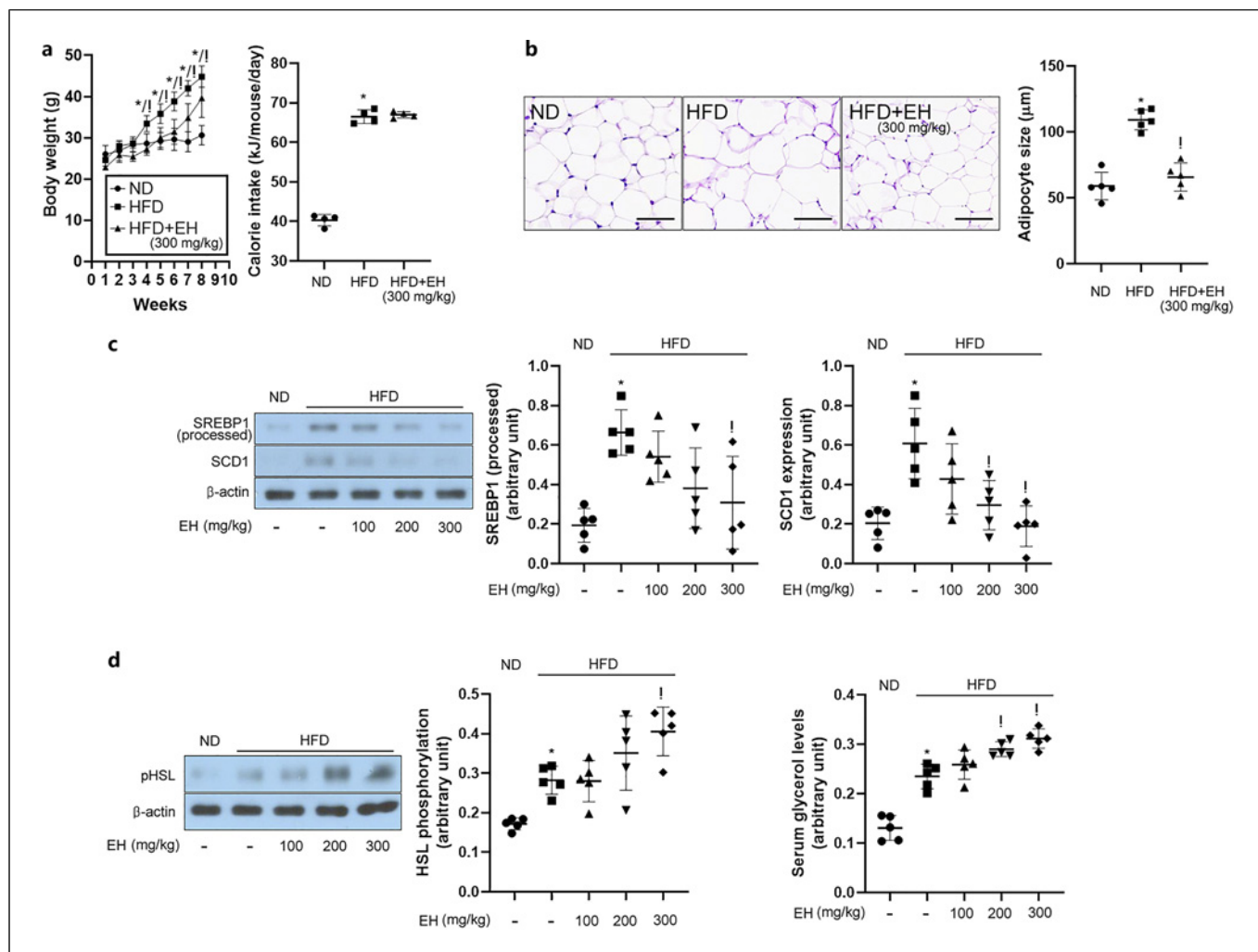


Fig. 1. EH improves obesity via the suppression of lipogenesis and upregulation of lipolysis-mediated reductions in body weight in HFD-fed mice. **a** Measurement of body weight gain and caloric intake during in vivo experiments. **b** H&E staining of visceral adipose tissue from experimental mice (each $n = 5$). Western

blotting of processed SREBP1 and SCD1 (**c**) and phosphorylated HSL (**d**) in visceral adipose tissue from experimental mice (each $n = 5$). The means $\pm$ SDs were calculated from five independent experiments. Significance ($p < 0.05$) *versus ND; [†]versus HFD. ND, normal diet; HFD, high-fat diet; H&E, hematoxylin and eosin.

been found to promote lipid buildup and decrease lipolysis in adipocytes [16]. PKA-mediated signaling can also cause p38 to lose its phosphate group [17]. In HFD-fed mice, EH administration led to an increase in PKA phosphorylation and a decrease in p38 phosphorylation in adipose tissue (Fig. 3a). Similar to the in vivo results, EH treatment of 3T3-L1 adipocytes dose-dependently increased PKA phosphorylation while inhibiting p38 phosphorylation (Fig. 3b). Furthermore, in 3T3-L1 adipocytes, H89, a PKA inhibitor, reduced the effects of EH on lipid accumulation, lipogenic protein expression, glycerol release, and phosphorylated p38 (Fig. 3c–e).

Western blotting and glycerol release assays were conducted without including the preadipocyte group because the focus was on assessing the effects of EH on mature adipocytes (Fig. 3d, e).

A High Dose of EH Causes Adipocyte Apoptosis without Inflammation

Targeting adipocyte apoptosis has been suggested as a potential therapeutic approach for treating obesity [18]. In our study, a high dose (100 μ g/mL) of EH reduced the viability of 3T3-L1 adipocytes and increased caspase 3 activity (Fig. 4a). However, the

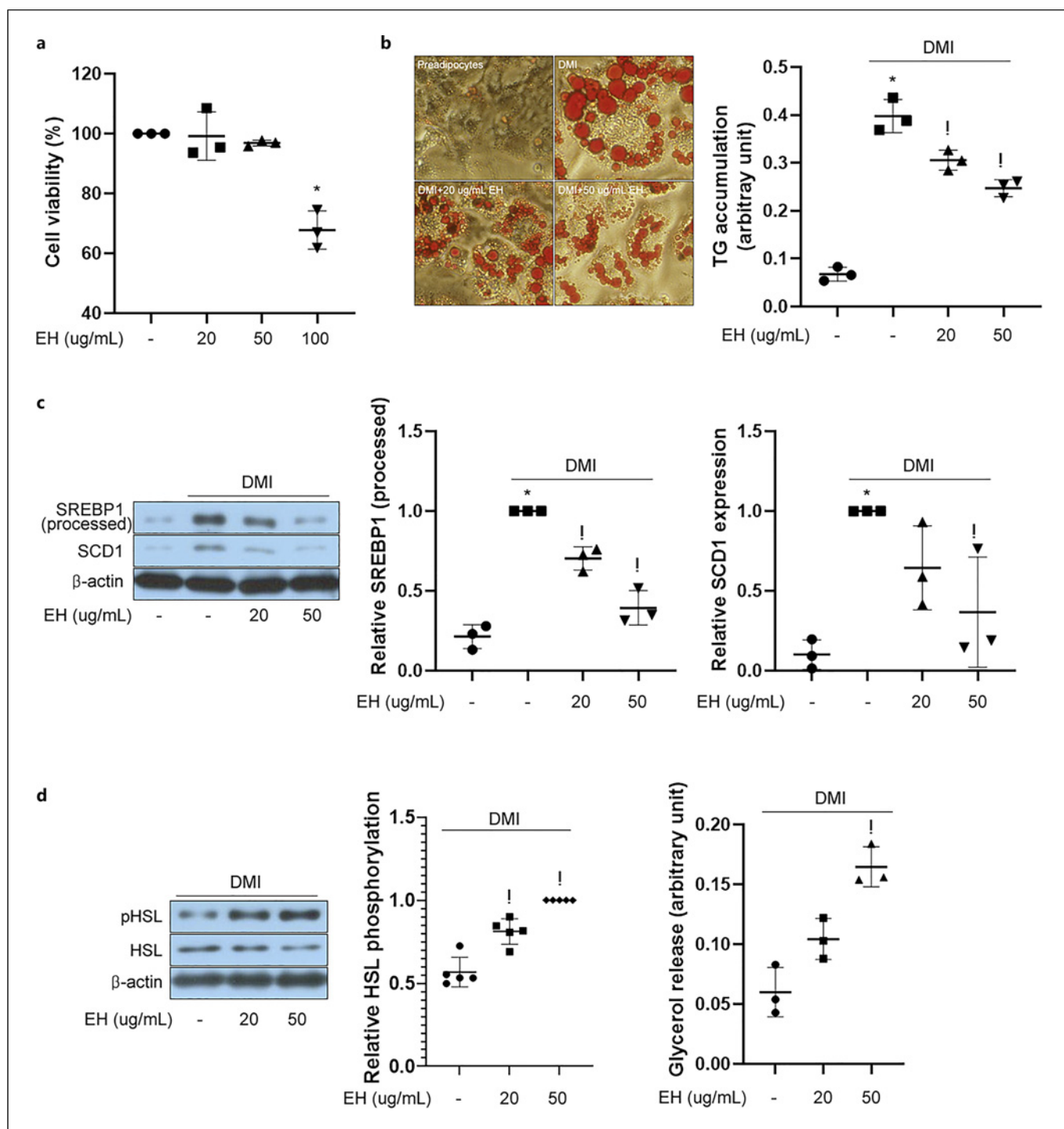
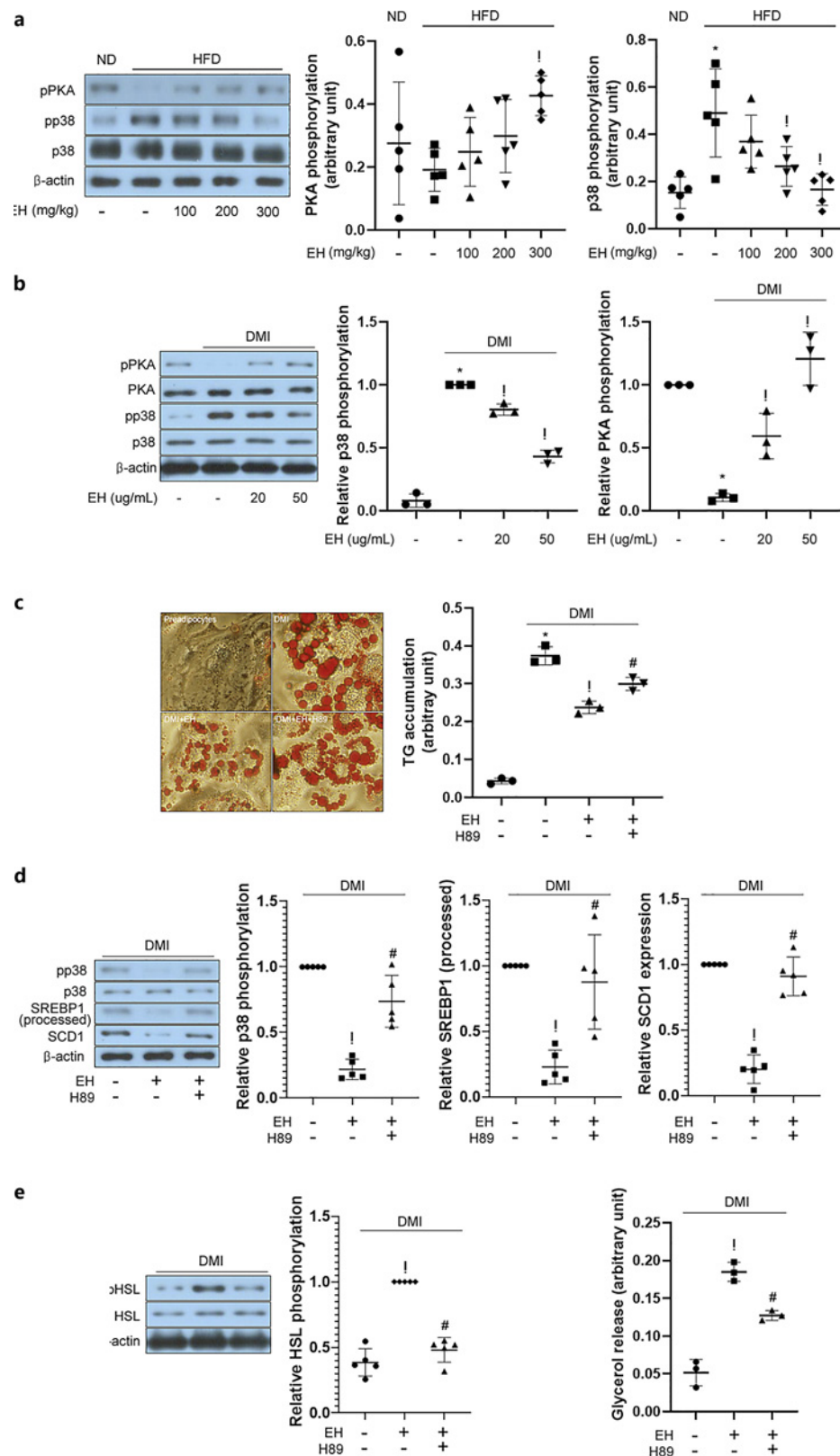


Fig. 2. EH attenuates lipid accumulation and enhances lipolysis in cultured adipocytes. **a** Cell viability assay in differentiated 3T3-L1 adipocytes treated with EH (0–100 µg/mL) for 24 h. Oil red O staining (**b**) and Western blotting of processed SREBP1 and SCD1 (**c**) in 3T3-L1 adipocytes treated with EH (0–50 µg/mL) for 24 h.

d Western blotting of phosphorylated HSL and the glycerol release assay in 3T3-L1 adipocytes. The means ± SDs were calculated from three or five independent experiments. Significance ($p < 0.05$) *versus control (not treated); !versus DMI. DMI: 1 µM insulin, 0.5 mM IBMX, and 0.5 µg/mL DMI. DMI, dexamethasone.

Fig. 3. EH ameliorates lipid deposition through PKA-regulated p38-dependent signaling in cultured adipocytes. **a** Western blot analysis of phosphorylated PKA and p38 in the visceral adipose tissue of experimental mice (each $n = 5$). **b** Western blot analysis of phosphorylated PKA and p38 in 3T3-L1 adipocytes treated with EH (0–50 $\mu\text{g/mL}$) for 24 h. Oil red O staining (**c**) and Western blotting of phosphorylated p38, processed SREBP1 and SCD1 (**d**) in 3T3-L1 adipocytes treated with EH (50 $\mu\text{g/mL}$) and/or H89 (10 μM) for 24 h. **e** Western blotting of phosphorylated HSL and the glycerol release assay in 3T3-L1 adipocytes treated with EH (50 $\mu\text{g/mL}$) and/or H89 (10 μM) for 24 h. The means $\pm$ SDs were calculated from three or five independent experiments. Significance ($p < 0.05$) *versus ND, control (not treated); † versus HFD or DMI; #versus DMI plus EH. DMI: 1 μM insulin, 0.5 mM IBMX, and 0.5 $\mu\text{g/mL}$ DMI. DMI, dexamethasone.



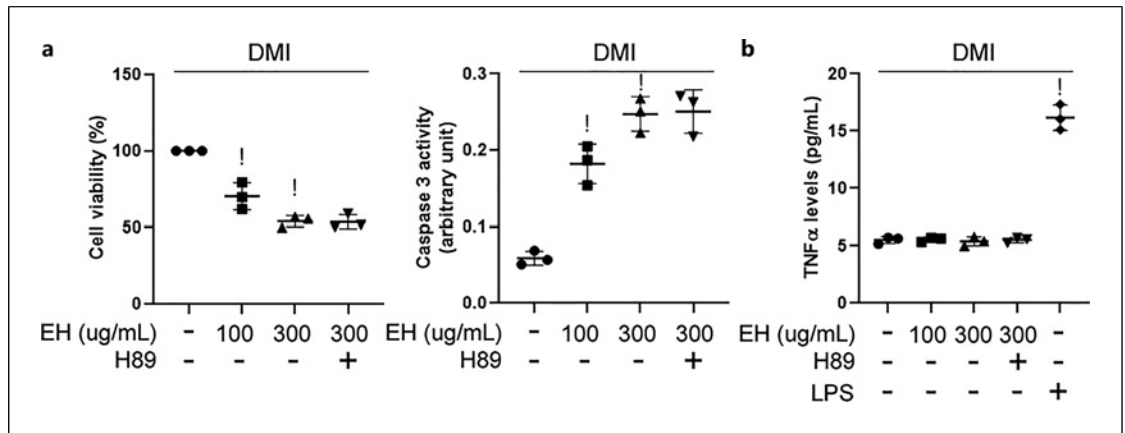


Fig. 4. EH causes adipocyte apoptosis without inflammation. **a** Cell viability assay and caspase 3 activity assay in differentiated 3T3-L1 adipocytes treated with EH (0–300 $\mu\text{g/mL}$) and/or H89 (10 μM) for 24 h. **b** ELISA of TNF α in the culture media of 3T3-L1 adipocytes treated with EH (0–300 $\mu\text{g/mL}$), LPS (1 $\mu\text{g/mL}$), and/or H89 (10 μM) for 24 h. Means $\pm$ SDs were calculated from three independent experiments. Significance ($p < 0.05$) ¹versus DMI. DMI: 1 μM insulin, 0.5 mM IBMX, and 0.5 $\mu\text{g/mL}$ DMI. DMI, dexamethasone.

administration of EH did not stimulate the release of the proinflammatory cytokine TNF α from 3T3-L1 adipocytes. In contrast, LPS, which served as a positive control [19], typically increased TNF α secretion (Fig. 4b). Notably, treatment with H89 did not affect the effects of EH on cell viability or caspase 3 activity (Fig. 4a). These experiments did not include the preadipocyte group because the goal was to assess the effects of EH on mature adipocytes (Fig. 4).

LU Attenuates Lipid Accumulation and p38 Phosphorylation through PKA Signaling

We further investigated the effects of LU, a main compound in EH [12], on lipid deposition and p38 phosphorylation to confirm that LU plays a crucial role in the impacts of EH on adipocytes. LU treatment suppressed lipid accumulation and phosphorylated p38 expression in 3T3-L1 adipocytes. However, H89 reversed all these changes (Fig. 5). The glycerol release assay excluded the preadipocyte group to specifically assess how EH affects mature adipocytes (Fig. 5b).

Discussion

To effectively treat obesity, it is important to reduce lipid accumulation by suppressing lipogenesis and inducing lipolysis in adipocytes [20, 21]. In this study, we confirmed the antiobesity effect of EH using in vitro and in vivo experimental models of obesity. Our study yielded novel findings on the effect of EH on obesity. First, in

mice fed an HFD, EH reduced body weight and adipocyte size in visceral adipose tissue by inhibiting lipogenesis and promoting lipolysis. Second, in fully differentiated 3T3-L1 adipocytes, EH treatment decreased lipid accumulation and stimulated lipolysis. Third, the PKA/p38 mechanism plays a role in the effect of EH on adipocyte lipid metabolism. Fourth, high concentrations of EH induced apoptosis without inflammation, independent of the PKA/p38 pathway.

Initially, we examined the impact of EH administration on weight gain and caloric intake associated with obesity in mice fed an HFD. Our results showed that EH treatment led to a decrease in weight gain caused by an HFD without affecting caloric intake, and the size of adipocytes in the visceral adipose tissue of the experimental mice that were fed an HFD was reduced by EH treatment. In addition, we observed that EH administration inhibited lipogenesis and stimulated lipolysis. These findings were also consistent in cultured mouse adipocytes, where EH administration suppressed lipogenesis in a concentration-dependent manner and stimulated lipolysis, similar to the results of the in vivo experiments. Taken together, these results suggest that EH has the potential to be an effective antiobesity agent.

PKA plays a critical role in lipid metabolism for maintaining energy homeostasis, particularly in the regulation of adipogenesis and lipolysis in adipocytes [15]. Liu et al. [22] discovered that PKA activation reduces adipogenesis, while suppressing PKA activity enhances adipogenic signaling in 3T3-L1 preadipocytes.

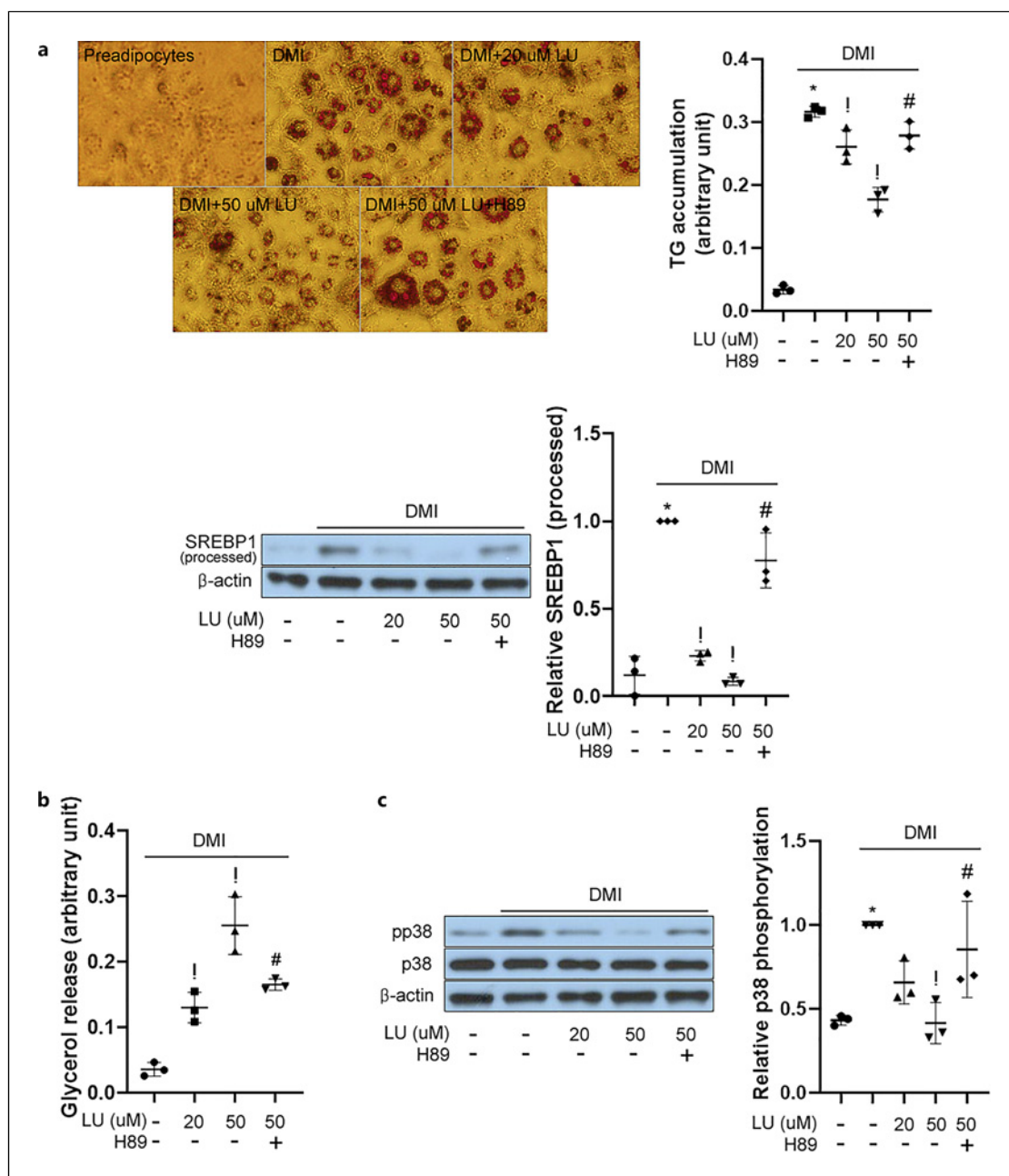


Fig. 5. LU suppresses lipid accumulation and enhances lipolysis in cultured adipocytes through the PKA/p38 pathway. **a** Oil red O staining and Western blotting of processed SREBP1 in 3T3-L1 adipocytes treated with LU (0–50 μ M) and/or H89 (10 μ M) for 24 h. **b** Glycerol release assay in 3T3-L1 adipocytes treated with LU (0–50 μ M) and/or H89 (10 μ M) for 24 h. **c** Western blotting

of phosphorylated p38 in 3T3-L1 adipocytes treated with LU (0–50 μ M) and/or H89 (10 μ M) for 24 h. The means $\pm$ SDs were calculated from three independent experiments. Significance ($p < 0.05$) *versus control (not treated); ! versus DMI; # versus DMI plus 50 μ M LU. DMI: 1 μ M insulin, 0.5 mM IBMX, and 0.5 μ g/mL dexamethasone.

PKA promotes lipolysis by phosphorylating HSL, which contacts lipid droplets in adipocytes, as well as perilipin, which stimulates lipolysis [23, 24]. Furthermore, recent studies have shown that the myokine musclin can de-

crease lipid accumulation and increase lipolysis in mouse adipocytes through PKA-mediated signaling [17]. Our current research indicated that treatment with EH increases PKA phosphorylation in 3T3-L1 adipocytes in a

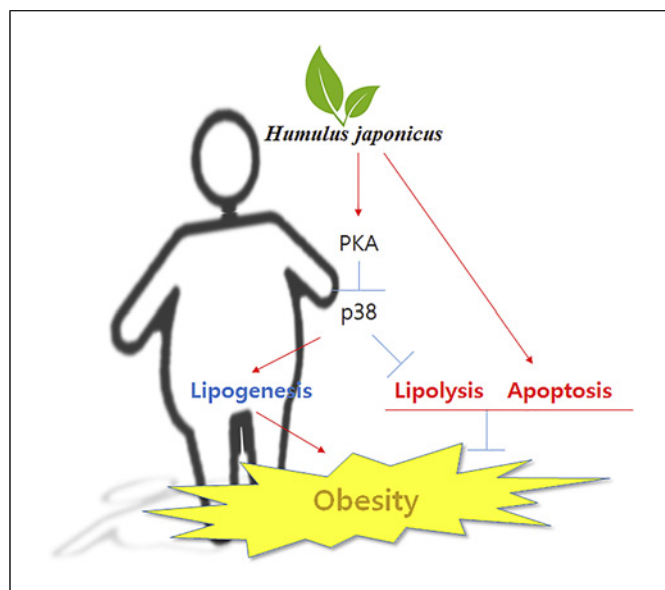


Fig. 6. Schematic diagram of the effects of EH on lipid metabolism and apoptosis in adipose tissue.

dose-dependent manner. Moreover, the effects of EH on lipogenesis and lipolysis were eliminated by H89, suggesting that the PKA-mediated pathway may play a role in the antiobesity effects of EH.

p38 MAPKs are known to react to a variety of stimuli, including ultraviolet light, heat and cold shocks, and inflammatory cytokines. They are also involved in cell differentiation, cell viability, and autophagy [25]. The p38 signaling pathway plays an important role in regulating adipogenesis. Engelman et al. [26] demonstrated that several p38 inhibitors suppress 3T3-L1 differentiation, resulting in reduced lipid accumulation. Elevated p38 activity is detected during human preadipocyte differentiation. Conversely, pharmacological inhibition of p38 prevents lipid accumulation in human adipocytes [27]. In addition, constitutive activation of p38-dependent signaling contributes to enhanced spontaneous adipogenesis in 3T3-L1 preadipocytes [28]. Therefore, appropriately suppressing p38 signaling could be a therapeutic approach for treating obesity. In the present study, we observed that treatment with EH reduced p38 phosphorylation in the visceral adipose tissue of HFD-fed mice and 3T3-L1 adipocytes. Treatment with H89 reversed the effects of EH on lipogenic lipid deposition, lipolysis, and p38 phosphorylation in 3T3-L1 adipocytes. These results suggest that PKA-regulated p38 signaling participates in

the effects of EH on lipogenesis and lipolysis in adipocytes.

To confirm that LU, known as the main active ingredient of EH, plays a major role in the antiobesity effect of EH, we investigated the effects of LU on adipocytes in the present study. Similar to EH, LU treatment ameliorated lipid deposition and stimulated lipolysis in 3T3-L1 adipocytes. Furthermore, the increase in p38 phosphorylation in 3T3-L1 adipocytes was mitigated by LU treatment. However, additional treatment with H89 abrogated all these changes caused by LU. These results suggest that the effects of EH on cultured adipocytes in this study were due to LU.

Conclusion

We have previously reported the effects of the aqueous extract of EH on lipogenesis in cultured adipocytes. The present study revealed that EH-containing LU is also able to inhibit lipogenesis and promote lipolysis, resulting in a decrease in the size of visceral adipose tissue by activating the PKA/p38 signaling pathway (Fig. 6). This observation indicates that EH has functional and therapeutic potential for attenuating obesity. Reducing adipocyte size is crucial in combating obesity. However, as adipose tissue loses its ability to store triacylglycerol, elevated levels of circulating free fatty acids may lead to complications such as lipid deposition in other tissues. Since our study focused solely on the effects of HE on adipocytes, further investigations are necessary to understand its impact on insulin target organs such as the liver and muscles in individuals with obesity. Moreover, while LU is recognized as the primary active component of EH [12], ongoing studies are examining its pharmacokinetic properties. This aspect is pivotal for assessing the future economic feasibility of anti-obesity drugs utilizing EH.

Statement of Ethics

The Institutional Animal Review Board at Chung-Ang University (Seoul, Korea; Approval No. A2022012) approved all the animal experiments. We will distribute our findings to the general public through open-access publications in peer-reviewed journals.

Conflict of Interest Statement

The authors declare no competing interests.

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Author Contributions

J.L.S., Y.J.K., S.S.P., and W.C.: conceptualization, investigation, and methodology. J.H.J. and T.W.J.: conceptualization and writing of the original draft. E.H.M. and A.M.A.: formal

analysis, validation, and writing – review and editing of the original and the revised draft. All authors approved the final version of the article. All authors are responsible for the overall integrity of the work.

Data Availability Statement

The data that support the findings of this study are not publicly available due to the possibility of infringement of research information of a specific company but are available from the corresponding author (J.H.J.) upon reasonable request.

References

- 1 Chopra S, Malhotra A, Ranjan P, Vikram NK, Singh N. Lifestyle-related advice in the management of obesity: a step-wise approach. *J Educ Health Promot*. 2020;9:239. https://doi.org/10.4103/jehp.jehp_216_20
- 2 Smith NR, Zivich PN, Frerichs L. Social influences on obesity: current knowledge, emerging methods, and directions for future research and practice. *Curr Nutr Rep*. 2020; 9(1):31–41. <https://doi.org/10.1007/s13668-020-00302-8>
- 3 Engin A. The definition and prevalence of obesity and metabolic syndrome. *Adv Exp Med Biol*. 2017;960:1–17. https://doi.org/10.1007/978-3-319-48382-5_1
- 4 Maitra SK, Rowland Payne CM. The obesity syndrome and acanthosis nigricans. Acanthosis nigricans is a common cosmetic problem providing epidemiological clues to the obesity syndrome, the insulin-resistance syndrome, the thrifty metabolism, dyslipidaemia, hypertension and diabetes mellitus type II. *J Cosmet Dermatol*. 2004;3(4): 202–10. <https://doi.org/10.1111/j.1473-2130.2004.00078.x>
- 5 Chiu CH, Ko MC, Wu LS, Yeh DP, Kan NW, Lee PF, et al. Benefits of different intensity of aerobic exercise in modulating body composition among obese young adults: a pilot randomized controlled trial. *Health Qual Life Outcomes*. 2017;15(1):168. <https://doi.org/10.1186/s12955-017-0743-4>
- 6 Sung B, Chung JW, Bae HR, Choi JS, Kim CM, Kim ND. *Humulus japonicus* extract exhibits antioxidative and anti-aging effects via modulation of the AMPK-SIRT1 pathway. *Exp Ther Med*. 2015;9(5):1819–26. <https://doi.org/10.3892/etm.2015.2302>
- 7 Yu BC, Yang MC, Lee KH, Kim KH, Choi SU, Lee KR. Two new phenolic constituents of *Humulus japonicus* and their cytotoxicity test *in vitro*. *Arch Pharm Res*. 2007;30(11): 1471–5. <https://doi.org/10.1007/BF02977373>
- 8 Jung TW, Kim HC, Shin YK, Min H, Cho SW, Kim ZS, et al. *Humulus japonicus* stimulates thermogenesis and ameliorates oxidative stress in mouse adipocytes. *J Mol Endocrinol*. 2019;63(1):1–9. <https://doi.org/10.1530/JME-19-0010>
- 9 Kang EJ, Kim HJ, Choi JH, Noh JR, Kim JH, Lee IB, et al. *Humulus japonicus* extract ameliorates collagen-induced arthritis in mice through regulation of overall articular inflammation. *Int J Mol Med*. 2020;45(2): 417–28. <https://doi.org/10.3892/ijmm.2019.4417>
- 10 Chung YH, Bang JS, Kang CM, Goh JW, Lee HS, Hong SM, et al. Aqueous extract of *Humulus japonicus* attenuates hyperlipidemia and fatty liver in obese mice. *J Med Food*. 2018;21(10):999–1008. <https://doi.org/10.1089/jmf.2017.4135>
- 11 Peng Y, Sun Q, Xu W, He Y, Jin W, Yuan L, et al. Vitexin ameliorates high fat diet-induced obesity in male C57BL/6J mice via the AMPK α -mediated pathway. *Food Funct*. 2019;10(4):1940–7. <https://doi.org/10.1039/c9fo00148d>
- 12 Cho W, Park SY, Oh H, Abd El-Aty AM, Hacimuftuoglu A, Kim DS, et al. *Humulus japonicus* extract ameliorates hepatic steatosis through the ppara-mediated suppression of alcohol-induced oxidative stress. *J Med Food*. 2023;26(3):193–200. <https://doi.org/10.1089/jmf.2022.K.0093>
- 13 Porter AG, Janicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999;6(2):99–104. <https://doi.org/10.1038/sj.cdd.4400476>
- 14 Haider N, Larose L. Harnessing adipogenesis to prevent obesity. *Adipocyte*. 2019;8(1): 98–104. <https://doi.org/10.1080/21623945.2019.1583037>
- 15 London E, Bloyd M, Stratakis CA. PKA functions in metabolism and resistance to obesity: lessons from mouse and human studies. *J Endocrinol*. 2020;246(3): R51–64. <https://doi.org/10.1530/JOE-20-0035>
- 16 Leiva M, Matesanz N, Pulgarin-Alfaro M, Nikolic I, Sabio G. Uncovering the role of p38 family members in adipose tissue physiology. *Front Endocrinol*. 2020;11:572089. <https://doi.org/10.3389/fendo.2020.572089>
- 17 Choi SW, Oh H, Park SY, Cho W, Abd El-Aty AM, Hacimuftuoglu A, et al. Myokine muscle alleviates lipid accumulation in 3T3-L1 adipocytes through PKA/p38-mediated up-regulation of lipolysis and suppression of lipogenesis. *Biochem Biophys Res Commun*. 2023;642:113–7. <https://doi.org/10.1016/j.bbrc.2022.12.056>
- 18 Zhang Y, Huang C. Targeting adipocyte apoptosis: a novel strategy for obesity therapy. *Biochem Biophys Res Commun*. 2012; 417(1):1–4. <https://doi.org/10.1016/j.bbrc.2011.11.158>
- 19 Chirumbolo S, Franceschetti G, Zoico E, Bambace C, Cominacini L, Zamboni M. LPS response pattern of inflammatory adipokines in an *in vitro* 3T3-L1 murine adipocyte model. *Inflamm Res*. 2014;63(6): 495–507. <https://doi.org/10.1007/s00011-014-0721-9>
- 20 Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. *Annu Rev Nutr*. 2007;27:79–101. <https://doi.org/10.1146/annurev.nutr.27.061406.093734>
- 21 Saponaro C, Gaggini M, Carli F, Gastaldelli A. The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis. *Nutrients*. 2015;7(11):9453–74. <https://doi.org/10.3390/nu7115475>
- 22 Liu M, Dai Y, Li Y, Luo Y, Huang F, Gong Z, et al. Madecassoside isolated from *Centella asiatica* herbs facilitates burn wound healing in mice. *Planta Med*. 2008; 74(8):809–15. <https://doi.org/10.1055/s-2008-1074533>
- 23 Clifford GM, Londos C, Kraemer FB, Vernon RG, Yeaman SJ. Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation of rat adipocytes. *J Biol Chem*. 2000;275(7):5011–5. <https://doi.org/10.1074/jbc.275.7.5011>
- 24 Holm C. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Biochem Soc Trans*. 2003;31(Pt 6):1120–4. <https://doi.org/10.1042/bst0311120>

- 25 Segales J, Perdiguero E, Munoz-Canoves P. Regulation of muscle stem cell functions: a focus on the p38 MAPK signaling pathway. *Front Cell Dev Biol.* 2016;4:91. <https://doi.org/10.3389/fcell.2016.00091>
- 26 Engelman JA, Lisanti MP, Scherer PE. Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis. *J Biol Chem.* 1998;273(48):32111–20. <https://doi.org/10.1074/jbc.273.48.32111>
- 27 Aouadi M, Jager J, Laurent K, Gonzalez T, Cormont M, Binetruy B, et al. p38MAP Kinase activity is required for human primary adipocyte differentiation. *FEBS Lett.* 2007; 581(29):5591–6. <https://doi.org/10.1016/j.febslet.2007.10.064>
- 28 Engelman JA, Berg AH, Lewis RY, Lin A, Lisanti MP, Scherer PE. Constitutively active mitogen-activated protein kinase kinase 6 (MKK6) or salicylate induces spontaneous 3T3-L1 adipogenesis. *J Biol Chem.* 1999;274(50):35630–8. <https://doi.org/10.1074/jbc.274.50.35630>