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Piperine reduces hair oiliness by inhibiting adipogenesis of hair stem cells

Minyoung Im[1†], Nackhyoung Kim[1†], Ui-Hyun Park[1], Hyeon Ho Heo[1] and Soo-Jong Um[1*]

Abstract

Piperine, an alkaloid compound in black pepper (*Piper nigrum*), has beneficial bioactivities. Specifically, piperine inhibits adipogenesis in 3T3-L1 cells by suppressing the transcriptional activity of PPARγ. Control of hair oiliness, which is related to adipogenic regulation, is important to prevent hair loss. Excessive sebum from the sebaceous gland (SG) can cause acne, folliculitis, or irritated skin by clogging pores. To investigate the in vivo function of piperine in SG, we used mice fed a high-fat diet (HFD). The HFD increased the size and Oil Red O (ORO) staining intensity of SG, which were significantly reduced by piperine. The HFD also upregulated the expression of sebocyte-associated genes, including PPARγ target genes, an effect reversed by piperine. In CD34/CD49f double-positive hair follicle bulge stem cells isolated from mouse vibrissae, piperine inhibited cellular adipogenesis, likely via transcriptional repression of related genes. Furthermore, piperine reduced the thickness of subcutaneous fat. In human dermal papilla cells, piperine inhibited cellular adipogenesis, as shown by the reduction in ORO staining and the downregulation of PPARγ target genes. In conclusion, piperine can be used to reduce hair greasiness by suppressing adipogenesis in hair stem cells.

Keywords Black pepper (*Piper nigrum*), Piperine, Adipogenesis, Bulge stem cells, High-fat diet, Dermal papilla cells

Introduction

A hair follicle, considered a mini-organ in the skin, has a multilayered structure consisting of the hair bulge, hair matrix, dermal papilla, hair shaft, hair sheath, pili muscle, and sebaceous gland (SG). The regulation of hair oiliness by the SG is crucial for nourishing the hair and protecting the skin [1]. Sebum from the SG, a natural oil composed of lipids, wax esters, and other substances, moisturizes the hair and acts as a barrier against external pollutants or microorganisms [2]. Because sebum maintains hair follicles and skin, both deficient and excess sebum can lead to harmful skin conditions. Sebum deficiency can lead to dry skin surface or thinning of the hair shaft, eventually resulting in hair loss. Conversely, excessive sebum secretion can trigger acne, seborrheic dermatitis, folliculitis, and irritated skin by clogging pores [3].

SG formation occurs mainly during hair follicle neosynthesis, and the size of SGs differs depending on the stage of the hair cycle. Sebocytes, lipocytes in the SG, originate from the epidermal bulge layer of the hair follicle [4]. The development of sebocytes involves the migration of Blimp1-positive bulge stem cells (BSCs) from the bulge region to the epithelium of the SG [5, 6]. Subsequently, BSCs begin to differentiate into sebocytes in the lower layer of the SG. As the sebocytes mature, the cells migrate upward and colonize the upper layer of the SG. The differentiation and maturation of sebocytes are controlled by tight regulation of gene expression [6–8].

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Transcription factors such as Myc, C/EBP, PAR2, LEF1, and PPARγ regulate sebocyte differentiation during SG development [9–12]. PPARγ, a peroxisome proliferator-activated receptor, not only activates genes associated with adipogenesis in preadipocytes but also increases lipid accumulation in mature adipocytes [13, 14]. Because a high-fat diet (HFD) can increase both the number of adipocytes and the amount of lipids within an adipocyte, diets with a positive effect on PPARγ have been investigated [15–17]. However, diet-mediated regulation of sebocytes via PPARγ has not been a focus of research.

Piperine, an alkaloid compound extracted from black pepper, is a highly reactive natural substance that can be converted to piperic acid and piperidine [18]. Piperine has anticancer, anti-inflammatory, antimicrobial, anti-hypertensive, antioxidant, and anti-obesity effects [18, 19]. Piperine inhibited adipogenesis in 3T3-L1 murine embryonic fibroblasts [20, 21]. The effect of piperine on obesity has been investigated in the context of insulin resistance, the gut microbiome, and caloric restriction in mice [22–24]. However, whether piperine directly affects physiological functions in sebocytes is unknown. We investigated the function of piperine in hair oiliness via regulation of adipogenic differentiation. We performed a histological analysis of the mouse skin SG and adipogenic differentiation assays using mouse BSCs and human dermal papilla cells (hDPCs). Piperine downregulated PPARγ adipogenic target genes during the adipogenesis of stem cells.

Materials and methods

Mice and dietary experiments

Three-week-old male C57BL/6J mice were purchased from KOATECH (Pyeongtaeksi, South Korea). Mice were housed at a controlled temperature (20 ± 1 °C) under a 12 h light/dark cycle and relative humidity of 45 ± 5%. Mouse experiments were approved by the Institutional Animal Care and Use Committee of Sejong University and were conducted according to the ARRIVE guidelines. The experiments were approved on August 03, 2018, and the approval was renewed on December 08, 2020 (Approval number: SJ-20180803E2).

For the dietary experiments, mice were fed a commercial diet for 18 weeks: ND (AIN-93G, Ziegler, Brussels, Belgium) and HFD (TD-06414, Envigo, IN, USA), containing 7% and 60% fat, respectively. The mice were provided free access to food and water. Synthetic ND or HFD feed was prepared by DooYeolBiotech (Seoul, South Korea) with the inclusion of 0.05% piperine (P49007, Sigma, MA, USA), determined through diet experiments involving varied amounts of piperine [25, 26]. Mouse body weight and food consumption were measured every 3 days. For tissue analysis, mice were euthanized by CO_2 in a 10 L chamber, and the CO_2 administration was maintained for 5 min at a flow rate equal to 35% of the chamber volume per minute. Euthanasia was confirmed by examining eye discoloration and lack of respiration.

Preparation of OCT frozen sections

Mouse skin was fixed with cold 4% paraformaldehyde (PFA, P6148, Sigma, MA, USA) for 10 min and then washed with PBS (pH 7.2, 21600010, Gibco, NY, USA). Fixed skin samples were embedded in OCT compound FSC 22 Clear (3801480, Leica Biosystems, Baden-Württemberg, Germany), and stored at −80 °C. Frozen samples were sectioned to 10–14 μm thickness. Slides were fixed in cold 4% PFA for 5 min, washed with PBS, and stored at −80 °C.

Hematoxylin & Eosin (H&E) staining

Skin-tissue slides were stained with hematoxylin (HHS-16, Sigma) for 1 min. After washing with PBS, sections were transferred to eosin (318906, Sigma) for 10 s. Next, slides were washed three times in distilled water, 95% ethanol, and 100% ethanol. After mounting the slides, bright-field images were captured using a Leica microscope (DM IL system, Leica Microsystems, Wetzlar, Germany).

Immunohistochemistry (IHC)

Tissue slides were permeabilized with PBS containing 0.5% Triton X-100 (X100, Sigma) for 15 min and blocked in PBS containing 5% bovine serum albumin (A2153, Sigma) and 0.1% Triton X-100 for 1 h. After washing with PBS, antigen retrieval was performed by boiling in Tris-EDTA (pH 9.0, ab93684, Abcam, Cambridge, UK) for 10 min. The slides were incubated with the primary antibody overnight at 4 °C in blocking solution. After washing with PBS, the slides were incubated for 2 h with the secondary antibody. After washing with PBS, the slides were stained with Hoechst 33,342 (H3570, Invitrogen, CA, USA) for 3 min. After a final wash, the slides were mounted in mounting medium (Vectashield, H-1000, Vector Laboratories, CA, USA). The following antibodies were used: anti-Pparγ (1:200, sc-7273, Santa Cruz Biotechnology, TX, USA), anti-Adipoq (1:200, CSB-PA07956A0Rb, Cusabio, TX, USA), anti-rabbit IgG secondary-Alexa Fluor 488 (1:500, A-11,008, Invitrogen), and anti-mouse IgG secondary-Alexa Fluor 568 (1:500, A-11,004, Invitrogen).

Culture of hair follicle stem cells

Murine BSCs were isolated from a 12-week-old male mouse. Vibrissae hair follicles were surgically isolated from the face of the mouse. The Bulge region was physically separated from vibrissae using micro forceps. Ten bulges were cultured in a coated 12-well plate (30,012, SPL, Kyonggi-do, South Korea). The culture medium for

BSCs was made by mix of 3:1 ratio of Dulbecco's modified Eagle's medium (DMEM, 12,100,046, Gibco) and one part DMEM/Nutrient Mixture F12 (DMEM/F12, 11320033, Gibco) supplemented with 10% fetal bovine serum (FBS, 12,483,020, Gibco) and B-27 supplement (17,504,044, Gibco). Human epidermal growth factor (10 ng/mL, PHG0263, Gibco) was added every 2 days. The cells were incubated at 37 °C in 5% CO_2. To identify BSCs, cells were fixed in 4% PFA, blocked, and immunostained with antibodies against CD34 (1:200, GTX28158, Genetex, CA, USA) and CD49f (1:200, GTX100565, Genetex). hDPCs (C-12,071, PromoCell, Heidelberg, Germany) were purchased with pre-designed medium (C-26,502, PromoCell) and incubated following the manufacturer's instructions. hDPCs passaged five or fewer times were used in all experiments.

MTT assay

For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, cells were plated in 96-well culture plates (30096, SPL) at 4×10^4/100 μL per well. After the cells attached, 50 μL DMSO (D2650, Sigma) or piperine was added. Then, 50 μL MTT (21,795, Cayman Chemical, MI, USA) in DMSO (1 μg/mL) was added, and the plates were incubated for 3 h at 37 °C. The medium was removed, and formazan was solubilized using DMSO. The absorbance at 540 nm was measured using a spectrophotometer (Eon Microplate Spectrophotometer, BioTek Instruments, VT, USA).

Adipogenic differentiation

Adipogenesis medium used for BSCs consisted of 1 μM dexamethasone (D4902, Sigma), 0.5 mM IBMX (I5879, Sigma), and 10 μg/mL insulin (I6634, Sigma) with or without piperine in DMEM supplemented with 10% FBS. Every 2 days after the first 2 days, cells were replaced with fresh DMEM supplemented with 10% FBS, 1 μM dexamethasone, 0.5 mM IBMX, 10 μg/mL insulin, and 100 μM piperine. After 11 days, the cells were harvested. For adipogenic differentiation of hDPCs, 0.2 mM indomethacin was added to the BSC medium. During the 14 days of adipogenesis process, cells were provided fresh BSC medium plus 0.2 mM indomethacin (405,268, Sigma) and 50 or 100 μM piperine or DMSO every 2 days.

Oil Red O (ORO) staining

ORO staining was performed using OCT-frozen-section slides and 12-well plates containing differentiated BSCs or hDPCs. The ORO stock solution was made by 0.5% w/v Oil-Red-O (O0625, Sigma) in isopropanol (34863, Sigma). ORO working solution was a 9:1 mixture of ORO stock solution and distilled water. For skin tissue slides, OCT frozen sections were washed in cold PBS to eliminate ice and washed with 60% isopropanol. Slides were dipped in ORO working solution for 15 min, washed with tap water, and stained with hematoxylin. Finally, the slides were mounted in 50% glycerol (GB0232, BioBasic, Ontario, Canada).

After adipogenesis, cells in 12-well plates were fixed with 10% formalin in phosphate buffer for 10 min at room temperature. The cells were then washed with PBS and stained with ORO working solution. After four washes with distilled water, the cells were observed under a microscope. For quantification, pure isopropanol was added to dissolve the ORO, and the absorbance at 500 nm was measured using a spectrophotometer.

AP staining

The AP activity of hDPCs was assayed using the Alkaline Phosphatase Staining Kit (AB284936, Abcam) according to the manufacturer's instructions. After washing with PBS, the cells were fixed with 4% PFA in PBS and incubated for 1 min at room temperature. The plates were exposed to a staining solution composed of AP buffer and were incubated in the dark for 30 min. The plates were washed and maintained in PBS.

RNA extraction and quantitative PCR (RT-qPCR)

Total RNA was isolated from tissues or cells using TRIzol (15596026, Invitrogen) reagent according to the manufacturer's instructions. cDNA was synthesized from 1 μg RNA using M-MLV Reverse Transcriptase (28025013, Invitrogen) and random oligo deoxyribonucleotides hexamers (48190011, Invitrogen). RT-qPCR was performed using the Thermocycler CFX96 Real-Time PCR Detection System (BR008923, Bio-Rad, CA, USA), SYBR Green PCR Mixture (ORA™ SEE qPCR Green ROX L Mix, QPD0501, HighQu, Baden-Württemberg, Germany), and the primers listed in Supplementary Information (Additional file: Table S1). Gene expression levels were normalized to that of GAPDH as the internal standard.

Statistics

Data are expressed as means ± standard deviation of at least three independent experiments. The control and experimental groups were compared by paired Student's *t*-test. A value of $p<0.05$ (*), < 0.01 (**), or <0.001 (***) was considered indicative of statistical significance.

Results

Piperine reduces lipid accumulation in the SG of mice fed a high-fat diet

Because piperine is ingestible, we used mice fed a high-fat diet (HFD) to evaluate the effect of piperine on sebaceous gland (SG) physiology. The mice were divided into four groups: mice fed a normal diet (ND), piperine-supplemented normal diet, HFD, or piperine-supplemented HFD. During the 18-week dietary experiment, the ND

group showed higher food intake compared to the other three groups (Additional file 1: Fig. S1A). However, energy intake was greatest in HFD-fed mice (Additional file 1: Fig. S1B), suggesting that high energy intake does not necessarily correlate with the quantity of food consumed. Similarly, the HFD group exhibited the most significant increase in body weight, while piperine supplementation resulted in reduced body weight in both the ND and HFD groups (Additional file 1: Fig. S1C). The hair on the back of the mice was oily in the HFD group (Fig. 1A). The oily hair appeared to be thinning because it was in clumps. However, the piperine-supplemented diet decreased hair oiliness and reduced its greasy appearance, compared with the HFD. For histologic analysis, back skin tissue was dissected and sectioned after embedding in optimal cutting temperature (OCT) compound. Hematoxylin and eosin (H&E) staining revealed that the HFD increased the size of the SG (Fig. 1B). Piperine restored the size of the SG to the level in the ND group. To investigate lipid accumulation, tissues were stained with Oil Red O (Fig. 1C). Quantification using Image J showed that the ORO-stained area was increased by the HFD and decreased by piperine. Therefore, these data suggest that piperine suppresses HFD-induced lipid accumulation in the SG.

Piperine downregulates adipogenic genes associated with SG development

To investigate the molecular mechanism underlying the anti-adipogenic effect of piperine, mouse dorsal skin was surgically dissected without subcutis. The dermis was separated from the epidermis using Dispase II (Sigma) and then subjected to total RNA extraction and cDNA synthesis. Quantitative real-time PCR (qPCR) revealed that the HFD increased the expression of genes related to the SG, including *Pparγ*, *Fabp4*, *Lpl*, *Adipoq*, and *Fasn*, in addition to *Blimp1* (a key modulator of sebocyte homeostasis) (Fig. 2A). Piperine significantly suppressed the

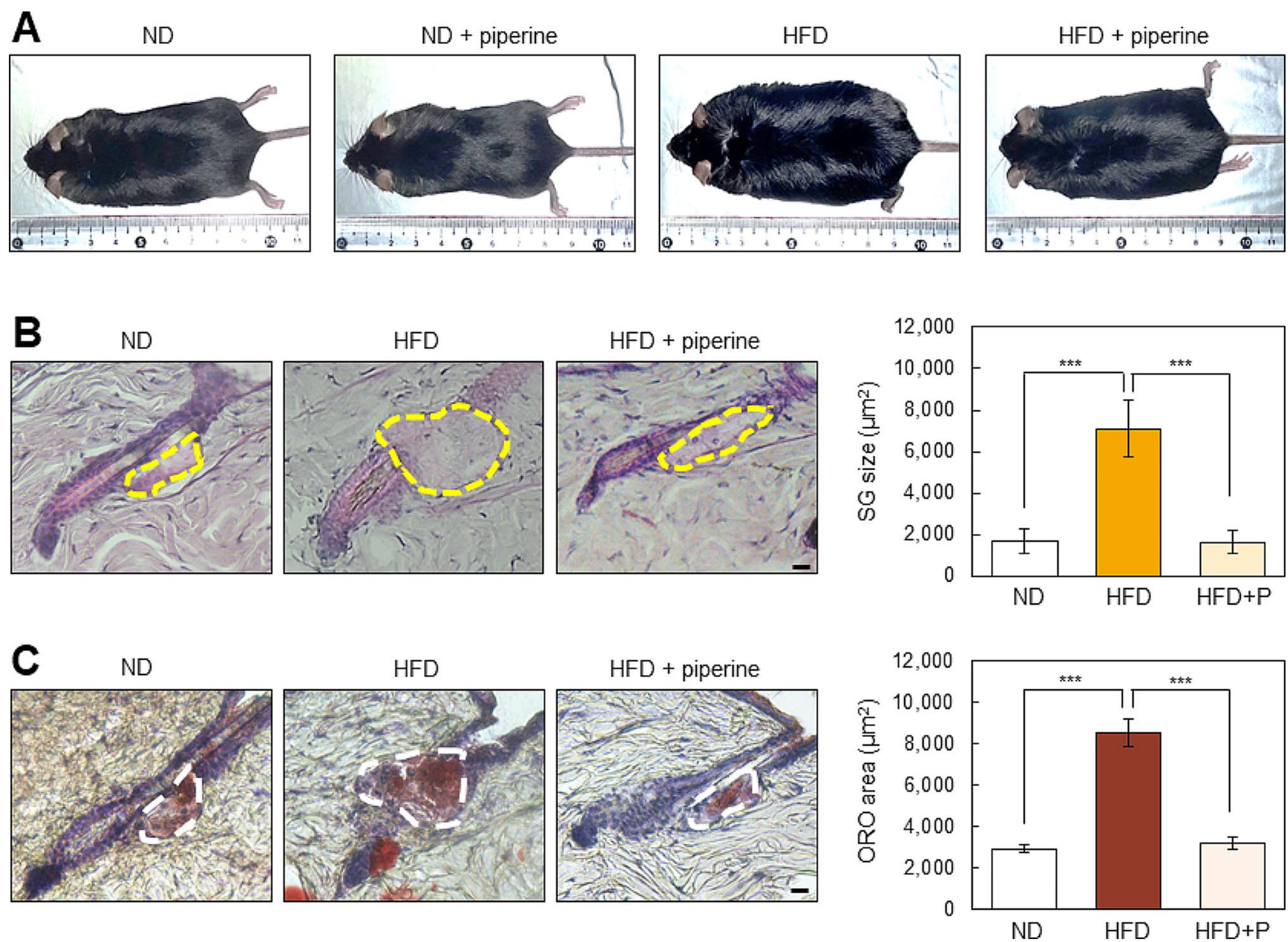

Fig. 1 Effect of piperine on body weight and hair oiliness in mice fed a HFD. (**A**) Photographs of a representative mouse of each group after 18 weeks of feeding a normal diet (ND), piperine-supplemented ND, high-fat diet (HFD), or piperine-supplemented HFD. (**B**) Effect of piperine on sebaceous gland (SG) size. Back skin samples were stained with H&E. Yellow dashed line: SG. (**C**) Oil Red O (ORO) staining and quantification of SGs in back skin. ORO staining was followed by H&E staining to visualize hair follicles. White dashed line: SG. Scale bars, 75 μm. Image J was used to quantitate the SG size. Results are means ± SD ($n = 5$, ***$p < 0.001$)

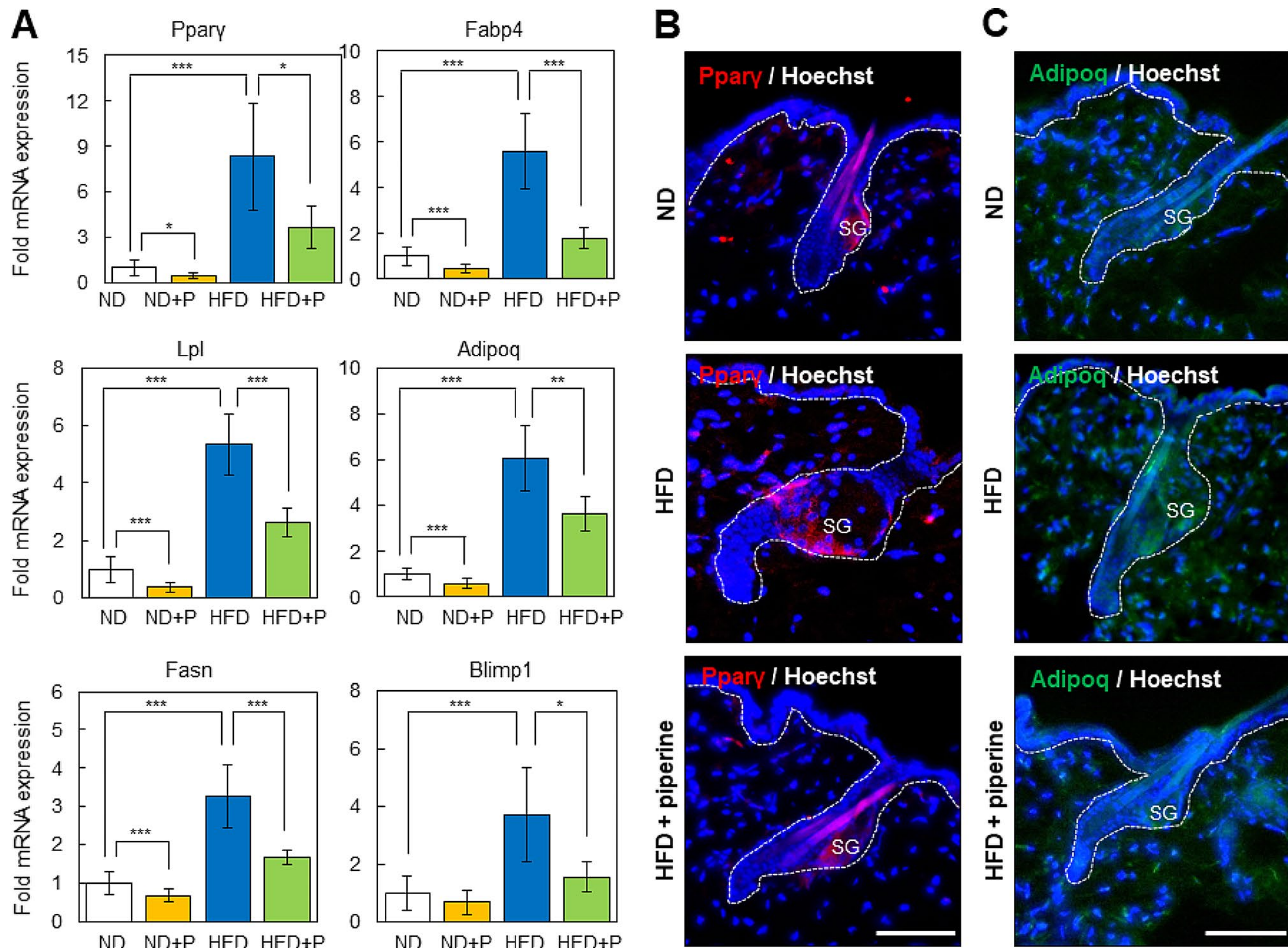

Fig. 2 Downregulated expression of adipogenic genes by piperine. (**A**) Effect of piperine on the expression of adipogenic genes. Total RNA from skin samples of mice fed the indicated diets was extracted and subjected to cDNA synthesis followed by qPCR. *Pparγ*, *Fabp4*, *Lpl*, and *Adipoq*: PPARγ target genes. *Blimp1*, *Pparγ*, and *Fasn*: SG markers. Gene expression was normalized to *Gapdh*, as the internal control. Results are means ± SD ($n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$). (**B**, **C**) Effect of piperine on the tissue expression of *Pparγ* (**B**) and *Adipoq* (**C**). Fluorescence immunohistochemistry was performed using skin tissues from the ND, HFD, and piperine-supplemented HFD groups. Scale bars, 75 μm. Dashed white lines, epidermal–dermal boundary

expression of these genes, albeit not to the level in the ND group. The protein levels of PPARγ and Adipoq near the SG were increased by the HFD but decreased by piperine (Fig. 2B, C). These data suggest that piperine inhibits lipid accumulation around the SG, likely by suppressing the expression of adipogenic PPARγ target genes.

Piperine inhibits adipogenesis of BSCs

Because pre-sebocytes are derived from BSCs of the hair follicle, BSCs were physically isolated from murine vibrissae for differentiation into sebocytes. As markers of BSCs, the protein levels of Cd34 and Cd49f were evaluated by immunocytochemical staining (Additional file 1: Fig. S2). The optimal concentration of piperine for the cellular differentiation assays was 100 μM, as determined by MTT assay (Fig. 3A). Piperine significantly reduced the intensity of ORO staining of BSCs in differentiation medium (Fig. 3B). The antagonistic effect of piperine on lipid accumulation was confirmed by measuring the absorbance at 500 nm of ORO-stained lipids extracted using isopropanol (Fig. 3C). RT-qPCR was performed to determine the effect of piperine on the expression of the following adipogenic genes associated with sebocyte homeostasis: *Pparγ*, *Fasn*, and *Blimp1*. These genes were markedly downregulated by piperine (Fig. 3D). Therefore, the effect of piperine on BSC adipogenesis is similar to that on SG development in HFD-fed mice.

Piperine reduces the thickness of hypodermal fat

In addition to the SG, hypodermal fat also affects the oiliness of hair follicles via secretion of chemokines such as Adipoq, IL-6, and IL-8, which stimulate the differentiation of pre-sebocytes or sebum production from the SG. In the HFD group, the subcutaneous layer was enlarged with an increased number of subcutaneous adipocytes (Fig. 4A). However, the thickness of the hypodermis in the skin of the piperine-supplemented HFD group was significantly reduced to the level in the ND group

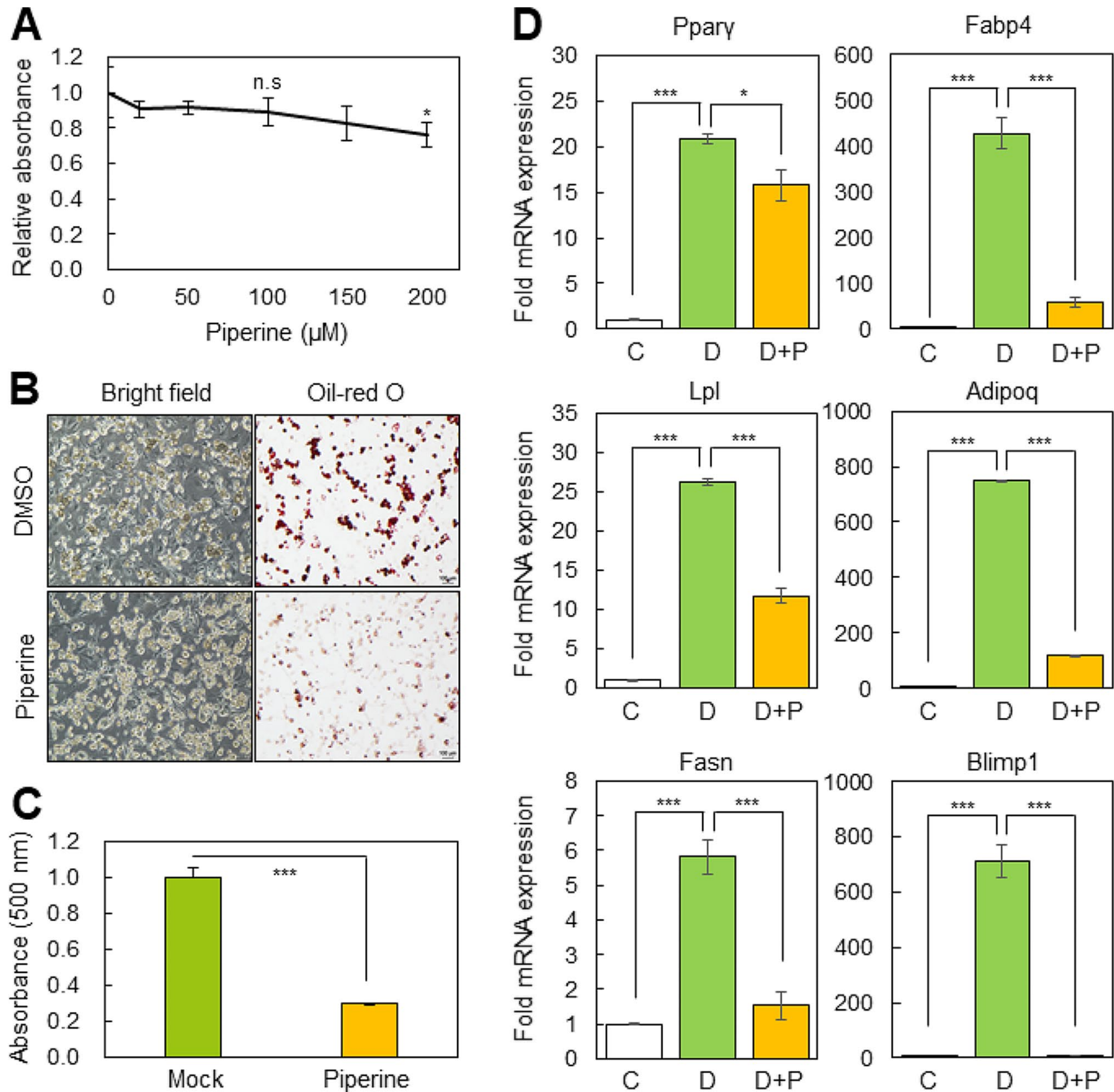

Fig. 3 Antagonistic effect of piperine on the adipogenesis of BSCs. The data represent means ± SD ($n = 3$, *$p < 0.05$ and ***$p < 0.001$). (**A**) Effect of piperine on BSC viability. Cytotoxicity was determined by MTT assay. (**B**) Effect of piperine on Oil Red O (ORO) staining of BSCs. Adipogenesis was induced in adipogenic differentiation medium for 14 days. (**C**) Quantification of ORO staining. ORO-stained lipids were extracted using isopropanol and quantitated by measuring the absorbance at 500 nm using a spectrophotometer. (**D**) Downregulation of adipogenic genes by piperine. After induction of adipogenesis of BSGs by piperine, the expression of PPARγ target and SG marker genes was evaluated by RT-qPCR. C, culture medium; D, differentiation medium; P, piperine (100 μM)

(Fig. 4B). Hypodermal adipocytes are derived from proliferative dermal fibroblasts, such as dermal papilla cells.

Piperine inhibits the adipogenic differentiation of hDPC

To investigate the hypodermis-specific function of piperine, hDPCs were isolated and confirmed by alkaline phosphatase (AP) staining (Additional file 1: Fig. S3A). MTT assays of hDPCs were performed to determine the optimal concentration of piperine for the adipogenesis assay (Additional file 1: Fig. S3B). hDPCs were cultured in adipogenic medium, and condensed cells tended to differentiate into adipocytes, as visualized by ORO staining and quantified by measuring the absorbance (Fig. 5A). Piperine markedly suppressed adipogenesis and lipid accumulation (Fig. 5B). According to RT-qPCR (Fig. 5C), piperine downregulated the expression of *Pparγ*, *LPL*,

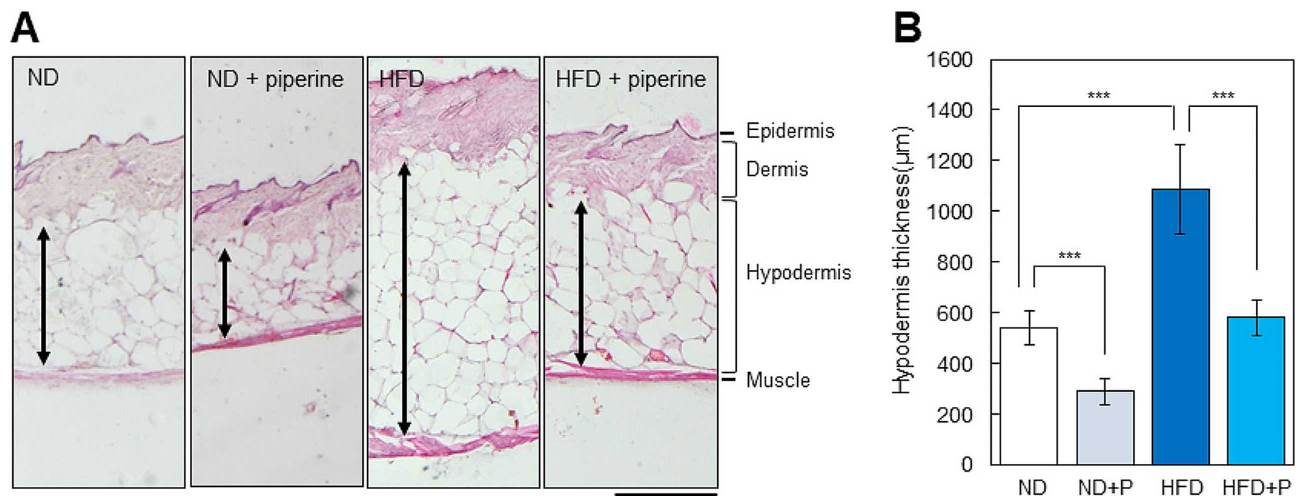

Fig. 4 Effect of piperine on hypodermal thickness. (**A**) H&E staining of mouse back skin. Arrow lines, hypodermal fat layers; scale bars, 500 µm. (**B**) Quantification of hypodermal thickness using Image J. The data represent means ± SD ($n = 5$, ***$p < 0.001$)

and *Fabp4* in DPCs, as in BSCs. These data suggest that piperine suppresses hypodermal lipid accumulation by inhibiting adipogenesis and the expression of adipogenic genes in DPCs.

Discussion

Obesity is a global health issue, while hair loss is a serious concern in terms of quality of life. Although hair condition is affected by skin oiliness, obesity is not thought to be closely related to hair loss. Because an enlarged SG is associated with androgenic alopecia, obesity accompanied by hypertrophy of sebocytes may cause hair loss due to overactive SGs [27]. Notably, studies with obese mice have observed excessive sebum secretion resulting in greasy hair [28]. Oily hair was consistently observed in an HFD-induced overweight mouse model investigating obesity-related hair loss [29]. Nevertheless, piperine, by effectively suppressing the increase in SG size, exhibited a potential preventive effect against hair loss caused by overactive sebaceous glands. In this study, we analyzed the effect of orally administered piperine. Despite concerns about its spicy taste affecting appetite, food consumption did not significantly differ among groups. Investigating piperine's impact on digestion and absorption could provide insights into its function in the digestive system. Additionally, exploring the effectiveness of piperine through direct skin application would enhance practical understanding against hypodermal expansion.

Our findings showed that piperine inhibits sebocyte differentiation and expansion. Interestingly, the size of the SG in the piperine-supplemented HFD group was reduced to the size in the ND group, but the expression levels of *Pparγ* and its target genes were not reduced. The higher expression levels of adipogenic genes in the piperine-supplemented HFD group compared with the ND group may not explain the piperine-mediated SG inhibition. By performing dietary experiments and ORO staining, we investigated the effect of piperine on adipogenic differentiation. However, piperine may have a function in mature sebocytes or adipocytes via unidentified mechanisms. Further investigation of mature adipocytes or of piperine treatment after an HFD may provide insight into the function of piperine during lipid production in sebocytes or adipocytes. The thickness of the hypodermis was significantly reduced by piperine in the ND and HFD groups. Body weight was reduced in the piperine-supplemented ND group compared with the ND group, suggesting that piperine modulates adipocyte biology.

Piperine suppresses the transcriptional activity of PPARγ [20]. However, the physical interaction between piperine and PPARγ has not been addressed in terms of piperine's function as a PPARγ antagonist. Transcriptional activation or repression by PPARγ is regulated principally by histone acetylation [30, 31]. Upregulation of PPARγ target genes is dependent on the enzymatic activity of CBP/P300 and LSD1 [32, 33]. Histone methylation, which represses transcription, can be removed by LSD1, whereas the CBP/P300 complex adds an acetyl group to histones. Transcriptional downregulation by PPARγ has been associated with SIRT1, a deacetylase enzyme [32, 34, 35]. In this context, the transcriptional regulation of piperine by PPARγ may be related to the regulation of histone acetylation. Piperine regulates the expression of adipogenic and lipolytic genes by modulating histone modifications to suppress adipogenesis of 3T3-L1 cells [21]. In this study, we have documented that piperine inhibits adipogenesis and the transcription of related genes in hair stem cells (HSCs). Computational analyses have identified potential interaction partners of piperine, including IL-1β, IL-6, NF-κB, JNK, and Cox-2

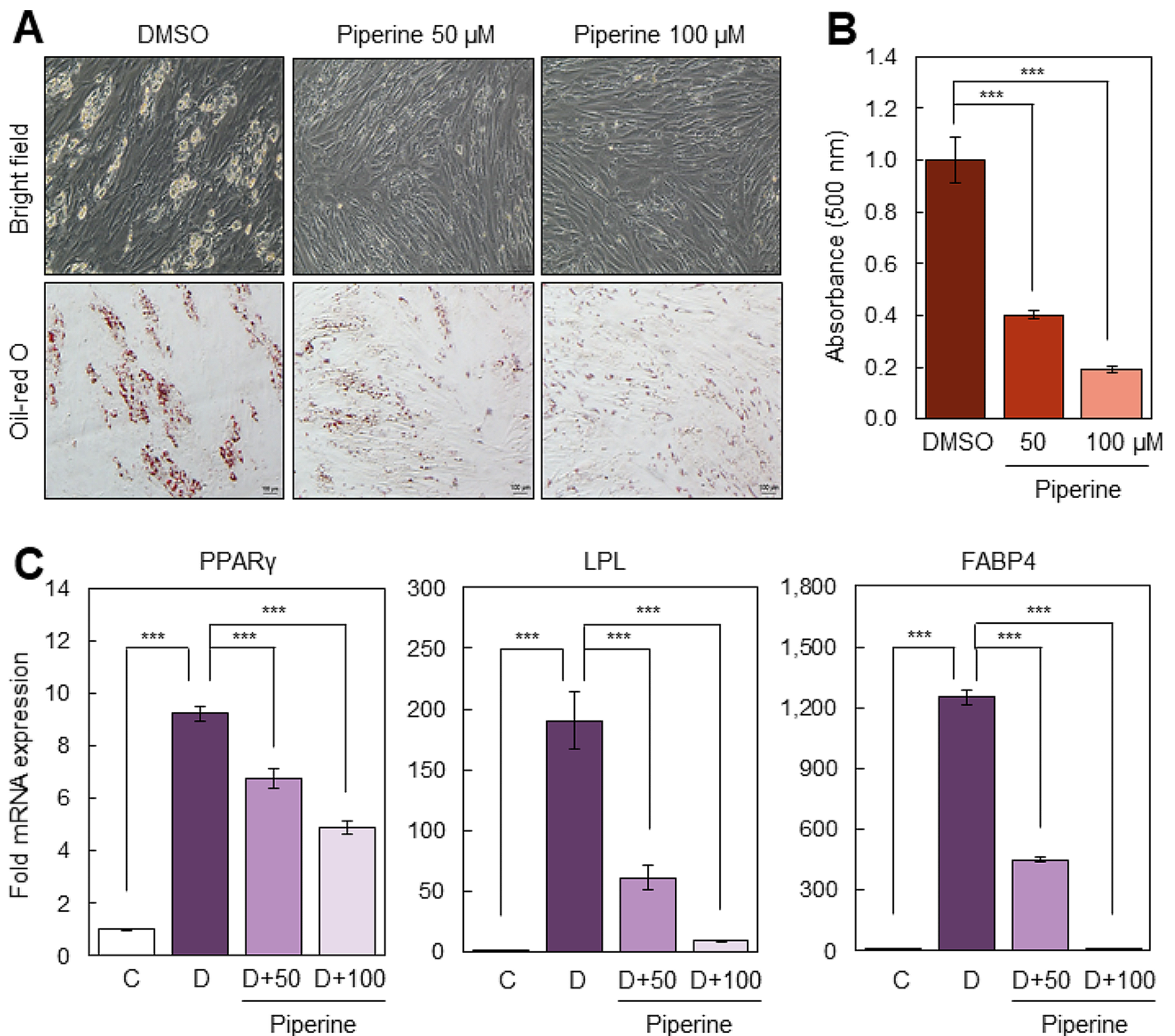

Fig. 5 Suppression of hDPC adipogenesis by piperine. Statistical results are means ± SD ($n=3$, ***$p<0.001$). (**A**) Micrographs of ORO-stained adipogenic hDPCs. Effects of 50 and 100 μM piperine were evaluated. (**B**) ORO staining was quantitated by measuring the absorbance at 500 nm using a spectrophotometer. (**C**) Effect of piperine on the expression of PPARγ target genes by RT-qPCR after differentiation of hDPCs in the presence of piperine. C: culture medium; D: differentiation medium; D + 50: 50 μM; D + 100: 100 μM piperine

[36, 37]. While the validation of piperine's interaction with IL-6 suggests its role in immune regulation [37], its association with transcription regulators during sebocyte differentiation of HSCs remains unknown. Uncovering the piperine interactome could provide valuable insights into its impact on transcription and adipogenesis.

Since there is no established positive control for reducing hair oiliness in the existing literature, we did not have a specific control for our study. Our findings indicate that piperine reduces hair oiliness by inhibiting the adipogenesis of hair stem cells, potentially through the downregulation of PPARγ activity. In the absence of a defined positive control, we propose that both synthetic and natural compounds with PPARγ antagonism could be considered suitable controls. It's worth noting that while both piperine and PPARγ antagonists inhibit the transcriptional activity of PPARγ, the mechanism of action for piperine involves indirect suppression, which differs from classical PPARγ antagonists. Based on our data, piperine could be considered as a positive control in studies focused on developing reagents to reduce hair oiliness.

Piperine has multiple beneficial biological functions, including an anti-inflammatory function in the skin, which may be related to its effect on adipogenesis inhibition [38, 39]. This is because obesity-induced

inflammation is triggered by secreted adipokines [40–42]. Sebocytes in the SG secrete pro-inflammatory mediators such as IL-6, IL-8, and TNF-α [43, 44]. In HFD-fed mice, the mRNA levels of Il-6 and Il-1β were upregulated, an effect reversed by piperine (data not shown). Because there is no therapy for skin inflammation due to obesity, piperine has promise for non-chronic skin inflammation. Taken together, our findings suggest that piperine has therapeutic potential for hair loss by controlling hair oiliness and inflammation.

Abbreviations

PPARγ	Peroxisome proliferator-activated receptor gamma
SG	Sebaceous gland
HFD	High-fat diet
ND	Normal diet
ORO	Oil Red O
BSC	bulge stem cell
hDPC	human dermal papilla cell
OCT	Optimal cutting temperature
H&E	Hematoxylin and eosin
IHC	Immunohistochemistry
ICC	Immunocytochemistry
RT-qPCR	Reverse transcription-quantitative PCR
AP	Alkaline phosphatase
PFA	Paraformaldehyde
PBS	Phosphate-buffered saline
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
IBMX	3-Isobutyl-1-methylxanthine
DMEM	Dulbecco's Modified Eagle Medium

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00889-4.

Supplementary Material 1. Additional files: Table S1

Supplementary Material 2. Additional files: Fig. S1-S3

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Author contributions
Data collection: M Im, N Kim, UH Park, HH Heo; experimental design: UH Park, SJ Um; analysis and interpretation of the data: N Kim, UH Park, SJ Um; writing manuscript: M Im, N Kim, SJ Um; overall supervision: SJ Um. All authors read and approved the final manuscript.

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Data availability
All study data are included in the article and Additional file. Data will be made available on request.

Declarations

Ethics approval and consent to participate
Mouse experiments were approved by the Institutional Animal Care and Use Committee of Sejong University and were conducted according to the ARRIVE guidelines (Authorization Number: SJ-20180803E2).

Competing interests
All authors have no competing interests to declare.

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