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ORIGINAL ARTICLE

4-Hydroxyderricin, as a PPARγ Agonist, Promotes Adipogenesis, Adiponectin Secretion, and Glucose Uptake in 3T3-L1 Cells

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Abstract Adipocyte differentiation plays a pivotal role in maintaining the production of small-size adipocytes with insulin sensitivity, and impaired adipogenesis is implicated in insulin resistance. 4-Hydroxyderricin (4-HD), a phytochemical component of *Angelica keiskei*, possesses diverse biological properties such as anti-inflammatory, antidiabetic, and antitumor. In the present study, we investigated the effects of 4-HD on adipocyte differentiation. 4-HD promoted lipid accumulation in 3T3-L1 cells, upregulated both peroxisome proliferator-activated receptor (PPAR)-γ mRNA and protein expression, and acted as a ligand for PPARγ in the luciferase assay. Moreover, 4-HD increased the mRNA and protein expression levels of adiponectin. Additionally, it promoted insulin-dependent glucose uptake into 3T3-L1 adipocytes and increased Akt phosphorylation and glucose transporter (GLUT) 4 mRNA expression. In summary, these findings suggest that 4-HD, which promoted adipogenesis and insulin sensitivity in 3T3-L1 cells, might be a phytochemical with potent insulin-sensitizing effects.

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Keywords 4-Hydroxyderricin · *Angelica keiskei* · Adipocyte · Adiponectin · Glucose uptake · PPARγ

Abbreviations

4-HD	4-Hydroxyderricin
aP2	Adipocyte fatty acid-binding protein 2
CCAAT	Cytosine–cytosine–adenosine–adenosine–thymidine
C/EBP	CCAAT/enhancer binding protein
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
HKR	HEPES-Krebs–Ringer
HMW	High molecular weight
IBMX	1-Methyl-3-isobutylxanthine
LMW	Low molecular weight
MCP-1	Monocyte chemoattractant protein-1
PBS	Phosphate-buffered saline
PPAR	Peroxisome proliferator-activated receptors
Pref1	Preadipocyte factor-1
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TG	Triglyceride
TRO	Troglitazone
TNFα	Tumor necrosis factors α
TZD	Thiazolidinedione
XA	Xanthoangelol

Introduction

Type 2 diabetes, a disorder involving chronic hyperglycemia, has become a global public health problem [1]. Type 2 diabetes begins with insulin resistance, a condition in which cells do not respond properly to insulin and therefore, fail to take up glucose from the bloodstream [2].

Adipose tissue, a highly insulin-responsive tissue, is crucial for whole body glucose homeostasis through its lipids storage and endocrine function [3, 4]. Alterations of adipose tissue, i.e. lipodystrophy (deficiency of fat) and obesity (excessive fat) are related with insulin resistance and hyperglycemia [5]. In the obese state, adipocytes become enlarged and less responsive to insulin [6]. Besides, enlarged adipocytes secret proinflammatory factors, such as tumor necrosis factors (TNF) α and monocyte chemoattractant protein (MCP)-1, which lead to insulin resistance and hyperglycemia [7]. Thus, maintenance of adipose function is critical in the regulation of glucose metabolism. Adipocyte differentiation or adipogenesis, a process during which preadipocytes become mature adipocytes, increases the number of insulin sensitive adipocytes. Impaired adipocyte differentiation, as indicated by decreased gene expression of adipogenesis markers and enlarged adipocyte size, is suggested to contribute to obesity associated insulin resistance [8, 9]. Therefore, the regulation of adipocyte differentiation is highly correlated with insulin resistance and metabolic syndromes.

Adipocyte differentiation is a complex process involving a series of transcription activators including Peroxisome proliferator-activated receptor (PPAR)γ and CCAAT/enhancer- binding proteins (C/EBP)α [10]. PPARγ, a member of the superfamily of nuclear receptors, is specifically expressed at high levels in adipose tissue, and plays a central role in adipocyte differentiation [11, 12]. PPARγ ligands such as the thiazolidinedione (TZD) class of drugs have been used clinically for the treatment insulin resistance [13]. TZD enhance insulin sensitivity through promoting adipocyte differentiation and elevating production of insulin-sensitizing adipocytokines such as adiponectin in adipocytes [14, 15]. TZD significantly elevate plasma adiponectin concentrations in insulin-resistant humans and rodents [14]. Adiponectin is predominately produced by adipocytes and directly sensitizes the body to insulin [16, 17]. Adiponectin levels are lower in animals and humans with insulin resistance and diabetes than they are in the healthy [18, 19]. Moreover, the treatment of ob/ob mice with adiponectin leads to decreased serum glucose [20]. Adiponectin is classified into three different full-length forms: low-molecular-weight (LMW, trimer), middle-molecular-weight (MMW), high-molecular-weight (HMW) forms, and all three circulate in the serum. Of the three forms, HMW adiponectin is better correlated with improvement in insulin sensitivity and low levels of HMW adiponectin is closely associated with type 2 diabetes compared to the other forms [21, 22].

Angelica keiskei, a Japanese herb, is consumed as a healthy vegetable and contains numerous phytochemicals including chalcone, flavanone, and coumarin [23]. Of all these bioactive substances, two chalcones named 4-hydroxyderricin (4-HD) and xanthoangelol (XA) occur in abundance and have been reported to exert several bioactivities including antitumor [24], antidiabetic [25, 26], and anti-inflammatory [27]. In this study, we investigated the effects of 4-HD and XA on adipocyte differentiation using 3T3-L1 cells, a well-established model of adipocyte differentiation. Our results showed that 4-HD but not XA promoted adipocyte triglyceride (TG) accumulation by activating PPARγ receptors and promoting PPARγ gene and protein expression levels. Moreover, treatment of 3T3-L1 cells with 4-HD enhanced not only total adiponectin secretion but also HMW adiponectin expression, which is better correlated with improvement of insulin sensitivity. Additionally, 4-HD increased insulin-dependent glucose uptake by adipocytes.

Materials and Methods

Materials

4-HD and XA were isolated from ethyl acetate extract of roots of *A. keiskei* using the method established by Baba *et al.* [28] and dissolved in dimethyl sulfoxide (DMSO). For all experimental groups, the final DMSO concentrations in the administered compounds were maintained at 0.1 %. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), Nacalai Tesque (Kyoto, Japan), or Wako Pure Chemicals (Osaka, Japan).

Cell Culture

The 3T3-L1 fibroblasts and monkey CV1 kidney cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured separately in Dulbecco's modified Eagle's medium (DMEM, high glucose) supplemented with 10 % fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified 5 % CO_2 atmosphere at 37 °C.

Two days after reaching confluence, 3T3-L1 were differentiated with basic medium plus 0.25 μM dexamethasone, 10 μg/mL insulin, 0.5 mM 1-methyl-3-isobutylxanthine for 48 h. Then the media was replaced with growth medium (basic medium containing 5 μg/mL insulin) every 2 days, as previously described [29–31]. XA or 4-HD was added throughout the experimental period at the indicated concentrations. DMSO (0.1 %) is used as the vehicle control. Adiponectin produced by the 3T3-L1 adipocytes in the medium was measured using the Mouse Adiponectin enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Oil Red O Staining

Cells were fixed with 10 % formalin for 1 h and stained with 0.5 % Oil Red O solution for 60 min. After washing with PBS, the cells were photographed. Then Oil Red O retained in the cells was eluted with 100 % isopropanol and the absorbance was measured at 490 nm.

Nile Red staining

Cells were washed once by PBS, then added with lipophilic fluorescent dye Nile Red (5 μg/ml) and nuclear fluorescent dye Hechst 33342 (10 μM)/PBS. After incubation at 37 °C for 10 min, the fluorescence was visualized by fluorescence microscopy and photographed.

Glucose Uptake

The rate of cellular uptake of 2-deoxy-D-[3*H*] glucose (Amersham Biosciences, Piscataway, NJ, USA) was measured as described previously [29–31]. On day 3 after cell differentiation in the presence or absence of 4-HD, 3T3-L1 cells were washed twice and incubated with serum-free DMEM. After 18 h, cells were incubated with HEPES-Krebs–Ringer (HKR) buffer containing 0.1 % bovine serum albumin with or without 100 nM insulin for 20 min at 37 °C. Glucose uptake was initiated by the addition of 2-deoxy-D-[3*H*] glucose at a final concentration of 0.5 μCi/mL to each well. After 10 min, the supernatant was discarded, the cells were washed twice with cold PBS, and then solubilized in 0.1 N sodium hydroxide (NaOH). The radioactivity of cell lysate was then measured using a scintillation counter and normalized to the protein concentrations.

mRNA Extraction and Quantitation

Total RNA was isolated using a commercially available reagent (Sepasol-RNA I Super G, Nacalai Tesque) and reverse transcribed with the M-MLV Reverse Transcriptase (Promega) in accordance with the manufacturer's instructions. The quantification of mRNA expression levels of target genes was performed using the LightCycler system (Roche Diagnostics, Mannheim, Germany) with SYBR Green fluorescence signals. The primers were as follows:

mC/EBPα (forward, 5′-TGGACAAGAACAGCAACGA C-3′ and reverse, 5′-TCACTGGTCA ACTCCAGCAC-3′), *mPPARγ* (forward, 5′- GGAGATCTCCAGTGATATCGA CCA-3′ and reverse, 5′-ACGGCTTCTACGGATCGA AACT-3′), adipocyte fatty acid-binding protein 2 (*maP2*, forward, 5′-AAGACAGCTCCTCCTCGAAGGTT-3′ and reverse, 5′-TGACCAAA TCCCCATTTACGC-3′), adiponectin (forward, 5′-TACAACCAACAGAATCATTATG ACGG -3′ and reverse, 5′-GAAAGCCAGTAAATAGAG TCGTTGA-3′), glucose transporter 4 (*mGlut4*, forward, 5′-TAGGAGCTGGTGTGGTCAATACG-3′ and reverse, 5′-TAAAAGGGAAGG TGTCCGTCG-3′), preadipocyte factor 1 (*mPref1*, forward, 5′-GTGACCCCCAGTATGG AT TC-3′ and reverse, 5′-AGGGAGAACCATTGATCA CG-3′), and *m36B4* (forward, 5′-TGTGTGTCTGCAGATCG GGTAC-3′ and reverse, 5′-CTTTGGCGGGATTAGTCG AAG- 3′).

All target genes were normalized to the housekeeping gene *m36B4*.

Western Blotting

Cells were lysed in lysis buffer containing 20 mM Tris-hydrochloride (HCl, pH 7.5), 150 mM sodium chloride (NaCl), 1 % Triton X-100, and a protease inhibitor cocktail (Nacalai Tesque), followed by centrifugation (15,000×*g*) at 4 °C for 10 min. The protein concentration of the supernatant was measured using the Bio-Rad DC protein assay. After denaturing in sodium dodecyl sulfate (SDS), equal amounts of protein were separated using 10 % SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride transfer membranes (GE Healthcare, Buckinghamshire, UK). The membranes were incubated with primary antibodies (anti-PPARγ, or anti-β-actin antibody, or anti-Akt, Cell Signaling Technology, MA, USA; anti-Phospho-Akt (S473), R&D systems, Minneapolis, MN) at 4 °C overnight, blocked with 5 % skim milk in PBS, and then incubated with the secondary antibody (Santa Cruz Biotechnology, CA, USA) for 1 h. The secondary antibody staining was visualized using a chemiluminescent horseradish peroxidase (HRP) substrate (Millipore, MA, USA). The adiponectin multimerization in the supernatant of the 3T3-L1 cells was measured as described previously [29–31].

Transfection and Luciferase Assay

Monkey CV-1 cells were transfected with p4xUASg-tk-luc (a reporter plasmid), pM-hPPARγ (an expression plasmid for a chimera protein of the GAL4 DNA-binding domain and human PPARγ-ligand binding domain), and pRL-CMV (an internal control for normalizing transfection efficiency) using Lipofectamine (Invitrogen, CA, USA) for 6 h. The transfected CV-1 cells were incubated with 4-HD for 24 h and then subjected to luciferase assays using a Dual-luciferase reporter gene Assay system (Promega) according to the manufacturer′s protocol.

Statistical Analysis

All data are presented as means at standard error of the mean (SEM) and were analyzed using unpaired *t*-tests and

one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test when variances were heterogeneous. Differences were considered significant at $P < 0.05$.

Results

4-HD Stimulated Differentiation of 3T3-L1 Preadipocytes

To evaluate the effects of 4-HD and XA on adipocyte differentiation, we first determined whether they promoted TG accumulation in 3T3-L1 preadipocytes (Fig. 1a). Differentiation of 3T3-L1 preadipocytes was induced in the presence of XA or 4-HD, and troglitazone (TRO) was used as a positive control. On day 10, we performed Oil-Red-O staining and Nile red staining, which demonstrated that 4-HD but not XA stimulated TG accumulation in 3T3-L1 cells, characterized by increased lipid droplets (Fig. 1b–d). Next, we measured adipogenic (aP2 and C/EBPα) and preadipocyte (Pref1) maker gene expression in 3T3-L1 cells treated with XA or 4-HD. Both C/EBPα and aP2 gene levels (Fig. 2a, b, respectively) were significantly elevated while Pref1 mRNA expression (Fig. 2c) declined in 4-HD-treated 3T3-L1 cells. The expression levels of all three genes remained unchanged in XA-treated 3T3-L1 cells compared with the control. These results (Fig. 2a–c) suggest that 4-HD treatment promoted differentiation of 3T3-L1 preadipocytes.

4-HD Increased Expression Levels of PPARγ in 3T3-L1 Cells and Activate PPARγ Signaling

The presence and activation of PPARγ, a member of the nuclear hormone receptor superfamily, is required for adipogenesis both *in vivo* and *in vitro* [32]. To

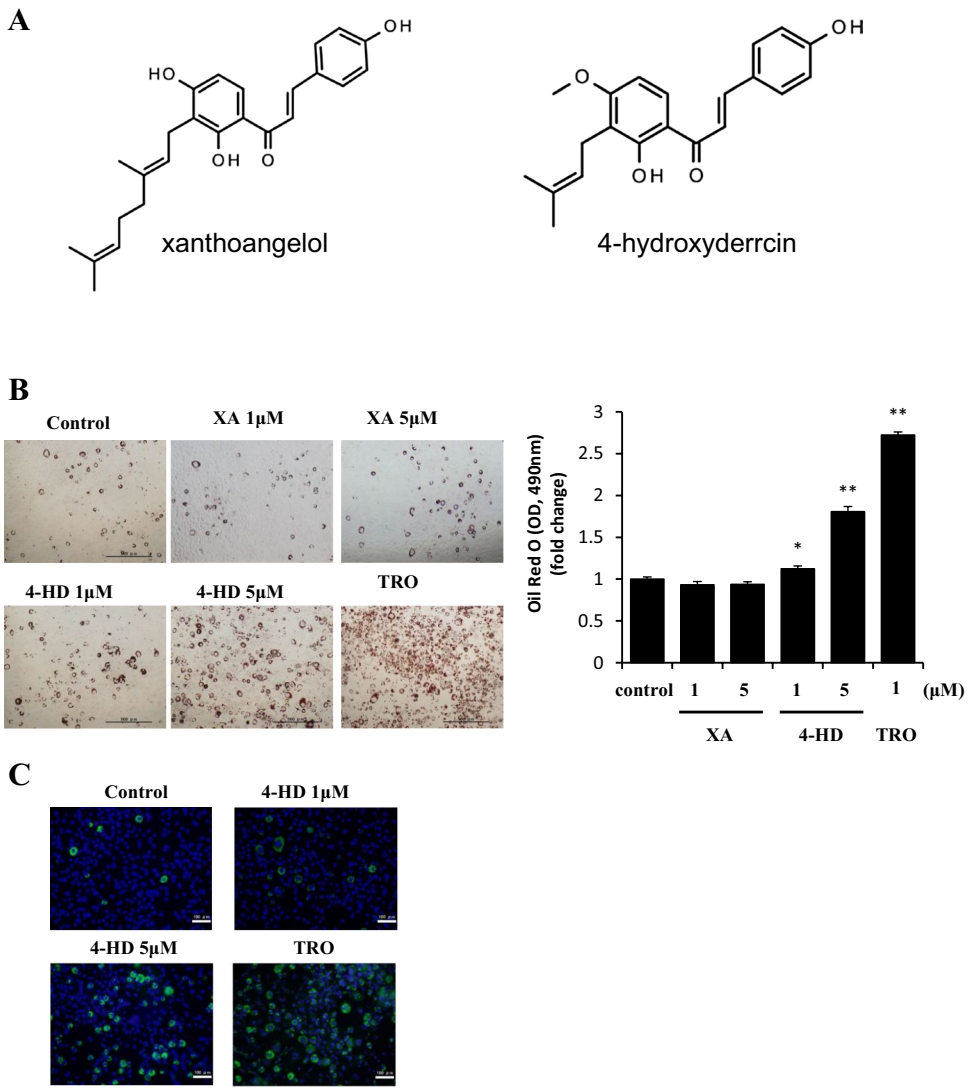

Fig. 1 4-Hydroxyderricin (4-HD)-induced adipogenesis in 3T3-L1 cells. **a** Chemical structure of xanthoangelol (XA) and 4-HD. **b** Oil Red O staining of 3T3-L1 cells treated with XA, 4-HD (1 and 5 μM), or Tro (1 μM). **c** The cells were stained with Oil Red O and eluted with isopropyl alcohol, then qualified at 490 nm. Values are means ± SEM of 4–5 samples; $*p < 0.05$ and $**p < 0.01$ compared with control. **d** Nile Red staining of 3T3-L1 cells treated with 4-HD (1 and 5 μM), or Tro (1 μM)

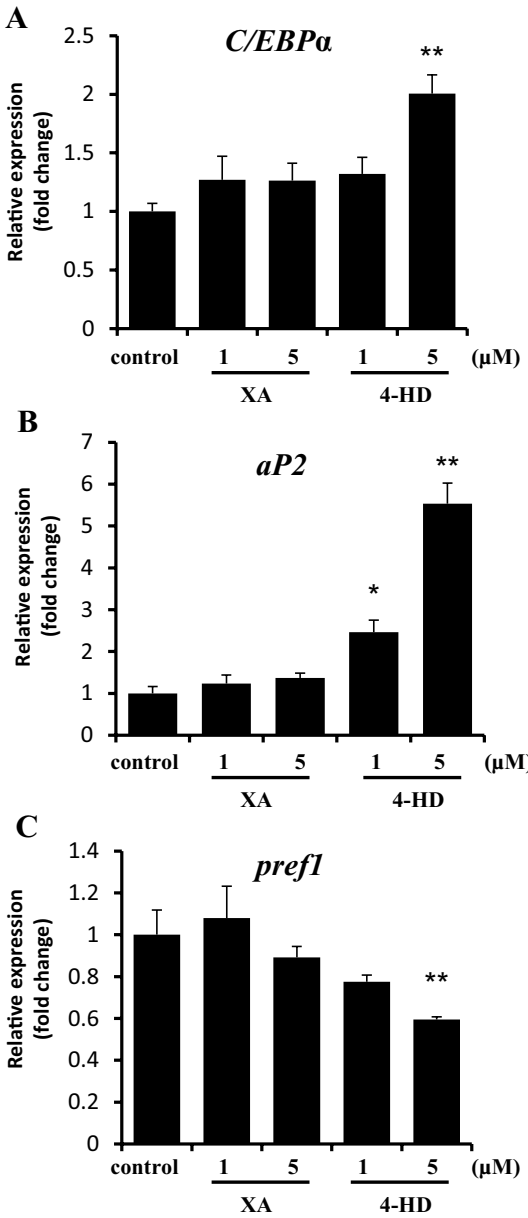

Fig. 2 4-Hydroxyderricin (4-HD) elevated the expression of adipogenesis marker genes, cytosine–cytosine–adenosine–adenosine–thymidine (CCAAT)/enhancer binding protein (*C/EBPα*) and adipocyte fatty acid binding-protein 2 (*aP2*) and downregulated that of preadipocyte marker gene, preadipocyte factor 1 (*Pref1*) in 3T3-L1 cells. **a** *C/EBPα*, **b** *aP2*, and **c** *Pref1* gene expression levels in 3T3-L1 cells treated with xanthoangelol (XA) and 4-HD (1 and 5 μM) were normalized to 36B4 mRNA expression levels. Values are means ± SEM of 3–4 samples; *$p < 0.05$ and **$p < 0.01$ compared with control

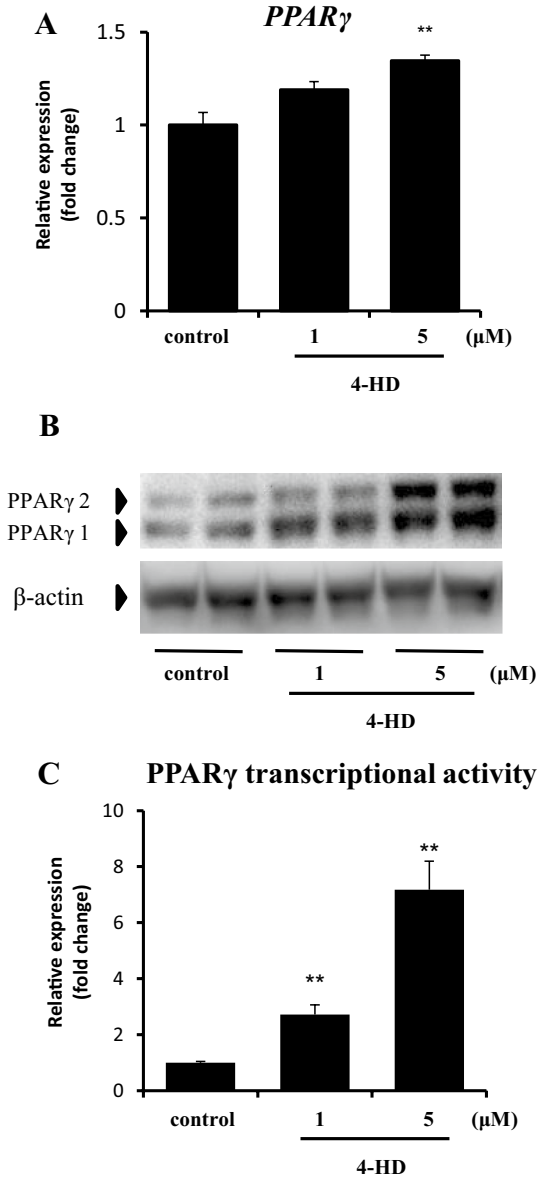

Fig. 3 4-Hydroxyderricin (4-HD) increased peroxisome proliferator-activated receptors (PPAR)-γ mRNA and protein expression levels and activated PPARγ. 3T3-L1 cells were induced to differentiate and then incubated in growth medium with or without 4-HD. mRNA expression of **a** PPARγ was measured on day 6, and **b** protein expression of PPARγ was determined on day 10. **c** Effect of 4-HD on PPARγ activity was measured using luciferase assay. Values are means ± SEM of 5 samples; *$p < 0.05$ and **$p < 0.01$ compared with control

elucidate the effects of 4-HD on PPARγ, we first investigated whether 4-HD increased the expression levels of PPARγ mRNA and protein. Treatment with 4-HD greatly upregulated PPARγ mRNA (including PPARγ1 and 2), and PPARγ1 and PPARγ2 protein expression (Fig. 3a, b, respectively) in 3T3-L1 cells compared with control cells. Next, we examined whether 4-HD served as a ligand for PPARγ by performing a luciferase assay. As shown in Fig. 3c, 4-HD but not XA (data not shown) at concentrations of 1 and 5 μM significantly activated PPARγ by 2.5- and 6.5-fold higher, respectively, than no treatment did. The results indicate that 4-HD did not

only promote PPARγ mRNA and protein expression but also served as a ligand of PPARγ.

4-HD Increased Adiponectin mRNA Expression and Secretion in 3T3-L1 Cells

The level of adiponectin, an insulin-sensitizing adipocytokine derived from adipocytes, increases during adipogenesis. Therefore, next, we determined the effects of 4-HD on adiponectin expression in 3T3-L1 cells. Treatment of 3T3-L1 cells with 5 μM 4-HD significantly enhanced adiponectin gene expression during differentiation with a 3-fold increase on day 6 compared to untreated cells (Fig. 4a). Consistent with the mRNA expression, the secretion of adiponectin in the medium of cells exposed to 4-HD was significantly upregulated on day 10 (Fig. 4b). Interestingly, 4-HD effectively enhanced the secretion of HMW adiponectin, which is better correlated with improvement of insulin sensitivity compared with the other forms of adiponectin (Fig. 4c). These data suggest that 4-HD treatment during adipocyte differentiation promoted adiponectin mRNA expression and protein secretion in adipocytes.

4-HD Promoted Insulin-Stimulated Glucose Uptake and GLUT4 mRNA Expression in 3T3-L1 Cells

To elucidate the effects of 4-HD on glucose utilization in 3T3-L1 adipocytes, we examined insulin-independent- and -dependent glucose uptake in 4-HD-treated adipocytes. Following treatment with 4-HD, insulin-dependent glucose uptake was significantly upregulated while insulin-independent glucose uptake remained unchanged (Fig. 5a). 4-HD treatment also increased the phosphorylation of Akt, a major protein kinase involved in insulin-stimulated glucose transport, in adipocytes. Besides, the mRNA expression of GLUT4, a key regulator of insulin-stimulated glucose uptake and whole-body glucose homeostasis was markedly elevated in 3T3-L1 cells treated with 4-HD (Fig. 5b). The results demonstrate that 4-HD increased insulin-stimulated glucose uptake accompanied by the upregulation of mRNA level of GLUT4 and Akt phosphorylation in adipocytes.

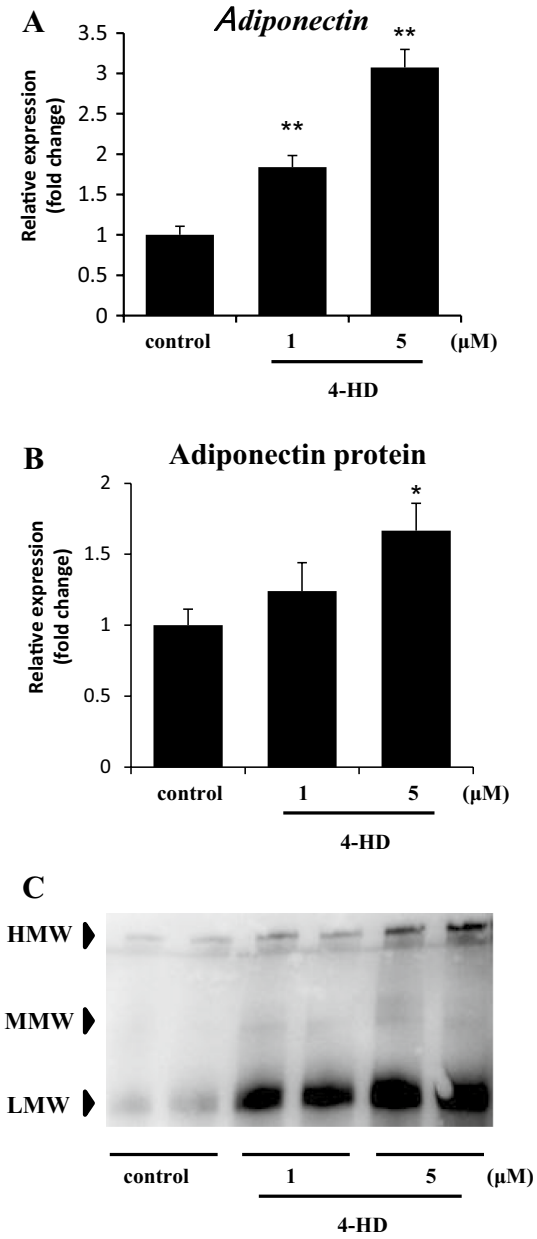

Fig. 4 4-Hydroxyderricin (4-HD) promoted adiponectin mRNA expression and secretion. 3T3-L1 cells were induced to differentiate and then incubated in growth medium with or without 4-HD. **a** mRNA expression of adiponectin was measured on day 6. **b** Total and **c** high-molecular-weight (HMW) adiponectin in 3T3-L1 cell supernatants were measured on day 10. Values are means ± SEM of 5–6 samples; $*p < 0.05$ and $**p < 0.01$ compared with control

Discussion

Insulin-responsive adipose tissue plays a key role in regulating insulin sensitivity and the risk for diabetes by its fats storage and endocrine functions [3, 33]. Adipocyte differentiation contributes to increasing insulin sensitive adipocytes, which are thought to be more insulin sensitive in comparison with large adipocytes. Failure of adipocyte differentiation might contribute to insulin resistance and type 2 diabetes [8]. In the present study, we examined the effects of 4-HD on adipogenesis in 3T3-L1 cells. 4-HD significantly enhanced adipogenesis as evidenced by the elevated TG accumulation of the 3T3-L1 cells. The mRNA expression of adipogenic markers such as C/EBPα and aP2 were significantly upregulated while the gene levels of the preadipocyte marker *Pref1* significantly decreased in adipocytes

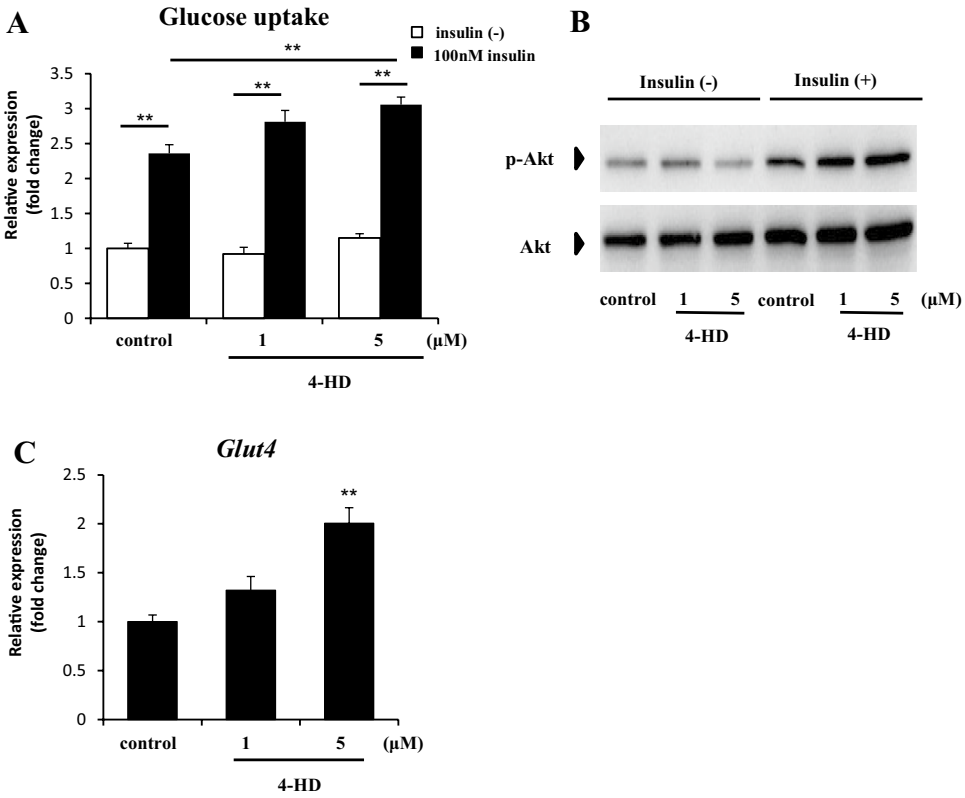

Fig. 5 4-Hydroxyderricin (4-HD) stimulated insulin-dependent glucose uptake and upregulated mRNA expression of glucose transporter 4 (GLUT4) and protein levels of AKT phosphorylation. 3T3-L1 cells were induced to differentiate, and then incubated in growth medium with or without 4-HD. **a** 2-Deoxyglucose uptake into 4-HD treated cells was determined on day 3. **b** The level of AKT phosphorylation and the expression of total protein were measured by immunoblotting. On day 3 after differentiation, the cells were washed with serum free medium for three times and incubated in serum free medium overnight, then stimulated with 3.4 nM insulin and extracted for immunoblot assay. **c** mRNA expression of GLUT4 was measured on day 6. Values are means ± SEM of 5–6 samples; $*p < 0.05$ and $**p < 0.01$ compared with control

treated with 4-HD. Based on these results, we proposed that 4-HD exhibited adipogenic effects in 3T3-L1 cells.

PPARγ, the major regulator involved in adipocyte differentiation, has been reported to be required for the differentiation of adipose tissue *in vivo* and *in vitro* [32]. In the present study, 4-HD elevated both mRNA and protein expression levels of PPARγ in 3T3-L1 cells during differentiation. Additionally, 4-HD upregulated PPARγ activity in the luciferase assay and the results suggest that the effect of 4-HD on adipogenesis might be mediated by the increase in ligand activation of PPARγ.

In addition, some interesting observations needed further discussion in the study. One is that, XA, with the same main structure as 4-HD, exhibited weaker adipogenic activity, although similar effects of XA and 4-HD at the same concentration on promoting glucose uptake in L6 cell and anti-inflammation in LPS-stimulated RAW cells were observed before [26, 27]. The diverse effects of XA and 4-HD on adipocyte differentiation might contribute to the difference of structure on the side hydrocarbon chain, which leads to distinct PPARγ ligand activity.

Another observation is that our results are contrary to the conclusion of Zhang *et al*. [34] who reported that 4-HD suppressed adipocyte differentiation of 3T3-L1 cells at a concentration of 5 μM. This discrepancy might be due to differences in exposure time (2 *vs* 3 days) and the differentiation inducer cocktail. In our study, we used the most common agents for differentiation, including dexamethasone, insulin, and 1-methyl-3-isobutylxanthine [35, 36]. Besides of these common agent, ascorbic acid was also listed in Zhang′s study. Although ascorbic acid has been reported to promote adipocyte differentiation in previous study [37], some study has shown that ascorbic acid suppress adipocyte differentiation [38]. Therefore, ascorbic acid might effect adipogenic activity of 4-HD.

PPARγ agonists such as TZD are used in the therapy of insulin resistance and type 2 diabetes [12]. Treatment of patients with diabetes using TZD is accompanied with elevated plasma adiponectin, which contributes to enhancing insulin sensitivity [14, 15]. Adiponectin is predominantly produced in white adipose tissue and increases during adipocyte differentiation. In the present study, treatment of 3T3-L1 cells with 4-HD also upregulated both mRNA and protein expression levels of adiponectin. Moreover, we firstly shown that 4-HD increased not only total adiponectin protein production but also HMW adiponectin secretion, which is the active form. The HMW adiponectin proportion rather than total adiponectin is a more accurate reflection of the association of adiponectin with insulin resistance [20]. Collectively, these results suggest that 4-HD might increase adiponectin production in adipose tissue via activation of PPARγ signaling.

Pioglitazone belongs to the TZD class of drugs and potently promotes glucose utilization in adipose tissue by

increasing the uptake and metabolism of glucose via activation of regulatory genes involved in glucose transport (GLUT4) and metabolism of lipids (fatty acid synthase, FAS) and phosphoenolpyruvate carboxykinase (PEPCK) [39]. GLUT4 plays a pivotal role in insulin-dependent glucose uptake and is impaired during insulin resistance [40]. Insulin promotes glucose transport in adipocytes mainly by enhancing the translocation of GLUT4 from intracellular sites to the membrane [41]. Akt is a major effector of the insulin response and capable of stimulating the translocation of GLUT4 to membrane [42]. In our study, 4-HD significantly increased insulin-dependent but not insulin-independent glucose uptake in 3T3-L1 cells during differentiation at a concentration of 5 μM. 4-HD treatment also elevated GLUT4 gene expression and Akt phosphorylation in 3T3-L1 adipocytes. Our results suggest that 4-HD increased insulin-dependent glucose transport by elevating GLUT4 expression and Akt phosphorylation. Two hours treatment of 3T3-L1 adipocytes with 20 μM 4-HD was reported to promote GLUT4 translocation to the plasma membrane [43]. These results suggest that 4-HD promotes insulin-stimulated glucose uptake though Akt/GLUT4 signaling pathway. 4-HD was reported to elevate glucose uptake in L6 myotubes by stimulating GLUT4 translocation with Akt-independent manner [26]. It suggests that 4-HD might promote glucose uptake via different mechanisms in different cell types.

Previous studies have demonstrated that 4-HD was rapidly absorbed in mice and distributed preferentially to adipose tissue [44], and decreased blood glucose in KK-Ay mice [25]. They suggest that 4-HD might promote glucose uptake and increase insulin sensitivity in adipose tissue of mice. In addition, TZD promote adipocyte differentiation by generating small insulin sensitive adipocytes [45]. Moreover, adipose tissue distribution influences metabolic disorder, the ratio of visceral adipose tissue and subcutaneous adipose tissue is positively associated with insulin resistance [46]. PPARγ agonist rosiglitazone was reported to elevate glucose uptake and intracellular metabolism in subcutaneous fat through redistribution of triacylglycerol from visceral adipose tissue to subcutaneous adipose tissue in a PPARγ-dependent way [47]. Nevertheless, the effects of 4-HD on glucose metabolism, generation of small adipocytes within adipose tissue and redistribution of fat between different adipose depots *in vivo* remain to be clarified in future.

Taken together, we demonstrated that 4-HD promoted adipocyte differentiation by activating PPARγ signaling. Additionally, it stimulated adiponectin production in adipocytes and enhanced insulin-dependent glucose uptake into adipocytes. These results collectively suggest that 4-HD might be a potent phytochemical with the potential to regulate adipocyte function.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing financial interests.

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