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Humulus japonicus stimulates thermogenesis and ameliorates oxidative stress in mouse adipocytes

Tae Woo Jung[1], Hyoung-Chun Kim[2], Yong Kyoo Shin[1], Hyeyoung Min[3], Seong-Wan Cho[4], Zi Soo Kim[1], Su Mi Han[1], A M Abd El-Aty[5,6], Ahmet Hacımüftüoğlu[6] and **Ji Hoon Jeong[1]**

[1]Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul, Republic of Korea
[2]Neuropsychopharmacology and Toxicology Program, College of Pharmacy, Kangwon National University, Chunchon, Republic of Korea
[3]College of Pharmacy, Chung-Ang University, Seoul, Republic of Korea
[4]Department of Pharmaceutics & Biotechnology, Konyang University, Daejeon, Republic of Korea
[5]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[6]Department of Medical Pharmacology, Medical Faculty, Ataturk University, Erzurum, Turkey

Correspondence should be addressed to J H Jeong: jhjeong3@cau.ac.kr

Abstract

An aqueous extract of *Humulus japonicus* (AH) has been documented to ameliorate hypertension and non-alcoholic fatty liver disease (NAFLD). Here, we investigated the effects of an aqueous extract of AH on thermogenesis and palmitate-induced oxidative stress in adipocytes. To verify the effect of AH on browning, we measured the expression levels of specific markers in 3T3-L1 adipocytes using qPCR and Western blotting, respectively. To assess the role of oxidative stress, cells were stained with DCFDA and observed by fluorescence microscopy. AH increased the expression of brown adipose tissue-specific markers. Additionally, it induced fatty acid oxidation and lipolysis and suppressed both lipogenic markers and lipid accumulation. Furthermore, AH ameliorated hydrogen peroxide-induced oxidative stress. Enhanced expression of these markers contributed to fat browning, fatty acid oxidation and lipolysis of 3T3-L1 adipocytes *via* the AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor delta (PPARδ) signaling pathways. Moreover, AMPK and PPARδ resulting in protective effects of AH against oxidative stress. In sum, AH could promote the browning, lipolysis and thermogenesis in 3T3-L1 adipocytes and would suppress the hydrogen peroxide-induced oxidative stress and lipogenesis during differentiation. We therefore suggest that AH could be used as a potential candidate for treating obesity and related metabolic disorders.

Key Words

- *Humulus japonicas*
- browning
- fatty acid oxidation
- lipogenesis
- lipolysis
- adipocytes

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Introduction

Adipose tissue has multiple metabolic functions and plays a crucial role in homeostatic control of energy balance. Depending on the macroscopic appearance, adipose tissue has been featured to be white or brown. White adipose tissue (WAT) is composed of white adipocytes and primarily serve as an energy storage of neutral lipids. WAT stores excess energy for usage by other tissues during energy depletion. Brown adipose tissue (BAT) plays a prominent role in thermoregulation. In response to a variety of external conditions, cells strongly expressing

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uncoupling protein 1 (UCP1), which induced structural and functional changes in WAT to brown adipocytes (Lim *et al.* 2012). UCP1 dissipates the mitochondrial proton motive force and increases thermogenesis in BAT. Cold shock and β3-adrenergic receptor activation by various external conditions may induce the appearance of brown adipocytes in WAT through remodeling of WAT adipocyte progenitors (Qiang *et al.* 2012). Recent advances in the treatment of obesity have revealed that WAT can demonstrate BAT-like phenotypes under certain external factors. Various pharmacological and dietary compounds have been suggested to increase energy expenditure and prevent lipid accumulation in mammals (Baboota *et al.* 2014, Roberts *et al.* 2014). For instance, capsaicin, β-isoaminobutyric acid (BAIBA) and irisin can stimulate the expression of browning-specific genes in adipocytes (Baboota *et al.* 2014, Roberts *et al.* 2014, Zhang *et al.* 2014*a*).

Owing to its multiple properties, *Humulus japonicas* has been reported to exert antimutagenic, antibacterial, antioxidant, anticancer and anti-inflammatory activities (Park *et al.* 1994, 1995, Hwang *et al.* 2009, Lee *et al.* 2012). It was reported that methanolic extract of *H. japonicus* could ameliorate atherosclerosis *via* suppression of the pro-atherogenic factors and lipid accumulation in the aortic endothelium of apolipoprotein E-deficient mice (Lim *et al.* 2016). Recently, the aqueous extract of *H. japonicus* (AH) was shown to alleviate hyperlipidemia and hepatic lipid accumulation in high-fat diet (HFD)-fed mice (Chung *et al.* 2018). However, the effects of AH on browning and oxidative stress in adipocytes have not yet been reported. Thus, in this study, we aimed to evaluate the effects of AH on stimulation of BAT-like phenotype in 3T3-L1 adipocytes. We further investigated the effects of AH on lipogenesis, lipolysis and oxidative stress and explored its molecular mechanisms.

Materials and methods

Ethical approval

This work does not contain any studies with human participants or animals performed by authors.

Cell culture, reagents and antibodies

We performed cell culture according to our previous protocol with modification (Jung *et al.* 2018*a*,*b*). Mouse preadipocytes (3T3-L1; ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Cells were cultured in a humidified atmosphere containing 5% CO_2 at 37°C. Two days after full confluence, cells were differentiated by incubation in DMEM containing insulin (1 µM), IBMX (0.5 mM) (Sigma), and dexamethasone (0.5 µg/mL) for 2 days and then in DMEM supplemented with insulin (1 µM) for 3 days. Subsequently, cells were maintained in and refed every 3 days with a culture medium containing insulin (1 µM) pending further experimental use (10 days after the initiation of differentiation). Differentiated 3T3-L1 cells were treated with AH (0–100 µg/mL) (I-woo Agricultural Company, Seoul, Republic of Korea) for 24 h.

Sample preparation

Sample preparation was carried out according to our previous protocol with modification (Jung *et al.* 2018*a*,*b*). The lyophilized water extract was prepared using dried *H. japonicus* that was obtained from Woori Oriental Medicine Materials, and authenticated by Dr. Yuan Lu Sun of Solvit P&F (Seoul, Republic of Korea). Dried *H. japonicus* (650 g) was mixed with distilled water (15 L) and boiled for 4 h at 100°C in duplicate. The extract was filtered, evaporated up to 15.6% and lyophilized using a lyophilizer (Labconco, Freezone 1L) at 5 mmHg and −50°C. The lyophilized powder was stored at −30°C.

Western blotting analysis

Western blotting was performed according to (Jung *et al.* 2018*a*,*b*) with modification. Differentiated 3T3-L1 cells were harvested and total proteins were extracted with a lysis buffer (PRO-PREP; Intron Biotechnology, Seoul, Republic of Korea) for 60 min at 4°C. Nuclear and cytosolic protein extracts were prepared using a protein fractionation kit (Biovision, Mountain View, CA, USA). Protein samples (40 µg) were subjected to 12% SDS-PAGE, transferred to a nitrocellulose membrane (Amersham Bioscience, Westborough, MA, USA) and probed with the indicated primary antibodies followed by secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology). Signals were visualized using enhanced chemiluminescence (ECL) kits (Amersham Bioscience).

RNA extraction and quantitative real-time PCR

RNA extraction and quantitative real-time PCR were assayed according to the protocol of (Jung *et al.* 2018*c*)

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with modification. Total RNA was extracted from the harvested hepatocytes using TRIzol reagent (Invitrogen). Gene expression was measured by quantitative real-time PCR (qPCR) using the fluorescent TaqMan 5′nuclease assay on an Applied Biosystems 7000 sequence detection system (Foster City, CA, USA). qPCR was performed using cDNA as a template, 2× TaqMan Master Mix, and 20× premade TaqMan gene expression assays (Applied Biosystems). The qPCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. PCR primer mixes for mouse *Ucp-1* (Applied Biosystems; Mm01244861_m1), *Pgc1α* (Applied Biosystems; Mm01208832_m1), *Prdm16* (Applied Biosystems; Mm00712556_m1), *Cpt1* (Applied Biosystems; Mm01231183_m1), *Aco* (Applied Biosystems; Mm00801417_m1) and *Fabp3* (Applied Biosystems; Mm02342495_m1) were used. The mRNA expression of β-actin was quantified as an endogenous control using the following primers: 5′-CGATGCTCCCCGGGCTGTAT-3′ and 5′-TGGGGTACTTCAGGGTCAGG-3′.

Transfection of cells with siRNAs for gene silencing

siRNA transfection was conducted according to a previously modified protocol (Jung *et al.* 2018*c*). siRNA oligonucleotides (20 nM) specific for AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor delta (PPARδ) were purchased from Santa Cruz Biotechnology. To suppress gene expression, cell transfection was performed using Lipofectamine™ 2000 (Invitrogen), according to the manufacturer's instructions. In brief, cells were grown to 60–70% confluence, followed by serum starvation for 12 h after 3T3-L1 cell differentiation. Cells were then transfected with validated siRNA or scramble siRNA at a final concentration of 20 nM in the presence of the transfection reagent. Thirty-six hours post transfection, cells were harvested for protein extraction and additional analysis.

Measurements of acetyl-CoA and ATP levels

We measured acetyl-CoA and ATP content according to (Jung *et al.* 2018*c*) with modification. Intracellular levels of acetyl-CoA were measured in differentiated 3T3-L1 cells using a PicoProbe acetyl-CoA assay kit™ (Abcam), whereas the intracellular ATP levels were measured using an ATP assay kit™ (Abcam), according to the manufacturer's protocols.

Oil red O staining and triglyceride (TG) assay

Oil red O staining was performed according to the protocol described in our previous report with modification (Jung *et al.* 2018*a*,*b*). Differentiated 3T3-L1 cells were stained using Oil red O to measure the accumulated cellular neutral lipids, including TG. After fixation with 10% formalin for 40 min, hepatocytes were stained with Oil red O solution (Sigma) for 1 h at 37°C. Oil red O-stained TG was quantified by adding isopropanol to each sample (Yamaguchi *et al.* 2017). The mixtures were gently agitated at 25°C for 8 min. Then, 100 µL of isopropanol-extracted samples were analyzed using a spectrophotometer at 510 nm.

Determination of intracellular ROS accumulation

We measured the cellular ROS levels according to the protocol modified from previous work (Jung *et al.* 2018*a*,*b*). Oxidative stress in adipocytes was evaluated using 5-(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein

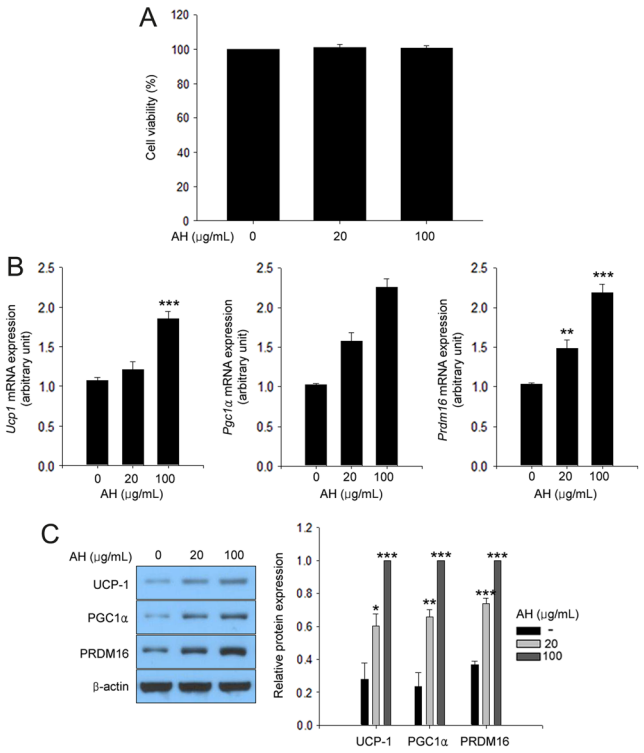

Figure 1
AH stimulates adipocyte browning. (A) Viability of 3T3-L1 adipocytes treated with AH at various concentrations (0, 20 and 100 µg/mL) for 24 h measured by MTT assay. (B) Quantitative real-time-PCR assay of *Ucp1*, *Pgc1α* and *Prdm16* in 3T3-L1 adipocytes treated with various concentrations of AH for 24 h. (C) Western blotting analysis of UCP-1, PGC1α and PRDM16 in 3T3-L1 adipocytes treated with various concentrations of AH for 24 h. Mean ± S.E.M. was obtained from three separate experiments. ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ compared to the controls.

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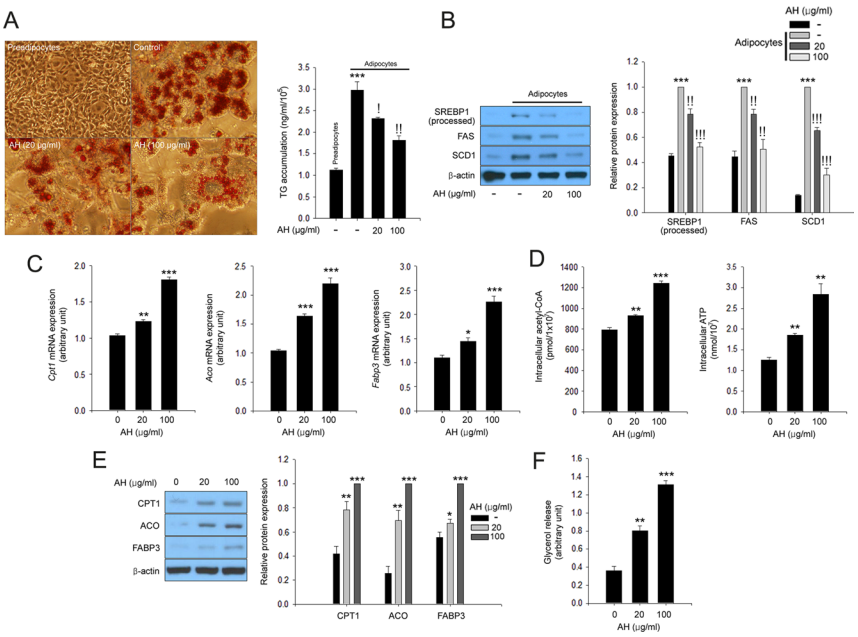

Figure 2
AH suppresses lipogenesis during differentiation and increases fatty acid oxidation and lipolysis in 3T3-L1 adipocytes. (A) Oil red O staining in preadipocytes and differentiated 3T3-L1 cells treated with AH (0, 20 and 100 μg/mL) for 24 h. Quantitative analysis of lipid accumulation was performed using an isopropanol extraction method. (B) Western blotting analysis of processed SREBP1, FAS and SCD1 in 3T3-L1 adipocytes treated with AH (0, 20 and 100 μg/mL) for 24 h. (C) Quantitative real-time-PCR assay of *Cpt1*, *Aco* and *Fabp3* in 3T3-L1 adipocytes treated with AH (0, 20 and 100 μg/mL) for 24 h. (D) Intracellular acetyl Co-A and intracellular ATP levels were measured in 3T3-L1 adipocytes treated with AH (0, 20 and 100 μg/mL) for 24 h. Western blotting analysis of CPT1, ACO and FABP3 (E) and lipolysis (F) in 3T3-L1 adipocytes treated with AH (0, 20 and 100 μg/mL) for 24 h. Mean ± S.E.M. was calculated from data obtained from three independent experiments. ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ compared to controls. !!!$P < 0.001$, !!$P < 0.01$ and !$P < 0.05$ compared to 3T3-L1 adipocytes. A full colour version of this figure is available at https://doi.org/10.1530/JME-19-0010.

diacetate, acetyl ester (CM-H_2DCFDA, Sigma). 3T3-L1 adipocytes were treated with 5 μM DCFDA for 1 h. Following treatment, cells were stabilized by incubation with DMEM containing 10% FBS for 10 min. Cellular fluorescence levels were measured using a microplate reader set at an excitation wavelength of 485 nm and emission wavelength of 535 nm.

Dosage information

Differentiated 3T3-L1 cells were treated with AH (0-100 μg/mL) for 24 h. Notably, fully differentiated 3T3-L1 cells did not show any sign of toxicity when treated with various concentrations of AH (0–100 μg/mL) for 24 h or 48 h.

Statistical analysis

Results were presented as absolute values (means ± S.E.M.). All experiments were performed in triplicate. Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. All analyses were performed using SPSS/PC (v 13.0 for Windows; SPSS).

Results

AH increased the expression of BAT markers in 3T3-L1 adipocytes

To investigate the effect of AH on the conversion of 3T3-L1 adipocytes into brown adipocytes, we treated fully differentiated 3T3-L1 cells with various concentrations of AH (0-100 μg/mL) for 48 h after the cell viability assay. AH treatment did not significantly affect cell viability at a concentration rate of 100 μg/mL (Fig. 1A). Furthermore, AH markedly increased the mRNA expression of BAT markers (Ucp1, PR domain containing 16 (PRDM16), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)) in 3T3-L1 adipocytes in a dose-dependent fashion (Fig. 1B). Furthermore, treatment of 3T3-L1 adipocytes with AH increased the expression of UCP1, PRDM16 and PGC-1α proteins in a dose-dependent manner (Fig. 1C).

AH regulated fat metabolism in adipocytes

As lipid metabolism plays a crucial role in browning in adipose tissue (Heeren & Scheja 2018), we next examined whether AH could affect lipogenesis, fatty acid oxidation and lipolysis in 3T3-L1 adipocytes. Treatment of 3T3-L1 adipocytes with AH suppressed the expression of lipogenesis-associated enzymes (sterol regulatory element-binding protein 1 (SREBP1), FAS and stearoyl-CoA desaturase 1 (SCD1)) and decreased the lipid content (Fig. 2A and B), whereas it increased the expression of β-oxidation markers (carnitine palmitoyltransferase 1 (CPT1), ACO, and fatty acid-binding protein 3 (FABP3)), as well as cellular levels of acetyl-CoA and ATP (Fig. 2C, D and E). Additionally, AH treatment induced glycerol releases from differentiated 3T3-L1 cells in a dose-dependent way (Fig. 2F), suggesting augmented lipolysis.

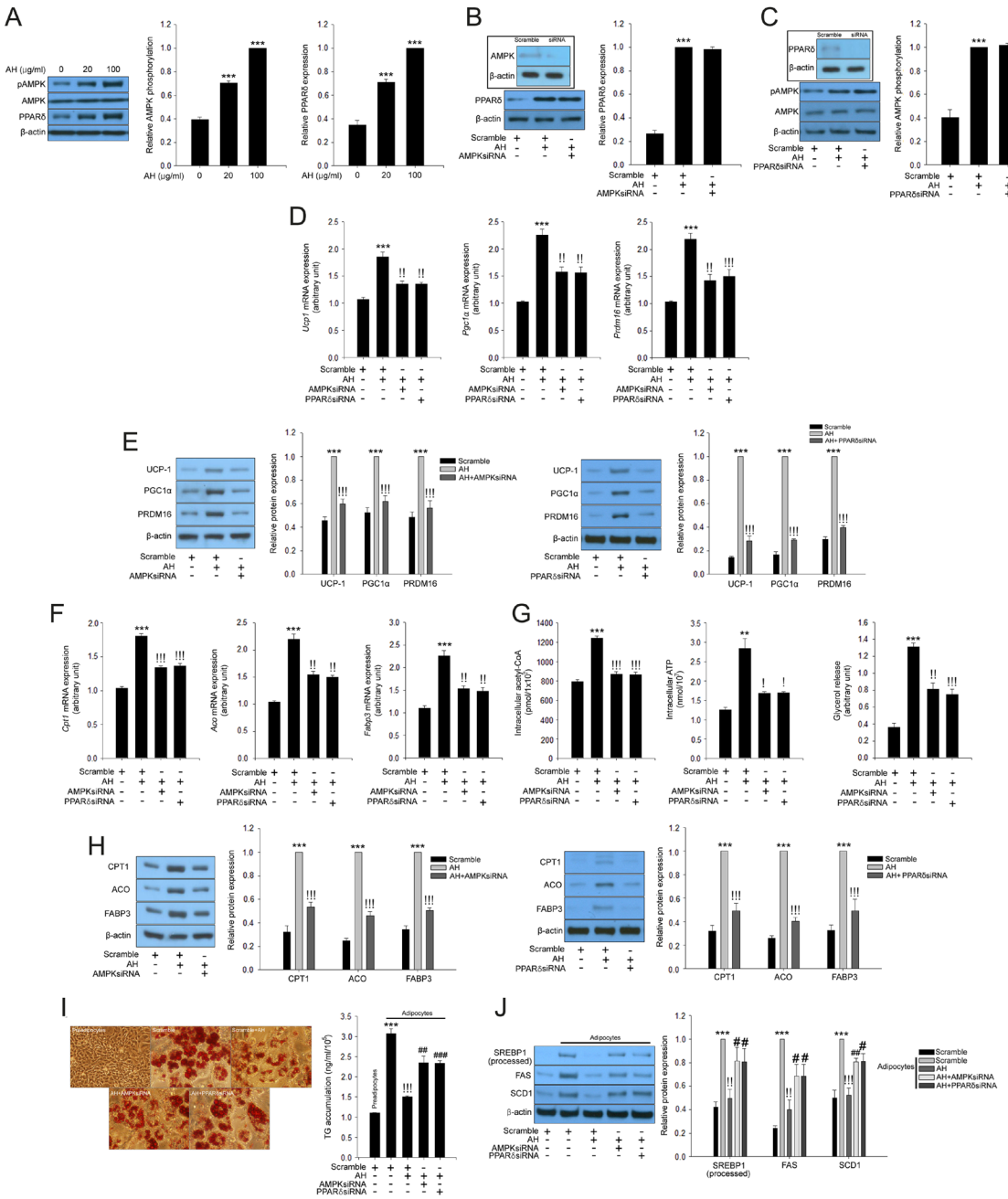

Figure 3

AH increases browning, fatty acid oxidation and lipolysis and suppresses lipogenesis during differentiation in 3T3-L1 adipocytes through AMPK or PPARδ pathway. (A) Western blotting analysis of AMPK phosphorylation in 3T3-L1 adipocytes treated with AH (0, 20 and 100 μg/mL) for 24 h. (B) Western blotting analysis of PPARδ expression in scrambled or AMPKsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. (C) Western blotting analysis of AMPK phosphorylation in scrambled or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. Quantitative real-time-PCR assay of *Ucp1*, *Pgc1α* and *Prdm16* (D) and Western blotting analysis of UCP1, PGC1α and PRDM16 expression (E) in scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. Quantitative real-time-PCR assay of *Cpt1*, *Aco* and *Fabp3* (F) and intracellular acetyl Co-A and intracellular ATP levels (G) were measured in scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. (H) Western blotting analysis of CPT1, ACO and FABP3 expression in scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. (I) Oil red O staining in preadipocytes and differentiated 3T3-L1 cells scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. Quantitative analysis of lipid accumulation was performed using an isopropanol extraction method. (J) Western blotting analysis of processed SREBP1, FAS and SCD1 in preadipocytes and differentiated 3T3-L1 cells scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. Mean ± S.E.M. was calculated from data obtained from three independent experiments. $^{***}P < 0.001$ and $^{**}P < 0.01$ compared to controls. $^{!!!}P < 0.001$, $^{!!}P < 0.01$, and $^{!}P < 0.05$ compared to AH treatment in 3T3-L1 adipocytes or control in 3T3-L1 adipocytes. $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared to controls in 3T3-L1 adipocytes. A full colour version of this figure is available at https://doi.org/10.1530/JME-19-0010.

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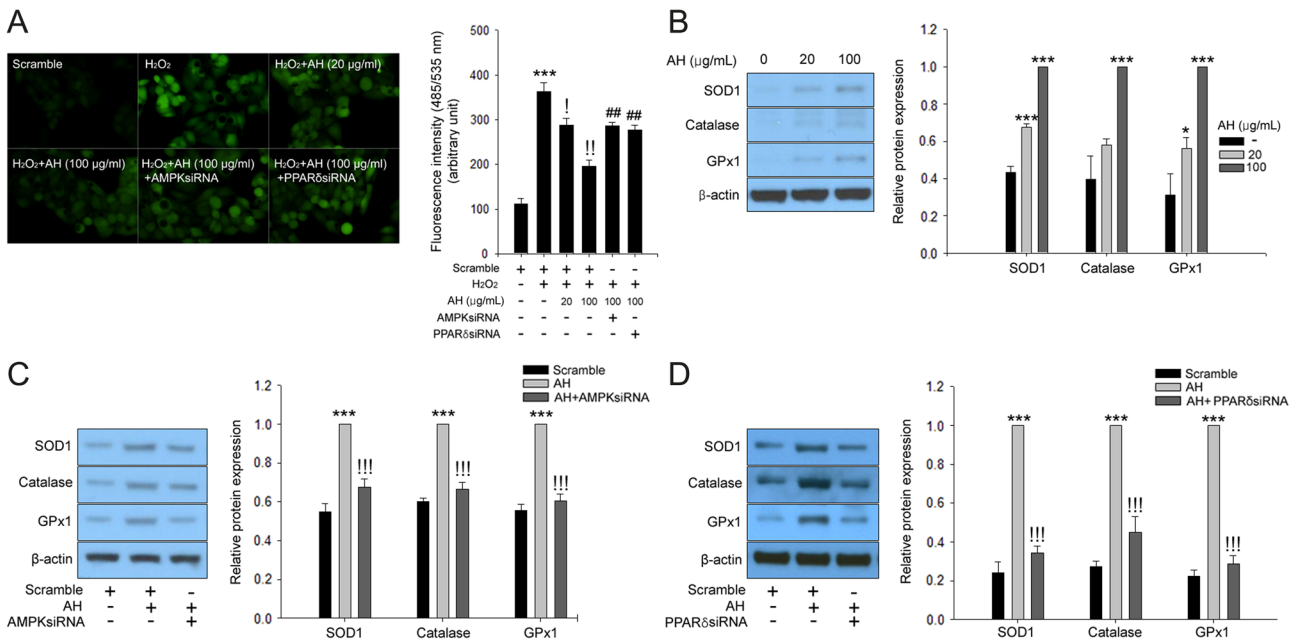

Figure 4

AH ameliorates oxidative stress through AMPK or PPARδ-mediated pathway. (A) ROS staining of preadipocytes and differentiated 3T3-L1 cells scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h. Western blotting analysis of SOD1, catalase and GPx-1 expression in preadipocytes and differentiated 3T3-L1 cells in the presence of AH (0, 20 and 100 μg/mL) (B) and in preadipocytes and differentiated 3T3-L1 cells scrambled, AMPKsiRNA or PPARδsiRNA-transfected 3T3-L1 adipocytes treated with AH for 24 h (C and D). Mean ± S.E.M. was calculated from data obtained from three independent experiments. ***$P < 0.001$ and *$P < 0.05$ compared to controls. !!!$P < 0.001$, !!$P < 0.01$ and !$P < 0.05$ compared to AH treatment or H_2O_2 treatment. ##$P < 0.01$ compared to H_2O_2 and AH treatment in 3T3-L1 adipocytes. A full colour version of this figure is available at https://doi.org/10.1530/JME-19-0010.

AH stimulated browning and β-oxidation via AMPK and PPARδ-mediated pathways

AMPK (Daval *et al.* 2006) and PPARδ (Goto *et al.* 2011) have been reported to stimulate fatty acid oxidation. Therefore, to identify the possible mechanisms associated with AH-mediated 3T3-L1 adipocyte browning, we therefore investigated the key lipid metabolic regulators – AMPK and PPARδ. Treatment of 3T3-L1 adipocytes with AH augmented AMPK phosphorylation and PPARδ expression in a dose-dependent manner (Fig. 3A). Interestingly, increased AMPK phosphorylation and PPARδ expression by AH did not affect the activity or expression of each other (Fig. 3B and C). Therefore, we hypothesized that AMPK and PPARδ might cause the induction of browning. Thence, we further investigated the effects of AMPK and PPARδ suppression on AH-induced BAT markers. siRNAs of AMPK and PPARδ mitigated the effects of AH on mRNA and protein expression of UCP1, PRDM16 and PGC1α in 3T3-L1 adipocytes (Fig. 3D and E). Moreover, siRNA against AMPK and PPARδ reversed AH effects on fatty acid oxidation markers, cellular acetyl-CoA, ATP contents and lipolysis (Fig. 3F, G and H). Furthermore, AMPK and PPARδ siRNA mitigated the effects of AH on lipid accumulation and expression of lipogenic proteins, such as processed SREBP1, FAS and SCD1 (Fig. 3I and J).

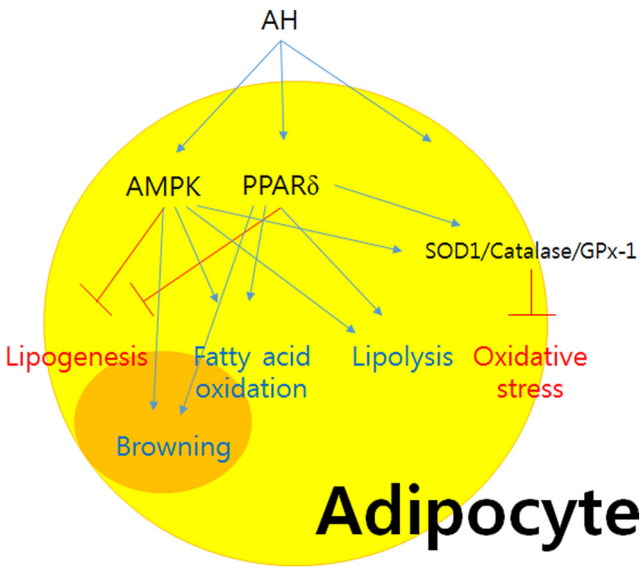

Figure 5

Schematic diagram of the effects of AH on adipocytes. A full colour version of this figure is available at https://doi.org/10.1530/JME-19-0010.

AH attenuated hydrogen peroxide-induced oxidative stress in differentiated 3T3-L1 cells

It was reported that oxidative stress could contribute to the development of obesity-associated metabolic disorders (Roberts & Sindhu 2009). Therefore, we evaluated the effects of AH on oxidative stress. AH ameliorated hydrogen peroxide (H_2O_2)-induced ROS production in a dose-dependent fashion. Furthermore, siRNA against AMPK and PPARδ weakened the inhibitory effects of AH on H_2O_2-induced ROS production (Fig. 4A). Treatment of 3T3-L1 cells with AH significantly increased the expression of antioxidant enzymes, such as superoxide dismutase 1 (SOD1), catalase and glutathione peroxidase 1 (GPx1) (Fig. 4B). However, siRNA against AMPK and PPARδ reversed these changes (Fig. 4C and D).

Discussion

Conversion of WAT into BAT as an energy consumption strategy is a promising approach for treating obesity and other metabolic diseases (Tseng *et al.* 2010). In the same context, improving thermogenesis in BAT or brown adipocyte-like features in WAT can be a crucial therapeutic approach for treating obesity and related diseases (Kiefer *et al.* 2012). Generally, 3T3-L1 adipocytes have been used as white adipocytes in *in vitro* model (Morrison & McGee 2015). In the present study, we verified that AH has the potential to stimulate brown adipocyte-like phenotype and ameliorate oxidative stress in fully differentiated 3T3-L1 adipocytes. Although Chung *et al.* have reported that AH can ameliorate hyperlipidemia and non-alcoholic fatty liver through regulation of lipogenesis (Chung *et al.* 2018), it remains unclear how AH ameliorates obesity. In this study, treatment with AH significantly augmented UCP1, PRDM16 and PGC-1α expression. These results suggest a possible role of AH in stimulating thermogenesis and browning, since UCP1, PRDM16 and PGC-1α have been shown to contribute to the recruitment of beige cells to WAT. It has been documented that SREBP1, a crucial transcription factor for lipogenesis, plays a pivotal role in lipid metabolism (Ito *et al.* 2013). FAS and SCD1 are representative lipogenesis-associated genes. FAS regulates the *de novo* biosynthesis of long-chain fatty acids (Wakil 1989), whereas CD1 contributes to the biosynthesis of monounsaturated fatty acids, the precursors of triglycerides (Xu *et al.* 2015). Herein, we elucidated that AH treatment mitigated lipogenesis through inhibition of processed SREBP1, FAS and SCD1 expression. AH treatment also abrogated lipid accumulation. Hence, lipogenesis and lipid accumulation (common features of obesity) were suppressed in adipocytes treated with AH. It has been verified that CPT1, ACO and FABP3 play critical roles in fatty acid oxidation. Here, AH treatment increased the mRNA expression of these three genes in adipocytes. Furthermore, the cellular levels of acetyl-CoA and ATP, products of fatty acid oxidation, were also increased following AH treatment. These results showed that AH stimulated fatty acid oxidation in adipocytes; the finding which may indicate that AH could downsize adipocytes *via* suppression of lipogenesis, as well as induction of thermogenesis and fatty acid oxidation.

AMPK is a key metabolic regulator for maintaining cellular energy homeostasis (Hardie *et al.* 2012), by controlling catabolic and anabolic pathways (Zhang *et al.* 2009). AMPK plays a crucial role in various biological processes, such as cell survival, cell migration, autophagy and energy metabolism (Mihaylova & Shaw 2011). Notably, AMPK plays a central role in the interplay between fatty acid metabolism and thermogenesis in adipose tissues (Day *et al.* 2017). It was reported that activation of AMPK stimulates fatty acid oxidation and suppresses fatty acid synthesis (Carlson & Kim 1973, Fullerton *et al.* 2013). Moreover, AMPK regulates the biogenesis of mitochondria *via* activation of the PGC1α-mediated pathway (Jager *et al.* 2007). AMPK activation promotes thermogenesis in BAT and WAT through PGC1α-dependent signaling (Zhang *et al.* 2014*b*) and suppresses white adipocyte differentiation (Zhou *et al.* 2009). Therefore, it is necessitated to explore the molecular mechanisms, by which AH-mediated browning in white adipocytes. We have found that treatment with AH significantly augmented AMPK phosphorylation in differentiated 3T3-L1 cells. Moreover, AMPK inhibition mitigated the effects of AH on thermogenesis, fatty acid oxidation and lipolysis. These results suggested that AMPK might play a significant role in AH-mediated stimulation of adipocyte browning and anti-obesity effects.

PPARδ belongs to the PPAR subfamily that also includes PPARα and PPARγ (Desvergne & Wahli 1999). These receptors regulate transcription through specific binding to PPAR response element (PPRE) in the promoter of target genes (Mangelsdorf *et al.* 1995). PPARs control diverse metabolic processes and activated by fatty acids (Lee *et al.* 1995). It has to be noted that PPARδ is highly expressed in various metabolically active tissues. A previous study suggested that dietary fatty acids could regulate the expression of adipose differentiation-related protein (ADRP), a lipid-coating protein, by very low-density lipoproteins through the PPARδ-mediated pathway (Chawla *et al.* 2003). PPARδ activation increased

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the expression of fatty acid oxidation-related genes (CPT1, ACO and long-chain acyl-CoA dehydrogenase (LCAD)), as well as thermogenic genes (UCP-1 and UCP-3) in BAT (Reilly & Lee 2008). Yu *et al.* showed that fish oil could stimulate lipolysis via PPARδ-dependent signaling (Yu *et al.* 2010). These studies provide evidence that PPARδ plays an important role in lipid metabolism and could be a potential candidate for targeting metabolic disorders. In the present study, AH treatment markedly induced PPARδ expression in 3T3-L1 adipocytes. Hence, suppression of PPARδ mitigated AH-induced thermogenesis, fatty acid oxidation and lipolysis. These results suggest that AH stimulated adipocyte browning through the PPARδ-mediated pathway.

Furthermore, current data indicated that AH ameliorated oxidative stress in 3T3-L1 adipocytes. Elevated oxidative stress observed in obese patients has been suggested to contribute to the development of metabolic syndrome, such as obesity, insulin resistance, hypertension and dyslipidemia (Roberts & Sindhu 2009). Increased levels of ROS in adipocytes might be attributed to reduced antioxidant enzymes, such as SOD, catalase and GPX (Furukawa *et al.* 2004). Therefore, AH can be used as a therapeutic approach for treating metabolic diseases via attenuating redox potential in WAT.

In conclusion, our results indicated that AH may play various roles in the stimulation of browning and lipid metabolism, and attenuation of oxidative stress in white adipocytes through AMPK or PPARδ-dependent pathways (Fig. 5). AH may constitute a new therapeutic strategy for the treatment of metabolic complications of obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

T W J, H C K, A A E, Y K S, H Y M, S W C, Z S K, S M H and J H J: substantial contribution to the conception and design; T W J and J H J: acquisition of data or analysis and interpretation of data; H C K and A M A: drafting and revising the MS. All authors approved the final version of the manuscript. T W J and J H J are responsible for the integrity of the work as a whole.

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