RESEARCH ARTICLE

Ursolic acid stimulates lipolysis in primary-cultured rat adipocytes

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Ursolic acid (UA) is a pentacyclic triterpenic acid with many biological functions naturally existing in many kinds of food. To investigate whether UA can accelerate lipolysis, primary-cultured rat adipocytes were treated with UA, and glycerol release in the culture medium was measured. UA stimulated lipolysis significantly. Furthermore, the lipolytic effect of UA was inhibited by the protein kinase A (PKA) specific inhibitor H89, suggesting that UA exerted its lipolytic function through the cAMP-dependent PKA pathway. Downstream targets of the PKA pathway, hormone-sensitive lipase (HSL) and perilipin A were checked, UA enhanced lipolysis by promoting the translocation of HSL from the cytosol to the lipid droplets and inhibiting the expression of perilipin A. Additionally, adipose triglyceride lipase (ATGL), a novel rate-limiting lipase in the lipolytic catabolism, was upregulated by UA. UA-induced expression of ATGL could not be blocked by H89, suggesting that ATGL upregulation is not regulated by the PKA pathway. These findings suggest that UA significantly stimulates lipolysis by translocating HSL, decreasing perilipin A expression by the PKA pathway, and up-regulating ATGL in primary cultured adipocytes. Thus, UA is a promising candidate for the treatment of obesity.

Keywords:
Adipose triglyceride lipase / Hormone-sensitive lipase / Lipolysis / Primary-cultured rat adipocytes / Ursolic acid

1 Introduction

Most weight reduction drugs have various degrees of side effects. Therefore naturally occurring bioactive compounds have attracted attention as potential alternatives. Ursolic acid (UA), a natural pentacyclic triterpenoid carboxylic acid, is widely distributed in various plants such as fruits and herbs. Increasing evidence, *in vitro* and *in vivo*, suggests that UA has many important biological effects, including anti-inflammatory [1, 2], anti-oxidative [3, 4], anti-mutagenic [5, 6], anti-carcinogenic [7], hepatoprotective [8, 9], anti-microbial [10], anti-atherosclerotic, and anti-hyperlipidemic effects [11, 12].

However, it is unclear whether UA could be applied to treat obesity. It is well known that lipolysis is an important mechanism involved in reducing body fat, and adipocyte lipolysis is lipase-catalyzed hydrolysis of triglycerides (TG) to glycerol and free fatty acids (FFA). Hormone-sensitive lipase (HSL) has been generally considered as a rate-limiting enzyme for lipolysis [13–15]. Under physiological conditions, its activity is driven mainly by phosphorylation of a serine residue through cAMP-dependent protein kinase A (PKA) [16]. Perilipin A, a predominant perilipin isoform in adipocytes, is the most prevalent PKA substrate in adipocytes, and a key regulator of lipolysis [17–20]. It interacts with the translocation of HSL from the cytoplasm to the lipid droplet surface during the stimulation of lipolysis *via* the PKA pathway. Recent research has shown that HSL is

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Abbreviations: ATGL, adipose triglyceride lipase; **f-DMEM,** phenol red-free DMEM; **FFA,** free fatty acids; **HSL,** hormone-sensitive lipase; **MTT,** 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide; **PCV,** packed cell volume; **PPAR,** peroxisome proliferator-activated receptor; **PKA,** protein kinase A; **TG,** triglyceride; **UA,** ursolic acid

not a unique rate-limiting enzyme for lipolysis in adipose tissue. Because diglycerides, but not TG, accumulate in non-obese phenotypic HSL knock-out mice [21–24], there must be other lipases in adipose tissue that preferentially hydrolyze the first ester bond of the TG molecule. Zimmermann *et al.* [25] reported that adipose triglyceride lipase (ATGL) is another key enzyme involved in TG hydrolysis. ATGL catalyzes the initial step in TG lipolysis, while HSL degrades diglyceride to monoacylglycerol and FFA. Therefore, perilipin A and HSL regulated by the PKA pathway and ATGL were selected as molecular targets to explore the mechanisms of UA-induced lipolysis. Peroxisome proliferator-activated receptor (PPAR) was first found by Issemann and Green in 1990 [26]. PPAR-γ is a target for a long-term lipolytic effect, and down-regulation of PPAR-γ could significantly stimulate lipolysis [27]. Therefore, the PPAR-γ pathway was also selected to explore the possible mechanisms of UA-induced lipolysis.

In this paper, we examined possible molecular targets for the anti-obesity function of UA. Based on these studies, we propose that UA may have a promising application for bodyweight reduction in humans.

2 Materials and methods

2.1 Animals

Sprague–Dawley rats were bought from Shanghai Slac Laboratory Animal. The animals were subject to a 12:12 h light:dark cycle, with regular rodent chow and water *ad libitum*, and were housed in cages individually in an environmentally controlled room at room temperature ($21 \pm 2.0^{\circ}C$) and relative humidity ($50 \pm 5\%$). The experimental protocols were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, and conducted in compliance with the animal-use guidelines (SYXK (Hei) 2006-010).

2.2 Chemicals

Phenol red-free DMEM (f-DMEM) and type I collagenase were purchased from Sigma-Aldrich (St. Louis, MO); HEPES, BSA, and adenosine were bought from Amersco (Solon, OH); the glycerol assay kit was bought from Applygen Technologies (Beijing, China); UA (98% purity) was bought from Nanjing Zelang Medical Technology and dissolved in DMSO. Polyclonal antibodies against rat PPAR-γ and rat β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA); polyclonal antibodies against rat HSL and rat perilipin A were obtained from Abcam (Cambridge, UK); rat ATGL antibody was from Cell Signaling Technology (Danvers, MA); H89 was from Beyotime Institute of Biotechnology (Nantong, China); Rosiglitazone (PPAR-γ agonist) was from Cayman Company (Ann Arbor, MI); Isoprel, which can stimulate lipolysis by activating the PKA pathway and is usually used as a lipolytic agent, was from Shanghai Harvest Pharmaceutical (Shanghai, China).

2.3 Isolation and culture of primary rat adipocytes

Adipocytes were isolated from epididymal fat pads of Sprague–Dawley rats (160–225 g) [28]. The fat pads were washed by PBS (pH 7.4) with 100 U/mL penicillin and 100 U/mL streptomycin three times, then were minced into 1–3 mm^3 cubes, and digested in 15 mL Krebs–Ringer buffer containing 25 mM HEPES, 1 mg/mL type I collagenase, 1% BSA, 120 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 15 mM $NaHCO_3$, 200 nM adenosine, pH 7.4. After a 40 min incubation at 37°C in a water bath shaken at 100 cycles/min, the cells were filtered through a 1000 μm nylon mesh, and washed three times in pre-warmed serum-free f-DMEM (containing 5 mM glucose, 200 nM adenosine, 1% BSA). Adipocytes floating in the tube were centrifuged at $200 \times g$ for 3 min. Packed adipocytes were diluted in 1% BSA f-DMEM to generate a cell suspension and incubated for 2 h at 37°C before treatment. For experiments involving the effect of various agents, adipocytes were cultured in 1% BSA f-DMEM in a humidified incubator with an atmosphere of 5% CO_2 at 37°C, with or without agents, in sterile tissue culture microplates.

2.4 Cytotoxicity assay

To establish the maximal non-cytotoxic dose of the UA, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) test was used [29]. Briefly, twofold serial dilutions of UA in f-DMEM medium were incubated at 37°C with adipocytes grown in 96-well tissue culture microplates. After 24 h, the MTT test was performed and the absorbance was read at 550 nm using a microplate reader (BIO-RAD, model 550, Hercules, CA). Medium with vehicle alone was used as the control group. Cell viability was calculated from the absorbance value, using control wells as 100%.

2.5 Lipolysis assay

A total of 20 μL of packed adipocytes were suspended in 500 μL f-DMEM. After the treatment, glycerol release in the culture medium was measured as described previously [30] Briefly, cells were centrifuged at $100 \times g$ for 30 s, the medium was collected and incubated at 70°C for 10 min to inactivate residual lipases. Glycerol released into the medium was determined by the glycerol assay kit (GPO Trinder reaction) at 490 nm. The medium was centrifuged at $10\,000 \times g$ for 5 min at 4°C before determination. Lipolysis data were expressed as micromoles of glycerol released *per* milliliter packed cell volume (PCV).

2.6 Preparation of cytosolic and fat cake fractions from primary rat adipocytes

Following the treatment, the fat cells were washed three times with PBS, pH 7.4, and then packed by centrifugation at $200 \times g$ for 3 min. Protein extraction was carried out according to a previously published method [28], with appropriate modifications. The packed cells were lysed in ice-cold 50 mM Tris · Cl buffer, pH 7.4, containing 255 mM sucrose, 1 mM EDTA, 1 mM PMSF. The cell lysate was incubated on ice for 15 min to solidify the floating fat cake and then centrifuged at $13\,000 \times g$ for 15 min at 4°C. The solidified fat cake of intracellular lipid droplets floated on top of the tube. The cytosolic fraction was aspirated from below the solidified fat cake and added to the sample buffer. The fat cake fraction was centrifuged again at $13\,000 \times g$ at 4°C for 15 min, and the residual cytosol was discarded. The fat layer was suspended in sample buffer containing 5% w/v SDS and the solution was vortexed thoroughly. After re-centrifugation, the fat cake protein extract was aspirated from below the floating fat layer. The samples were heated to 100°C for 5 min and cleaned at $12\,000 \times g$ for 10 min, prior to loading on SDS-PAGE.

2.7 Total protein extraction

The adipocytes were washed three times with PBS after treatment, and then added to protein lysis buffer and vortexed vigorously. Following lysis, the lysate was centrifuged at $13\,000 \times g$ for 15 min at 4°C. The protein extract below the layer of the fat cake was collected and stored at −80°C before use.

2.8 Western blotting analysis

Protein content was determined by a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). For immunoblotting of perilipin A, an equal concentration of fat cake extract was loaded and separated by electrophoresis on 8% SDS-PAGE. For analysis of HSL, an identical concentration of protein extracted from the cytosolic and fat cake fractions was separated by electrophorisis on 8% SDS-PAGE [31]. For immunoblotting of ATGL and PPAR-γ, equivalent concentrations of total protein extracts were separated by 8% SDS-PAGE; β-actin was used as the loading control. After electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes at 4°C and immunoblotted, as described previously [32]. Briefly, the membranes were blocked with 1% BSA in TBS-T buffer (150 mM NaCl, 20 mM Tris · Cl, pH 7.4, 0.05% Tween-20), and incubated overnight at 4°C with antibodies against HSL, ATGL, perilipin A, and PPAR-γ. The membranes were washed three times with TBS-T buffer for 10 min, incubated with rabbit IgG antibody for 1 h at 37°C, and then washed 3 times with TBS-T buffer. The blots were detected with alkaline phosphatase.

2.9 Analysis of PPAR-γ mRNA expression

PPAR-γ mRNA was determined by RT-PCR, as described previously [33]. Briefly, total RNA was isolated from adipocytes with TRIzol (GIBCO-BRL, Grand Island, NY), and 2 μg RNA was reverse transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Two microliters of each RT reaction was amplified in a 50 μL PCR system. The glyceraldehyde-3-phosphate dehydrogenase gene expression was used as a loading control.

2.10 Statistical analysis

Statistical analysis of data was performed using one-way ANOVA, followed by the *post hoc* multiple comparisons test. Data were expressed as mean ± SD, and p values less than 0.05 were considered statistically significant.

3 Results

3.1 Cell viability

Cytotoxicity was observed when the concentration of UA was at 400 μM (Fig. 1). Therefore, the non-cytotoxic doses, 25, 50 and 100 μM UA were chosen in subsequent studies.

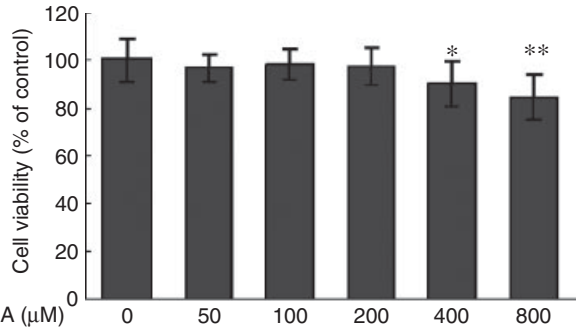

Figure 1. Effect of UA on the viability of primary rat adipocytes. Ten microliters of packed adipocytes were suspended in 200 μL f-DMEM with different concentrations of UA in the wells of a 96-well tissue culture microplate. After a 24 h incubation, MTT was added to the culture. After a 4 h incubation, the medium was aspirated from below the purple crystal formazan after 4 h incubation, 100 μL DMSO was added to each well, and the microplates were shaken until the purple crystal had been dissolved completely. The absorbance was detected as described in Section 2. Values are mean ± SD ($n = 4$). $^{*}p < 0.05$ and $^{**}p < 0.01$ were indicate statistical significance compared with controls.

3.2 UA increases glycerol release level in adipocytes

Primary rat adipocytes have been used widely as an *in vitro* model for the study of lipolytic regulation [28, 34]. Adipose tissue lipolysis is a catabolic process leading to the breakdown of TG stored in fat cells and the release of fatty acids and glycerol. The level of glycerol released in the culture medium of adipocytes is generally used to assess the lipolytic effect. In this study, the effect of different concentrations of UA on primary cultured adipocytes was examined by assaying for released glycerol. Treatment with UA increased lipolytic levels in a dose-dependent manner, after both 4 and 24 h of incubation (Fig. 2). UA at 50 μM and 100 M significantly stimulated lipolysis ($p<0.01$). The long-term lipolysis effect of UA (24 h) was weaker than that of the short-term (4 h) effect. The lipolytic effect of 100 μM UA at 4 h was 1.75-fold higher than that of the control group, but was lowered to a factor of 1.51 over the control group at 24 h. A similar result was also observed at 50 μM UA.

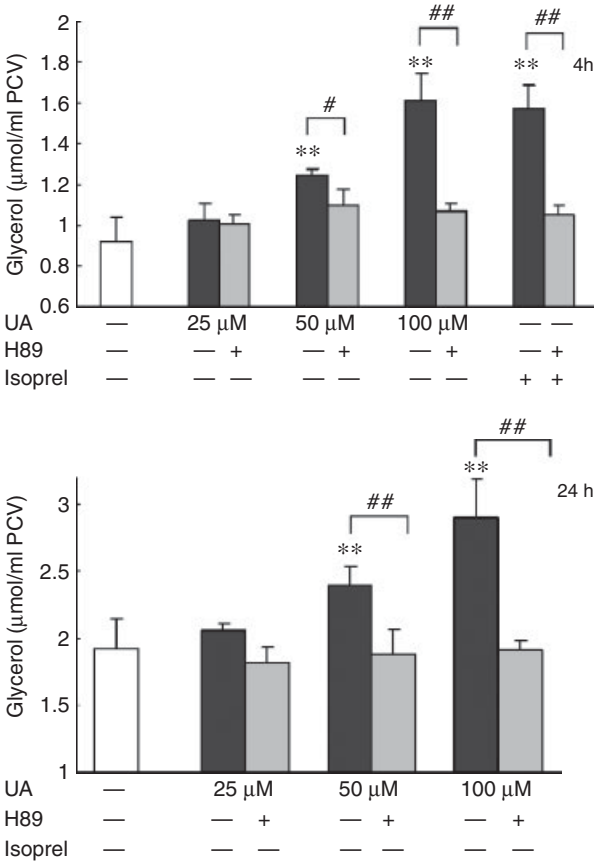

Figure 2. UA increased lipolysis in adipocytes. Adipocytes were treated with 25, 50, or 100 μM UA, with or without 10 μM H89, for 4 h (top panel) or 24 h (bottom panel). The cells were treated with H89 for 10 min before UA addition. Adipocytes treated with 10 μM isoprel for 30 min, with or without H89. After incubation, glycerol was detected as described in Section 2. The *Y*-axis represents glycerol release, the data are expressed as mean ± SD ($n=3$). $^{**}p<0.01$ indicates statistically significant differences between the UA-treated groups and the untreated groups; $^{\sharp}p<0.05$ and $^{\sharp\sharp}p<0.01$ reflect statistically significant differences between the UA-treated groups with or without H89.

3.3 UA promotes HSL translocation and down-regulates perilipin A

HSL is considered to be a key enzyme for TG breakdown in adipocytes. It acts *via* translocation from the cytosol to the lipid droplets [35, 36]. Perilipin A is a major structural protein that coats the surface of lipid droplets in adipocytes. A decrease in perilipin A can enhance adipocyte lipolysis [37, 38]. To determinate whether the localization of HSL and perilipin A in cells was altered after UA treatment, the expression of HSL in the cytosol and on the surface of the lipid droplets, and perilipin A were evaluated by Western blot analysis. UA facilitated significant HSL translocation from the cytosol to the lipid droplets in a dose-dependent manner at 4 h ($p<0.01$); the translocation of HSL was weaker at 24 h than that at 4 h (Fig. 3A). Perilipin A was down-regulated following incubation with UA for 4 and 24 h (Fig. 3C).

3.4 PKA participates in the lipolytic action of UA

H89, a PKA pathway-specific inhibitor, was applied to verify whether this pathway was involved in UA-induced lipolysis. Treatment with H89 blocked UA-induced lipolysis after 4 or 24 h incubation (Fig. 2A). H89 also blocked UA-induced translocation of HSL (Fig. 3B) and the decrease of perilipin A levels (Fig. 3D), suggesting that the PKA signal pathway is involved in the UA-induced lipolytic effect.

3.5 UA up-regulates ATGL expression

A novel adipocyte triglyceride lipase (ATGL) has been reported recently, providing a new target for weight reduction research [25]. To ascertain whether ATGL participated in UA-induced lipolysis, ATGL protein expression in adipocytes was studied. ATGL was significantly up-regulated by UA in a dose-dependent manner at 4 and 24 h (Fig. 4A). This increase was greater at 24 h (1.77-fold greater than the control at 100 μM UA) than that at 4 h (1.33-fold greater than the control at 100 μM UA). Subsequently, to probe whether the UA-induced increase in ATGL expression could be regulated by the PKA signaling pathway, the effect of the inhibitor H89 was examined. The results indicated that H89 did not block UA-induced ATGL up-regulation (Fig. 4B), suggesting that the increased expression of ATGL is not regulated by the PKA signal pathway.

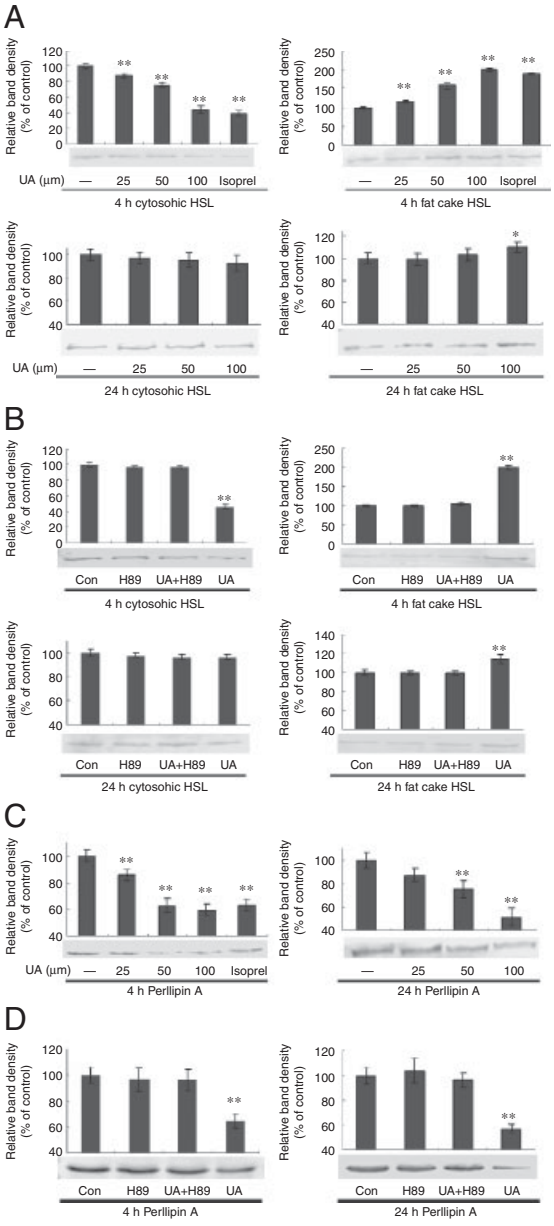

Figure 3. Effects of UA on HSL and perilipin A expression in primary-cultured rat adipocytes. A total of 100 μL of packed adipocytes were suspended in 1 mL f-DMEM with different reagents in 24-well tissue culture microplates. After incubation for 4 or 24 h different reagents (adipocytes with 10 μM isoprel were treated for 30 min), adipocytes were transferred to centrifuge tubes. Then the cells were washed three times with PBS, pH 7.4. After centrifugation for 3 min at 200 × *g*, the packed adipocytes were collected. (A) Cytosolic and fat cake fraction proteins were extracted for HSL translocation analysis as described in Section 2. (B) Immunoblotting of HSL with 100 μM UA and/or 10 μM H89 for analysis of its translocation. (C) Perilipin A in the fat cake fraction was detected. (D) Immunoblotting of perilipin A in the fat cake fraction with 100 μM UA and/or 10 μM H89. These figures are representative of three separate experiments. $^{*}p<0.05$ and $^{**}p<0.01$ were indicate statistical significance compared with controls (Con).

3.6 PPAR-γ is not involved in UA-induced lipolysis

To examine the function of PPAR-γ in adipocyte lipolysis, PPAR-γ mRNA and protein expression were measured following UA treatment. UA strongly down-regulated both the mRNA and protein levels of PPAR-γ at 4 and 24 h (Fig. 5A and B). However, activation of the PPAR-γ pathway with the PPAR-γ agonist, rosiglitazone, did not counteract UA-induced lipolysis (Fig. 5C). This observation suggests that PPAR-γ is not a prominent pathway involved in the lipolytic activity of UA. Interestingly, we also found that rosiglitazone decreased PPAR-γ mRNA and protein expression. This may be a feedback mechanism for balancing the transcriptional activity of PPAR-γ in response to activating ligands that trigger the degradation of the protein by proteasome 26S [39].

4 Discussion

Kim *et al* [40] have shown recently that UA enhances lipolysis in rat fat cells, but the molecular mechanisms of this effect are not clear. Our study is the first investigation of the potential molecular mechanism of lipolysis stimulated by UA in primary-cultured rat adipocytes. Our results indicate that UA effectively increased lipolytic levels in a dose-dependent manner, an observation that is supported by the results of Kim *et al*. [40]. It is well known that adipocyte lipolysis is catalyzed by adipose tissue lipases in sequential steps, leading to the formation of FFA and glycerol. HSL was commonly considered as the rate-limiting lipase [13–15], until a novel key enzyme for TG hydrolysis, ATGL, was reported recently [25]. It has been shown that ATGL and HSL are responsible for more than 95% of the TG hydrolase activity in adipose tissue [41]. We therefore examined the change of HSL and ATGL expression in the presence of UA. For maximal lipolysis, not only the activation of HSL, but also its translocation to the substrate, the lipid droplet, was required. Our study showed that cytosolic HSL was transferred significantly to the surface of the lipid droplet, suggesting that the enhanced lipolysis was partially through the translocation of HSL by UA. The translocation of HSL was weaker after the 24 h treatment, compared with the 4 h treatment with UA, most likely because the cAMP-dependent PKA pathway mainly regulates short-term lipolysis of HSL [42, 43].

Perilipin A, which functions as a ''barrier'' between stored neutral lipid and lipases in the basal state, is anchored to the surface layer of the lipid droplet in adipocytes. Londos *et al*. have found that perilipin A protected the TG from the hydrolysis action of soluble intracellular lipases [44]. This barrier hypothesis for perilipin A was also supported by Rydén *et al*. [37]. Their study showed that tumor necrosis factor-α enhances lipolysis through down-regulation of the expression of perilipin. In addition, over-expression of perilipin A blocked the ability of tumor necrosis factor-α to increase lipolysis in 3T3-L1 adipocytes

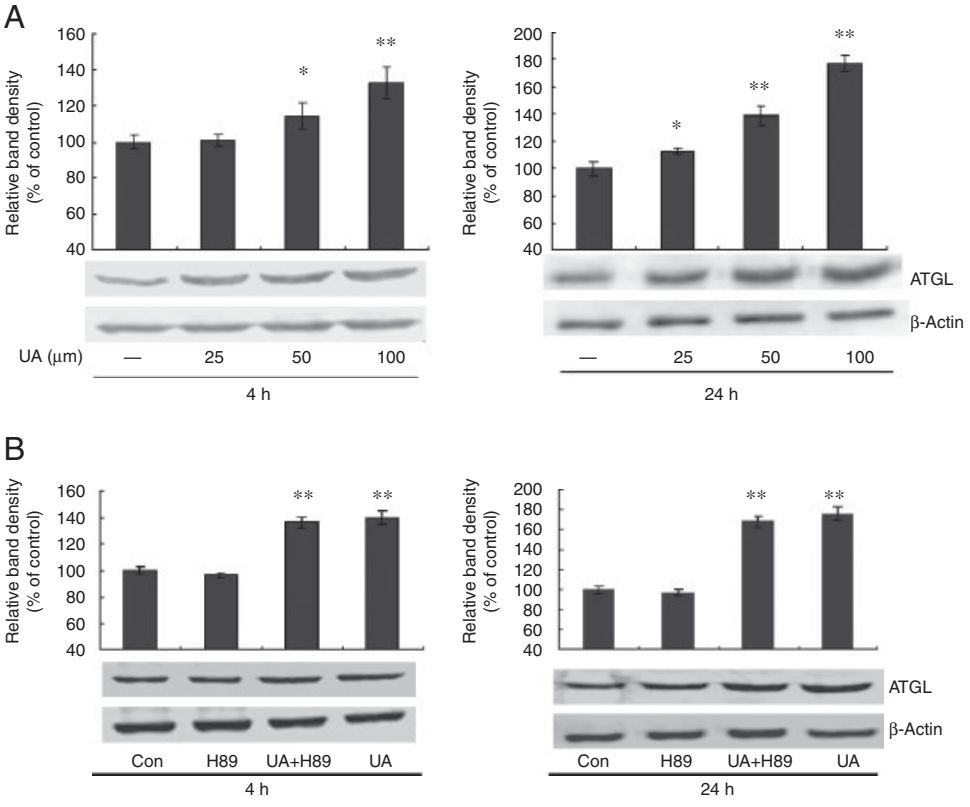

Figure 4. Effect of ATGL on UA-induced lipolysis in primary-cultured rat adipocytes. A total of 100 μL of packed adipocytes were suspended in 1 mL f-DMEM with different consentrations of UA in each well of 24-well tissue culture plates. After incubation for 4 or 24 h, adipocytes were transferred to centrifuge tubes, and the adipocytes were washed three times with PBS, pH 7.4. After centrifugation at $200 \times g$ for 3 min, the packed adipocytes were collected. (A) Total protein was extracted for immunoblotting to determine UA-induced ATGL protein expression. (B) Immunoblotting of ATGL with 100 μM UA and/or 10 μM H89. These figures are representative of three separate experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ indicate statistically significant differences when compared with controls (Con).

[45]. We found that UA down-regulated perilipin A protein expression at the surface of lipid droplets, compared with untreated adipocytes. These results strongly support the hypothesis that UA enabled the HSL to approach the droplet more readily to exert its lipolytic action, because the ''barrier'' functions of perilipin A became weaker.

In addition, the lipolytic effect of UA, the translocation of HSL, and the reduction of perilipin A were all blocked by H89, suggesting that the PKA pathway was responsible for UA-induced lipolysis.

ATGL is a novel, rate-limiting lipase in the lipolytic catabolism of stored fat in adipose tissue [25]. Over-expression of ATGL in 3T3-L1 adipocytes increased basal lipolysis [46]. UA up-regulated ATGL protein expression in a dose-dependent manner, indicating that ATGL is another target for UA-induced lipolysis. Miyoshi *et al.* reported that ATGL was regulated in PKA-stimulated lipolysis by controlling serine 517 of perilipin A [47]. Our experiments showed that the PKA pathway is not involved in the UA-induced increase in ATGL expression, since H89, a specific inhibitor of PKA, did not block this process.

Pinent *et al* [27] have shown that PPAR-γ is a target in lipolysis. In our study, however, PPAR-γ was found not to be responsible for the lipolytic effect observed in adipocytes subjected to UA, because when UA-treated adipocytes were incubated with rosiglitazone, a specific agonist of PPAR-γ, the lipolytic effect was not prevented, although PPAR-γ was down-regulated.

Effective enhancement of lipolysis in adipocytes might achieve the purpose of rapid weight reduction. Lipolysis, however, is usually accompanied by the release of large quantities of FFA into blood plasma. Excess FFA in blood plasma is one of the most crucial reasons for hyperlipemia, insulin resistance, type-2 diabetes, and hepatic injury. Therefore, the use of weight reduction drugs, by increasing lipolysis, is usually accompanied by side effects. However, scientific studies have proved that UA could lower blood cholesterol (44%), and β-lipoprotein levels (50%) in experimental animals [11, 12]. Jayaprakasam *et al.* have reported that UA decreases lipid accumulation in the liver of high-fat fed C57BL/6 mice [48]. This observation implied that UA decreased blood lipids and regulated lipid metabolism by affecting hepatic lipid oxidation and lipogenesis. They also found that UA improved insulin resistance and prevented type-2 diabetes *via* increasing insulin sensitivity, insulin secretion, and protecting the islet architecture. Therefore, UA was a favorable selection for weight reduction, with many other activities. Moreover, their experiment also showed that UA decreased body weight in high-fat fed C57BL/6 mice [48]. Our study proved that UA decreases the TG content in adipocytes. Thus, UA effectively reduced body TG content both *in vivo* and *in vitro*. In addition, taking anti-inflammatory [1, 2] and anti-oxidant [3, 4] activities into account, UA has the potential to treat the obesity problem.

In conclusion, our results revealed that UA stimulated lipolysis in primary-cultured adipocytes *via* activation of the

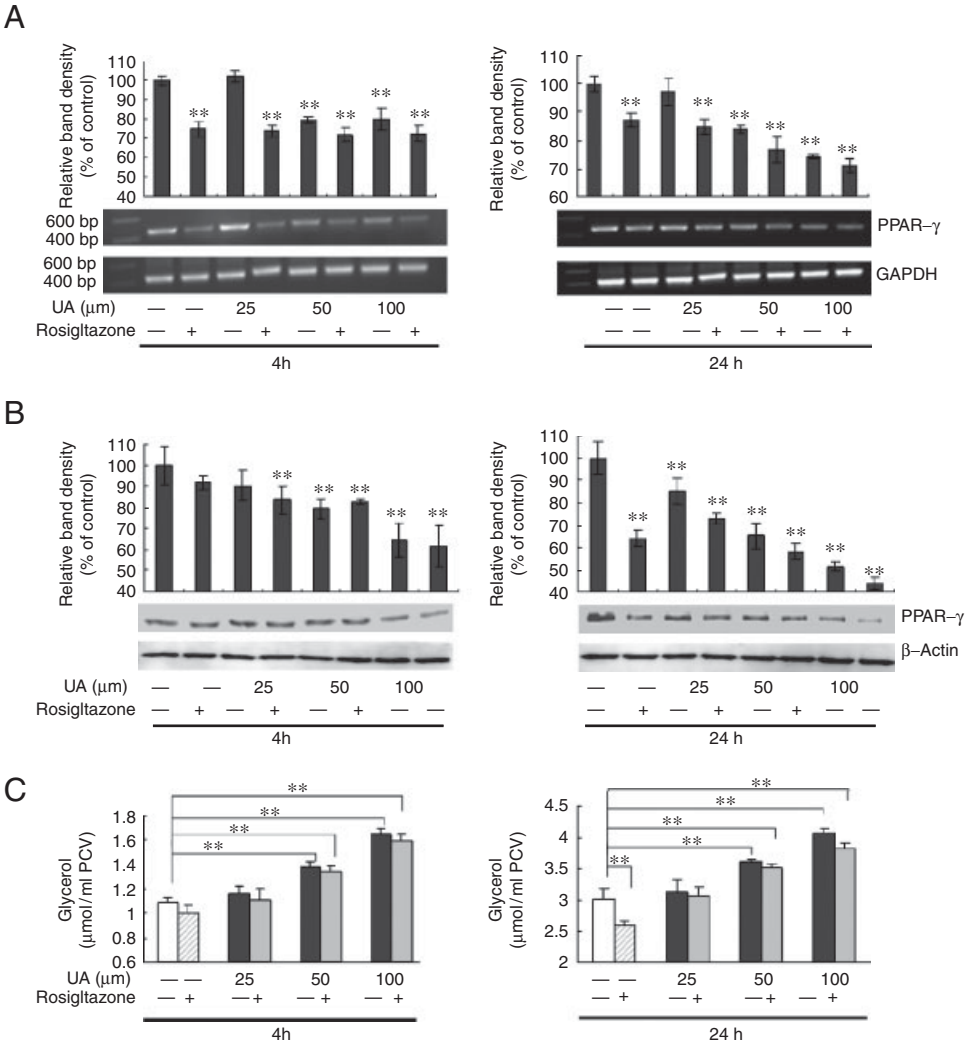

Figure 5. Effect of PPAR-γ on UA-induced lipolysis in primary-cultured adipocytes. Primary-cultured adipocytes were treated with UA in the presence or absence of 10 μM rosiglitazone. After 4 or 24 h incubation, adipocytes were transferred to centrifuge tubes, and washed three times with PBS, pH 7.4. Following centrifugation at 200 × *g* for 3 min, the packed adipocytes were collected. Total RNA and protein was extracted for PPAR-γ analysis as described in Section 2. (A) and (B) show PPAR-γ gene expression and protein expression, respectively. These data are representative of data from three separate experiments. (C) represents glycerol release. *Y*-axis data are expressed as mean ± SD ($n = 3$). $^{**}p < 0.01$ indicates statistically significant differences compared with controls.

PKA pathway and up-regulation of ATGL. The effects of UA implicate its potential therapeutic use for obesity.

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The authors have declared no conflict of interest.

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