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Resveratrol regulates lipolysis via adipose triglyceride lipase

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Abstract

Resveratrol has been reported to increase adrenaline-induced lipolysis in 3T3-L1 adipocytes. The general aim of the present work was to gain more insight concerning the effects of *trans*-resveratrol on lipid mobilization. The specific purpose was to assess the involvement of the two main lipases: adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), in the activation of lipolysis induced by this molecule. For lipolysis experiments, 3T3-L1 and human SGBS adipocytes as well as adipose tissue from wild-type, ATGL knockout and HSL knockout mice were used. Moreover, gene and protein expressions of these lipases were analyzed. Resveratrol-induced free fatty acids release but not glycerol release in 3T3-L1 under basal and isoproterenol-stimulating conditions and under isoproterenol-stimulating conditions in SGBS adipocytes. When HSL was blocked by compound 76-0079, free fatty acid release was still induced by resveratrol. By contrast, in the presence of the compound C, an inhibitor of adenosine monophosphate-activated protein kinase, resveratrol effect was totally blunted. Resveratrol increased ATGL gene and protein expressions, an effect that was not observed for HSL. Resveratrol increased fatty acids release in epididymal adipose tissue from wild-type and HSL knockout mice but not in that adipose tissue from ATGL knockout mice. Taking as a whole, the present results provide novel evidence that resveratrol regulates lipolytic activity in human and murine adipocytes, as well as in white adipose tissue from mice, acting mainly on ATGL at transcriptional and posttranscriptional levels. Enzyme activation seems to be induced via adenosine monophosphate-activated protein kinase.

Keywords: Resveratrol; Adipose triglyceride lipase; Hormone-sensitive lipase; 3T3-L1

1. Introduction

Obesity, a disease characterized by an excessive accumulation of fat in white adipose tissue, is reaching epidemic proportions worldwide and is associated with an increased risk of premature death. The prevalence of obesity in Western societies has increased dramatically in recent years. As a result, scientific research is constantly looking for new molecules that could be used as functional ingredients to prevent or to treat overweight and obesity. In recent years, a great deal of interest has focussed on resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring diphenolic compound, synthesized in a wide variety of plant species in response to stress and fungal infection [1,2] and mostly present in grapes and drinks derived from them, such as, grape juice or red wine.

Apart from its well-known positive effects on cardiovascular health, resveratrol has been shown to reduce body weight [3,4] and body fat accumulation [5,6]. However, very little is known concerning the mechanisms of action underlying these effects. Picard et al. [7] found that resveratrol decreased TG accumulation in 3T3-L1 adipocytes by increasing adrenaline-induced lipolysis. In line with this study, Szkudelska et al. [8] observed that resveratrol increased epinephrine-induced lipolysis in rat adipocytes.

Lipolysis is one of the most important metabolic pathways regulating adipose tissue weight and obesity. The two major enzymes that catabolize triglycerides (TGs) are hormone-sensitive lipase (HSL) and adipose TG lipase (ATGL) [9–11]. HSL is the most active enzyme against diglycerides (DG), which are hydrolyzed 10 times faster than TG. On the other hand, ATGL selectively performs the first step in TG hydrolysis resulting in the formation of DG and free fatty acids (FFAs). Its activity against TG is 10 times more specific than that against DG. Differences between these two lipases are also found in their localization. Whereas HSL is present in the cytosol, ATGL is localized in the cytosol and also on lipid droplets.

With regard to the molecular mechanisms regulating lipase activities, considerable differences exist between ATGL and HSL. Both enzymes are stimulated by the binding of catecholamines to their respective β-adrenergic receptors via phosphorylation events. Phosphorylation of HSL by protein kinase A (PKA) leads to its translocation from the cytosol to the lipid droplet surface. PKA also phosphorylates perilipin on the lipid droplet [12–14], resulting in enhanced stimulated lipolysis [15]. ATGL activity is highly stimulated

Abbreviations: RSV, resveratrol; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; AMPK, AMP-activated protein kinase; ISO, isoproterenol; CC, compound C; SGBS cells, Simpson-Golabi-Behmel Syndrome cells; FFA, free fatty acids.

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by an activator protein termed CGI-58. The localization of CGI-58 does, however, depend on phosphorylation of perilipin. In its basal state, CGI-58 is mostly bound to perilipin, leading to incomplete or absent activation of ATGL activity. In its activated state, perilipin is phosphorylated and releases CGI-58, which is then available for ATGL activation. Apart from this classical regulatory pathway depending on PKA, there are some other alternative signaling pathways that may activate lipolysis, such as, the 5-adenosine monophosphate (AMP) activated protein kinase (5′-AMPK) pathway. Experiments with adipocytes have suggested that AMPK inhibits HSL activation through phosphorylation at Ser-565 [16–22]. Moreover, it has been shown that after the activation of AMPK by 5′-aminoimidasole-4-carboxamide-1-β-D-ribofuranoside, ATGL protein content and TG hydrolase activity are significantly enhanced [23,24]. Finally, it has been proposed that resveratrol can activate AMPK [25,26].

Taking all this into account, the general aim of the present work was to gain more insight into the effects of *trans*-resveratrol on lipid mobilization. The specific purpose was to assess the involvement of the two main lipases, ATGL and HSL, in the activation of lipolysis induced by this molecule, by assessing its effects on the expression and activity of these enzymes.

2. Methods and materials

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (BRL Life Technologies, Grand Island, NY, USA). Compound C (CC) was obtained from Sigma (P5499; Sigma, St. Louis, MO, USA) and the specific inhibitor of HSL (HSLi) 76-0079 (NNC 0076-0000-0079) was from Novo Nordisk (Denmark). *Trans*-resveratrol was purchased from Cayman Chemicals (Ann Arbor, Michigan, USA).

Commercial kits for analyzing FFAs and free glycerol were supplied by WAKO and Sigma, respectively [NEFA-HR [Wako Chemicals USA Inc., Richmond, VA, USA] and F6428 [Sigma]). TGs were determined by Infinity TGs reagent (Thermo Scientific, Rockford, IL, USA). Protein concentrations of cell extracts were measured with the Bradford protein assay (Bio-Rad, Hercules, CA, USA) and bicinchoninic acid (BCA) reagent (Thermo Scientific, Rockford, IL, USA), for Western blot and cultured cell protein determinations.

2.2. Experimental design

The first part of this study was carried out in cultured adipocytes. For this purpose, a murine cell line of adipocytes (3T3-L1) and human adipocytes (Simpson-Golabi-Behmel Syndrome [SGBS]) were used. The cells were cultured in a manner that is described later. 3T3-L1 mouse embryo fibroblasts were obtained from American Type Culture Collection (Manassas, VA, USA). SGBS human adipocytes were provided by Novo Department of Pediatrics and Adolescent Medicine, University of Ulm.

The second part of this study was performed in male 3-month-old wild-type (WT), ATGL knockout (ATGL KO) and HSL knockout (HSL KO) mice. Wild-type mice are littermates (C57BL/6 background). The knockout mice were self-bred and generated by targeted homologous recombination as previously described [27,28]. They were fully backcrossed (at least five times) to the C57BL/6 background. Mice were bred and housed under controlled temperature (22°C) and lighting (12:12 h light–dark cycle) and had free access to standard mouse chow diet (Ssniff Spezialitäten GmbH, Soest, Germany) and to tap water. They were killed by cervical dislocation, and epididymal adipose tissue was removed. The protocols of experiments were reviewed and approved by the local ethical commission for investigation of animals.

2.3. Cell culture

3T3-L1 preadipocytes were cultured in DMEM containing 10% fetal calf serum (FCS). Two days after confluence (day 0), the cells were stimulated to differentiate with DMEM containing 10% FCS, 10 μg/ml insulin, 0.5 mM isobutylmethylxanthine (IBMX) and 1 μM dexamethasone for 2 days. On day 2, the differentiation medium was replaced by 10% fetal bovine serum/DMEM medium containing 0.2 μg/ml insulin and incubated for 2 days, followed by culturing with 10% FCS/DMEM medium for an additional period of 10 days (day 12). 3T3-L1 mouse embryo fibroblasts were obtained from American Type Culture Collection.

SGBS adipocytes were cultured in DMEM/Ham's F12 containing 10% FCS. Two days after confluence (day 0), the cells were stimulated to differentiate with DMEM/F12 containing 20 nM human insulin, 0.01 mg/ml transferrin, 0.1 μM cortisol, 200 pM triiodothyronine (T_3), 500 μM IBMX, 2 μM rosiglitazone (BRL) and 0.25 μM dexamethasone for 4 days. On day 4, differentiation medium was replaced by DMEM/F12 medium containing 20 nM human insulin, 0.01 mg/ml transferrin, 0.1 μM cortisol, 200 pM T_3, 500 μM IBMX, 2 μM BRL and 0.25 μM dexamethasone and incubated for 3 days (until day 7). On day 7, the medium was replaced by DMEM/F12 medium containing 20 nM human insulin, 0.01 mg/ml transferrin, 0.1 μM cortisol, 200 pM T_3 and 2 μM BRL for an additional period of 4 days (day 11).

At day 12 (3T3-L1) and day 11 (SGBS), >90% of cells developed mature adipocytes with visible lipid droplets. All media contained 1% penicillin streptomycin (10,000 U/ml), and the media for differentiation and maturation contained 1% (vol/vol) of biotin and pantothenic acid. Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

2.4. Measurement of triacylglycerol content in adipocytes

For TG extraction, mature cells were washed extensively with phosphate-buffered saline (PBS) and incubated three times with 500 μl of hexane/isopropanol (2:1). The total volume was then evaporated by vacuumed centrifugation, and the pellet was resuspended in 200 μl Triton X-100 in 1% distilled water. Afterward, TG were disrupted by a sonicator, and the content was measured by a commercial kit. For protein determinations, cells were lysed in 0.3N NaOH, 0.1% sodium dodecyl sulfate. Protein measurements were performed using the BCA reagent. This experiment was repeated three times.

2.5. In vitro lipolysis in 3T3-L1 and SGBS cells

To study the effect of different concentrations of resveratrol on basal and stimulated lipolysis, fully differentiated cells were incubated in DMEM containing 2% fatty acid–FBS albumin (Sigma) with 100 μM of resveratrol (dissolved in 95% ethanol), either in the presence or absence of 10 μM isoproterenol. Additionally, in order to inhibit AMP kinase and HSL activities, 3T3-L1 cells (in this case at day 8 after differentiation) were incubated in the presence of 5 μM of the CC and SGBS cells in the presence of 25 μM of 76-0079 (HSLi), respectively. When HSLi was tested, a dose of 200 μM of resveratrol was also used. After incubation periods of 12, 24 and 48 h in 3T3-L1 or 12 and 24 h in SGBS, aliquots of the medium were removed and analyzed for FFA and glycerol. Experiments performed in 3T3-L1 adipocytes were repeated three times, and experiments in SGBS cells, two times.

2.6. In vitro lipolysis in isolated epididymal white adipose tissue

Epididymal fat pads from 3-month-old WT, ATGL KO and HSL KO mice were surgically removed and washed several times with PBS. Tissue pieces (≈20 mg) were incubated in DMEM containing 2% fatty acid-free bovine serum albumin for 8 h, under basal conditions in the presence or in the absence of 100 μM of resveratrol (diluted in 95% ethanol) at 37°C. Thereafter, aliquots of the medium were removed and analyzed for FFA and glycerol content. For protein determinations, fat pads were washed extensively with PBS and lysed in 0.3N NaOH, 0.1% sodium dodecyl sulfate. Protein measurements were performed using the BCA reagent. This experiment was repeated three times.

2.7. Extraction and analysis of RNA and quantification by reverse transcription polymerase chain reaction

Mature adipocytes grown in 6-well plates (3T3-L1) and 12-well plates (SGBS) were incubated with either 0.1% ethanol (95%) or resveratrol (100 μM, diluted in 95% ethanol) on day 11 (SGBS) or 12 (3T3-L1) of differentiation. After 24 h of treatment, RNA samples were extracted using TRIzol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The integrity of the RNA extracted from all samples was verified and quantified using an RNA 6000 Nano Assay (Thermo Scientific, Wilmington, DE, USA). RNA samples were then treated with DNase I kit (Applied Biosystems Inc., Foster City, CA USA) to remove any contamination with genomic DNA.

One microgram of total RNA in a total reaction volume of 20 μl was reverse transcribed using the complement DNA Archive Kit (Applied Biosystems Inc.) according to the manufacturer's protocols. Reactions were incubated initially at 25°C for 10 min and subsequently at 37°C for 120 min and 85°C for 5 min.

Relative HSL and ATGL messenger RNA (mRNA) levels were quantified using real-time polymerase chain reaction (PCR) with an iCycler–MyiQ Real-Time PCR Detection System (Bio-Rad). β-Actin mRNA levels were similarly measured and served as the reference gene. The PCR reagent mixture consists of 1 μl of each complement DNA (10 pmol/μl), SYBR Green Master Mix (Applied Biosystems) and the upstream and downstream primers (900 nM each). Specific primers were synthesized commercially (for 3T3-L1: Tib Molbiol Berlin, Germany, and for SGBS: Eurofins MWG Operon, Ebersberg, Germany), and the sequences were as follows:

Murine HSL: 5′-GGT GAC ACT CGC AGA AGA CAA TA-3′ (forward); 5′-GCC GCC GTG CTG TCT CT-3′ (reverse)
Murine ATGL: 5′-CAC TTT AGC TCC AAG GAT GA-3′ (forward); 5′-TGG TTC AGT AGG CCA TTC CT-3′ (reverse)
Murine β-actin: 5′-ACG AGG CCC AGA GCA AGA G-3′ (forward); 5′-GGT GTG GTG CCA GAT CTT CTC-3′ (reverse)
Human HSL: 5′- TAC CGC AGC CTA GTG CAC AC -3′ (forward); 5′- AGA TGG TCT GCA GGA ATG GC -3′ (reverse)

Human ATGL: 5′-CGT GGA TCC CCG CCC TTG CTG GCG TTG C-3′ (forward); 5′-CCC TCG AGT CTC AGC AGG TCC GTG GG-3′ (reverse)
Human β-actin: 5′- AGC CAT GTA CGT AGC CAT CCA -3′ (forward); 5′- TCT CCG GAG TCC ATC ACA ATG -3′ (reverse)

PCR parameters were as follows: initial 2 min at 50°C, denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for HSL and β-actin and 62°C for ATGL for 15 s and extension at 60°C for 30 s. All sample mRNA levels were normalized to the values of β-actin, and the results expressed as fold changes of threshold cycle (Ct) value relative to controls using the $2^{-\Delta\Delta Ct}$ method [29]. This experiment was repeated two times.

2.8. Western blot analysis

Cells were washed with PBS and lysed in buffer composed of 0.25 M sucrose, 1 mM dithiothreitol, 1 mM EDTA and protease inhibitor (1 μM). Cells were then disrupted by a sonicator, and cell lysates were centrifuged and the infranatant was collected. An aliquot was used to determine protein concentration using the Bradford method. Thirty-microgram of protein was separated on 10%-polyacrylamide gels and then electroblotted to polyvinyl difluoride (PVFD; Roth) membranes (Amersham Pharmacia Biotech, NJ, USA). Blots were blocked with 5% dry milk in Tris–saline–Tween-20 (TST) for 1 h at 4°C for LC3 and overnight at 4°C for HSL and ATGL. Then blots were incubated with antibodies for HSL (1:2000) and ATGL (1:1000) at room temperature for 1 h. Antibodies for HSL were self-made [27], and those for ATGL were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Blots were washed in TST and incubated with antirabbit peroxidase-conjugated secondary antibody (1:10000) for 1 h at room temperature. Next, blots were washed in TST and developed by chemiluminescent detection with use of a standard kit (ECL; Amersham Pharmacia Biotech). ATGL and HSL expression was quantified by densitometry with a scanner equipped with a transilluminator and BIO IMAGE analysis software (Millipore, Leiden, the Netherlands). This experiment was repeated two times.

2.9. Statistical analysis

Results are presented as mean±standard error of the mean. Statistical analysis was performed using SPSS 16.0 (SPSS Inc. Chicago, IL, USA). Statistical analysis was determined by Student's unpaired *t* test (two-tailed). Statistical significance was set at the $P<.05$, .01 and .001 levels.

3. Results

3.1. Effects of resveratrol on lipolysis in 3T3-L1 and SGBS adipocytes

TG content was reduced in 3T3-L1 mature adipocytes by resveratrol (100 μM) after the three experimental periods (12, 24 and 48 h) (Fig. 1). Resveratrol treatment led to a significantly higher release of FFA after the three treatment periods analyzed (Fig. 2A). However, glycerol release in this experiment was not altered by resveratrol (data not shown). After 24 h, resveratrol also induced higher FFA release in this cell line under isoproterenol stimulation (Fig. 2B).

Resveratrol treatment had no effect on FFA release in basal conditions in SGBS cells after both 12 and 24 h of treatment (Fig. 3). However, it induced higher FFA release after 12 and 24 h of treatment when lipolysis was stimulated with isoproterenol (Fig. 3).

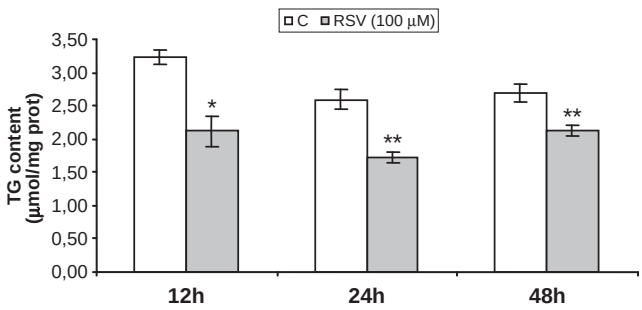

Fig. 1. TG accumulation in 3T3-L1 adipocytes after 12, 24 and 48 h of 100-μM RSV treatment. Results are expressed as means±S.E.M., shown by vertical bars (*$P<.05$; **$P<.01$). The experiment was repeated three times. C, control; RSV, resveratrol.

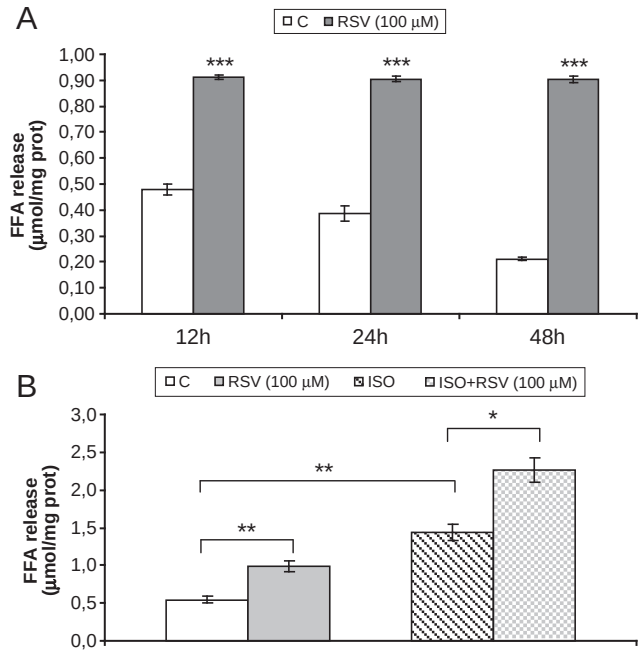

Fig. 2. FFA release in 3T3-L1 adipocytes (A) under basal conditions after 12, 24 and 48 h of 100-μM RSV treatment and (B) under basal and ISO stimulation conditions after 24 h of RSV treatment. Results are expressed as means±S.E.M., shown by vertical bars (***$P<.01$). The experiment was repeated three times. C, control; RSV, resveratrol; ISO, isoproterenol.

In order to better understand the influence of resveratrol on HSL, additional experiments were carried out. Thus, the effects of this molecule were assessed in SGBS cells in which HSL was inhibited by HSLi. In the cells treated with this compound for 24 h, a significantly higher FFA release ($P<.05$) was observed with 200 μM of resveratrol under isoproterenol-stimulated conditions (Fig. 4).

In another set of experiments, the activity of AMPK was inhibited by the CC in 3T3-L1 adipocytes. In the presence of this compound, the increase produced in FFA release by resveratrol in isoproterenol-stimulated cells was blocked (Fig. 5).

3.2. Effects of resveratrol on ATGL and HSL expressions in 3T3-L1 and human SGBS adipocytes

After 24 h of treatment with 100 μM of resveratrol, ATGL expression was enhanced in both cell lines, 3T3-L1 and SGBS adipocytes. By contrast, HSL expression was not altered in these

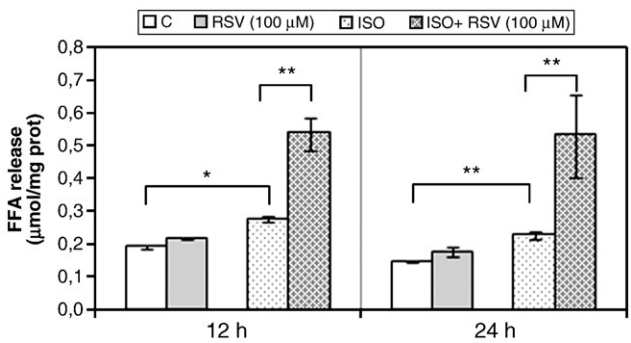

Fig. 3. FFA release in SGBS adipocytes under basal and ISO stimulated conditions after 12 and 24 h of 100-μM RSV treatment. Results are expressed as means±S.E.M., shown by vertical bars (*$P<.05$; **$P<.01$). The experiment was repeated two times. C, control; RSV, resveratrol; ISO, isoproterenol.

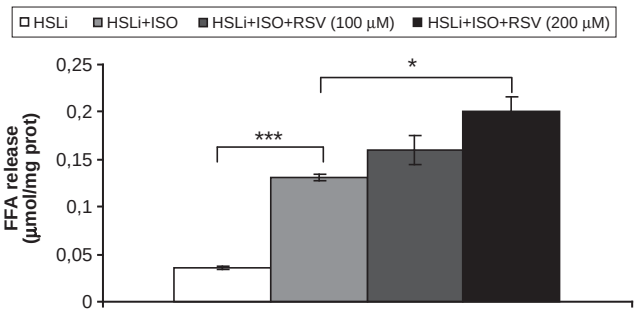

Fig. 4. FFA release in SGBS adipocytes in the presence of HSL inhibitor after 24 h of treatment with 100 and 200 μM of RSV. Results are expressed as means±S.E.M., shown by vertical bars (*P<.05). The experiment was repeated two times. C, control; RSV, resveratrol; ISO, isoproterenol; HSLi, HSL inhibitor.

adipocytes (Fig. 6A, B). According to these results, when protein expression was measured by Western blot in 3T3-L1 adipocytes, ATGL protein content was significantly higher (12-fold) in the resveratrol-treated cells than in the control 3T3-L1 cells, but HSL protein content remained unchanged (Fig. 7A, B).

3.3. Effects of resveratrol on lipolysis in ATGL and HSL KO mice

When adipose tissue from WT and HSL KO mice was incubated for 8 h with resveratrol, FFA release was significantly increased. Contrastingly, no changes were observed in tissues of ATGL KO mice (Fig. 8).

4. Discussion

As explained in the "Introduction" section, it has been proposed that resveratrol can reduce fat accumulation, at least in part by increasing lipid mobilization in adipocytes [7,8]. As far as we know, the involvement of the two main lipases, ATGL and HSL, in this effect has not been described.

The results obtained in the present study show that resveratrol regulates TG storage and lipolysis in murine 3T3-L1 adipocytes. These cells showed increased fatty acid release after the addition of this polyphenol, and the effect was maintained over different time periods (12, 24 and 48 h of treatment). It is important to emphasize that increased release of FFA was also induced by resveratrol in SGBS adipocytes. This issue is of great importance because these cells are human adipocytes, and thus, the obtained results are closer to human beings than those obtained in 3T3-L1 adipocytes.

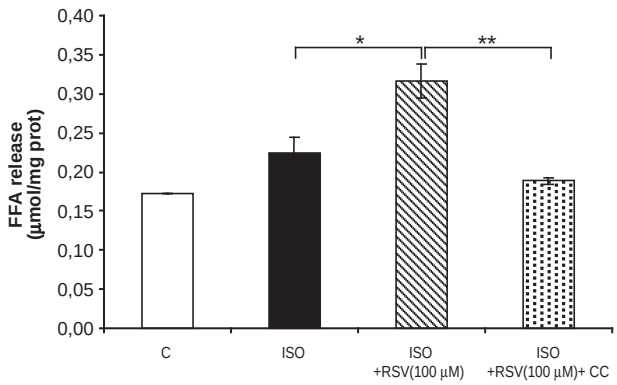

Fig. 5. FFA release in 3T3-L1 adipocytes in the presence of AMPK inhibitor after 24 h of treatment with 100 μM of RSV. Results are expressed as means±S.E.M., shown by vertical bars (*P<.05; **P<.01). The experiment was repeated three times. C, control; RSV, resveratrol; ISO, isoproterenol.

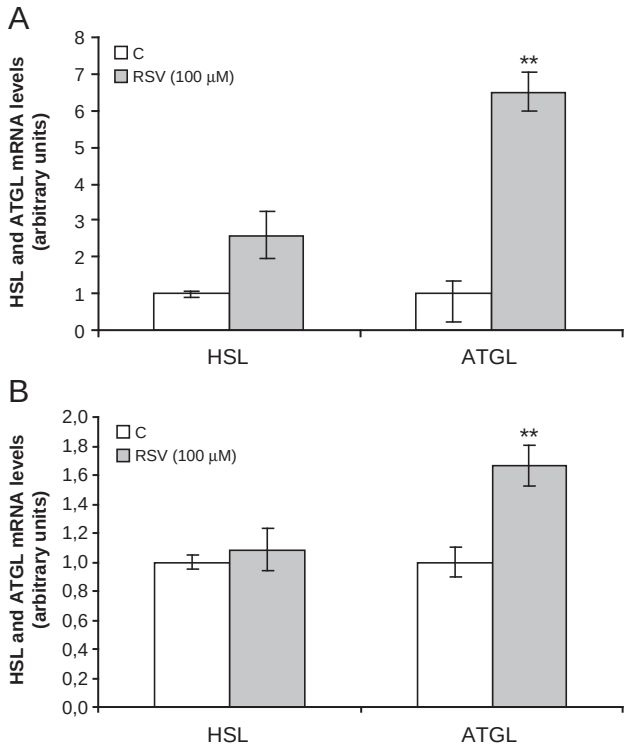

Fig. 6. ATGL and HSL mRNA levels measured in 3T3-L1 (A) and SGBS (B) adipocytes after 24 h of 100-μM RSV treatment. Values are expressed as relative to the control and results are given as means±S.E.M., shown by vertical bars (**P<.01). The experiment was repeated two times. C, control; RSV, resveratrol.

The reduction of the TG content observed in 3T3-L1 adipocytes after the three incubation periods is in good accordance with the above-described effects on lipolysis. These findings are in line with those reported by Picard et al. [7] in 3T3-L1 adipocytes.

Interestingly, in the present study, FFA release was increased by resveratrol, but glycerol remained unchanged in both cell lines. Considering both that ATGL acts mainly on TGs, thus releasing FFA, and that the lipolytic action of HSL, which is coordinated to that of MGL, results in FFA and glycerol release, the present results suggest that resveratrol acts preferentially on ATGL. These results are not in accordance with the study published by Szkudelska et al. [8], where resveratrol increased the glycerol release in adipocytes isolated from rat adipose tissue stimulated by adrenaline. An important difference between the experiment reported by Szkudelska et al. and the present study is that the amounts of cAMP in the incubation medium would presumably reach higher levels in our experiment. Szkudelska et al. stimulated lipolysis by using 1 μM epinephrine, a molecule that acts not only on β-adrenoceptors, thus leading to increased cAMP, but also on α_2-adrenoceptors, which are coupled to the Gi protein that reduces cAMP production. However, in the present study, lipolysis was stimulated by using isoproterenol, a molecule that only acts on β-adrenoceptors, at a dose 10-fold higher (10 μM). As a result, it could be thought that, in Szkudelska et al.'s study, a smaller amount of cAMP leads to a lower activation of HSL. Thus, the increased production of DG due to ATGL activation could result in increased degradation of this lipid species by HSL, the following lipase acting downstream in the lipolytic pathway. Nevertheless, with the lipolytic cascade fully activated by a high concentration of isoproterenol in our experimental conditions, the increased amount of DG is not likely to be additionally degraded by HSL, thus resulting in no increased glycerol production.

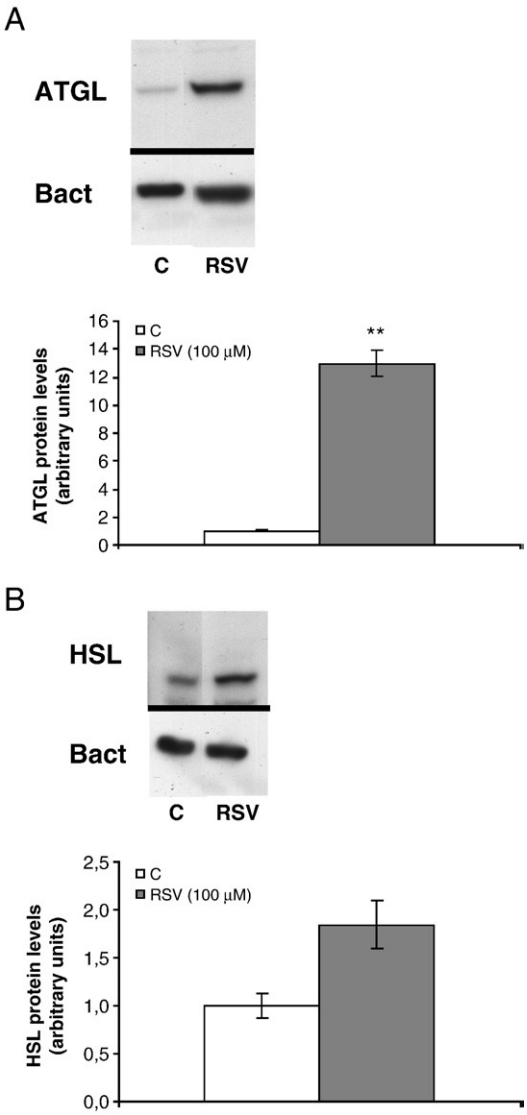

Fig. 7. ATGL (A) and HSL (B) Western blots showing protein content in 3T3-L1 after 24 h of 100-μM RSV treatment (**P<.01). The experiment was repeated two times. Bact, β-actin; C, control; RSV, resveratrol.

In order to gain more support concerning the hypothesis, that resveratrol acts via ATGL, additional experiments were performed. Thus, the effects of resveratrol on FFA release were tested in adipocytes after blocking HSL activity with the compound 76-0079.

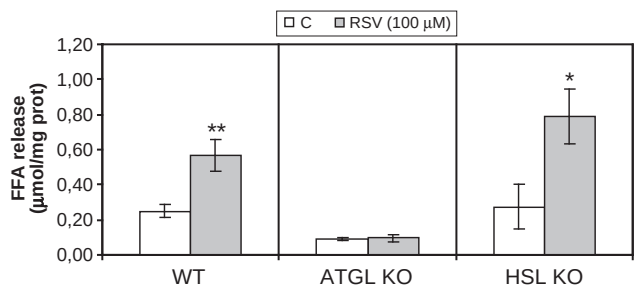

Fig. 8. FFA release in epididymal adipose tissue from WT, ATGL KO and HSL KO mice after 8 h of 100-μM RSV treatment. Results are expressed as means±S.E.M., shown by vertical bars (**P<.01). The experiment was repeated three times. C, control; RSV, resveratrol.

The increase in FFA release observed in SGBS adipocytes under these experimental conditions demonstrates that resveratrol was able to induce TG breakdown, despite the lack of HSL activity. These results suggest again that the enzyme responsible for the release of FFA induced by resveratrol was ATGL.

Different lipolytic regulatory pathways have been described in the literature. Although the involvement of AMPK is so far a controversial issue, it has been proposed that this enzyme activates ATGL [23]. With regard to AMPK, some authors have recently shown that this enzyme is a target for resveratrol [30,31]. Thus, it has been observed that AMPK-deficient mice were resistant to the effects of this polyphenol. Resveratrol increased the metabolic rate, insulin sensitivity, and glucose tolerance and reduced fat mass in WT mice but not in AMPK-deficient mice.

Taking all that into account, the effects of resveratrol in 3T3-L1 adipocytes incubated with an inhibitor of AMPK were assessed in the present study. This AMPK inhibitor diminished the increase in FFA release produced by resveratrol when isoproterenol was included in the incubation medium as a lipolytic stimulator. These results show that FFA release induced by resveratrol is mediated by AMPK. Moreover, considering that, as previously explained, AMPK activates ATGL but not HSL, these data reinforce the hypothesis that ATGL is the main target for the lipolytic effect of resveratrol.

The experiments performed in KO mice provide data in the same line. The lack of increase in FFA release observed in mice not showing ATGL activity demonstrates that this lipase is a key enzyme for resveratrol effects. By contrast, when resveratrol was added to adipose tissue from HSL KO mice, FFA release was induced as efficiently as in WT mice, suggesting that HSL does not play a crucial role in resveratrol effect.

Although important regulatory mechanisms of lipases take place at the level of phosphorylation, other regulatory mechanisms also take place at transcriptional and/or translational levels. In order to check whether resveratrol acts at these levels, gene and protein expressions of both lipases were assessed in the present study. As observed in the lipolytic experiments, ATGL seems to be the main target for resveratrol, because while its mRNA and protein amounts were increased, no changes were observed in HSL.

Taking as a whole, the present results provide novel evidence that resveratrol regulates lipolytic activity in human and murine adipocytes, as well as in white adipose tissue from mice, acting mainly on ATGL at transcriptional and posttranscriptional levels. AMPK seems to be involved in enzyme activation. Although further studies in this field are needed, the present results suggest that resveratrol may perhaps represent a potential new molecule in the obesity treatment.

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References

[1] Langcake P, Pryce R. A new class of phytoalexins from grapevines. Experientia 1977;33:115–52.
[2] Signorelli P, Ghidoni R. Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. J Nutr Biochem 2005;16:449–66.
[3] Baur J, Pearson K, Price N, Jamieson H, Lerin C, Kalra A, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006;444:337–42.
[4] Ahn J, Cho I, Kim S, Kwon D, Ha T. Dietary resveratrol alters lipid metabolism-related gene expression of mice on an atherogenic diet. J Hepatol 2008;49: 1019–28.

[5] Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109–22.
[6] Rivera L, Morón R, Zarzuelo A, Galisteo M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. Biochem Pharmacol 2009;77:1053–63.
[7] Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 2004;429:771–6.
[8] Szkudelska K, Nogowski L, Szkudelski T. Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes. J Steroid Biochem Mol Biol 2009;113: 17–24.
[9] Jenkins C, Mancuso D, Yan W, Sims H, Gibson B, Gross R. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 2004;279:48968–75.
[10] Villena J, Roy S, Sarkadi-Nagy E, Kim K, Sul H. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J Biol Chem 2004;279:47066–75.
[11] Zimmermann R, Strauss J, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 2004;306:1383–6.
[12] Anthonsen M, Rönnstrand L, Wernstedt C, Degerman E, Holm C. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. J Biol Chem 1998;273:215–21.
[13] Su C, Sztalryd C, Contreras J, Holm C, Kimmel A, Londos C. Mutational analysis of the hormone-sensitive lipase translocation reaction in adipocytes. J Biol Chem 2003;278:43615–9.
[14] Holm C. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Biochem Soc Trans 2003;31:1120–4.
[15] Maeda N, Funahashi T, Hibuse T, Nagasawa A, Kishida K, Kuriyama H, et al. Adaptation to fasting by glycerol transport through aquaporin 7 in adipose tissue. Proc Natl Acad Sci U S A 2004;101:17801–6.
[16] Corton J, Gillespie J, Hawley S, Hardie D. 5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells. Eur J Biochem 1995;229:558–65.
[17] Garton A, Campbell D, Carling D, Hardie D, Colbran R, Yeaman S. Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. Eur J Biochem 1989;179: 249–54.
[18] Garton A, Yeaman S. Identification and role of the basal phosphorylation site on hormone-sensitive lipase. Eur J Biochem 1990;191:245–50.
[19] Roepstorff C, Vistisen B, Donsmark M, Nielsen J, Galbo H, Green K, et al. Regulation of hormone-sensitive lipase activity and Ser563 and Ser565 phosphorylation in human skeletal muscle during exercise. J Physiol 2004;560:551–62.
[20] Sullivan J, Brocklehurst K, Marley A, Carey F, Carling D, Beri R. Inhibition of lipolysis and lipogenesis in isolated rat adipocytes with AICAR, a cell-permeable activator of AMP-activated protein kinase. FEBS Lett 1994;353:33–6.
[21] Watt M, Holmes A, Pinnamaneni S, Garnham A, Steinberg G, Kemp B, et al. Regulation of HSL serine phosphorylation in skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab 2006;290:E500–8.
[22] Watt M, Steinberg G, Chan S, Garnham A, Kemp B, Febbraio M. Beta-adrenergic stimulation of skeletal muscle HSL can be overridden by AMPK signaling. FASEB J 2004;18:1445–6.
[23] Gaidhu M, Fediuc S, Anthony N, So M, Mirpourian M, Perry R, et al. Prolonged AICAR-induced AMP-kinase activation promotes energy dissipation in white adipocytes: novel mechanisms integrating HSL and ATGL. J Lipid Res 2009;50: 704–15.
[24] Yin W, Mu J, Birnbaum M. Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis In 3T3-L1 adipocytes. J Biol Chem 2003;278:43074–80.
[25] Zang M, Xu S, Maitland-Toolan K, Zuccollo A, Hou X, Jiang B, et al. Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. Diabetes 2006;55: 2180–91.
[26] Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. Proc Natl Acad Sci U S A 2007;104:7217–22.
[27] Haemmerle G, Zimmermann R, Hayn M, Theussl C, Waeg G, Wagner E, et al. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. J Biol Chem 2002;277:4806–15.
[28] Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, et al. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. Science 2006;312:734–7.
[29] Livak K, Schmittgen T. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402–8.
[30] Hwang J, Kwon D, Yoon S. AMP-activated protein kinase: a potential target for the diseases prevention by natural occurring polyphenols. Nat Biotechnol 2009;26: 17–22.
[31] Um J, Park SJ, Kang H, Yang S, Foretz M, McBurney M, et al. AMPK-deficient mice are resistant to the metabolic effects of resveratrol. Diabetes 2010;59:554–63.