ORIGINAL

Resveratrol exerts anti-obesity effects in high-fat diet obese mice and displays differential dosage effects on cytotoxicity, differentiation, and lipolysis in 3T3-L1 cells

Chih-Chun Chang¹⁾, Keng-Yang Lin^{2), 3)}, Kang-Yu Peng²⁾, Yuan-Ji Day⁴⁾ and Li-Man Hung^{2), 3)}

¹⁾ Department of Clinical Pathology, Far Eastern Memorial Hospital, New Taipei, Taiwan

²⁾ Department of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan

³⁾ Center for Healthy and Aging Research, Chang Gung University, Taoyuan, Taiwan

⁴⁾ Department of Anesthesiology, Chang Gung Memorial Hospital, Taoyuan, Taiwan

Abstract. Studies on resveratrol in a wide range of concentrations on obese mice and adipose cells are necessary to comprehend its range of diverse and contradictory effects. In this study, we examined the anti-obesity effects of resveratrol on high-fat diet (HFD)-induced obese mice at dosages ranging from 1 to 30 mg/kg treatment for 10 wk. We also evaluated the effects of resveratrol on cytotoxicity, proliferation, adipogenic differentiation, and lipolysis of 3T3-L1 cells at concentrations ranging from 0.03 to 100 μ M. In HFD obese mice, resveratrol treatment for 10 wk without decreased calories intake significantly attenuated HFD-induced weight gain in a dose-dependent manner. Resveratrol treatment also protected against HFD-induced lipid deposition in adipose tissues and liver. In cultured 3T3-L1 preadipocytes, high dosage (10 to 100 μ M) resveratrol treatment produced cytotoxicity in both preadipocytes and mature adipocytes. In contrast, low concentration resveratrol treatment (1 to 10 μ M) significantly inhibited the capacity of 3T3-L1 cells differentiated into mature adipocytes. Low dose resveratrol treatment also downregulated peroxisome proliferator-activated receptor gamma (PPAR γ) and perilipin protein expressions in differentiated adipocytes. Additionally, tumor necrosis factor alpha (TNF α)-induced lipolysis was inhibited by low concentration resveratrol treatment in mature adipocytes. At concentrations of 10-100 μ M, resveratrol exerted cytotoxicity. In contrast, at concentrations of 1-10 μ M resveratrol inhibited adipogenic differentiation in preadipocytes and suppressed lipolysis in mature adipocytes. Our results suggest that resveratrol possessed anti-obesity effects by induction of cytotoxicity at high dosage and that it influences preadipocyte differentiation and mature adipocyte lipolysis at low concentration.

Key words: Resveratrol, Obesity, Cytotoxicity, Adipogenic differentiation, Lipolysis

OBESITY has become an ongoing health concern not only in industrialized but also in some developing countries in these past few decades. Many clinical studies have disclosed that obesity dramatically contributes to various chronic diseases including atherosclerosis, hypertension, metabolic syndrome, stroke, and specific forms of cancer [1-4]. Obesity is characterized by adipogenesis and lipogenesis. Adipogenesis is known as the hyperplastic transformation from undifferentiated pre-adipocytes to mature adipocytes and, lipogenesis, the conversion and accumulation of lipid droplets in adi-

pocytes from free fatty acids in bloodstream. Emerging evidence has indicated that uncontrolled adipogenesis and lipogenesis lead to obesity and may provide a potential target in prevention and treatment of obesity-related disorders [5]. Adipogenesis and lipogenesis are complex interconnections taking place among the differentiation and proliferation of pre-adipocytes and synthesis of fatty acids [6]. The adipogenic and lipogenic capacities of adipose tissue depend on a group of transcription factors [7, 8]. Peroxisome proliferator-activated receptor gamma (PPAR γ), one of the regulators in lipid and glucose homeostasis, is also involved in regulating adipocyte-specific protein expressions. Previous investigations have demonstrated both expression and transactivation of PPAR γ were associated with development of insulin resistance and ultimately obesity [9-11]. In addition, obesity was closely associated with inflammatory stress. It was reported that obesity increased

Submitted Sep. 19, 2015; Accepted Nov. 5, 2015 as EJ15-0545
Released online in J-STAGE as advance publication Dec. 23, 2015
Correspondence to: Li-Man Hung, Ph.D., Department of Biomedical Sciences, College of Medicine, Chang Gung University, No. 259, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33302, Taiwan (R.O.C.). E-mail: lisahung@mail.cgu.edu.tw
Chih-Chun Chang and Keng-Yang Lin contributed equally to this work.

infiltration of macrophages in adipose tissue, releasing numerous pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF- α), and interleukins, which consequently contributed to progressive exacerbation of insulin resistance and eventually, metabolic syndrome [12, 13].

Resveratrol (3,4,5-trihydroxystilbene), a natural bioactive phytochemical mainly found in grapes and berries, has been previously identified as a potent anti-oxidation [14-16] and anti-inflammation [17, 18] agent. It has been demonstrated that resveratrol possesses broad bioactivities in prevention of cardiovascular disease [19-22], cancer progression [23-25], neurodegenerative disorders [26, 27], and metabolic syndrome [28-30]. Recently, growing interest in resveratrol has been targeted on its potential effects against obesity and other complicated disorders [31]. It was also indicated that resveratrol down-regulated PPAR γ expression in attenuation of obesity [32, 33]. The detailed mechanism of resveratrol, nevertheless, needs to be further elucidated in terms of the composition of various cellular activities and effects exerted against obesity. To clarify the anti-obesity effects of resveratrol, we evaluated its morphological and biochemical profiles using high fat diet-fed mice with different dosage of resveratrol supplement. *In vitro* study was also conducted to evaluate the dosage effects of resveratrol on cytotoxicity, proliferation, differentiation, and lipolysis in 3T3-L1 cells. The present study was aimed to provide a more generalized and deepening understanding with respect to the attenuation of obesity by resveratrol treatment.

Materials and Methods

Animal experiments

Male C57BL/6C mice (10 wk old, 23-25 g in body weight) were purchased from BioLASCO Taiwan Co., Ltd (Taiwan, ROC). All animals were housed individually in an environmentally-controlled room of 12h/12h light-dark cycle and provided with *ad libitum* access to food and water. All experimental procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the Chang Gung University at Taiwan. The mice were randomly divided into five groups: mice on chow diet (Laboratory Autoclavable Rodent Diet 5010; 13.5% of calories from fat) served as normal control group (CON), mice fed with high-fat diet (Research Diet

12451; 45.3% of calories from fat) treated with vehicle (0.9% NaCl) for 10 weeks (HFD), mice on HFD treated with resveratrol 1 mg/kg (HFR1), 10 mg/kg (HFR10), and 30 mg/kg (HFR30) for 10 wk. Resveratrol was dissolved in DMSO and diluted by 0.9% NaCl then was administered as oral gavage daily. All mice were fasted overnight (8 hr) and were sacrificed at chow or HFD feeding for 10 wk after being anesthetized by chloral hydrate at a dosage of 448 mg/kg, and blood plasma, adipose tissues, heart, and liver were collected and stored at -80°C for further examination.

Serum biochemical analysis

Blood samples were obtained by retro-orbital bleeding under anesthesia. Glucose levels were determined upon blood collection using SureStep Glucometer Kit (LifeScan Inc.). Separation of serum was performed by centrifugation at $9,300 \times g$ for 10 min at 4°C and stored at -80°C before analysis. Serum insulin concentration was measured using an enzyme-linked immunosorbent assay (ELISA) commercial kit (Mercodia, Uppsala, Sweden). Serum cholesterol, triglyceride, and non-esterified fatty acid levels were determined by colorimetric assay commercial kits (Randox, Crumlin, UK), respectively. All examinations were performed in accordance with the manufacturer's protocols.

Histological examinations

Subcutaneous, epididymal and brown adipose tissues were dissected and fixed in 4% paraformaldehyde and subsequently embedded in paraffin. Paraffin sections were trimmed at a thickness of 10 μ m and stained with hematoxylin and eosin (H&E) for morphologic evaluation. The 3T3-L1 adipocyte was fixed in 4% paraformaldehyde and subjected to the Oil-Red-O staining for lipid content assessment. Images were observed and photographed in randomly chosen microscopic fields.

Culture and differentiation of 3T3-L1 cells

3T3-L1 pre-adipocytes were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% of fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO $_2$. While 3T3-L1 cells were grown to confluence, cells were stimulated by differentiation medium consisting of 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 1 μ M dexamethasone (DEX) and incubation for 8 days and then insulin (10 μ g/mL) was added at the last 2 days, at which time

more than 90% of cells were mature adipocytes with accumulated fat droplets (confirmed by Oil-Red-O staining). For evaluation of anti-proliferative and anti-differentiated effects of resveratrol, 3T3-L1 cells were cultured in 96-well plates and treated with varied concentrations of resveratrol (0.03 to 100 μ M) for 7 and 8 days, respectively.

Cell viability assay

Viability of 3T3-L1 cells with or without resveratrol treatment was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, MO) method. Briefly, each cell culture was added with 50 μ L of 5 mg/mL MTT in phosphate-buffered saline (PBS) solution and maintained for 2 hr at 37°C in a humidified atmosphere with 5% CO₂. After incubation, dimethyl sulfoxide (DMSO) was added to each well. Absorbance was detected by chemical well spectrophotometry at 570 nm (Epoch, Bio-Tek Co., Winooski, VT) for evaluation of cell viability.

Lipolysis assay

3T3-L1 pre-adipocytes were seeded in 24-well plates and incubated in the differentiation medium for 14 days to develop into mature adipocytes as described above. Mature adipocytes treated with varied concentrations of resveratrol (0.03 to 100 μ M) in combination with or without recombinant mouse TNF α (R&D Systems, Inc., Minneapolis, MN) and incubated for 24 hr. The incubation medium was collected and the contents of glycerol release from 3T3-L1 cells were determined using Free Glycerol Reagent kit (Sigma-Aldrich Chemical Co) based on the manufacturer's instructions.

Western blot analysis

Cells were collected and extracted in lysis buffer containing 20 mM Tris-HCl (pH 7.4), 50 mM glycerophosphate, 2 mM ethylenediaminetetraacetic acid

(EDTA), 20 mM sodium fluoride (NaF), 2 mM sodium orthovanadate (Na₃VO₄) and 1 mM phenylmethylsulfonyl fluoride (PMSF), 1% Triton-100, and 1% β -mercaptoethanol. Cell protein extracts (25 μ g) were loaded on 10% SDS-polyacrylamide gels for electrophoresis, followed by transfer onto PVDF membranes and then blotted with PPAR γ and perilipin antibodies. Protein levels were determined by chemiluminescent detection and analyzed by densitometric method.

Statistical analysis

Results are presented as the mean $\pm$ S.E. One-way ANOVA coupled with Student's *t* test was performed in comparison of group means. *P* < 0.05 was considered significant.

Results

Resveratrol attenuated body weight gain in HFD-induced obese mice in a dose-dependent manner

Mice on HFD feeding for 10 wk showed significant body weight gain with lower calorie intake compared to chow diet control mice (Table 1). Treatment with resveratrol at concentrations of 1 to 30 mg/kg for 10 wk significantly attenuated HFD-induced obesity in a dose-dependent manner compared to non-treated HFD mice (Table 1). We further evaluated the tissue weight of a variety of vital organs. There was a noticeable weight gain in all kinds of adipose tissue (including subcutaneous, epididymal, and brown adipose tissues) in HFD mice compared to chow diet control mice (Table 2). Resveratrol (30 mg/kg) treatment significantly mitigated the weight augmentation of subcutaneous and epididymal adipose tissues in HFD mice, but not that of brown adipose tissue (Table 2). Notably, resveratrol also reduced liver weight at dosage of 30 mg/kg compared to non-treated HFD mice (Table 2).

To evaluate the effect of resveratrol on morphology of adipose tissue in high fat diet-fed mice, biopsy

Table 1 Dosage effects of resveratrol on body weight and caloric intake in mice fed with high-fat diet for 10 weeks

	CON	HFD	HFR1	HFR10	HFR30
Body weight (g)	29.36 $\pm$ 0.70	44.40 $\pm$ 1.07*	36.22 $\pm$ 0.43 [†]	34.04 $\pm$ 31.29 [†]	34.76 $\pm$ 1.96 [†]
% of initial body weight	115.98 $\pm$ 1.42	169.36 $\pm$ 3.26*	142.88 $\pm$ 2.63 [†]	134.47 $\pm$ 8.40 [†]	136.43 $\pm$ 8.95 [†]
Caloric intake (cal/g/day)	471.41 $\pm$ 21.69	319.29 $\pm$ 9.75*	346.13 $\pm$ 18.74	329.09 $\pm$ 8.69	362.66 $\pm$ 19.40 [†]

Data are presented as mean $\pm$ SE for n=10-11 in each group. * *p* < 0.05, vs. CON. [†] *p* < 0.05, vs. HFD. CON, control; HFD, high fat diet; HFR1, HFD mice treated with resveratrol 1 mg/kg; HFR10, HFD mice treated with resveratrol 10 mg/kg; HFR30, HFD mice treated with resveratrol 30 mg/kg. Resveratrol was dissolved in DMSO and diluted by 0.9% NaCl then was administered as oral gavage daily.

Table 2 Dosage effects of resveratrol on tissue weight in mice fed with high-fat diet for 10 weeks

Tissue weight (g)	CON	HFD	HFR1	HFR10	HFR30
Heart	0.12 ± 0.01	0.14 ± 0.01	0.12 ± 0.00 [†]	0.12 ± 0.01	0.12 ± 0.01
Liver	1.08 ± 0.08	1.19 ± 0.10	1.06 ± 0.04	0.90 ± 0.08	0.93 ± 0.04 [†]
Adipose tissues (AT)					
Subcutaneous AT	0.35 ± 0.08	2.16 ± 0.36 [*]	1.33 ± 0.12	1.03 ± 0.22 [†]	1.21 ± 0.26 [†]
Epididymal AT	0.38 ± 0.05	2.67 ± 0.13 [*]	1.90 ± 0.13 [†]	1.75 ± 0.43	1.58 ± 0.30 [†]
Brown AT	0.06 ± 0.01	0.16 ± 0.02 [*]	0.13 ± 0.02	0.11 ± 0.02	0.13 ± 0.03
Muscle	0.29 ± 0.02	0.38 ± 0.02	0.35 ± 0.02	0.31 ± 0.01 [†]	0.35 ± 0.01
Kidney	0.38 ± 0.01	0.43 ± 0.04	0.40 ± 0.02	0.33 ± 0.02	0.37 ± 0.03

Data are presented as mean ± SE for n=5-6 in each group. ^{*}*p* < 0.05, vs. CON. [†]*p* < 0.05, vs. HFD. CON, control; HFD, high fat diet; HFR1, HFD mice treated with resveratrol 1 mg/kg; HFR10, HFD mice treated with resveratrol 10 mg/kg; HFR30, HFD mice treated with resveratrol 30 mg/kg.

of subcutaneous (Fig. 1A), epididymal (Fig. 1B), and brown (Fig. 1C) adipose tissue was performed and observed in a microscopic view with H&E staining. Enlargement of lipid droplets was observed in every kinds of adipose tissue mentioned above in HFD mice compared with mice on chow diet feeding. Resveratrol treatment at concentrations of 10 and 30 mg/kg in the HFD group showed remarkable reduction in fat droplet accumulations of every kind of adipose tissue when compared with HFD non-treated group (Fig. 1).

Resveratrol induced cytotoxicity and suppressed adipogenesis in 3T3-L1 cells

To determine whether the anti-obese effects of resveratrol produce cytotoxicity in adipose tissues, the pre-adipocyte and maturing pre-adipocyte were treated with wide range of resveratrol (0.03-100 μM) for 2 and 8 days, respectively, and survival rate was examined by MTT assay. The results showed that resveratrol treatment significantly decreased the survival rate of 3T3-L1 pre-adipocytes to 70.13±1.10%, 35.67±6.29%, and 23.63±5.04% in concentrations of 10, 30, and 100 μM compared with control, respectively (Fig. 2A). Also, the survival rate of mature adipocytes treated with 10, 30, and 100 μM resveratrol for 8 days was decreased to 90.53±1.99%, 3.23±1.02%, and 1.51±0.38% with statistical significance in comparison with the control group, respectively (Fig. 2B).

There was minimal cytotoxicity of resveratrol in either pre-adipocytes or mature adipocytes treated with low concentrations of resveratrol (0.03 to 10 μM) (Fig. 2). We subsequently evaluated the effect of subtoxic doses of resveratrol on 3T3-L1 cell proliferation and adipogenic differentiation with growth curve and Oil-Red-O stained experiments, respectively. The results show that only 10 μM of resveratrol treatment for 7 days

significantly inhibited 3T3-L1 pre-adipocytes proliferation compared to non-treated control group (Fig. 2C), suggesting that the growth inhibitory effect may be induced by the cytotoxicity of resveratrol. In addition, resveratrol treatment for 8 days significantly suppressed adipogenic differentiation in concentrations of 1 to 10 μM in dose dependence, when compared with the control group (Fig. 2D and 2E).

It was known that both PPARγ and perilipin played essential roles in regulation of adipogenesis and lipogenesis. Thus, western blot analysis was performed to estimate the effect of resveratrol on protein expressions of PPARγ and perilipin in differentiated 3T3-L1 cells. Our results showed that PPARγ and perilipin protein expressions were significantly down-regulated in resveratrol-treated mature adipocytes with dosage of 3 to 10 μM in comparison with the control group (Fig. 3A and 3B).

Resveratrol inhibited lipolysis in mature 3T3-L1 adipocytes

Lipolysis implies the breakdown of triacylglycerols in adipocytes and subsequently release of glycerol and fatty acids. To evaluate whether resveratrol affects lipolysis in maturing 3T3-L1 adipocytes, the amount of glycerol release was examined in cultured medium. Isoproterenol (10 μM) treatment for 24 hr led to a remarkably higher released of glycerol in mature 3T3-L1 adipocytes. In contrast, resveratrol treatment at concentrations of 0.03 to 10 μM for 24 hr triggered a significant inhibition in adipose lipolysis in a dose-dependent manner (Fig. 4A).

Treatment of TNFα also dramatically increased glycerol release in mature adipocytes. In contrast, resveratrol (0.1 to 10 μM) and TNFα (10 ng/mL) co-treatment for 24 hr resulted in significantly antagonized TNFα-induced lipolysis in mature adipocytes (Fig. 4B).

Fig. 1 Dosage effect of resveratrol on cellular morphology of adipose tissues in high fat diet-fed mice. Subcutaneous (A), epididymal (B), and brown (C) adipose tissues were sectioned and observed with H&E staining in HFD obese mice with or without resveratrol treatment for 10 wk. CON, age-matched chow diet feeding for 10 wk; HFD, high-fat diet feeding for 10 wk; HFR1, HFD mice treated with resveratrol 1 mg/kg; HFR10, HFD mice treated with resveratrol 10 mg/kg; HFR30, HFD mice treated with resveratrol 30 mg/kg for 10 wk.

Fig. 2 Dosage effect of resveratrol (RSV) on cytotoxicity, proliferation, and adipogenesis in 3T3-L1 cells. Resveratrol decreased survival rate was examined by MTT assay in pre-adipocyte (A) and mature adipocyte (B). Anti-proliferative effect of resveratrol was examined by cell growth curve (C). Dosage effect of resveratrol on adipogenic differentiation was evaluated with Oil-Red-O staining (D) and the quantified ratios were shown with normalization by control group (E). Data are expressed as the mean $\pm$ SE, $n=5$ in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control.

Fig. 3 Resveratrol (RSV) down-regulated PPAR γ and perilipin protein expressions in 3T3-L1 mature adipocytes. Western blot with PPAR γ (A) and perilipin (B) was performed and the quantified ratios are shown with normalization by control group. Data are expressed as the mean $\pm$ SE, $n=5$ in each group. * $p < 0.05$, *** $p < 0.001$ vs. control.

Fig. 4 Resveratrol (RSV) suppressed lipolysis was examined in 3T3-L1 mature adipocytes under both basal (A) and TNF α -stimulated (B) conditions. Quantified ratios are shown with normalization by control group. Data are expressed as the mean $\pm$ SE, $n=5$ in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control, # $p < 0.05$ vs. TNF α treatment alone. ISO, isoproterenol.

Discussion

Resveratrol has recently become available in tablet form and is recommended as a dietary supplement, with commercial resveratrol available at between 50 μg and 300 mg per dosage. A 25 mg oral dose results in peak plasma levels of resveratrol at about 2 μM and a plasma half-life of 9.2 ± 0.6 hr in human subjects [34]. This pharmacokinetic study shows that the bioavailability of resveratrol is very low and only a small proportion reaches plasma and tissues. Interestingly, despite its low bioavailability, resveratrol exerts a wide range of biological effects that could benefit human health

[19-31]. The functional discrepancies may result from different concentrations of resveratrol and treatment period lengths that different cells are exposed to in different experimental settings. Therefore it is necessary to have a comprehensive grasp of the diverse and contradictory effects of resveratrol. Additionally, studies of resveratrol in a wide range of concentrations on adipose cells can provide a more generalized and deepening understanding with respect to the attenuation of obesity by resveratrol treatment. In the present study, we found that resveratrol exhibited anti-obesity effects on high-fat diet-induced obese mice in a dose-dependent manner. The anti-obesity effect of resveratrol was most likely through attenuation of lipid deposition in adipose tissues (including subcutaneous and epididymal fat) but not *via* influence of calorie intake. High concentration of resveratrol treatment (10 to 100 μ M) significantly induced cytotoxicity in both 3T3-L1 preadipocytes and mature adipocytes. Resveratrol also inhibited preadipocyte proliferation at concentrations of 10 μ M. These results indicate that the anti-proliferative effects of resveratrol may result from exertion of cytotoxicity but not through direct growth inhibition. At sub-toxic concentrations (1 to 10 μ M) of resveratrol treatment significantly suppressed adipogenic differentiation and lipolysis in preadipocytes and matured adipocytes. Accordingly, protein expression of PPAR γ , a main adipogenesis-inducing regulator, was down-regulated by 0.3 to 10 μ M resveratrol treatment, suggesting that resveratrol inhibited adipocyte differentiation and lipolysis by modulating of PPAR γ protein expressions. Taken together, these results indicate that the anti-obesity mechanisms of resveratrol could be involved with cell viability, cell proliferation, intracellular lipid accumulation, and lipolysis in adipocytes and pre-adipocytes, depending on the treatment dosage.

With regard to cytotoxicity, resveratrol significantly reduced cell survival rate at concentrations of 10 to 100 μ M in both 3T3-L1 pre- and mature adipocytes. Consistent with our findings, it has been reported that resveratrol-stimulated cell apoptosis at concentrations of 50 and 100 μ M [35]. Our observations also found mature adipocytes to be more sensitive to resveratrol treatment than pre-adipocytes. Adipogenesis is known as a hyperplastic transformation from undifferentiated pre-adipocytes to mature adipocytes and, lipogenesis, the convert and accumulation of lipid droplets in adipocyte from free fatty acids. Regarding the anti-adipogenic effects, several studies have demonstrated that

resveratrol inhibited adipogenesis at concentrations of 20 to 100 μ M and in the treatment period length (24 hr to 8 days) [36-39]. Our results show that resveratrol attenuated adipogenesis and lipogenesis at concentrations of 1 to 10 μ M which is much less than the above published studies. Our results suggest that resveratrol directly suppressed adipogenic differentiation and lipid droplets accumulation at concentrations below 10 μ M. In contrast, at high concentrations (10 to 100 μ M) resveratrol treatment seemed to lead to cell death instead of induction of anti-differentiated effects.

Lipolysis implies the breakdown of triacylglycerols in adipocytes, and subsequently leads to release of glycerol and fatty acids. The effects of resveratrol on lipolysis have been studied *in vitro*, *ex vivo*, and *in vivo* [40, 41]. One study demonstrated that resveratrol (100 μ M) failed to promote lipolysis in epididymal adipocytes of rat [40]. Another study suggested that treatment of resveratrol (100 μ M) for 12, 24, or 48 hr significantly promoted lipolysis in 3T3-L1 adipocytes and also exerted synergistic action with epinephrine [41]. It is not clear whether this discrepancy is due to differential response of rat epididymal adipocytes *versus* 3T3-L1 cells. Our results show that resveratrol treatment at concentrations of 0.03 to 10 μ M for 24 hr not only inhibited lipolysis in basal conditions but also when lipolysis was stimulated with TNF α in 3T3-L1 matured adipocytes. Inhibition of adipose lipolysis may prevent ectopic lipid accumulation in non-adipose tissues such as liver. Our *in vivo* study also showed that resveratrol significantly decreased liver weight at concentrations of 30 mg/kg in high-fat diet obese mice, suggesting that resveratrol could attenuate obesity-associated liver steatosis. It is noteworthy that while 3T3-L1 mature adipocytes exposed to high concentrations of resveratrol (above 10 μ M) exhibited a dramatic reduction in cell survival rate which may have confounded its anti-lipolysis action. Corresponding to the opposing effects of resveratrol at low (from our study) *versus* high (from a study by others) concentrations on lipolysis of 3T3-L1 cells is unclear and would be of great interest for future studies.

Our study revealed distinct and much more dynamic actions of resveratrol on both high-fat diet obese mice and 3T3-L1 cells conferred by a wide range of concentrations, as compared to previous studies [35-41]. At low concentrations (below 10 μ M), resveratrol appears to inhibit adipogenic differentiation and lipolysis. At high concentrations (above 10 μ M), however, resveratrol decreased cell survival rate in pre- and mature

adipocytes as well as the proliferation rate of preadipocytes. Our study also disclosed that resveratrol elicited anti-obese effects by inhibition of adipogenic differentiation and lipid droplet accumulation as well as by stimulation of cytotoxic effects on adipocytes. The present study provides valuable guidelines for using resveratrol in patients with obesity.

Acknowledgements

This work was supported financially by research grants

from Chang Gung Memorial Hospital (CMRPD1A0122, CMRPD1A0203 and CMRPD1C0463), Chang Gung University (EMRPD1D0881), and Ministry of Science and Technology (MOST103-2320-B-182-008) of Taiwan to Dr. Li-Man Hung.

Disclosure

The authors declare that they have no conflict of interests.

References

1. Kannel WB, Cupples LA, Ramaswami R, Stokes J, Kreger BE, et al. (1991) Regional obesity and risk of cardiovascular disease; the Framingham Study. *J Clin Epidemiol* 44: 183-190.
2. Field AE, Coakley EH, Must A, Spadano JL, Laird N, et al. (2001) Impact of overweight on the risk of developing common chronic diseases during a 10-year period. *Arch Intern Med* 161: 1581-1586.
3. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, et al. (2006) Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 295: 1549-1555.
4. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348: 1625-1638.
5. Gregoire FM, Smas CM, Sul HS (1998) Understanding adipocyte differentiation. *Physiol Rev* 78: 783-809.
6. He Y, Li Y, Zhao T, Wang Y, Sun C (2013) Ursolic acid inhibits adipogenesis in 3T3-L1 adipocytes through LKB1/AMPK pathway. *PLoS One* 8: e70135.
7. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 79: 1147-1156.
8. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM (2000) Transcriptional regulation of adipogenesis. *Genes Dev* 14: 1293-1307.
9. Zhang B, Berger J, Zhou G, Elbrecht A, Biswas S, et al. (1996) Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor gamma. *J Biol Chem* 271: 31771-31774.
10. Rangwala SM, Rhoades B, Shapiro JS, Rich AS, Kim JK, et al. (2003) Genetic modulation of PPARgamma phosphorylation regulates insulin sensitivity. *Dev Cell* 5: 657-663.
11. Shiomi Y, Yamauchi T, Iwabu M, Okada-Iwabu M, Nakayama R, et al. (2015) A novel Peroxisome Proliferator-activated Receptor (PPAR) α agonist and PPAR γ antagonist, Z-551, ameliorates high-fat diet-induced obesity and metabolic disorders in mice. *J Biol Chem* 290: 14567-14581.
12. Gouranton E, Romier B, Marcotrichino J, Tourniaire F, Astier J, et al. (2014) Visfatin is involved in TNF α -mediated insulin resistance via an NAD(+)/Sirt1/PTP1B pathway in 3T3-L1 adipocytes. *Adipocyte* 3: 180-189.
13. Finucane OM, Reynolds CM, McGillicuddy FC, Roche HM (2012) Insights into the role of macrophage migration inhibitory factor in obesity and insulin resistance. *Proc Nutr Soc* 71: 622-633.
14. Huang JP, Huang SS, Deng JY, Chang CC, Day YJ, et al. (2010) Insulin and resveratrol act synergistically, preventing cardiac dysfunction in diabetes, but the advantage of resveratrol in diabetics with acute heart attack is antagonized by insulin. *Free Radic Biol Med* 49: 1710-1721.
15. Akar F, Pektas MB, Tufan C, Soylemez S, Sepici A, et al. (2011) Resveratrol shows vasoprotective effect reducing oxidative stress without affecting metabolic disturbances in insulin-dependent diabetes of rabbits. *Cardiovasc Drugs Ther* 25: 119-131.
16. Xu Y, Nie L, Yin YG, Tang JL, Zhou JY, et al. (2012) Resveratrol protects against hyperglycemia-induced oxidative damage to mitochondria by activating SIRT1 in rat mesangial cells. *Toxicol Appl Pharmacol* 259: 395-401.
17. Ghanim H, Sia CL, Abuaysheh S, Korzeniewski K, Patnaik P, et al. (2010) An antiinflammatory and reactive oxygen species suppressive effects of an extract of Polygonum cuspidatum containing resveratrol. *J Clin Endocrinol Metab* 95: E1-E8.
18. Palsamy P, Sivakumar S, Subramanian S (2010) Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. *Chem Biol Interact* 186: 200-210.
19. Das DK, Sato M, Ray PS, Maulik G, Engelman RM, et

- al. (1999) Cardioprotection of red wine: role of polyphenolic antioxidants. *Drugs Exp Clin Res* 25: 115-120.
20. Wu JM, Wang ZR, Hsieh TC, Bruder JL, Zou JG, et al. (2001) Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine. *Int J Mol Med* 8: 3-17.
21. Delmas D, Jannin B, Latruffe N (2005) Resveratrol: preventing properties against vascular alterations and ageing. *Mol Nutr Food Res* 49: 377-395.
22. Ruan BF, Lu XQ, Song J, Zhu HL (2012) Derivatives of resveratrol: potential agents in prevention and treatment of cardiovascular disease. *Curr Med Chem* 19: 4175-4183.
23. Bishayee A (2009) Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. *Cancer Prev Res(Phila)* 2: 409-418.
24. Whitlock NC, Baek SJ (2012). The anticancer effects of resveratrol: modulation of transcription factors. *Nutr Cancer* 64: 493-502.
25. Juan ME, Alfara I, Planas JM (2012) Colorectal cancer chemoprevention by trans-resveratrol. *Pharmacol Res* 65: 584-591.
26. Albani D, Polito L, Signorini A, Forloni G (2010) Neuroprotective properties of resveratrol in different neurodegenerative disorders. *Biofactors* 36: 370-376.
27. Li F, Gong Q, Dong H, Shi J (2012) Resveratrol, a neuroprotective supplement for Alzheimer's disease. *Curr Pharm Des* 18: 27-33.
28. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, et al. (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127: 1109-1122.
29. Liu L, Wang Y, Lam KS, Xu A (2008) Moderate wine consumption in the prevention of metabolic syndrome and its related medical complications. *Endocr Metab Immune Disord Drug Targets* 8: 89-98.
30. Beaudeau JL, Nivet-Antoine V, Giral P (2010) Resveratrol: a relevant pharmacological approach for the treatment of metabolic syndrome? *Curr Opin Clin Nutr Metab Care* 13: 729-736.
31. Xia X, Weng J (2010) Targeting metabolic syndrome: candidate natural agents. *J Diabetes* 2: 243-249.
32. Costa Cdos S, Rohden F, Hammes TO, Margis R, Bortolotto JW, et al. (2011) Resveratrol upregulated SIRT1, FOXO1, and adiponectin and downregulated PPAR γ 1-3 mRNA expression in human visceral adipocytes. *Obes Surg* 21: 356-361.
33. Baile CA, Yang JY, Rayalam S, Hartzell DL, Lai CY, et al. (2011) Effect of resveratrol on fat mobilization. *Ann N Y Acad Sci* 1215: 40-47.
34. Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK (2004) High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos* 32: 1377-1382.
35. Rayalam S, Della-Fera MA, Yang JY, Park HJ, Ambati S, et al. (2007) Resveratrol potentiates genistein's anti-adipogenic and proapoptotic effects in 3T3-L1 adipocytes. *J Nutr* 137: 2668-2673.
36. Hu P, Zhao L, Chen J (2015) Physiologically achievable doses of resveratrol enhance 3T3-L1 adipocyte differentiation. *Eur J Nutr* 54: 569-579.
37. Carpena C, Gomez-Zorita S, Deleruyelle S, Carpena MA (2015) Novel strategies for preventing diabetes and obesity complications with natural polyphenols. *Curr Med Chem* 22: 150-164.
38. Bai L, Pang WJ, Yang YJ, Yang GS (2008) Modulation of Sirt1 by resveratrol and nicotinamide alters proliferation and differentiation of pig preadipocytes. *Mol Cell Biochem* 307: 129-140.
39. Fisher-Posovszky P, Kukulus V, Tews D, Unterkircher T, Debatin KM, et al. (2010) Resveratrol regulates human adipocyte number and function in a Sirt1-dependent manner. *Am J Clin Nutr* 92: 5-15.
40. Szkudelska K, Nogowski L, Szkudelski T (2009) Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes. *J Steroid Biochem Mol Biol* 113: 17-24.
41. Lasa A, Schweiger M, Kotzbeck P, Churrua I, Simón E, et al. (2012) Resveratrol regulates lipolysis via adipose triglyceride lipase. *J Nutr Biochem* 23: 379-384.