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Resveratrol improves insulin signaling in a tissue-specific manner under insulin-resistant conditions only: in vitro and in vivo experiments in rodents

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ARTICLE INFO

Article history:
Received 26 January 2011
Accepted 15 August 2011

ABSTRACT

Resveratrol (RSV) has various metabolic effects, especially with relatively high-dose therapy. However, the ability of RSV to modulate insulin signaling has not been completely evaluated. Here, we determined whether RSV alters insulin signaling in insulin-responsive cells and tissues. The effects of RSV on insulin signaling in 3T3-L1 adipocytes under both insulin-sensitive and insulin-resistant states and in insulin-sensitive tissues of high fat–fed diet-induced obese (DIO) mice were investigated. Insulin-stimulated insulin receptor substrate–1 tyrosine phosphorylation (Y612) was suppressed in RSV-treated adipocytes compared with untreated adipocytes, as was the insulin-stimulated Akt phosphorylation (Ser473). However, under an insulin-resistant condition that was made by incubating 3T3-L1 adipocytes in the conditioned medium from lipopolysaccharide-stimulated LAW264.7 cells, RSV reduced inducible nitric oxide synthase expression and IκBα protein degradation and improved insulin-stimulated Akt phosphorylation (Ser473). In DIO mice, relatively low-dose RSV (30 mg/kg daily for 2 weeks) therapy lowered fasting blood glucose level and serum insulin, increased hepatic glycogen content, and ameliorated fatty liver without change in body weight. The insulin-stimulated Akt phosphorylation was decreased in the liver and white adipose tissue of DIO mice, but it was completely normalized by RSV treatment. However, in the skeletal muscle of DIO mice, insulin signaling was not improved by RSV treatment, whereas the phosphorylation of adenosine monophosphate–activated protein kinase α (Thr172) was improved by it. Our results show that RSV enhances insulin action only under insulin-resistant conditions and suggest that the effect of RSV may depend on the type of tissue being targeted and its metabolic status.

Clinical trial registration number: not applicable.

Author contributions: Wonyoung Kang and Hyun Ju Hong: design and conduct of the study, data collection and analysis, data interpretation. Jian Guan, Dong Geon Kim, and Eun-In Yang: conduct of animal studies and data collection. Gwanpyo Koh and Doekbae Park: design of cell experiments, data analysis, data interpretation. Chang Hoon Han and Young-Jae Lee: design of the study, regular checking, analysis and interpretation of data, and manuscript writing. Dae-Ho Lee: design and conduct of the study, data collection and analysis, data interpretation, and manuscript writing.

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doi:10.1016/j.metabol.2011.08.003

1. Introduction

Resveratrol (RSV; 3,5,4′-trihydroxystilbene) is a natural phytoalexin produced by a variety of plants, such as grapes, peanuts, and mulberries, and notably found in red wine in its 2 isomers, *trans* and *cis* [1]. Recent studies suggest that RSV, by activating silent mating type information regulation 2 homolog 1 (SIRT1), mimics the effects of caloric restriction in laboratory animals [2,3]. The action of RSV has also been intensively studied in chronic metabolic diseases because caloric restriction and/or SIRT1 is involved in longevity, fuel metabolism, and other aging-related processes [4]. Treatment of high fat (HF)–fed mice with RSV at a high dose (400 mg/[kg d]) decreased total body fat content and increased whole-body insulin sensitivity, aerobic capacity and exercise endurances, and adaptive thermogenesis on cold exposure [5]. These effects are most likely due to an induction of genes for oxidative phosphorylation and mitochondrial biogenesis, which are thought to be mediated by an increase in peroxisome proliferator-activated receptor-γ coactivator-α (PGC-1α) activity of skeletal muscle and brown adipose tissue [3,5]. Resveratrol also has a wide range of pharmacological effects: antioxidant activity [6], inhibition of platelet aggregation [7], induction of nitric oxide production [8], modulation of the synthesis of hepatic apolipoprotein and lipids [9], and anti-inflammatory action [10,11]. Thus, these data opened a very promising perspective of the potential use of RSV and its analogues in preventing and/or treating chronic metabolic disorders such as obesity and diabetes.

Recent studies demonstrated the diversity of RSV's effects on insulin signaling in cultured cells [12-15]. Resveratrol was shown to reduce insulin-induced glucose conversion to total lipids and glucose oxidation, as well as to attenuate the antilipolytic action in adipose cells [12]. In human primary myotubes and muscle-derived cell lines, RSV inhibits the insulin signaling pathway at the level of class IA phosphoinositide 3-kinase catalytic subunits p110α and p110β with the impairment of downstream Akt-FOXO signaling induced by insulin without changes in mitogen-activated protein kinase (MAPK) activation [13]. In contrast, another study demonstrated that RSV could inhibit both Akt and MAPK signaling pathways in hepatocytes by inhibiting the insulin-stimulated interaction between insulin receptor substrate–1 (IRS-1) and phosphoinositide 3-kinase and between IRS-1 and growth-factor-receptor–bound protein 2 [14]. The inhibitory action of RSV on insulin signaling appeared to be independent of SIRT1 action [13-15]. Interestingly, overexpression of SIRT1 in C2C12 myotubes improves insulin sensitivity under insulin-resistant conditions [16]. Furthermore, experimental evidences revealed that high doses of RSV or its analogues caused a significant improvement in insulin sensitivity in various murine models with insulin resistance [3-5,17,18]. However, it is unclear whether the improvement of insulin sensitivity by RSV therapy was due to a secondary effect resulting from the less weight gain in the RSV-treated obese mice compared with vehicle-treated obese mice [4,5]. Taken together, these data suggest that RSV's effect on insulin action may depend on metabolic states and have tissue/cell specificity. In the present study, we aimed to investigate the effects of RSV on insulin signaling in 3T3-L1 adipocytes under 2 different conditions; a normal insulin-sensitive state and an inflammatory conditioned insulin-resistant state. We also evaluated the integrated effect of RSV on insulin action by assessing insulin signaling in insulin-sensitive tissues of the HF-fed obese mice.

2. Methods

2.1. Materials

Trans-RSV and carboxyl methyl cellulose were purchased from Sigma (St Louis, MO). The vendors for the antibodies are as follows: anti-phospho-insulin receptor (pIR) (Tyr1162/1163) was from Invitrogen (Camarillo, CA); anti-insulin receptor β was from BD Biosciences (Camarillo, CA); anti-phospho-Akt (Ser473), anti-phospho-Akt (Thr308), anti-phospho-adenosine monophosphate–activated protein kinase (AMPK) α (Thr172), and anti-AMPK antibody were from Cell Signaling Technology (Beverly, MA); anti-β-actin (monoclonal Ab) was from Sigma; anti-Akt, anti-inducible nitric oxide synthase (iNOS), anti-IκBα (C21), anti-phospho-IκBα (Ser32), and anti-rabbit immunoglobulin G (IgG) or anti-mouse IgG horseradish peroxidase–linked secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Venders of reagents and media used in cell experiments are as follows: Dulbecco modified Eagle medium (DMEM) was from Hyclone (Novato, CA); fetal bovine serum (FBS), bovine serum, trypsin, and penicillin-streptomycin (p/s) were from GIBCO (Carlsbad, CO); lipopolysaccharide (LPS), 3-isobutyl-1-methylxanthine, dexamethasone, and insulin were from Sigma. All other reagents and chemicals were purchased from commercial sources and were of reagent grade or better.

2.2. RAW264.7 cell culture and preparation of conditioned media

A murine macrophage cell line, RAW264.7, provided by Korean Cell Line Bank (Seoul, Korea), was cultured in DMEM supplemented with 10% FBS and 1% p/s at 37°C in 5% CO_2 humidified atmosphere. Inflammatory media were prepared as LPS-treated conditioned media (L/CM), which were obtained from the culture media of RAW264.7 cells treated with 1 μg/mL of LPS for 24 hours. Control media were prepared from RAW264.7 using the same method without the LPS stimulation. The L/CM and CM were filtered before incubating mature adipocytes.

2.3. 3T3-L1 adipocytes culture and insulin signaling study

3T3-L1 preadipocytes were provided by American Type Culture Collection and were cultured in DMEM supplemented with 10% bovine serum and 1% p/s at 37°C in 5% CO_2 humidified atmosphere. At the day of postconfluence (day 0), media were changed to differentiation initiation media (10% FBS, 1% p/s, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 0.5 μmol/L dexamethasone, and 10 μg/mL insulin in DMEM) and were incubated for 48 hours. At day 2, differentiation initiation media were changed to differentiation progression media (10% FBS, 1% p/s, and 10 μg/mL insulin in DMEM) and incubated for 48 hours. At days 4 and 6, cells were cultured in DMEM containing 10% FBS and 1% p/s and were maintained until complete differentiation. Mature 3T3-L1 adipocytes at days 8 to 12 were used for the study.

Mature 3T3-L1 adipocytes were incubated in the usual maintenance media or L/CM with or without RSV at concentrations of 10, 25, and 50 μmol/L for 24 hours. Cells were starved and stimulated with insulin at 10 μg/mL for 30 minutes.

2.4. *Animal experiments*

Male C57BL/6N mice (5 weeks old) were supplied by Orient (Songham, Korea). The animals were housed in 24°C ± 1°C at 50% humidity with a 12-hour light/12-hour dark cycle. All animal experiments were carried out in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Jeju National University (approval no. 20090018).

The mice were divided into 3 groups of 12 mice each. The experimental groups were as follows: the control mice were maintained on a normal chow diet that consisted of 12.5% lipids, 63.2% carbohydrates, and 24.3% proteins (kilocalories) (Orient) (control group); for HF-fed diet-induced obese (DIO)

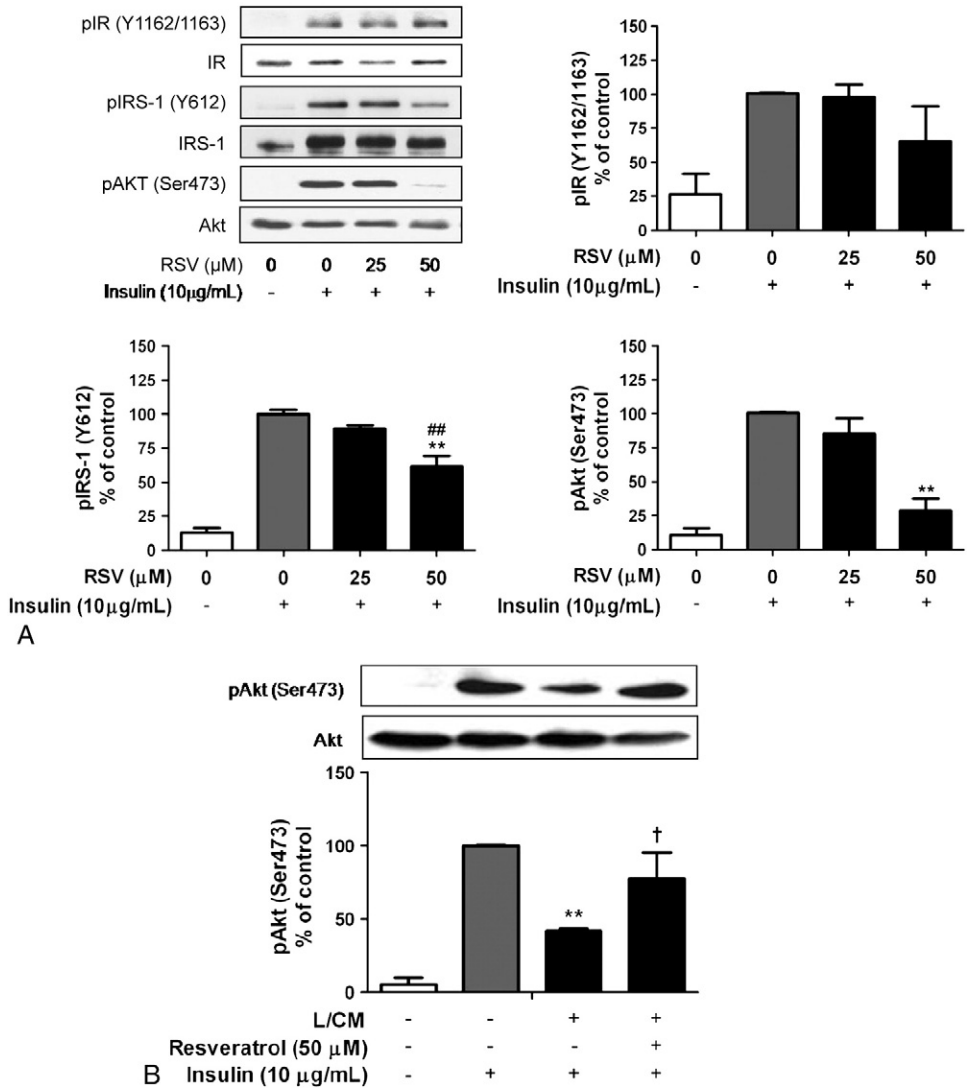

Fig. 1 – The divergent effects of RSV on insulin signaling in cultured adipocytes. Mature 3T3-L1 adipocytes were incubated in the usual maintenance media or the inflammatory conditioned media (L/CM) (prepared as in "Methods") with or without RSV at the concentrations indicated for 24 hours. Afterward, the cells were starved and stimulated with insulin (10 μg/mL for 30 minutes). The representative immunoblotting images among, at least, 3 experiments are presented for insulin-stimulated site-specific phosphorylation of IR, IRS-1, and Akt. For quantification, phosphoproteins were normalized to the corresponding total proteins except for IRS-1. Note: The IRS-1 antibody reacts more strongly with phosphorylated IRS-1 protein. Normalized phosphorylation levels of the proteins were expressed as the percentage of insulin-stimulated control adipocytes (% of control). Each value represents the mean ± SE of at least 3 experiments. A, Resveratrol's effect in insulin-sensitive adipocytes. $^{**}P < .01$ vs insulin-stimulated control adipocytes. $^{\#\#}P < .01$ vs the insulin-stimulated adipocytes exposed to RSV treatment at 25 μmol/L. B, Resveratrol's effect in insulin-resistant adipocytes. $^{**}P < .01$ vs the insulin-stimulated adipocytes not exposed to L/CM and RSV. $^{\dagger}P < .05$ vs the insulin-stimulated adipocytes incubated in L/CM without RSV.

group (HF group), mice were given a commercial HF diet (with 58% kcal from fat, Research Diets, D12331) for 20 weeks starting at 6 weeks of age; for RSV-treated DIO group (HF + RSV group), the mice were fed the same HF diet maintained as the HF group for 20 weeks with an additional treatment with RSV (by oral gavage 30 mg/[kg d]) during the last 2 weeks. In addition, in a separate experiment, we studied the effect of RSV on insulin signaling in normal chow-fed control mice under similar experimental conditions to compare the effect of RSV with HF-fed mice.

2.5. *Metabolic measurement and insulin signaling studies*

Body weights were measured once a week. At the end of the study, blood glucose levels were measured after a fast of 16 hours using OneTouch Horizon (LifeScan). Serum insulin was measured by enzyme-linked immunosorbent assay (Crystal Chem). Tissue glycogen content was measured using a commercial kit (Abcam).

For insulin signaling studies in vivo, the mice were injected intraperitoneally with saline or with human regular insulin (Humulin R, Lilly) at 5 U/kg of body weight. Ten minutes later, the mice were killed by decapitation; and blood was collected. Afterward, liver, skeletal muscle (gastrocnemius), and white adipose tissue (WAT) were rapidly isolated, blotted dry, snap-frozen in liquid nitrogen, and stored at –80°C until processing.

2.6. *Western blot analysis*

Cells or frozen tissues were homogenized in cold lysis buffer (20 mmol/L Tris-HCl [pH 7.4], with 5 mmol/L EDTA [pH 8.0], 10 mmol/L $Na_2P_4O_7$, 100 mmol/L NaF, 2 mmol/L Na_3VO_4, 1% NP-40, 13.2 μg/mL aprotinin, 13.2 μg/mL leupeptin, 1 mmol/L phenylmethylsulfonyl fluoride). The homogenates were centrifuged at 10 000*g* for 20 minutes at 4°C. The supernatants were stored at –80°C until analysis. The protein concentration was determined by using a BCA protein assay kit (DC protein assay; Bio-Rad).

Aliquots of 60 μg protein from tissue homogenate or from cell lysate of mature 3T3-L1 adipocytes were resolved using 10% (vol/vol) sodium dodecyl sulfate polyacrylamide gel electrophoresis and were transferred onto a polyvinylidene difluoride membrane (Immobilon P; Millipore, Billerica, MA) for 100 minutes at 110 V. The transferred membrane was incubated in blocking buffer (20 mmol/L Tris-HCl [pH 7.6] with 137 mmol/L NaCl and 0.05% Tween-20 supplemented with 5% [wt/vol] nonfat dry milk [TTBS]) for 1 hour, washed in TTBS for 10 minutes 4 times, and then incubated with primary antibodies for several hours. The primary antibodies were diluted 1000-fold with TTBS. After washing with TTBS, the membranes were incubated with anti-rabbit IgG or anti-mouse IgG horseradish peroxidase–linked secondary antibodies at 1:10 000 or 1:5000 dilution in TTBS for 40 minutes. Reactive bands were visualized using enhanced chemiluminescence reagents (Intron Biotechnology). The band intensities of the proteins were analyzed by using National Institutes of Health Image J 1.42 software, and the levels of phosphorylation were normalized by the total protein amounts.

2.7. *Quantitative real-time polymerase chain reaction*

RNA was extracted from frozen liver tissue using the RNeasy mini kit (Qiagen). For real-time polymerase chain reaction (PCR), first-strand complementary DNA (cDNA) was synthesized from 1 μg total RNA using Advantage RT-for-PCR Kit (Clon Tech). Relative messenger RNA levels were determined by real-time PCR using Brilliant II SYBR Green QPCR Master Mix kit (Stratagene) and an Mx3000P thermal cycler (Stratagene). All cDNA levels where normalized to the level of ubiquitin cDNA. Primer sequences used in these experiments were from a published article [19].

2.8. *Histological examination*

The livers were isolated from saline-injected mice and fixed in 10% formaldehyde. The fixed tissues were embedded in paraffin and were sectioned (4.0 μm). The sections were stained with hematoxylin-eosin and were examined under light microscope to determine the grades of fatty change in liver.

2.9. *Statistical analysis*

All results were presented as mean ± SE. Comparisons among the groups in animal study were carried out using 1-way analysis of variance (SPSS program, version 12.0.0; SPSS, Chicago, IL), whereas results of the cell experiments were analyzed by an unpaired Student *t* test (unless otherwise indicated). *P* values < .05 were considered significant.

3. Results

3.1. *RSV has an inhibitory action on insulin signaling pathway in 3T3-L1 adipocytes*

To evaluate the effects of RSV on insulin signaling, mature 3T3-L1 adipocytes were cultured in the usual maintenance media in the presence or absence of RSV. Insulin-stimulated IRS-1 tyrosine phosphorylation (Y612) was suppressed in the RSV-treated adipocytes compared with nontreated adipocytes, as was the insulin-stimulated phosphorylation of Akt (Ser473) (Fig. 1A). However, the insulin-stimulated tyrosine phosphorylation (Y1162/1163) of IR was not affected by RSV treatment. The expressions of Akt, IR, and IRS-1 in the cells were unaltered by RSV therapy.

3.2. *RSV improves insulin signaling in adipocytes cultured in inflammatory conditioned media*

To investigate the effects of RSV on insulin signaling pathway under an insulin-resistant condition, mature 3T3-L1 adipocytes were cultured in L/CM in the presence or absence of RSV. The insulin-stimulated phosphorylation of Akt (Ser473) was significantly lower in L/CM-cultured adipocytes than in adipocytes cultured in the CM ($P < .001$) (Fig. 1B). In the presence of RSV, however, the phosphorylated Akt (Ser473) level in L/CM-cultured adipocytes was restored to normal level. These results suggest that RSV has an ability to ameliorate cellular insulin resistance that was induced by a proinflammatory conditioning strategy.

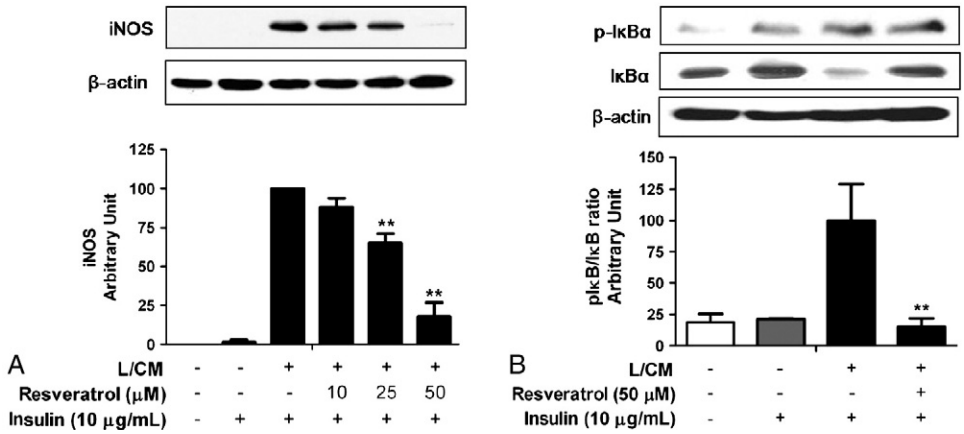

Fig. 2 – Effects of RSV on iNOS expression (A) and IκBα degradation (B) in adipocytes cultured in inflammatory media. Mature 3T3-L1 adipocytes were coincubated with indicated concentrations of RSV and L/CM for 24 hours and were treated with insulin (10 μg/mL) for 30 minutes. The iNOS expression was observed with Western blot analysis, and the values of densitometry were determined in relation to the concentration of β-actin. Values are expressed as mean ± SE. **P < .01 vs the insulin-stimulated adipocytes incubated in L/CM without RSV.

3.3. *RSV has an anti-inflammatory effect in adipocytes cultured in inflammatory conditioned media*

To evaluate the effects of RSV on inflammatory pathways, the protein expression levels of iNOS and IκBα were determined in the mature 3T3-L1 adipocytes that were cultured in L/CM in the presence or absence of RSV (Fig. 2). The level of iNOS expression was higher in adipocytes cultured in L/CM than in adipocytes cultured in the CM (Fig. 2A). Resveratrol, in a dose-dependent manner, suppressed the level of iNOS expression in adipocytes cultured in L/CM (Fig. 2A).

In adipocytes cultured in L/CM, the phosphorylation of IκBα (Ser32) was increased, whereas IκBα protein expression was significantly decreased (Fig. 2B). Resveratrol treatment (50 μmol/L) restored the level of IκBα expression in L/CM-cultured adipocytes without a significant effect on the level of phosphorylated IκBα (Ser32). The ratio of phospho-IκBα (Ser32) to total IκBα expression decreased significantly

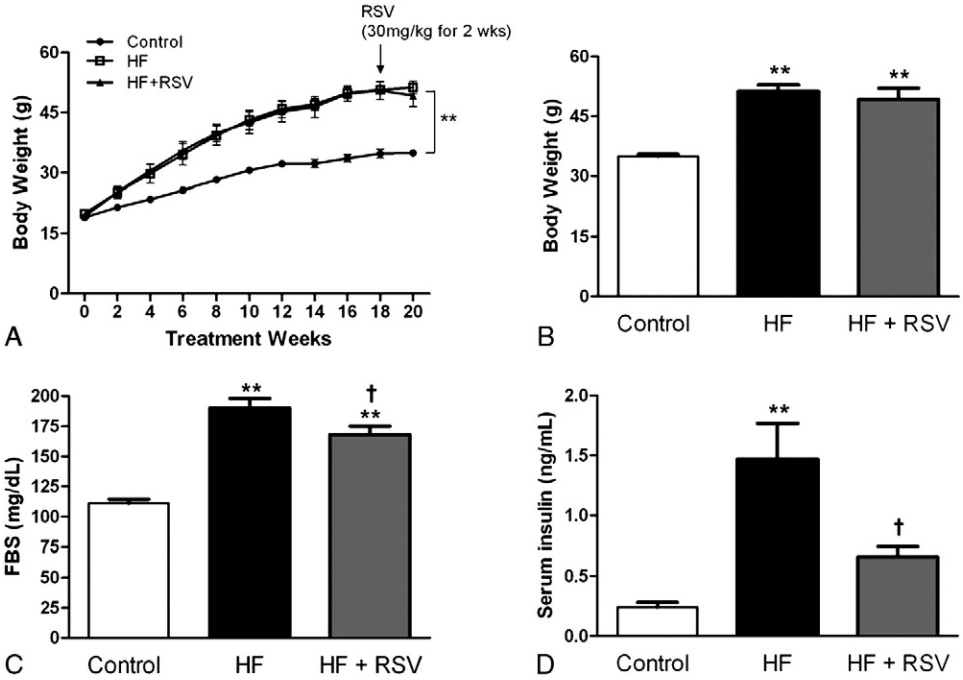

Fig. 3 – Effects of RSV on body weight, fasting blood glucose, and fasting serum insulin in HF-fed mice. The mice (6 weeks old) were fed with high-fat diets (HF, n = 12) or normal chow diet (control, n = 12) for 20 weeks. In the third group, HF-fed mice were treated with RSV (HF + RSV, n = 13). In this group, RSV (30 mg/kg daily) was administered with the HF diet during the last 2 weeks. Data are mean ± SE. **P < .01 vs control group. †P < .05 vs HF-fed mice. FBS indicates fasting blood glucose.

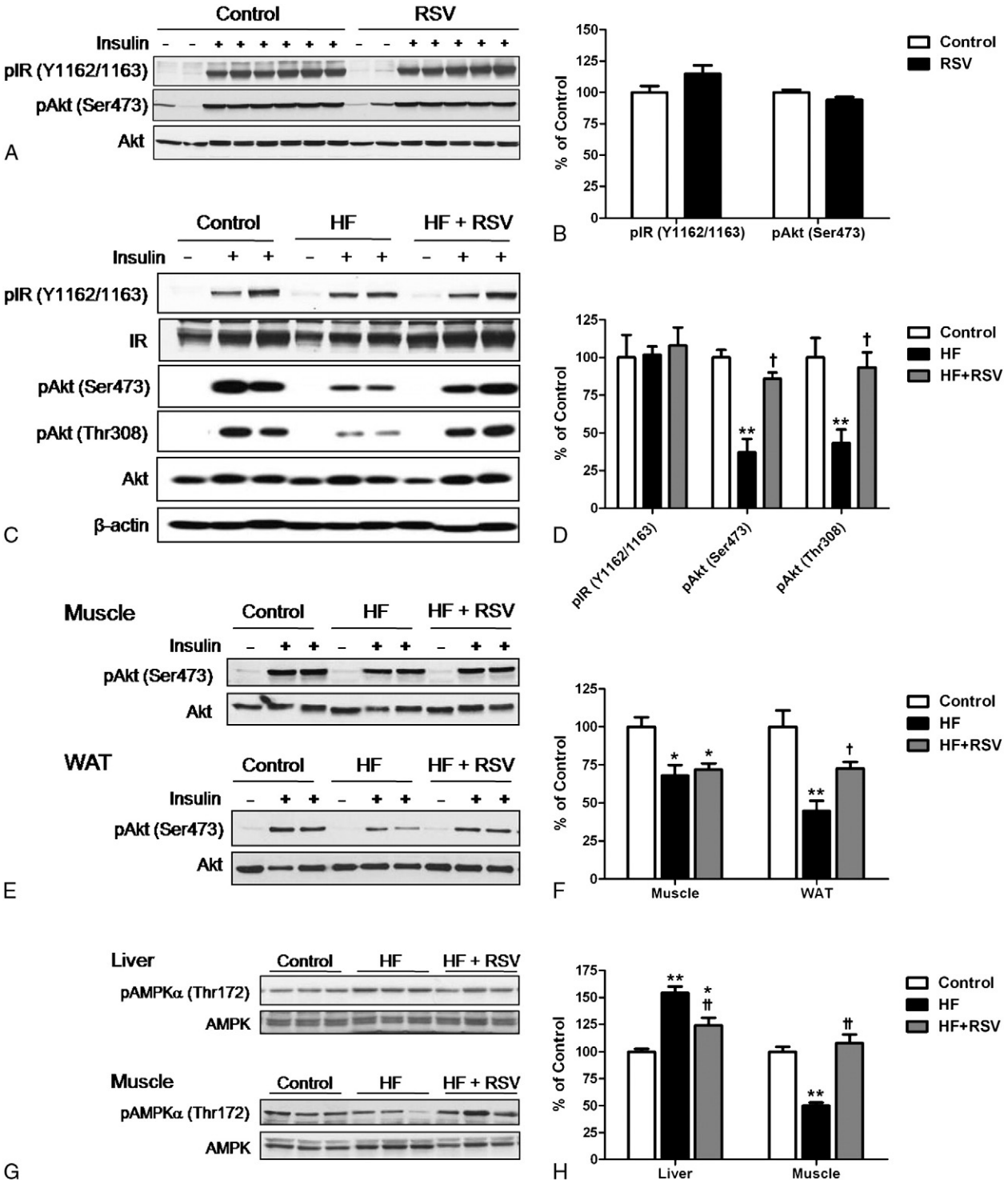

Fig. 4 – Effects of RSV on insulin signaling and AMPK*α* phosphorylation in insulin-sensitive tissues. For insulin signaling studies, after an overnight fast, the experimental mice were injected intraperitoneally with saline or 5 U/kg of human regular insulin. Ten minutes later, the tissues were removed. Tissue lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblot with antibodies as indicated. A and B, Insulin signaling studies performed in the liver of normal chow-fed mice after vehicle or RSV treatment (30 mg/kg daily for 2 weeks). C to F, Insulin signaling studies in the liver (C, D), WAT, and muscle (E, F) of the experimental mice including HF-fed DIO mice. G and H, AMPK*α* phosphorylation (Thr172) was evaluated in the liver and muscle of the experimental mice (n = 5-6 for each group). The immunoblots shown are representative images for each antibody. For each group, n = 2-3 for saline and n = 5-7 for insulin. The bands were quantitated using densitometry. For quantification, phosphoproteins were normalized to the corresponding total proteins. Data are means ± SE. *P < .05; **P < .01 vs insulin-stimulated control mice. †P < .05; ††P < .01 vs insulin-stimulated HF-fed mice.

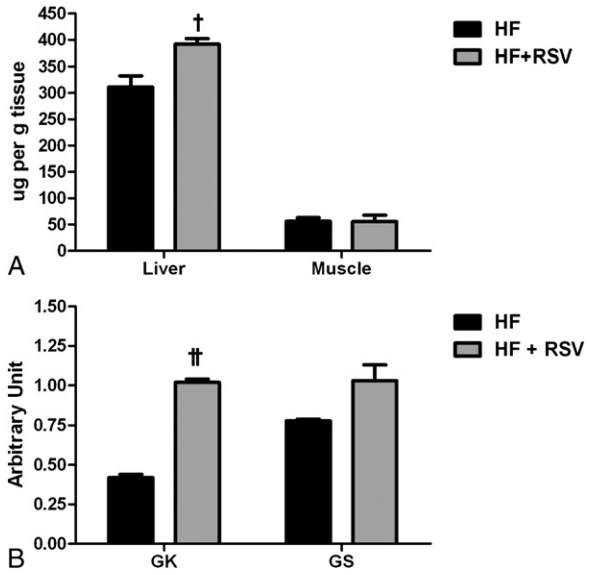

Fig. 5 – Effects of RSV on hepatic and muscular glycogen content and gene expression in the liver of HF-fed mice. A, Hepatic and muscular glycogen content. The experimental mice (n = 3-4 for each group) were killed in the morning after an overnight fast for the determination of glycogen levels in the liver and muscle extracts. B, Real-time PCR was performed with specific primers targeting the genes as indicated. Data are means ± SE. †P < .05; ††P < .01 vs HF-fed mice. GK indicates glucokinase; GS, glycogen synthase.

(P < .001) when the L/CM-cultured adipocytes were treated with RSV (Fig. 2B). The data show that RSV can suppress inflammatory pathways in adipocytes under certain inflammatory stimuli.

3.4. *RSV has a glucose-lowering effect in HF-fed DIO mice*

Previous studies have shown that RSV has antiobesity and insulin-sensitizing effects in murine obesity models with insulin resistance [4,5]. Because changes in body weight caused by high-dose RSV therapy may explain some differences in insulin action between control DIO mice and RSV-treated DIO mice, we treated DIO mice with a relatively low dose of RSV (30 mg/kg of body weight daily).

Compared with the control group, the HF-fed mice showed a significant increase in their body weight after 2 weeks of HF feeding; and the difference was maintained until the end of the study (Fig. 3A, B). However, there was no difference in body weight between the HF and HF + RSV groups throughout the study. These results indicate that RSV at 30 mg/kg of body weight does not affect body weight in HF-fed DIO mice.

Blood glucose and serum insulin in the fasting state were both significantly higher in the HF-fed DIO mice compared with the normal control mice. In addition, the RSV treatment in the HF-fed DIO mice significantly lowered both circulating glucose and insulin levels (Fig. 3C, D). These results indicate that RSV has an insulin-sensitizing effect in HF-fed DIO mice.

3.5. *RSV affects insulin action and AMPKα phosphorylation in a tissue-dependent manner in HF-fed DIO mice*

When we evaluated the effect of RSV on insulin signaling in the liver of normal chow–fed male mice, RSV treatment (30 mg/kg of body weight for 2 weeks) did not change the insulin-induced tyrosine phosphorylation (Y1162/1163) of IR and the insulin-induced Akt phosphorylation (Ser473) (Fig. 4A, B).

Afterward, we evaluated the effects of RSV on insulin action in HF-fed DIO mice. Although the insulin-stimulated

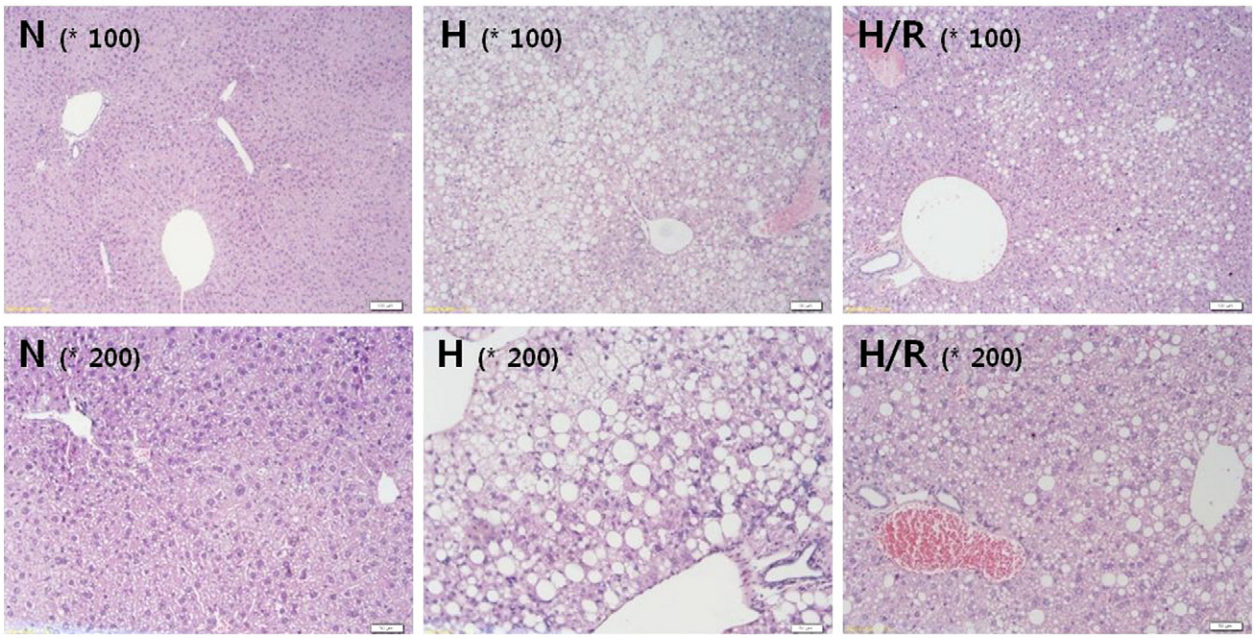

Fig. 6 – Effects of RSV on fatty changes of liver in HF-fed obese mice. At the end of the study (20th week), liver tissue was taken and fixed in 10% formaldehyde solution. Fatty changes were evaluated (hematoxylin-eosin staining, original magnification, ×100 and ×200).

tyrosine phosphorylation level of IR was not different between the experimental groups, the insulin-stimulated Akt phosphorylations (Ser473 and Thr308) in the liver homogenate were significantly decreased in the HF group compared with the control group. However, RSV treatment restored the insulin-stimulated Akt phosphorylations (Ser473 and Thr308) in HF-fed DIO mice (Fig. 4C, D). The insulin-stimulated Akt phosphorylation (Ser473) in WAT showed the same patterns as in the liver of the experimental mice. However, RSV did not improve insulin signaling in the skeletal muscle of HF-fed DIO mice (Fig. 4E, F).

We also investigated the phosphorylation of AMPKα (Thr172) in the skeletal muscle and liver of the experimental mice. The phosphorylated AMPKα (Thr172) was significantly decreased in the muscle of HF-fed DIO mice compared with the control mice. Resveratrol treatment in the DIO mice restored the phosphorylation of AMPKα (Thr172) in the skeletal muscle. As regards the liver, the phosphorylation of AMPKα (Thr172) was increased in the HF-fed DIO mice compared with the control mice. Resveratrol treatment in the HF-fed DIO mice decreased the hepatic AMPKα phosphorylation, but not to the level of the normal control mice (Fig. 4G, H).

3.6. *RSV increases glycogen content in the liver of HF-fed DIO mice*

To determine the effect of RSV on glucose metabolism in the liver of HF-fed DIO mice, hepatic glycogen content and the expression of related genes were analyzed. Resveratrol treatment in HF-fed DIO mice increased glycogen content in the liver, but not in the skeletal muscle (Fig. 5A) We also observed the increased expression of glucokinase gene and a tendency toward an increase in glycogen synthase gene expression in the RSV-treated HF-fed DIO mice compared with the non-treated HF-fed DIO mice (Fig. 5B). However, the hepatic expressions of gluconeogenic genes (glucose-6-phosphatase and phosphoenolpyruvate carboxykinase) were unaltered by RSV therapy in HF-fed DIO mice (data not shown).

3.7. *RSV ameliorates fatty changes in the liver of HF-fed DIO mice*

A histologic study was performed to observe RSV's effects on fatty changes in liver of HF-fed obese mice. As shown in Fig. 6, there were marked fatty changes in the liver isolated from the HF group compared with the control group. After 2 weeks of RSV therapy, the fatty changes were significantly ameliorated in the liver of the RSV-treated HF-fed DIO mice.

4. Discussion

In the present study, we first investigated the effects of RSV on insulin signaling in 3T3-L1 adipocytes under 2 different conditions; a normal state and an insulin-resistant state. Our results showed that RSV has an inhibitory effect on insulin signaling pathways downstream of IRS-1 in 3T3-L1 cells under the usual culture condition that does not provoke insulin resistance. This inhibitory effect of RSV on insulin signaling was also reported for other cell lines under the usual culture condition [12-14,20,21].

It is well established that proinflammatory signaling, mainly involving nuclear factor (NF)–κB pathway, is one of the important causes of insulin resistance in various pathophysiologic conditions. IκBα restricts NF-κB to cytoplasmic localization by forming a complex with it. With inflammatory signals, activated IκB kinase β (IKKβ) phosphorylates IκBα, resulting in its dissociation from NF-κB. The dissociated IκBα undergoes degradation, whereas the free NFκB translocates to the nucleus where it binds to specific promoter elements of target genes, including those encoding for tumor necrosis factor-–α, iNOS, and monocyte chemoattractant protein-1 (MCP-1) [22,23]. This leads to the activation of c-Jun N-terminal kinase (JNK), IKKβ, and other serine kinases. Accordingly, the inhibition of JNK1 or IKKβ prevents insulin resistance in cultured cells and mouse models [24-28]. Among the various molecules that are involved in both inflammation and insulin resistance, iNOS functions as both a downstream effector and an upstream enhancer of inflammation [29].

In the present study, RSV prevented the degradation of IκBα protein and caused a marked suppression of iNOS protein expression in 3T3-L1 adipocytes incubated in the conditioned medium from LPS-treated macrophage. In addition, RSV restored the insulin-stimulated phosphorylation of Akt (Ser473) in 3T3-L1 adipocytes incubated in the conditioned medium. The suppressive effect of RSV on the degradation of IκB is in accordance with a previous report [30]. Other researchers have reported that RSV acts on NF-κB by the inhibition of IKK, which results in the prevention of translocation of NF-κB into the nucleus [31] or through its sirtuin-like activity, which deacetylates NF-κB [11,32]. Some studies also showed that, in stimulated macrophages, the expression of iNOS and the release of nitric oxide are reduced by RSV [10,33,34]. Collectively, these findings suggest that RSV improves insulin signaling in adipocytes under certain insulin-resistant conditions by suppressing inflammatory pathways. Decreased SIRT1 activity has been observed in some tissues from human subjects and animals with insulin resistance [35]. During the revision of the present article, a study reported that RSV affects IRS1 (Ser307)/IRS2 (Thr348) phosphorylation status via inhibition of a protein kinase cascade, including Akt and JNK, in insulin-resistant cells [15]. However, further studies are required to clarify the molecular mechanism by which RSV regulates insulin singling in normal insulin-sensitive cells and in insulin-resistant cells.

We also evaluated the effects of relatively low-dose and short-term RSV therapy in HF-fed DIO mice. With this therapeutic strategy to avoid a secondary effect caused by changes in body weight, we demonstrated that RSV could lower both circulating glucose and insulin levels and enhance insulin signaling in the liver and WAT of this insulin-resistant animal model. In addition, RSV attenuated fatty changes in the liver isolated from the obese mice. Although these metabolic effects induced by RSV treatment seem to be independent of body weight change, there is a report showing that the treatment of obese Zucker rats with RSV at lower dosage (10 mg/kg daily) for 8 weeks decreases serum free fatty acid and abdominal fat content without body weight change

[36]. The limitation of our current study is that we did not measure body fat content and circulating free fatty acid.

Interestingly, our study found that the skeletal muscle of the HF-fed DIO mice showed different responses to the RSV treatment; the insulin-stimulated Akt phosphorylation (Ser473) did not improve, whereas the phosphorylation of AMPKα (Thr172) was restored to normal level. There seems to be some tissue differences in response to RSV [37]. Clear explanation for the different metabolic effects of RSV between the tissues is lacking. The different SIRT1 responses of liver and muscle to calorie restriction suggest different functions of SIRT1 in these tissues [38]. In addition, AMPK isoform activity after a long-term HF diet in mice is different between the 2 tissues [39]. Further studies will be necessary to define the differential functions of SIRT1 according to the tissues and their metabolic status.

In conclusion, our results show that RSV suppresses insulin signaling in adipocytes under insulin-sensitive state, whereas the agent enhances insulin signaling only when the cells are insulin resistant. Furthermore, RSV therapy improves insulin signaling in the liver and WAT, but not in the skeletal muscle, of HF-fed mice. These results suggest that the therapeutic effect of RSV may depend on the metabolic status and the type of tissue being targeted.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (grant number 2011-0010128).

Acknowledgment

The authors thank Minhye Kim for assistance in writing the manuscript.

Conflict of Interest

There are no conflicts of interest.

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