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Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice

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ABSTRACT

Resveratrol is a natural polyphenolic stilbene derivative found in a variety of edible fruits, including nuts, berries, and grape skin. Although resveratrol has been suggested to improve thermogenesis in the brown adipose tissues of obese animals, there have been no reports on the anti-adipogenic and anti-inflammatory effects of resveratrol in the white adipose tissues of obese animals. The primary aim of this study was to investigate whether resveratrol attenuates high-fat diet (HFD)-induced adipogenesis and inflammation in the epididymal fat tissues of mice and to explore the underlying mechanisms involved in this attenuation. In comparison with HFD-fed mice, mice fed with a 0.4% resveratrol-supplemented diet (RSD) showed significantly lower body weight gain (−48%), visceral fat-pad weights (−58%), and plasma levels of triglyceride, FFA, total cholesterol, glucose, tumor necrosis factor (TNF) α, and monocyte chemoattractant protein-1 (MCP1). Resveratrol significantly reversed the HFD-induced up-regulation of galanin-mediated signaling molecules (GalR1/2, PKCδ, Cyc-D, E2F1, and p-ERK) and key adipogenic genes (PPARγ2, C/EBPα, SREBP-1c, FAS, LPL, aP2, and leptin) in the epididymal adipose tissues of mice. Furthermore, resveratrol significantly attenuated the HFD-induced up-regulation of pro-inflammatory cytokines (TNFα, IFNα, IFNβ, and IL-6) and their upstream signaling molecules (TLR2/4, MyD88, Tirap, TRIF, TRAF6, IRF5, p-IRF3, and NF-κB) in the adipose tissues of mice. The results of this study suggest that resveratrol inhibits visceral adipogenesis by suppressing the galanin-mediated adipogenesis signaling cascade. It may also attenuate cytokine production in the adipose tissue by repressing the TLR2- and TLR4-mediated pro-inflammatory signaling cascades in HFD-fed mice.

1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene) is a natural polyphenolic compound belonging to the stilbene class of aromatic phytochemicals; this compound exists in both *cis* and *trans* forms [1]. The *trans*-form, which is present in significant amounts in a variety of edible fruits, including nuts, berries, and grape skin (and red wine derived thereof), has received attention because of its possible role in the prevention of diverse pathologic processes [1–3]. The cardioprotective [4,5], anti-cancer [6,7], anti-oxidant and anti-inflammatory [8] properties of resveratrol are well characterized. A recent study reported that resveratrol has an inhibitory effect on hyperglycemia-induced inflammation in human retinal pigment epithelial cells [9] and inhibits nuclear factor-κB (NF-κB) signaling in the sciatic nerves of rats with streptozotocin-induced diabetes [10].

Resveratrol exerts beneficial effects in the prevention and treatment of non-alcoholic fatty liver disease (NAFLD) through the modulation of lipid metabolism-related genes, such as fatty acid synthase (FAS), peroxisome proliferator-activated receptor alpha (PPARα), peroxisome proliferator-activated receptor gamma (PPARγ), paraoxonase 1 (PON1), type B scavenger receptor (CD36), and silent information regulation 2 homolog 1 (SIRT1), in the liver of mice fed an atherogenic diet [11]. Furthermore, two recent independent studies demonstrated that resveratrol restores glucose homeostasis in the liver [12] and improves mitochondrial function by increasing energy expenditure in the muscle and

Abbreviations: aP2, adipocyte protein 2; C/EBPα, CCAAT/enhancer binding protein alpha; RSD, resveratrol-supplemented diet; Cyc-D, cyclin D; DIO, diet induced obesity; E2F1, E2F transcription factor 1; FAS, fatty acid synthase; FFAs, free fatty acids; GalR1, galanin receptor 1; GalR2, galanin receptor 2; GAPDH, glyceraldehydes 3-phosphate dehydrogenase; HFD, high-fat diet; IFNα, interferon-alpha; IFNβ, interferon-beta; IL-6, interleukin 6; IRF5, interferon regulatory factor 5; LXR, liver X receptor; LPL, lipoprotein lipase; MCP1, monocyte chemoattractant protein-1; MyD88, myeloid differentiation primary response gene 88; ND, normal diet; NF-κB, nuclear factor-kappaB; PKCδ, protein kinase C delta; PPARγ2, peroxisome proliferator-activated receptor gamma 2; SREBP-1c, sterol regulatory element-binding factor 1; Tirap, toll-interleukin 1 receptor (TIR) domain-containing adaptor protein; TLR2, toll like receptor 2; TLR4, toll like receptor 4; TNFα, tumor necrosis factor-alpha; TRAF6, TNF receptor-associated factor 6; TRIF, TIR-domain-containing adapter-inducing interferon-β.

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brown adipose tissue of mice fed a high-fat diet (HFD) [13]. Both studies indicate that such effects may be related to the increased activity of peroxisome proliferative activated receptor gamma coactivator-1α (PGC-1α), which is probably linked to resveratrol-dependent SIRT1 activation.

The study of adipose tissue is central to the understanding of the metabolic abnormalities associated with obesity development [14]. In 3T3-L1 mouse preadipocytes, resveratrol shows inhibitory effects on adipocyte differentiation, which accompanies the down-regulation of several adipocyte-specific genes, such as those encoding PPARγ, FAS, CCAAT/enhancer binding protein-alpha (C/EBPα), sterol regulatory element binding proteins-1c (SREBP-1c), hormone-sensitive lipase (HSL), and lipoprotein lipase (LPL) [15]. White adipose tissue, particularly in the visceral area, is an active endocrine tissue that produces various inflammatory cytokines [16,17]. Dysregulation of the function and production of pro- and anti-inflammatory cytokines in the white adipose tissue of obese individuals may promote obesity-linked metabolic disorders. Some researchers have referred to this inflammatory state as "metaflammation," i.e., metabolically triggered inflammation [18].

Although resveratrol has been suggested to decrease adipocyte differentiation *in vitro*, there have been no reports on the anti-adipogenic effect of resveratrol in tissues of obese animals. Additionally, the protective effect of resveratrol against the HFD-induced activation of inflammatory responses in adipose tissue is unclear. The aim of this study was to investigate whether resveratrol attenuates the HFD-induced adipogenesis and inflammation in the visceral adipose tissue of mice. Possible involvements of the galanin-mediated adipogenic signaling pathway and the TLR2- and TLR4-mediated pro-inflammatory signaling cascades as mechanisms underlying the beneficial effects of resveratrol in the HFD-fed mice were evaluated.

2. Materials and methods

2.1. Animals and diets

Thirty male C57BL/6J mice (age, 9 weeks) were purchased from Orient Bio (Gyeonggi-do, Korea) and conditioned in the animal facility for 1 week. Mice were housed individually in standard plastic rodent cages in animal quarters maintained at 21 ± 2.0 °C and 50 ± 5% relative humidity with a light/dark cycle of 12 h. All the mice had *ad libitum* access to rodent chow and water for 1 week prior to division into the following 3 weight-matched groups (n = 10): normal diet (ND) group, high-fat diet (HFD) group, and 0.4% *trans*-resveratrol-supplemented diet (RSD) group (resveratrol was obtained from Sigma–Aldrich, Steinheim, Germany). The ND was a purified diet based on the AIN-76 rodent diet. The HFD was identical to the ND except for the addition of 200 g of fat/kg (170 g of lard plus 30 g of corn oil) and 1% (w/w) cholesterol. The RSD was identical to the HFD and contained 0.4% resveratrol. Diets were provided in the form of pellets for 10 weeks.

Food intake of the mice was recorded daily, and their body weights were monitored every week during the feeding period. At the end of the experimental period, animals were anesthetized with ether after a 12-h fasting period. To analyze plasma lipid levels, freshly collected blood samples were centrifuged at 2000 × g for 15 min at 4 °C. Livers and visceral fat-pads from 4 different regions (epididymal, perirenal, mesenteric, and retroperitoneal regions) were removed, rinsed with phosphate-buffered saline (PBS), and weighed. The plasma, liver, and visceral fat-pad samples were collected and stored at −70 °C until analysis. All mice were housed in a specific pathogen-free facility of Yonsei University, Seoul, Korea, and this study was approved by the Institutional Animal Care and Use Committee of Yonsei University.

2.2. Histological analysis

The epididymal fat pads were fixed in 10% buffered formalin and embedded in paraffin, cut at thicknesses of 5 μm, and later stained with hematoxylin and eosin (H&E), for the histological examination of fat droplets.

2.3. Biochemical analysis

Plasma concentrations of total cholesterol, triglyceride, free fatty acid (FFA), and glucose were determined enzymatically using commercial kits (Bio-Clinical System, Gyeonggi-do, Korea). Plasma insulin levels were measured through radioimmunoassay (Linco Research, Inc., St. Louis, MO, USA). The homeostasis model assessment of basal insulin resistance (HOMA-IR) was used to calculate an index from the product of the fasting concentrations of plasma glucose (mmol/L) and insulin (pmol/L) divided by 22.5. Lower HOMA-IR values indicated greater insulin sensitivity, whereas higher HOMA-IR values indicated lower insulin sensitivity (insulin resistance). Hepatic lipids were extracted as described by Folch et al. [19], and dried lipid residues were dissolved in 2 ml of ethanol. Cholesterol and triglyceride concentrations in hepatic lipid extracts were measured using the same enzymatic kits (Bio-Clinical System) used for plasma analysis. Plasma levels of tumor necrosis factor-alpha (TNFα) and monocyte chemoattractant protein-1 (MCP1) were measured using ELISA kits (ID Labs, MA, USA).

2.4. RNA extraction and semi-quantitative RT-PCR analysis

Total RNA was isolated from epididymal adipose tissue using Trizol (Invitrogen, CA, USA). Fat contained in tissue homogenates was removed by centrifugation for 10 min at 13,000 × g for 4 °C. 4 μg of the total RNA was reverse-transcribed using the Superscript II kit (Invitrogen), according to the manufacturer's recommendations. The GenBank accession numbers of the relevant templates and the forward (F) and reverse (R) primer sequences are shown in Table 1. Primers to amplify the 530-bp cDNA fragment encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed and used as an internal control. The cDNA served as a template in a 40-μl reaction mixture and was processed using the following protocol: an initial denaturation at 94 °C for 5 min, followed by 30–33 amplification cycles (94 °C for 30 s, 55–60 °C for 45 s, and 72 °C for 1 min) and 72 °C for 10 min. Next, 4 μl of each PCR reaction mixture was mixed with 1 μl of 6-fold-concentrated loading buffer and loaded onto a 2% agarose gel containing ethidium bromide.

2.5. Western blot analysis

Epididymal fat-pads were homogenized at 4 °C in an extraction buffer (100 mM Tris–HCl, pH 7.4, 5 mM EDTA, 50 mM NaCl, 50 mM sodium pyrophosphate, 50 mM NaF, 100 mM orthovanadate, 1% Triton X-100, 1 mM phenylmethanesulphonylfluoride, 2 mg/ml aprotinin, 1 mg/ml pepstatin A, and 1 mg/ml leupeptin). Tissue homogenates were centrifuged (13,000 × g, 20 min, 4 °C), and the resulting supernatants (whole-tissue extracts) were used for Western blot analyses. Protein concentrations were measured using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA). Protein samples (80 μg/lane) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Amersham, Buckinghamshire, UK). The membranes were then probed with

Table 1
Primer sequences and PCR conditions.

Gene description	Primers	Sequences (5′ → 3′)	T_m (°C)	Size (bp)
Adipogenesis related				
Galanin receptor 1 (GalR1)	F	CCAAGGGGGTATCCCAGTAA	55	147
	R	GGCCAAACACTACCAGCGTA		
Galanin receptor 2 (GalR2)	F	ATAGTGGTGGCATGCTGGAA	60	134
	R	AGGCTGGATCGAGGGTTCTA		
Protein kinase C delta (PKCδ)	F	CTGAGCGCTGCAAGAAGAAC	60	146
	R	TGGAAACTTTGCCTCCTCCT		
Cyclin D (Cyc-D)	F	TGGGAAGTTTTGTTGGGTCA	55	144
	R	TCCTTGTCCAGGTAATGCCA		
E2F transcription factor 1 (E2F1)	F	CCTCGCAGATCGTCATCATC	55	102
	R	AGAGCAGCACGTCAGAATCG		
Peroxisome proliferator-activated receptor gamma (PPARγ2)	F	TTCGGAATCAGCTCTGTGGA	55	148
	R	CCATTGGGTCAGCTCTTGTG		
CCAAT/enhancer binding protein alpha (C/EBPα)	F	TCGGTGCGTCTAAGATGAGG	55	187
	R	TCAAGGCACATTTTTGCTCC		
Sterol regulatory element binding transcription factor 1 (SREBP1c)	F	ATCGCAAACAAGCTGACCTG	55	115
	R	AGATCCAGGTTTGAGGTGGG		
Liver X receptor (LXR)	F	TCCTACACGAGGATCAAGCG	55	119
	R	AGTCGCAATGCAAAGACCTG		
Fatty acid synthase (FAS)	F	TTGCCCGAGTCAGAGAACC	55	171
	R	CGTCCACAATAGCTTCATAGC		
Lipoprotein lipase (LPL)	F	TGCCGCTGTTTTGTTTTACC	55	172
	R	TCACAGTTTCTGCTCCCAGC		
Fatty acid binding protein (aP2)	F	AGCATCATAACCCTAGATGG	55	128
	R	GAAGTCACGCCTTTCATAAC		
Leptin	F	CTCCAAGGTTGTCCAGGGTT	55	143
	R	AAAACTCCCCACAGAATGGG		
Inflammation-related				
Toll like receptor 2 (TLR2)	F	GAGCATCCGAATTGCATCAC	55	120
	R	TATGGCCACCAAGATCCAGA		
Toll like receptor 4 (TLR4)	F	TCGAATCCTGAGCAAACAGC	55	199
	R	CCCGGTAAGGTCCATGCTAT		
Myeloid differentiation primary response gene 88 (MyD88)	F	AAGAAAGTGAGTCTCCCCTC	55	149
	R	TCCCATGAAACCTCTAACAC		
TIR-domain-containing adaptor protein (Tirap)	F	GTGGCCGCTGGAGCAAAGAC	55	370
	R	TTGCCTCTGCCATCCACATA		
TNF receptor-associated factor 6 (TRAF6)	F	GCACAAGTGCCCAGTTGACAATGA	55	479
	R	AGTGTCGTGCCAAGTGATTCCTCT		
TIR-domain-containing adapter-inducing interferon-β (TRIF)	F	ATGGATAACCCAGGGCCT T	55	528
	R	TTCTGGTCACTGCAGGGGAT		
Interferon regulatory factor 5 (IRF5)	F	AATACCCCACCACCTTTTGA	55	191
	R	TTGAGATCCGGGTTTGAGAT		
NF-κB (p50)	F	CGCCAAAGTATAAGGATGTC	55	104
	R	GTAGAGAAAGGGTTTCGGTT		
NF-κB (p65)	F	AGCACAGATACCACCAAGAC	55	158
	R	TCAGCCTCATAGTAGCCATC		
TNF alpha (TNFα)	F	TGTCTCAGCCTCTTCTCATT	55	156
	R	AGATGATCTGAGTGTGAGGG		
Interferon alpha (IFNα)	F	ATGGCTAG(G/A)CTCTGTGCTTTCCT	55	500
	R	GGGCTCTCCAGA(T/C)TTCTGCTCTG		
Interferon beta (IFNβ)	F	TGGAGCAGCTGAATGGAAAG	55	122
	R	GAGCATCTCTTGGATGGCAA		
Interleukin 6 (IL-6)	F	ATGAAGTTCCTCTCTGCAAGAGACT	55	638
	R	CACTAGGTTTGCCGAGTAGATCTC		
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F	AGAACATCATCCCTGCATCC	55	321
	R	TCCACCACCCTGTTGCTGTA		

a 1:1000 dilution of primary antibody. Antibodies to the following proteins were purchased from the indicated sources: ERK, p-ERK (Thr202/Tyr204), and p-IRF3 (Ser396) were obtained from Cell Signaling Technology (Cell signaling, Beverly, MA, USA); IRF3 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). After incubation with the corresponding secondary antibody (Santa Cruz), signals were detected using a chemiluminescent detection system (Amersham) and quantified using Quantity One analysis software (Bio-Rad).

2.6. Statistical analysis

Data on the body weight gain, visceral fat-pad and liver weights, and plasma and hepatic biochemistries are presented as means ± SEM of 10 mice. RT-PCR and Western blot data are presented as means ± SEM of the results of triplicate analyses of RNA and protein samples, respectively, which were pooled from 10 mice per group. Data were analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple-range tests. Statistical significance was defined as $p < 0.05$. Statistical analysis of the data was performed using SPSS statistical software (Version 12.0).

3. Results

3.1. Body and visceral fat-pad weights

The HFD increased the body weight in mice, but supplementing the HFD with 400 mg resveratrol/kg diet suppressed the body weight gain (−48%, $p < 0.05$) (Fig. 1A) without affecting food intake (Fig. 1B). The food efficiency ratio in the RSD group was

significantly lower (−51%) than that in the HFD group (Fig. 1C). The relative weights of the epididymal (−57%), mesenteric (−56%), perirenal (−56%), and retroperitoneal (−62%) fat-pads in RSD mice were significantly lower than those in the HFD mice (Fig. 1D). The histological analysis of epididymal adipose tissue sections by H&E staining also showed smaller adipocytes in RSD-fed mice than in HFD-fed mice (Fig. 1E).

3.2. Plasma and hepatic biochemistry

Mice that received resveratrol showed significantly lower plasma concentrations of triglyceride (−23%), FFA (−25%), total cholesterol (−20%), TNFα (−41%), and MCP1 (−56%) in comparison with the values for HFD control mice. Resveratrol significantly reversed the HFD-induced elevations in plasma glucose (−17%)

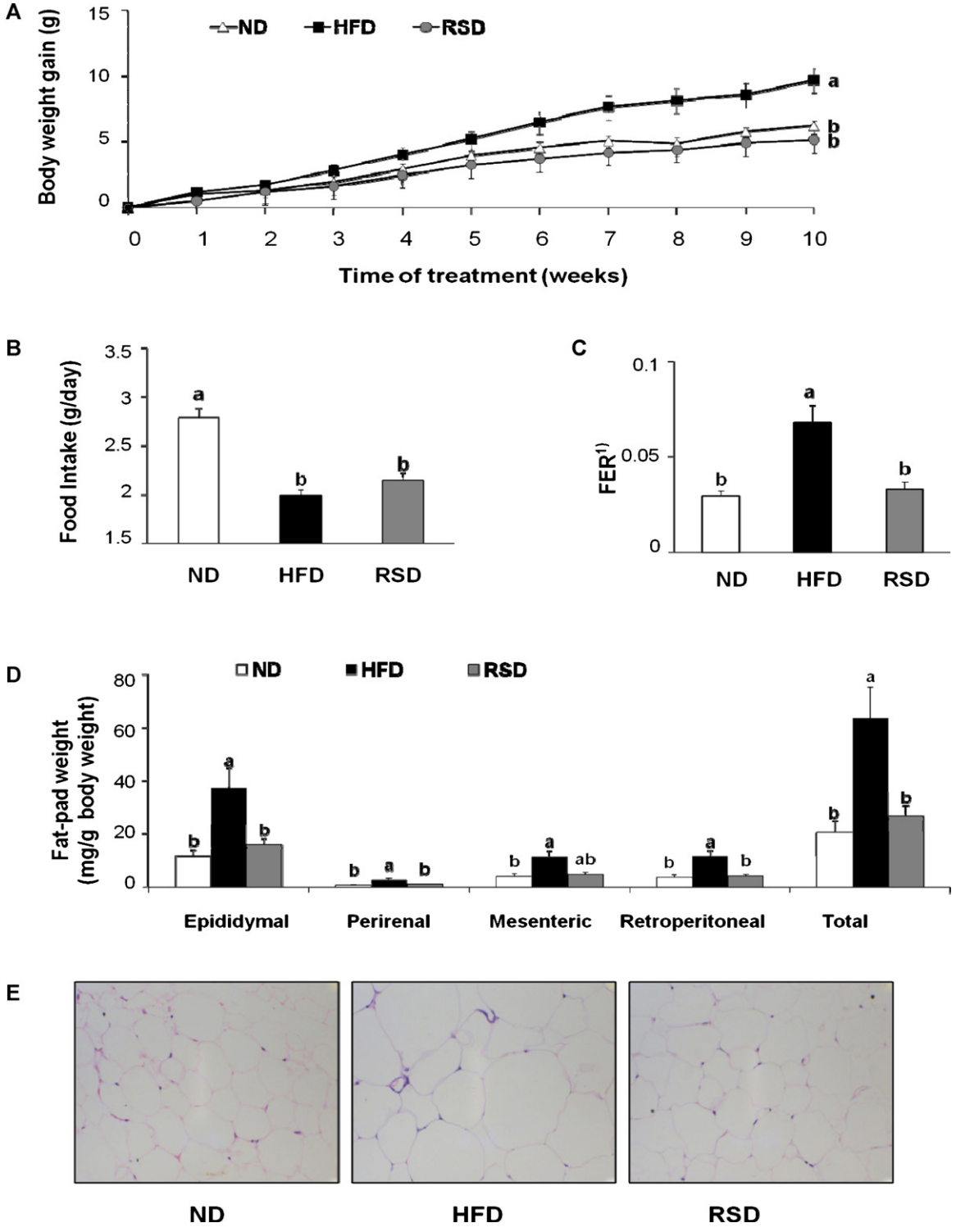

Fig. 1. Effects of resveratrol on body weight gain, food intake, FER[1)], visceral fat-pad weights, and histology of epididymal adipose tissue of mice fed a HFD. Animals were sacrificed after 10 weeks of ND and HFD consumption with or without 0.4% resveratrol (RSD or HFD, respectively). (A) Changes in body weight gain (g), (B) food intake, (C) FER, and (D) visceral fat-pad weights in each group are shown. (E) Representative histological images of the epididymal adipose tissue as assessed by H&E staining; magnification, 200×. Values are expressed as mean ± SEM ($n = 10$). Means without a common letter differ, $p < 0.05$. [1)] FER (food efficiency ratio) $= \frac{\text{Body weight gain for the experimental period (g)}}{\text{Food intake for the experimental period (g)}}$.

Table 2
Plasma and hepatic biochemistries of mice fed experimental diets.

Groups	ND	HFD	RSD
Plasma			
Triglyceride (mmol/L)	0.60 ± 0.05^{b}	0.84 ± 0.04^{a}	0.65 ± 0.04^{b}
Free fatty acid (mEq/L)	657 ± 26.5^{b}	999 ± 31.8^{a}	752 ± 38.9^{b}
Total cholesterol (mmol/L)	2.22 ± 0.12^{c}	3.86 ± 0.11^{a}	3.07 ± 0.18^{b}
Glucose (mmol/L)	3.79 ± 0.40^{c}	6.82 ± 0.35^{a}	5.62 ± 0.42^{b}
Insulin (pmol/L)	107 ± 6.54^{b}	187 ± 8.12^{a}	111 ± 7.90^{b}
HOMA-IR	17.9 ± 0.81^{b}	56.7 ± 2.77^{a}	27.9 ± 2.04^{b}
TNFα (pg/ml)	38.3 ± 4.87^{c}	105.8 ± 5.75^{a}	62.2 ± 5.34^{b}
MCP1 (pg/ml)	16.3 ± 2.29^{c}	80.5 ± 5.20^{a}	35.3 ± 5.22^{b}
Liver			
Liver weight (g/100 g body weight)	3.60 ± 0.10^{c}	5.00 ± 0.30^{a}	4.40 ± 0.20^{b}
Triglyceride (mg/g liver)	12.4 ± 0.99^{c}	31.4 ± 1.53^{a}	19.2 ± 0.66^{b}
Cholesterol (μmol/g liver)	36.3 ± 6.54^{c}	86.1 ± 7.25^{a}	62.2 ± 8.28^{b}

Values are expressed as mean ± SEM ($n = 10$). Values within a row without a common letter differ significantly, $p < 0.05$.
ND, normal diet group; HFD, high-fat diet group; RSD, 0.4% resveratrol-supplemented diet group.

and insulin (−40%) levels. The insulin sensitivity was increased significantly by resveratrol supplementation as indicated by 51% reduction in HOMA-IR. Similarly, resveratrol reversed the detrimental effects of HFD feeding on the hepatic accumulation of triglyceride (−39%) and cholesterol (−28%) ($p < 0.05$). The relative weight of the liver in RSD-fed mice was also significantly lower than that in HFD-fed mice (Table 2).

3.3. Expression of adipogenesis-related signaling molecules

We explored the potential mechanisms by which resveratrol may attenuate the HFD-induced activation of adipogenesis in the epididymal adipose tissue of mice. The mRNA levels of GalR1, GalR2, PKCδ, Cyc-D, and E2F1 in RSD mice were significantly lower than those in HFD mice (Fig. 2A). The mRNA levels of several adipogenic transcription factors, including PPARγ2, C/EBPα, SREBP-1c, and liver X receptor (LXR), in the visceral adipose tissue of RSD-fed mice were significantly lower than those in the HFD control mice. Additionally, the mRNA levels of key adipogenic target genes, including FAS, LPL, adipocyte protein 2 (aP2), and leptin, in RSD-fed mice were significantly down-regulated in comparison with their levels in HFD-fed mice (Fig. 2B). Results from the Western blot analysis of proteins isolated from epididymal adipose tissue confirmed that ERK phosphorylation (Thr202/Tyr204) in RSD-fed mice was significantly down-regulated in comparison with that in HFD-fed mice (Fig. 2C).

3.4. Expression of inflammation-related signaling molecules

Next, we tested whether resveratrol can attenuate HFD-induced activation of toll-like receptor (TLR)-mediated pro-inflammatory signaling in the epididymal adipose tissue of mice. The mRNA levels of TLR2 and TLR4 in RSD-fed mice were lower than those in HFD control mice ($p < 0.05$). Moreover, mRNA expression of myeloid differentiation primary response gene 88 (MyD88)-dependent adaptor molecules, such as MyD88, toll-interleukin 1 receptor domain containing adaptor protein (Tirap), and TNF receptor-associated factor 6 (TRAF6), in RSD-fed mice were down-regulated in comparison with their expression in HFD-fed mice ($p < 0.05$). Similarly, the mRNA level of a MyD88-independent adaptor molecule, TIR-domain-containing adapter-inducing interferon-1 β (TRIF), was also significantly lower in RSD mice than in HFD mice (Fig. 3A). As shown in Fig. 3B, the mRNA levels of pro-inflammatory transcription factors, such as interferon regulatory factor 5 (IRF5) and NF-κB (p50 and p65), in the epididymal adipose tissue of RDS-fed mice were significantly lower than the levels in HFD-fed mice. Furthermore, expression of the target cytokine genes, including those expressing TNFα, interferon-alpha (IFNα), interferon-beta (IFNβ), and interleukin 6 (IL-6), in the RSD mice were down-regulated in comparison with their expressions in the HFD mice ($p < 0.05$). Immunoblot results indicate that the phosphorylated IFN regulatory factor-3 (IRF3) level in the epididymal adipose tissue of RSD-fed mice was significantly lower than that in HFD-fed mice (Fig. 3C).

4. Discussion

The resveratrol dosage (0.4%) given to mice in this study was chosen on the basis of 2 previous reports: Lagouge et al. indicated that 0.4% resveratrol supplementation for 15 weeks significantly decreases body weight gain and visceral fat-pad weights in HFD-fed mice [13]. In contrast, supplementation of 0.04% resveratrol to the HFD for 6 months did not lead to a significant reduction in body weight but it improved health and survival of mice [12]. The daily resveratrol intake of the mice in our study (approximately 400 mg/kg body weight) was equivalent to an intake of approximately 32.4 mg/kg human body weight (1944 mg/60 kg person) when calculated on the basis of normalization to body surface area under the recommendations of the U.S. Food and Drug Administration (http://www.fda.gov/cder/cancer/animalframe.htm) and Reagan-Shaw et al. [20] when an extrapolation of animal dose to human dose is performed. Additionally, daily doses of commercial dietary supplements range from 50 to as high as 2000 mg *trans*-resveratrol (0.8–33 mg/kg body weight) for a 60-kg human [21–25].

In the present study, mice fed a diet supplemented with 0.4% resveratrol for 10 weeks showed significantly lower body weight gain (−48%) and visceral fat-pad weights (−58%) than the HFD-fed mice. These beneficial effects of resveratrol on body and visceral fat-pad weights were not because of decreased food intake, as the amount of food consumed per mouse was unchanged. The magnitudes of body weight gain and visceral fat-pad weight attenuations induced by 0.4% resveratrol supplementation were greater in the mice in our study than in the animals raised by Lagouge et al. (body weight gain: −48% vs. −30%, visceral fat-pad weight: −58% vs. −20%) despite the fact that the mice in the latter study were maintained on experimental diets for a longer period (10 weeks vs. 15 weeks) [13]. We further observed that supplementation with 0.4% resveratrol resulted in a significant reduction in plasma and hepatic triglyceride and cholesterol levels. These beneficial resveratrol-induced changes in the plasma and hepatic lipid profiles correlate with the findings of a previous report by Rivera et al., in which resveratrol (10 mg/kg body weight; administered for 8 weeks) was reported to be effective in reducing plasma levels of triglycerides, FFA, and cholesterol, and hepatic triglyceride levels in obese Zucker rats [26], however, this contrasts with the results of two independent studies on mice with diet-induced obesity [12,13].

Galanin is a neuropeptide that plays a role in food intake regulation by acting on the central nervous system in mammals [27,28]. Circulating serum levels of galanin are elevated in obese individuals relative to lean individuals, suggesting that elevated peripheral galanin levels may contribute to the development of obesity and obesity-associated impairments [29–31]. The possible association of the galanin-mediated signaling pathways in the manifestation of the HFD-induced activation of adipogenesis has been suggested in the epididymal adipose tissues of mice [32]. Prolonged consumption of a HFD leads to increased expression of galanin and its receptors, along with increased expression and/or activation of downstream molecules related to adipogenesis, such as PKCδ, Cyc-D, E2F1, and p-ERK, and key adipogenic transcription factors, such as PPARγ2 and its target gene, aP2, in epididymal

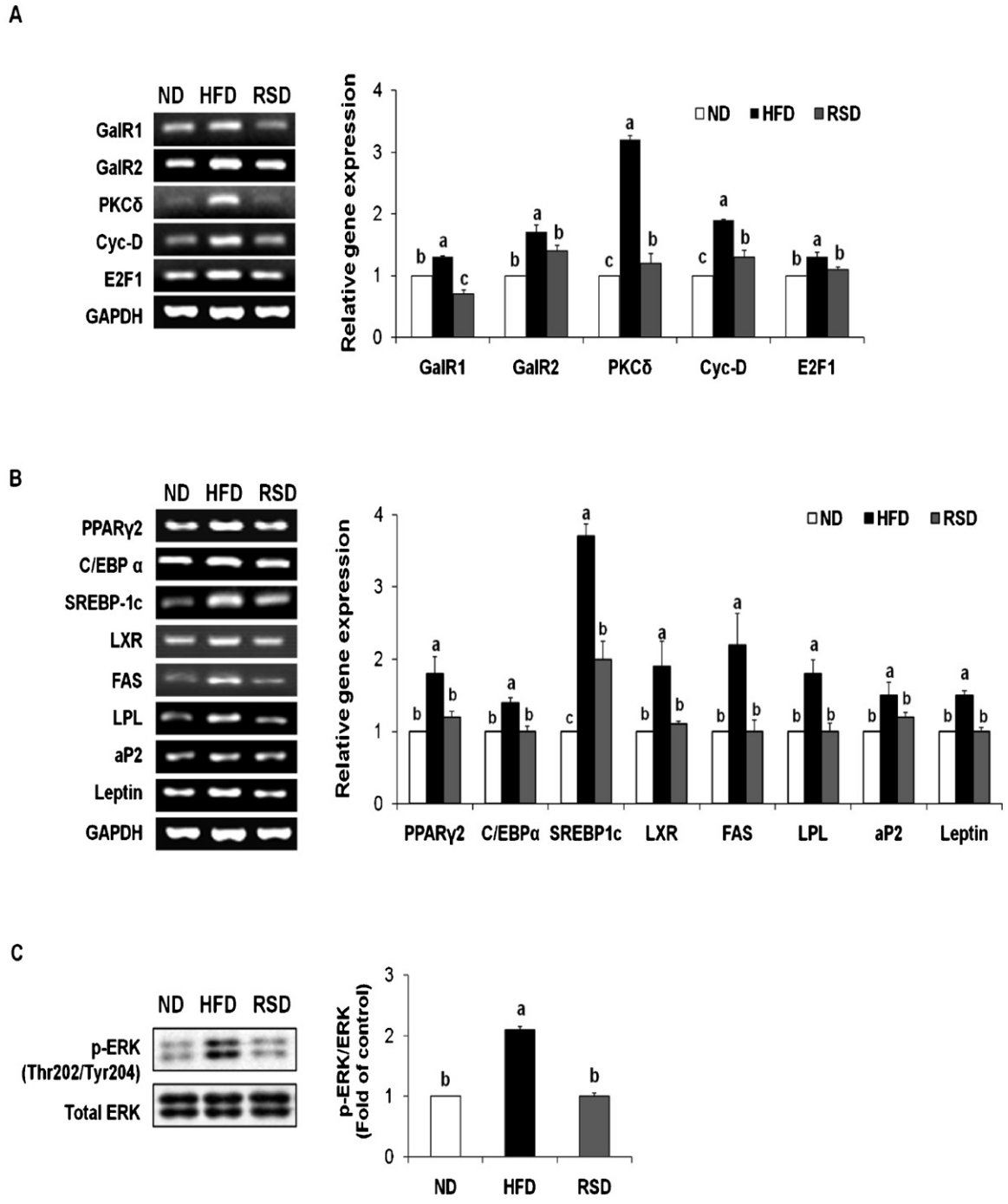

Fig. 2. RT-PCR (A and B) and Western blot (C) analyses of epididymal adipose tissues from the ND, HFD, and RSD groups showing the expression of molecules involved in galanin-mediated adipogenesis signaling. A representative image from 3 independent experiments of is shown in the left portion of the figure. The right portion of the figure shows the results of densitometric analysis of (A) upstream and (B) downstream molecules of galanin-mediated signaling. Data were normalized to the GAPDH mRNA levels and compared to ND group measurements, which were assigned a value of 1.0. (C) Protein levels of p-ERK were determined by Western blotting. The levels of p-ERK were normalized to those of total ERK. Each bar represents the mean ± SEM of 3 independent experiments of the RNA or protein samples pooled from 10 mice per group. Means without a common letter differ, $p < 0.05$.

adipose tissues of mice. The activation of ERK signaling promotes adipogenesis by enhancing the PPARγ2 and C/EBPα gene expressions during the differentiation of 3T3-L1 preadipocytes [33]. Another current study in the literature states that Cyc-D/E2F1 pathway lies downstream the mitogenically activated RAS/RAF/MEK/ERK cascade [34]. In the current study, resveratrol significantly reversed HFD-induced up-regulation of galanin-mediated adipogenic signaling molecules (GalR1, GalR2, PKCδ, Cyc-D, E2F1, and p-ERK) in the adipose tissues of mice (Fig. 2A). Furthermore, resveratrol significantly suppressed the expression of adipogenic transcription factors (PPARγ2, C/EBPα, SREBP-1c, and LXR) and their target genes (FAS, LPL, aP2, and leptin) in adipose tissues (Fig. 2B), which may have contributed to the lower visceral adiposity and weight gain in HFD-fed mice (Fig. 4).

Toll-like receptors (TLRs) are involved in the innate immune response, and their stimulation typically leads to the activation of pro-inflammatory processes in response to microbial pathogens [35]. In previous studies, TLR2 and TLR4 have been reported to

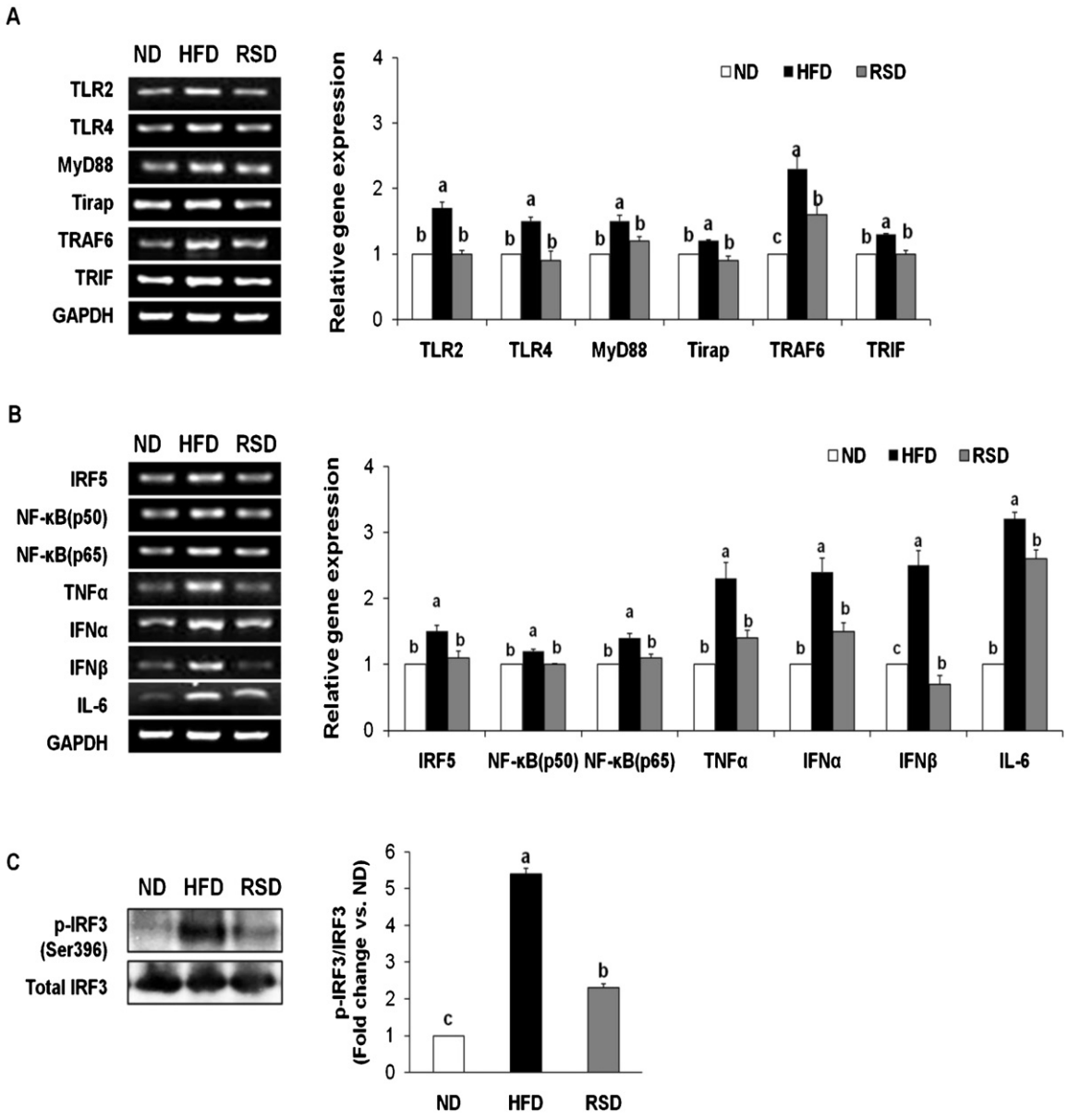

Fig. 3. RT-PCR (A and B) and Western blot (C) analyses of the expression of TLR-mediated signaling molecules of epididymal adipose tissues from ND, HFD, and RSD groups. A representative image from 3 independent experiments is shown in the left portion of the figure. The right portion of the figure shows the results of densitometric analysis of (A) upstream and (B) downstream molecules of TLR-mediated signaling. Data were normalized to GAPDH mRNA and compared to ND group measurements, which were assigned a value of 1.0. (C) Protein levels of p-IRF3 were determined by Western blotting. Levels of p-IRF3 were normalized to that of total IRF3. Each bar represents the mean ± SEM of 3 independent experiments of the RNA or protein samples pooled from 10 mice per group. Means without a common letter differ, $p < 0.05$.

recognize select host lipids and play important roles in the pathogenesis of noninfectious, inflammatory diseases of host deregulation, such as obesity [36–38]. Signaling through MyD88 by TLR2 or TLR4 results in NF-κB activation, which is mediated by TRAF6 and leads to the expression of pro-inflammatory cytokines such as TNFα and IL-6 [39]. Alternatively, signaling through TRIF by TLR4 induces the phosphorylation of the IRF3, which results in the expression of IFNα and IFNβ [39] (Fig. 4). Our results demonstrate that resveratrol significantly attenuates the HFD-induced up-regulation of pro-inflammatory cytokines such as TNFα, IFNα, IFNβ, and IL-6, and of their upstream signaling molecules, including TLR2, TLR4, MyD88, Tirap, TRIF, TRAF6, IRF5, p-IRF3, and NF-κB in epididymal adipose tissues of mice (Figs. 3B and 4). These decreased mRNA levels of pro-inflammatory cytokines in adipose tissues appear to be related to the lower levels of plasma TNFα and MCP1 in RSD mice (Table 2). In the present study, the plasma concentration of FFA, known as a ligand of TLR2 and TLR4 [40,41], was prominently decreased by resveratrol. These results together suggest that resveratrol may improve obesity-induced inflammation by both down-regulating TLR2 and TLR4 expression in epididymal adipose tissue and lowering plasma FFA level in mice maintained on a HFD.

In conclusion, the results of this study demonstrate that resveratrol may inhibit visceral adipogenesis through suppression of the galanin-mediated adipogenesis signaling cascade in mice fed a HFD, and it may also attenuate cytokine production in adipose tissue by repressing TLR2- and TLR4-mediated pro-inflammatory signaling cascades. These results reveal novel insights into resveratrol action in the epididymal adipose tissue of mice and add to the potential of this dietary polyphenol in the control of obesity and metaflammation.

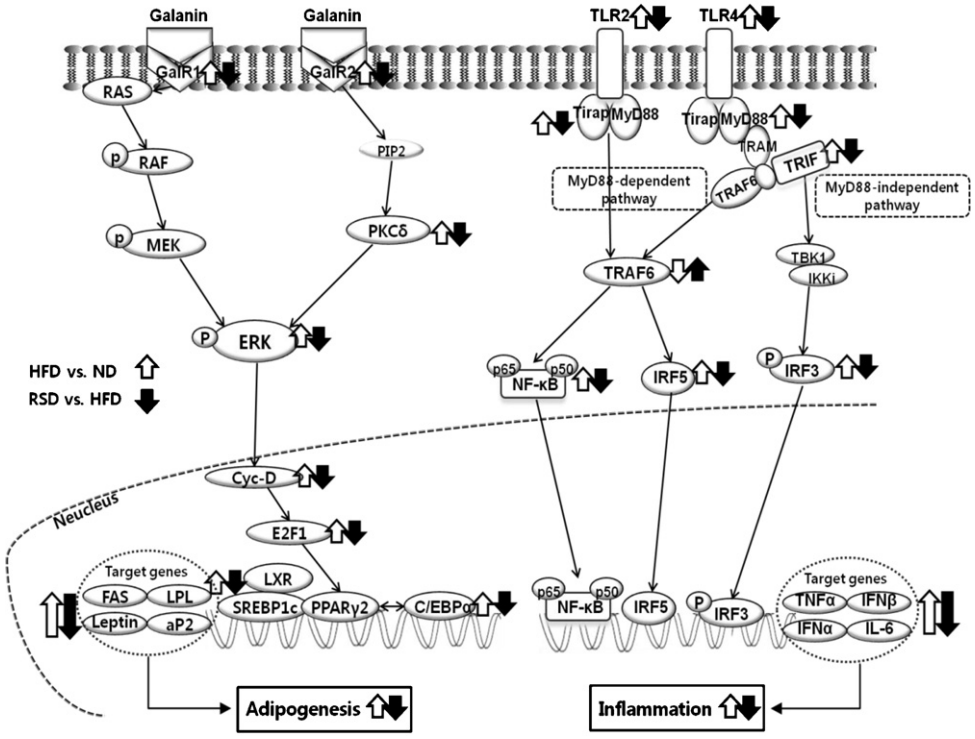

Fig. 4. The possible molecular mechanisms of dietary resveratrol in attenuating adipogenesis and inflammation induced by HFD. Dietary resveratrol reverses the HFD-induced changes in the GalR1, GalR2, PKCδ, and p-ERK protein expressions, along with the subsequent changes in the Cyc-D and E2F1 expressions, which are implicated in the galanin-mediated adipogenesis cascades, in the epididymal adipose tissue. The downstream adipogenic transcription factors (PPARγ2, C/EBPα, SREBP-1c, and LXR) and their target genes (FAS, LPL, aP2, and leptin) were also suppressed by resveratrol in the adipose tissues of HFD-fed mice. TLR4 uses MyD88-dependent and MyD88-independent pathways, whereas TLR2 signals only in the MyD88-dependent manner. The MyD88-dependent pathway uses TRAF6 and IRF5, leading to its nuclear translocation and co-operation with NF-κB. The MyD88-independent pathway uses TRIF in activating NF-κB in either a TRAF6-dependent or a TRAF6-independent mechanism. TRIF associates with TBK1 and IKKi, which in turn leads p-IRF3. Resveratrol reverses the HFD-induced changes in the expression of TLR2, TLR4, and downstream molecules (MyD88, Tirap, TRIF, TRAF6, IRF5, p-IRF3, and NF-κB), along with the subsequent changes in the cytokines (TNFα, IFNα, IFNβ, and IL-6), which are implicated in the TLR2/4-mediated pro-inflammatory signaling cascades, in the epididymal adipose tissue.

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References

[1] Fremont L. Biological effects of resveratrol. Life Sci 2000;66:663–73.
[2] Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997;275:218–20.
[3] Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 2006;5:493–506.
[4] Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res 2000;47:549–55.
[5] Das S, Alagappan VK, Bagchi D, Sharma HS, Maulik N, Das DK. Coordinated induction of iNOS-VEGF-KDR-eNOS after resveratrol consumption: a potential mechanism for resveratrol preconditioning of the heart. Vascul Pharmacol 2005;42:281–9.
[6] Atten MJ, Attar BM, Milson T, Holian O. Resveratrol-induced inactivation of human gastric adenocarcinoma cells through a protein kinase C-mediated mechanism. Biochem Pharmacol 2001;62:1423–32.
[7] El-Mowafy AM, Alkhalaf M. Resveratrol activates adenylyl-cyclase in human breast cancer cells: a novel, estrogen receptor-independent cytostatic mechanism. Carcinogenesis 2003;24:869–73.
[8] de la Lastra CA, Villegas I. Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochem Soc Trans 2007;35:1156–60.
[9] Losso JN, Truax RE, Richard G. Trans-resveratrol inhibits hyperglycemia-induced inflammation and connexin downregulation in retinal pigment epithelial cells. J Agric Food Chem 2010;58:8246–52.
[10] Kumar A, Sharma SS. NF-kappaB inhibitory action of resveratrol: a probable mechanism of neuroprotection in experimental diabetic neuropathy. Biochem Biophys Res Commun 2010;394:360–5.
[11] 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.
[12] Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006;444:337–42.
[13] 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.
[14] Vazquez-Vela ME, Torres N, Tovar AR. White adipose tissue as endocrine organ and its role in obesity. Arch Med Res 2008;39:715–28.
[15] Rayalam S, Yang JY, Ambati S, Della-Fera MA, Baile CA. Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. Phytother Res 2008;22:1367–71.
[16] Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006;17:4–12.
[17] Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6:772–83.
[18] Torres-Leal FL, Fonseca-Alaniz MH, Rogero MM, Tirapegui J. The role of inflamed adipose tissue in the insulin resistance. Cell Biochem Funct 2010;28:623–31.
[19] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509.
[20] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659–61.
[21] Harborne JB, Baxter H, Moss GP. 2163: Resveratrol. In: Phtochemical dictionary. Philadephia, PA: Taylor and Francis; 1999. p. 568.
[22] Greens G. In: Greens G, editor. Resveratrox dietary supplement (Product label). Fairfield; 2007.
[23] RevGenetics. Resveratrol. Take it. Live it. Belive it; 2007, http://wwww.rev-genetics.com/ [accessed 29.12.10].
[24] Inc SN. Resveratrol dietary supplement; 2007, http://www.gnc.com/family/index.jsp?categoryId=3972302&cp=3593190 [accessed 29.12.10].

[25] Williams LD, Burdock GA, Edwards JA, Beck M, Bausch J. Safety studies conducted on high-purity trans-resveratrol in experimental animals. Food Chem Toxicol 2009;47:2170–82.
[26] Rivera L, Moron 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.
[27] Bartfai T, Hokfelt T, Langel U. Galanin – a neuroendocrine peptide. Crit Rev Neurobiol 1993;7:229–74.
[28] Crawley JN. The role of galanin in feeding behavior. Neuropeptides 1999;33:369–75.
[29] Baranowska B, Radzikowska M, Wasilewska-Dziubinska E, Kaplinski A, Roguski K, Plonowski A, et al. leptin, galanin and insulin in women with polycystic ovary syndrome. Gynecol Endocrinol 1999;13:344–51.
[30] Baranowska B, Radzikowska M, Wasilewska-Dziubinska E, Roguski K, Borowiec M. Disturbed release of gastrointestinal peptides in anorexia nervosa and in obesity. Diabetes Obes Metab 2000;2:99–103.
[31] Baranowska B, Wasilewska-Dziubinska E, Radzikowska M, Plonowski A, Roguski K, Neuropeptide Y. Galanin, and leptin release in obese women and in women with anorexia nervosa. Metabolism 1997;46:1384–9.
[32] Kim A, Park T. Diet-induced obesity regulates the galanin-mediated signaling cascade in the adipose tissue of mice. Mol Nutr Food Res 2010;54:1361–70.
[33] Prusty D, Park BH, Davis KE, Farmer SR. Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARgamma) and C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem 2002;277:46226–32.
[34] Korotayev K, Chaussepied M, Ginsberg D. ERK activation is regulated by E2F1 and is essential for E2F1-induced S phase entry. Cell Signal 2008;20:1221–6.
[35] O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 2008;226:10–8.
[36] Fessler MB, Rudel LL, Brown JM. Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome. Curr Opin Lipidol 2009;20:379–85.
[37] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006;116:3015–25.
[38] Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. Biochem Biophys Res Commun 2006;346:739–45.
[39] Huang Q, Pope RM. Toll-like receptor signaling: a potential link among rheumatoid arthritis, systemic lupus, and atherosclerosis. J Leukoc Biol 2010;88:253–62.
[40] Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 2001;276:16683–9.
[41] Lee JY, Zhao L, Youn HS, Weatherill AR, Tapping R, Feng L, et al. Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. J Biol Chem 2004;279:16971–9.