

Effect of resveratrol on resistin and apelin gene expressions in adipose tissue of diabetic rats

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Background/aim: Adipose tissue plays a major role in glucose homeostasis. Dietary antioxidants such as resveratrol (RSV) may offer some protection against the early stage of diabetes mellitus and the development of complications. The present study investigated the effects of RSV on biochemical parameters and resistin and apelin gene expressions in adipose tissue of rats with type 2 diabetes.

Materials and methods: Diabetes was induced using a single dose of streptozotocin + nicotinamide. Three groups of diabetic rats were treated with different doses of RSV (1, 5, and 10 mg/kg body weight per day). Oxidative status, serum biochemical parameters, insulin, and HOMA index were measured. Finally, resistin and apelin gene expressions were determined in rats' adipose tissue using RT-PCR (qRT-PCR).

Results: A significant reduction in serum glucose level was observed in rats treated with 5 and 10 mg/kg per day RSV compared with the diabetic control. Resistin expression in adipose tissue was reduced in RSV-treated groups, while no significant changes were observed in apelin expression.

Conclusion: The results showed that RSV improves insulin sensitivity due to a simultaneous decrease in blood glucose and an increase in insulin. We concluded that RSV has potential hypoglycemic effect, probably by increasing insulin levels and changing the expression of resistin.

Key word: Apelin, diabetes mellitus, gene, resveratrol, resistin

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by increasing blood glucose and perturbation in the regulation of carbohydrate, protein, and lipid metabolisms (1,2). During the last 2 decades, prevalence of diabetes has increased, probably due to obesity, low physical activity, changes in life style in developed countries, and population aging (3).

Given that some herbal derivatives are capable of influencing DM, their use can serve as a therapeutic strategy. Resveratrol (RSV) ($C_{14}H_{12}O_3$) with 228 g/mol molecular weight and 253–255 °C melting temperature) is one of these compounds (4). It is found in different herbal species such as red grapes and raspberry; hence, it exists in normal human diets (5).

RSV reduces NF- κ B activity, but increases the activity of SIRT-1 deacetylase (6). On the other hand, cancers and chronic disorders are dependent on NF- κ B activation. This

chronic situation leads to muscular dystrophy and insulin resistance (7). Moreover, an increase in SIRT-1 activity reduces blood glucose levels, increases the consumption of glucose by muscles, adipose tissue, and the liver, and increases glycogen synthesis in the liver.

Some proteins secreted by adipose tissue (adipocytokine) that are essential for insulin production from pancreatic cells and consequently contribute to improvement of DM symptoms are characterized. Some studies have shown changes in the expression level or function of these proteins in response to supplements or natural compounds such as RSV that may play an important role in the pathology of insulin resistance and diabetes (8,9).

Resistin and apelin are two of these proteins with 114 and 77 amino acids, respectively. Some studies showed that the expression of resistin gene, called Retn, decreased during fasting (10,11) and increased after feeding (11). It

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was also shown that resistin gene expression in cell culture decreased following the addition of RSV in adipocytes (12). Therefore, it can be concluded that RSV could strongly affect insulin sensitivity (12) and consequently affect resistin expression, since expression of resistin in adipose tissues is strongly regulated by insulin. It is especially important as resistin plasma concentration is high in obese and hyperinsulinemic humans and mice (13).

It has been reported that in the absence of insulin, mRNA of apelin reduces in adipocytes (14), and, as stated in previous studies, apelin increase in adipose tissue prevents lipid aggregation and ROS production in adipocytes (15). Since apelin regulates appetite, it may influence pathogenesis of insulin resistance, DM, and obesity (16,17).

Oxidative stress is a characteristic of DM. It is demonstrated that RSV is a compound with three antioxidant properties (18); it competes with Q coenzyme and reduces oxidative complex, traps constructed ROS in aerobic conditions, and prevents peroxidation of lipids.

Since apelin and resistin may play a role in the reduction of diabetic symptoms, especially in reducing insulin resistance, and increasing blood glucose, the effect of compounds that may influence their expression could be useful in preventing DM or employing therapeutic strategies to treat it. Thus, the aim of the present study was to investigate the effects of RSV on the expression of these adipokine genes.

The present study particularly investigated the effects of RSV on the expression of resistin and apelin, as adipocytokines, in an animal model of type 2 diabetes. Furthermore, its effects on biochemical, antioxidant, and oxidant parameters in the serum of diabetic rats were studied.

2. Materials and methods

2.1. Animals and diabetes induction

Male Wistar rats (5–8 weeks old, weighing 180–250 g, $n = 50$) were purchased from Razi Institute (Iran). The protocol of the study was approved by the Ethical Committee of Hamadan University of Medical Sciences, Hamadan, Iran. The animals were housed under controlled temperature (30 ± 2 °C) and humidity condition ($60 \pm 5\%$) in polypropylene cages under 12-h light and dark cycles in the Central Animal House, Hamadan University of Medical Sciences, Hamadan, Iran. They all received a standard pellet diet and water. The rats were maintained for 7 days to adjust the conditions before starting the experiments. Diabetes induction was performed using streptozocin (STZ, 60 mg/kg body weight) and nicotine amide (NA, 120 mg/kg body weight). Using a glucometer, the researchers measured blood glucose after 7 days of

injection to confirm diabetes in rats. The animal groups in this study included control (healthy, common diet), diabetic control, and three diabetic groups that received 1, 5, and 10 mg/kg per day of RSV for 30 days. At the end of treatment there were eight rats in each group; the rats were fasted for 24 h, anesthetized using 50 mg/kg ketamine, and finally sacrificed. An abdominal incision was made and blood was collected from the jugular vein. Serum was separated and stored at -20 °C and adipose tissues were washed with normal saline and stored at -80 °C until biochemical and gene expression (real-time PCR) analyses were performed.

2.2. Analyzing the biochemical parameters

Total antioxidant capacity (TAC) in serum samples was determined using a ferric reducing antioxidant power assay (FRAP) (19). Malondialdehyde (MDA), a marker for lipid peroxidation, was determined using the thiobarbituric acid fluorometric method (20). Moreover, total oxidant status (TOS) was determined by measuring ferric ions oxidized in the presence of various oxidants, using xylenol orange (21).

Using an immunoassay kit (Alpco, USA), serum insulin was assayed. HOMA, an insulin resistance measure, was calculated using the formula $[\text{insulin } (\mu\text{U/mL}) \times \text{glucose (mmol/L)}] / 22.5$ (22). Serum glucose, cholesterol (Chol), triglycerides (TAG), and high density lipoprotein cholesterol (HDL-C) were determined using a Pars Azmun kit (Iran), and low density lipoprotein cholesterol (LDL-C) was calculated using the formula $[\text{Chol} - (\text{TG}/5 + \text{HDL-C})]$ (23). Serum thiol (SH) groups were assessed using DTNB (2,2-dithionitrobenzoic acid) colorimetric method.

2.3. RNA analysis and qRT-PCR

Using TRIzol, total RNA was extracted from adipose tissue. Quantity and quality of extracted RNA were determined using a NanoDrop (BioTek, Epoch, USA) and 1% agarose gel electrophoresis, respectively. Amplification of resistin, apelin, and 18s rRNA genes with 137, 107, and 151 bp lengths was confirmed by running 5 μL of PCR products on 1% agarose gel electrophoresis.

A Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (K1622) was utilized to synthesize cDNA, using a random hexamer primer. Quantitative real-time PCR analysis was performed on this cDNA using the SYBR Green method (SYBR Premix Ex Taq 2 Kit, TakaRa No. RR820L) on a BioRad system. The designed primers for resistin (NM_144741.1) and apelin (NM_031612.3) amplification and their properties are listed in Table 1. The temperatures and times of reaction are presented in Table 2. Finally, 1% gel electrophoresis was used in order to confirm cDNA amplification with proper size.

Cycle threshold (Ct) was also determined for the studied genes in all experimental groups. Finally, the

Table 1. Primers used in the amplification of resistin, apelin, and 18s rRNA genes.

Gene	Primer	Sequence of primer	Primer length	GC%	Tm	Product length
Resistin	F	GACGGTTGATTGAGAACTGA	20	45	51.7	137
	R	TTGTGTATTTCCAGACCCTC	20	45	51.1	
Apelin	F	TCCTCTTGATGATGTCCTTTC	21	42.9	61.3	107
	R	TCCTCTTGATGATGTCCTTTC	21	45	62.6	
18S rRNA	F	GTAACGCGTTGAACCCATT	20	54.8	64.5	151
	R	CCATCCAATCGGTAGTAGCG	20	54.4	64.2	

F: Forward primer, R: Reverse primer, Tm: Melting temperature.

Table 2. Temperatures and times of the qRT-PCR reaction.

Real-time steps	Temperature	Duration
Initial activation	95 °C	30 s
40 cycles of:		
Denaturation	95 °C	5 s
Annealing	optimized annealing temperature	Depending on the gene
Extension	72 °C	30 s
Data acquisition	72 °C–95 °C	0.5 °C/0.05 s

researchers compared the relative differences in gene expression (using 18s rRNA as a reference gene) among the studied groups by calculating $\Delta\Delta Ct$ and $2^{-\Delta\Delta Ct}$ as described by Livak et al. (24).

2.4. Statistical analysis

The results are presented as means \pm SD. The obtained data between different groups were compared using one-way ANOVA followed by Tukey's test in SPSS (v. 16).

3. Results

3.1. Effects of RSV on biochemical parameters

The results of the study indicated that the level of the serum glucose increased significantly, while insulin levels decreased in diabetic rats (Table 3). Moreover, a significant reduction in serum glucose levels was observed in rats treated with 5 and 10 mg/kg per day RSV (but not

1 mg/kg per day) compared with the diabetic and control groups (Table 3). Moreover, HOMA, a marker for insulin resistance, decreased in rats treated with 5 and 10 mg/kg per day RSV.

Furthermore, a significant reduction in TAG was observed in diabetic rats treated with low and high doses of RSV compared with the control group (Table 4, $P < 0.05$). Other lipid parameters did not change significantly (Table 4).

As shown in Table 5, a significant reduction in TAC, thiol group (SH), and uric acid, and an increase in oxidant parameters (MDA and TOS) were observed in diabetic rats. TAC levels increased in diabetic rats treated with all different doses of RSV, while SH and uric acid increased in groups treated with 5 and 10 mg/kg RSV. Treatment with 5 and 10 mg/kg RSV caused a reduction in MDA and

Table 3. Serum glucose and insulin levels and HOMA index in experimental groups.

Factor	Cont	Dia	Dia + RSV 1 (mg/kg)	Dia + RSV 5 (mg/kg)	Dia + RSV10 (mg/kg)
Glucose (mg/dL)	92.0 \pm 10.8	303.3 \pm 92.1 ^a	271.37 \pm 77.55 ^b	192.50 \pm 84.80 ^b	190.33 \pm 68.63 ^b
Insulin (μ U/dL)	11.17 \pm 1.06	7.23 \pm 1.15 ^a	8.13 \pm 0.97	9.52 \pm 1.05 ^b	9.83 \pm 0.86 ^b
HOMA	2.51 \pm 0.37	5.46 \pm 2.09 ^a	5.52 \pm 1.92	3.73 \pm 1.91 ^b	4.77 \pm 1.84 ^b

a: $P < 0.001$ compared with the control group (Cont), b: $P < 0.001$ compared with the diabetic group (Dia).

Table 4. Serum lipid parameters in experimental groups.

Factor	Cont	Dia	Dia + Rsv 1 (mg/kg)	Dia + Rsv 5 (mg/kg)	Dia + Rsv 10 (mg/kg)
TAG (mg/dL)	129.71 ± 28.47	91.00 ± 21.30	67.62 ± 29.61 ^a	108.28 ± 51.93	81.78 ± 26.43 ^a
Chol (mg/dL)	175.86 ± 9.80	177.50 ± 10.71	173.37 ± 13.66	165.43 ± 15.35	181.33 ± 18.67
HDL-C (mg/dL)	44.14 ± 6.72	48.87 ± 8.35	46.37 ± 7.09	40.57 ± 9.58	48.33 ± 5.33
LDL-C (mg/dL)	105.77 ± 9.97	110.42 ± 8.16	113.47 ± 9.21	103.20 ± 12.98	116.64 ± 10.32

Cont: control group, Dia: diabetic rats via STZ and NA, Dia + RSV 1, 5, and 10: diabetic groups received 1, 5, and 10 mg/kg Resveratrol, TAG: Triglyceride, Chol: Cholesterol.

^a P < 0.05 Compared with the control group (Cont), ^b P < 0.05 Compared with the diabetic group (Dia).

Table 5. Levels of antioxidant and oxidant parameters in experimental groups.

Parameter	Cont	Dia	Dia + Rsv 1 (mg/kg)	Dia + Rsv 5 (mg/kg)	Dia + Rsv 10 (mg/kg)
TAC (mmol/mL)	0.22 ± 0.02	0.12 ± 0.01 ^{a*}	0.15 ± 0.01	0.18 ± 0.02 ^{b*}	0.19 ± 0.04 ^{b*}
SH (mmol/mL)	0.38 ± 0.06	0.25 ± 0.04 ^{a*}	0.30 ± 0.06	0.33 ± 0.04 ^{b*}	0.33 ± 0.07 ^{b*}
MDA (µm/L)	0.48 ± 0.12	1.24 ± 0.19 ^{a*}	1.21 ± 0.18	0.85 ± 0.13 ^{b**}	0.69 ± 0.12 ^{b**}
TOS (mmol/mL)	1.60 ± 0.14	1.95 ± 0.18 ^{aΔ}	1.8 ± 0.17	1.53 ± 0.18 ^{bΔ}	1.44 ± 0.22 ^{b*}
Uric acid (mg/dL)	2.10 ± 0.78	1.13 ± 0.28 ^{aΔ}	1.22 ± 0.50	1.24 ± 0.28 ^{bΔ}	1.34 ± 0.52 ^{bΔ}

Cont: control group, Dia: diabetic rats via STZ and NA, Dia+RSV 1, 5, and 10: diabetic groups received 1, 5, and 10 mg/kg Resveratrol, TAC: Total antioxidant capacity, SH: thiol group, MDA: Malondialdehyde, TOS: Total oxidant status.

a: compared with the control group (Cont), b: compared with the diabetic group (Dia), Δ : P < 0.05, * P < 0.01 ** P < 0.001.

TOS levels (Table 5). The effects of RSV on antioxidant and oxidant parameters are summarized in Table 5.

The body weight of rats significantly decreased 7 days after diabetes induction (P < 0.05). However, in comparison with the untreated diabetic group, body weight of the treated group increased after 30 days of treatment with RSV (P < 0.01).

3.2. Apelin and resistin gene expressions

The result of gel electrophoresis (1% agarose) confirmed amplification of the genes with correct sizes (Figure). Moreover, the results of ΔΔCt and 2^{-ΔΔCt} showed significant changes in resistin gene expression in all studied groups, while no significant changes were observed in the expression of apelin (Tables 6 and 7). An increase in resistin gene expression was also observed in the diabetic group compared with the normal control group (lower ΔCt, P < 0.001) (Table 6). Resistin expression decreased in all RSV-treated diabetic groups compared with the untreated diabetic group (higher ΔCt, P < 0.01) (Table 6).

Moreover, compared with the untreated diabetic group, changes in resistin expression in diabetic rats that received 1, 5, and 10 mg/kg RSV were 0.72-, 0.46-, and 0.33-fold, respectively, indicating a reduction in resistin expression by increasing the RSV dose (Table 7).

In the case of apelin expression, however, it was found that apelin expression increased in the diabetic group compared with the healthy control group, though the difference was not significant (Table 6). Moreover,

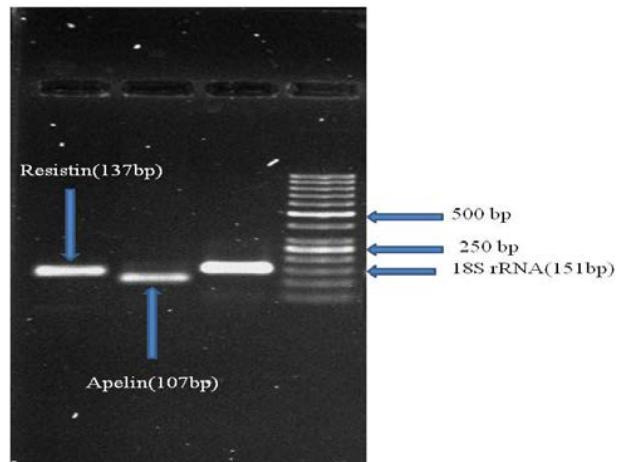


Figure. Amplification of resistin, apelin, and 18s rRNA with 137, 107, and 151 bp length was confirmed by running 5 µL of PCR products on 1% agarose gel electrophoresis.

Table 6. Mean \pm SD of \square Ct of studied genes in the healthy control group (n = 8), diabetic control group (n = 8), and RSV-treated diabetic rats (1, 5, and 10 mg/kg, n = 8).

Groups	Cont	Dia	Dia + Rsv 1	Dia + Rsv 5	Dia + Rsv 10
Resistin	5.33 \pm 0.73	1.59 \pm 1.10 ^a	2.07 \pm 1.11 ^b	2.71 \pm 0.51 ^b	3.15 \pm 0.68 ^b
Apelin	4.40 \pm 0.99	3.08 \pm 1.04	3.88 \pm 1.75	3.86 \pm 1.31	3.70 \pm 0.93

a: P < 0.001 compared with the control group (Cont), b: P < 0.01 compared with the diabetic group (Dia),

treatment with RSV did not change apelin expression significantly (Tables 6 and 7).

4. Discussion

RSV, an herbal component with antioxidant and inflammation properties, could prevent cancers and is capable of influencing DM (25,26). Considering the results of resistin expression and changes in blood glucose, insulin levels, and HOMA index, it is likely that RSV improved insulin resistance and consequently reduced DM symptoms.

A review of the related literature reveals that the effect of RSV on adipocytokines in diabetic models or cells is controversial. In 2011, Mercader et al. (27) showed that RSV decreased resistin gene expression by influencing oxidation pathways and lipogenesis in cultured 3T3-L1 cells. In another study, it was revealed that RSV can inhibit resistin and some other adipogenesis-involved genes (28), while another study suggested that RSV can increase resistin gene expression in cultured cells derived from adipose tissue (29).

Although no significant changes were observed in apelin expression levels in the present study (P = 0.419), in 2013 it was shown that RSV could increase expression of apelin in preadipocytes (30).

Weight gain is considered an important risk factor for diabetes and cardiovascular disease. Some studies have shown that RSV had no effect on body weight in animal models (rats) (31–34), while in the present study weight gain was observed after 30 days in all groups treated with RSV (P < 0.001).

Increase in blood glucose and disturbance in the regulation of carbohydrate metabolism are other DM characteristics caused by defects in insulin production and secretion in beta pancreatic cells or resistance of cells to insulin, or both (1). A reduction in blood glucose, glucose consumption by muscles, adipose tissue, and liver, and glycogen synthesis in liver have been reported as the results of treatment with RSV (35).

In 2006, Su et al. (36) observed 25% reduction in plasma glucose levels in rats treated with RSV compared with rats treated with exercise after 14 days.

A significant increase in sensitivity to insulin (P < 0.001) and decrease in blood glucose levels and plasma insulin level

(P < 0.001) have been shown in a study by Rivera et al. (37) on Zucker punch rats.

In another study, significant decrease in blood glucose, but not improvement in insulin resistance, was observed in elderly people with impaired glucose tolerance (38), which is in line with the result observed by Yoshino et al. (39) in women with normal glucose tolerance.

In the present study, a significant decrease in blood glucose levels and an increase in serum insulin near the normal level were observed in RSV-treated animals compared with untreated diabetic rats (P < 0.001). HOMA, an insulin resistance criterion, decreased in rats that received 5 and 10 mg/kg per day RSV, while it did not change significantly in rats that received 1 mg/kg per day dose.

In some studies, reduction in TAG was observed in Zucker punch rats that received RSV (37,38), while other studies have shown no significant changes in TAG and other lipid parameter in rats (32), or nonobese women with normal glucose tolerance (39). In the present study, significant changes (P < 0.05) in TAG levels were found in diabetic rats that received 1 and 10, but not 5 mg/kg per day RSV, while other lipid parameters (Chol, HDL-C, and LDL-C) did not change.

Increases in MDA, an indicator of oxidative stress, have been observed in patients with DM in various studies (40,41). MDA reduction by RSV was also found in other studies (42–45). In the current study, significant increases in antioxidant parameters (such as TAC, SH, and uric acid) and decreases in oxidant parameters (such as MDA and TOS) were observed in rats that received different doses of RSV.

Table 7. Fold change ($2^{-\Delta\Delta C_t}$) of resistin and apelin genes expression in the studied groups.

Groups	Resistin	Apelin
Diabetic/Healthy	13.36	2.5
Diabetic-RSV 1 mg/kg	0.72	0.57
Diabetic-RSV 5 mg/kg	0.46	0.58
Diabetic-RSV 10 mg/kg	0.33	0.65

To clarify the RSV antidiabetic mechanism in more detail, the researchers of the present study have recently demonstrated that RSV decreases visfatin and vaspin the other adipokine gene expressions in diabetic rats' adipose tissue (46). In another report, the authors of the current study showed that SNARE protein expression levels significantly decreased in diabetic rats, and it was found that treatment with RSV supplementation was associated with increased expression of these proteins (47).

In conclusion, the present study found that RSV decreased glucose and increased insulin levels, and these changes improved insulin resistance in DM. Moreover,

RSV increased antioxidant parameters and decreased oxidants. It can be implied that the reduction in resistin gene expression by RSV can be a mechanism for the antidiabetic effect of this compound. Finally, it should be noted that, in spite of the results found in this and other studies, further studies in diabetic patients are required to reveal more details regarding the discussed issues.

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