Effects of Resveratrol Supplementation and Exercise on Apoptosis, Lipid Profile, and Expression of Farnesoid X Receptor, Liver X Receptor and Sirtuin 1 Genes in the Liver of Type 1 Diabetic Rats

ABSTRACT

Background and objectives: Diabetes mellitus is the most common metabolic disorder in the world. Here, we evaluated effects of resveratrol supplementation alone and combined with exercise on blood glucose, insulin, lipid profile, apoptosis biomarkers, and expression of farnesoid X receptor (Fxr), liver X receptor (Lxr), and sirtuin 1 (Sirt 1) genes in the liver of type 1 diabetic rats.

Methods: Streptozotocin was used to induce type 1 diabetes in Wistar rats. The rats were randomly assigned into seven groups. After treatment with resveratrol alone or combined with exercise training, the animals were sacrificed and lipid profile and levels of blood glucose and insulin were measured. Hepatocyte apoptosis was assessed by measuring the level of Bax and Bcl2 proteins using enzyme-linked immunosorbent assay kits. Expression of Fxr, Lxr, and Sirt1 was evaluated using real-time polymerase chain reaction. Comparison of the mean levels of all variables between different groups was performed using one-way analysis of variance, at statistical significance level of 0.05.

Results: Resveratrol significantly reduced the level of blood glucose and insulin compared with the control groups (p<0.001). It also significantly affected the lipid profile (p<0.001). Diabetes was significantly associated with decreased expression of Sirt1, Lxr, and Fxr and increased hepatocyte apoptosis. Resveratrol significantly improved the expression of all three genes (p<0.01). Overall, resveratrol supplementation combined with exercise was more effective than other methods.

Conclusion: The results indicate that that combination of resveratrol therapy with exercise could be beneficial for diabetic patients. However, more studies are needed to confirm this finding.

Keywords: Diabetes Mellitus, Resveratrol, Exercise, Apoptosis, Sirtuin 1, Liver X Receptors.
INTRODUCTION
Diabetes mellitus, which is characterized with insulin resistance and chronic hyperglycemia, is a leading cause of mortality worldwide (1). In addition to hyperglycemia, diabetes may be associated with hyperlipidemia, hypertension, ketoacidosis, cardiovascular disease, and nonalcoholic fatty liver disease (NAFLD) (2).

In this regard, numerous studies have focused on molecular mechanisms of diabetes pathogenesis and its therapeutic strategies. Several risk factors such as obesity or overweight, impaired glucose tolerance, genetics, age, sedentary lifestyle, and ethnicity are thought to be responsible for development of diabetes (3).

Previous studies have demonstrated that diabetes mellitus can be associated with changes in expression of different genes, especially farnesoid X receptor (Fxr), liver X receptor (Lxr), and sirtuin 1 (Sirt1) (4). The Fxr gene codes for a ligand-activated transcription factor that is highly expressed in the liver and regulates genes related to lipoprotein and lipid metabolism. Increased serum level of cholesterol and triglyceride was reported in Fxr-deficient mice (5). The Sirt1 gene encodes for a NAD+-dependent deacetylase that regulates a wide variety of metabolic pathways, such as glucose-lipid metabolism, insulin secretion, oxidative stress, inflammation, and apoptosis (4, 6). Recent studies have demonstrated that Sirt1 plays an important role in glucose homeostasis and insulin sensitivity (4). The Lxr gene is considered as a sensor of cholesterol metabolism and lipid biosynthesis. Similar to Fxr and Sirt1, Lxr is involved in different cell-signaling pathways and regulates hepatic glucose production, inflammation, and lipogenesis (7).

Resveratrol (RES) is a natural antioxidant, which can be extracted from different plants such as grape and peanuts (8). Previous research reported protective effects of RES on the liver and cardiovascular system (9). Some studies reported its anti-apoptotic, anti-inflammatory, and anti-oxidative effects (10-12). More recently, a study has reported that RES improves liver function in rats with NAFLD by increasing the expression of hepatic Sirt1, Fxr, and Lxr (13). Therefore, RES can be considered as a therapeutic candidate for metabolic disorders, such as diabetes (14). Also, a recent study has shown that continuous and interval exercises can improve expression of Sirt1, Fxr, and Lxr genes in the liver of rats with NAFLD (13, 15).

Given the critical role of the Sirt1, Fxr, and Lxr genes in lipid and glucose metabolism, we assumed that RES supplementation alone or combined with interval and continuous exercises may exert beneficial effect on diabetic patients by improving Sirt1, Fxr and Lxr expression. This study was designed to evaluate effects of RES alone and combined with exercise on blood glucose and insulin levels, lipid profile, apoptosis biomarkers, and expression of Fxr, Lxr and Sirt1 in the liver of diabetic rats.

MATERIALS AND METHODS
Animals and diabetes induction
Wistar rats (age range: 40-45 weeks) weighting 250-300 g were obtained from laboratory animal research center at Islamic Azad University of Sari (Sari, Iran). The animals were housed in individual cages in a controlled environment, with access to standard laboratory food and water ad libitum. For the induction of diabetes, 50 mg/kg of streptozotocin (ZellBio, Germany) were injected intravenously to 49 rats (16). Streptozotocin treatment induces type 1 diabetes by ablation of beta cells. Two days after the injection, blood samples were taken to determine glucose concentration, and a glucose level of >250 mg/dl along with polyuria confirmed induction of diabetes. Diabetic rats were then randomly divided into seven experimental groups based on treatment regimens (seven animals per group): patient, saline, RES, continuous training (MIT), interval training (HIT), MIT+RES, and HIT+RES. Animals in the RES groups received 25 mg/kg RES daily by intraperitoneal injection (13).

Training program
Before the intervention, animals in the exercise groups were adapted with a rodent treadmill for 5 days (at speed of 10 m/min and 0% inclination for 5 min/day) (17, 18). The interval and continuous exercise programs were done according to a previous study (13).

Samples collection and measurements
Two days after the final exercise training, the animals were anesthetized with ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg). The liver
was removed and homogenized in phosphate buffer saline (pH 7.0) at 4 °C using a homogenizer (Hielscher, Germany). Next, the mixture was centrifuged at 12,000 rpm and 4 °C for 15 minutes (19). Supernatant was collected for Bax and Bcl2 measurement. Blood samples were collected from the abdominal aorta for measurement of blood insulin, glucose, and lipid profile. Blood insulin level was measured using the rat insulin enzyme-linked immunosorbent assay (ELISA) kit (ZellBio, Germany). Blood glucose, triglyceride, cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using an autoanalyzer apparatus (Parspars Azmoon Co., Iran).

**Bcl2 and Bax measurements**

The amount of Bcl2 and Bax proteins in the liver of all animals were measured using commercial ELISA kits (ZellBio, Germany). The Bax ELISA kit had a sensitivity of 1.95 pg/ml and detection range of 7.8-500 pg/ml, at detection wavelength of 450 nm. The Bcl2 ELISA kit had a sensitivity of 7.8 pg/ml and detection range of 31.2-2000 pg/ml, at detection wavelength of 450 nm.

**Real-time polymerase chain reaction (PCR)**

For real-time PCR assay, total RNA was extracted from the homogenized liver tissues using the RNX-Plus kit (SinaClon, Iran). Next, cDNA was synthesized using the Revert Aid Reverse Transcriptase kit (Thermo science, Germany). A Rotor Gene 6000 thermocycler (Corbett Research, Australia) and Real Q-PCR 29 Master Mix kit (Amplicon, Denmark) were utilized for amplifications in 40 cycles. Each reaction solution included 5 μl master mix and 100 nM of each primer. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene.

The obtained mRNA levels were normalized relative to the amount of GAPDH mRNA. Relative expression of studied genes was calculated using the \(2^{-\Delta\Delta Ct}\) method. Table 1 shows the sequences of the primers used in the real-time PCR assay.

### Table 1- Sequences of the primers used in the real-time PCR assay.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fxr</td>
<td>5/-AGTTGAGGAATTTGAGTGG-3/</td>
<td>5/-GATTGTGTATGGGAGTA-3/</td>
</tr>
<tr>
<td>Lxr</td>
<td>5/-CTGATTTCTCCTGGTCTCTGGTG-3/</td>
<td>5/-CACCCCTACCTTGGACTCTCT-3/</td>
</tr>
<tr>
<td>Sirt1</td>
<td>5/-GAGTTGTTGTAGTGGTGG-3/</td>
<td>5/-AAATTAGAGGTTGGTGGG-3/</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5/-AGTTCACCGCCACAGTCAAGG-3/</td>
<td>5/-CATACCTGACACGACATACC-3/</td>
</tr>
</tbody>
</table>

### Statistical analysis

Data were analyzed using SPSS software (version 19). All data are presented as means ± standard deviation (SD). Comparison of the mean of all variables between groups was performed using one-way analysis of variance (ANOVA) with post-hoc Tukey. A p-value of less than 0.05 was considered statistically significant.

### RESULTS

The mean levels of blood glucose and insulin in each group are presented in figures 1 and 2. The mean concentrations of blood glucose and insulin in the patient and saline groups were significantly higher than in other groups \((p<0.0001)\). In addition, RES supplementation alone or in combination with interval or continuous exercises significantly reduced the blood glucose and insulin levels compared with the patient and saline groups \((p<0.001)\). Combined therapy with RES and exercise was more effective in reducing blood glucose and insulin levels.

### Table 2- The lipid profile of rats in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Low-density lipoprotein (mg/dl)</th>
<th>High-density lipoprotein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92.07±10.16a</td>
<td>81.37±7.76a</td>
<td>23.03±6.54a</td>
<td>42.03±5.13a</td>
</tr>
<tr>
<td>Patient</td>
<td>208.67±17.48a</td>
<td>172.14±13.86a</td>
<td>66.30±7.99a</td>
<td>30.96±5.96a</td>
</tr>
<tr>
<td>Saline</td>
<td>206.09±20.26a</td>
<td>171.66±16.72a</td>
<td>65.91±14.14a</td>
<td>31.83±6.33a</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>135.16±26.41h</td>
<td>128.53±10.11h</td>
<td>42.01±7.54h</td>
<td>36.11±5.27a</td>
</tr>
<tr>
<td>Continuous exercise</td>
<td>131.13±15.21b</td>
<td>112.83±14.07h</td>
<td>34.49±7.18b</td>
<td>39.01±5.04ab</td>
</tr>
<tr>
<td>Interval exercise</td>
<td>128.01±3.61h</td>
<td>121.53±9.01h</td>
<td>38.34±6.21b</td>
<td>37.23±5.15b</td>
</tr>
<tr>
<td>Continuous exercise + resveratrol</td>
<td>117.97±18.39b</td>
<td>108.21±10.64b</td>
<td>31.96±6.36bc</td>
<td>40.81±5.83ab</td>
</tr>
<tr>
<td>Interval exercise + resveratrol</td>
<td>124.50±13.82b</td>
<td>119.80±24.97h</td>
<td>35.50±6.86c</td>
<td>38.61±4.43ab</td>
</tr>
</tbody>
</table>

\(p\)-value <0.0001 <0.0001 <0.0001 0.002

There was no significant difference in the mean level of variables between the groups with similar symbols (a-d). The mean level of variables was in this order: a>b>ab>b>bc>c>d.
The control group had significantly lower blood glucose and insulin levels compared with the other groups \( (p<0.01) \). 

**Table 2** shows the lipid profile of rats in all study groups. The patient and saline groups had significantly higher triglyceride, cholesterol, and LDL levels compared with other groups \( (p<0.001) \), while the mean level of HDL in the patients and saline groups was significantly lower than that in other groups \( (p<0.0001) \).

Moreover, RES and exercise training significantly increased HDL and significantly reduced triglyceride, cholesterol, and LDL levels in diabetic rats. This effect was more significant in rats, which had received combined therapy with RES and exercise training (**Table 2**).

The mean level of Bax and Bcl2 expression in hepatic tissue, as well as Bax/Bcl2 ratio in the liver of diabetic rats \( (p<0.001) \). In contrast, Bcl2 level was significantly increased after combined therapy with RES and interval or continuous training (**Table 3**).

A significant difference was found in the expression pattern of *Fxr, Lxr*, and *Sirt1* between the study groups (**Figures 3, 4, and 5**). The expression of *Fxr* was significantly higher in the control and HIT+RES groups compared with other groups \( (p<0.0001) \). However, the expression of this gene in the sham and saline groups was significantly lower than in other groups \( (p<0.001) \). The *Fxr* mRNA expression in the HIT and MIT groups was higher than in the saline groups (**Figure 3** \( (p<0.01) \).

The mRNA expression of *Lxr* was significantly elevated in the control, HIT+RES, and MIT+RES groups compared with other groups \( (p<0.0001) \). The mRNA expression of *Lxr* in the RES and exercise training groups was higher than that in the saline groups \( (p<0.001) \). The expression of *Sirt1* in the control and HIT+RES groups was significantly higher than that in the saline, MIT, HIT, and RES groups \( (p<0.001) \). Both exercises (MIT and HIT) significantly increased the expression of *Sirt1* compared with the saline and RES groups \( (p<0.001) \). Remarkably, the increase in mRNA levels of *Fxr, Lxr*, and *Sirt1* was more significant in the MIT+RES and HIT+RES groups than in the supplement-only or exercise-only groups \( (p<0.001) \) (**Figure 4**).

**DISCUSSION**

In this study, for the first time, the effect of RES supplementation alone and in combination with exercises was evaluated on *Sirt1, Lxr* and *Fxr* expression, blood glucose level, insulin level, lipid profile, and apoptosis markers in hepatic tissue of diabetic rats. Our findings revealed decreased expression of *Fxr, Lxr*, and *Sirt1* in the liver of diabetic rats. Many studies demonstrated that diabetes mellitus is associated with decreased expression of *Fxr, Lxr*, and *Sirt1* \( (4, 7, 20, 21) \). For example, Zhang et al. reported decreased
Expression of \( Fxr \) either at RNA or protein levels in the liver of diabetic rats (21). Although numerous studies reported downregulation of \( Fxr, Lxr, \) and \( Sirt1 \) genes in diabetes mellitus, the exact role of these genes in this disease remains unclear. These genes play a key regulatory role in glucose homeostasis as well as in lipid and lipoprotein metabolism (22).

Therefore, dysregulation of these genes may lead to insulin resistance and alterations in the glucose, lipid, and bile acid metabolism in diabetic patients (20).

In this study, we found increased levels of blood glucose, insulin, LDL, triglyceride, and cholesterol in diabetic rats. Furthermore, our results showed that diabetes is associated with increased hepatocyte apoptosis. Nascimento et al. demonstrated that \( Sirt1 \) suppression is associated with inflammation and apoptosis in the liver of rats with NAFLD (23). Therefore, reduced expression of \( Fxr, Lxr, \) and \( Sirt1 \) in the liver of diabetic rats may be the main contributor to these abnormalities. Previous studies also indicate that \( Fxr, Lxr, \) and \( Sirt1 \) genes agonists may serve as useful factors for the treatment of hyperglycemia, hyperlipidemia, and apoptosis in the liver of diabetic patients (21).
We found that the expression of Sirt1, Lxr, and Fxr was significantly increased in diabetic rats after treatment with RES. This effect was associated with a significant decrease in the blood glucose, insulin, LDL, triglyceride, and cholesterol levels. Moreover, we found that RES supplementation significantly decreased Bax/Bcl2 ratio, which suggest the critical role of this natural antioxidant in prevention of hepatic cells apoptosis. Similarly, several studies have shown that RES supplementation can improve the expression of Fxr, Lxr, and Sirt1 in different pathologies (24, 25). For example, Sevov et al. reported that RES induces Lxr expression in human monocyte-derived macrophages (26). Chen et al. reported that RES induces Sirt1-dependent apoptosis in 3T3-L1 preadipocytes by activating AMP-activated protein kinase (AMPK), suppressing AKT strain transforming activity, and inducing survivin expression (27). More recently, Hajighasem et al. have reported that RES supplementation increases the expression of Fxr, Lxr, and Sirt1 in the liver of rats with NAFLD and decreases hepatocyte apoptosis (13). Resveratrol reduces fat accumulation, induces apoptosis in a dose-dependent manner, and increases lipolysis in bovine muscle adipocytes.

The ability of RES to increase antioxidant defense and inhibit oxidative stress-induced damage may be due to the presence of flavonoids in the extract. In addition, presence of proanthocyanidin compounds in RES might also contribute to the antioxidant properties (28). In this research, we also observed that RES supplementation combined with exercise was more effective. Compared with RES supplementation alone, the combined therapy significantly improved the expression of Sirt1, Lxr, and Fxr genes and decreased blood glucose and insulin levels as well as hyperlipidemia and hepatocyte apoptosis. Several studies reported the positive effects of combined therapy with RES and different exercises on the liver.

For example, Faghihzadeh et al., found that combined use of RES and exercise were associated with a significant improvement in liver disease (29). Similarly, Tung et al. reported that combined therapy with RES and exercise increased the activity of antioxidant defense systems and decreased hepatic cells apoptosis (30). Also, Liao et al. reported that exercise training, RES supplementation, or their combination can significantly increase p-AMPK and Sirt1 expression and decrease p53 acetyl expression and Bax/Bcl-2 ratio in older mice (28).

The small number of rats in the study groups was a limitation of the present study. Further studies should be carried out to evaluate expression of these genes at the protein level. Since diabetes is significantly associated with oxidative stress and antioxidants depletion, evaluation of expression of antioxidant enzymes such as glutathione peroxidase, catalase, superoxide dismutase, as well as other oxidative stress biomarkers is valuable.

![Figure 4](image-url)  
**Figure 4** - Comparison of the mean Lxr mRNA levels between the study groups. Gene expression was detected by real-time PCR. There was no significant difference in the mean Lxr mRNA levels between the groups with similar symbols (a-d). The mean Lxr mRNA level was in this order: a>b>c>d. RES: Resveratrol; MIT: continuous exercise; HIT: Interval exercise; MIT+RES: Continuous exercise+Resveratrol; HIT+RES: Interval exercise+Resveratrol.
CONCLUSION
Our results revealed that diabetes mellitus is associated with decreased expression of Sirt1, Lxr, and Fxr, hyperlipidemia, and hepatocyte apoptosis. Moreover, combination of RES supplementation with exercise could be beneficial for diabetic patients. However, more studies are needed to confirm these findings.

ACKNOWLEDGMENTS
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DECLARATIONS
FUNDING
This study was supported by a grant provided by the Exercise Physiology Department of Islamic Azad University, Sari Branch, Sari, Iran.

Ethics approvals and consent to participate
This experiment was approved by the ethics committee of the Islamic Azad University, Sari Branch (IR.IAU.SARI.REC.1397.8).

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest regarding publication of this article

REFERENCES


