

## The effects of resveratrol on biochemical changes in fructose-induced insulin resistance

### *Fruktoz ile indüklenen insulin direncinde resveratrolün biyokimyasal deęişiklikler üzerine etkileri*

Nevin İlhan, Dilara Kaman, Necip İlhan

Firat University Medical Faculty, Department of Medical Biochemistry, Elazığ, Turkey

#### ABSTRACT

**Objectives:** The present study aimed to evaluate the influence of resveratrol (RSV) administration on biochemical parameters, free fatty acids (FFAs), cytokines and chemokines of rats administered high dose of fructose.

**Materials and methods:** Rats fed with fructoses that form an experimental animal model of the metabolic syndrome. Adult Wistar rats were divided into three groups of 5 rats each. In Groups 1, animals received starch-based control diet, while groups 2 and 3 rats were fed a high-fructose diet. Groups 3 animals additionally received RSV (10 mg/kg) daily for 20 days after 40 days fed with fructose. The levels of glucose, insulin, cholesterol and triglyceride were measured by using an enzymatic assay on an Auto analyzer. Serum levels of FFAs were measured by Gas Chromatography and cytokines such as MCP-1, IL-10, RANTES and eotaxin levels were measured by ELISA.

**Results:** Fructose-fed rats exhibited increased levels of glucose, insulin, HOMA-IR index and FFAs. Eotaxin, RANTES and TNF- $\alpha$  levels were also increased in fructose fed rats. RSV administration improved biochemical parameters as well as FFAs in an experimental animal model of the metabolic syndrome.

**Conclusion:** The benefits of RSV observed in this model suggest that therapeutic use of RSV may be thought in metabolic syndrome. *J Clin Exp Invest* 2011; 2 (4): 339-346

**Key words:** fructose diet, resveratrol, free fatty acids, cytokines, chemokines

#### INTRODUCTION

In recent years much research in humans and experimental animal models have focused on association between obesity, insulin resistance, dyslipidaemia and hypertension, and has been closely linked to the development of diabetes and cardiovascular disease.<sup>1</sup> The increased prevalence of insulin resistance is linked to the western diet and

#### ÖZET

**Amaç:** Bu çalışmada, fruktozun yüksek dozu verilen sıçanlarda resveratrolün (RSV), serbest yağ asitleri (SYA'leri), sitokinler ve kemokinler gibi biyokimyasal parametreler üzerine etkisinin deęerlendirilmesi amaçlandı.

**Gereç ve yöntem:** Metabolik sendromun bu deneysel hayvan modelinde sıçanlar fruktozla beslendi. Erişkin Wistar cinsi sıçanlar her birinde 5 sıçan olacak şekilde üç gruba ayrıldı. Grup 1, nişasta bazlı kontrol diyeti aldı. Grup 2 ve 3 sıçanlar yüksek fruktoz diyeti ile beslendi. 40 gün fruktoz ile beslenen Grup 3 sıçanlara daha sonra 20 gün boyunca günlük resveratrol (10 mg / kg) uygulaması yapıldı. Otoanalizörde enzimatik analiz yöntemleri kullanılarak glukoz, insülin, kolesterol ve trigliserid düzeyleri ölçüldü. SYA'leri Gaz Kromatografisi ile MCP-1, IL-10 RANTES ve eotaksin gibi sitokinlerin serum düzeyleri ise ELISA yöntemi ile ölçüldü. Fruktoz ile beslenen sıçanlarda artmış glukoz, insülin, HOMA-IR indeksi ve FFAs seviyeleri tespit edildi.

**Bulgular:** Eotaksin, RANTES ve TNF- $\alpha$  düzeyleri de fruktozla beslenen sıçanlarda artmıştı. Resveratrol uygulaması, metabolik sendromun bu deneysel hayvan modelinde biyokimyasal parametrelerin yanı sıra SYA'lerini de olumlu etkiledi.

**Sonuç:** Bu modelde gözlenen yararlı etkileri nedeniyle metabolik sendromda RSV'un tedavide kullanımı düşünülebilir.

**Anahtar kelimeler:** Fruktozlu diyet, resveratrol, serbest yağ asitleri, sitokinler, kemokinler

reduced physical activity. These metabolic changes are similar to metabolic syndrome described in humans by Reaven<sup>2</sup> and fructose-fed rats are often used to evaluate drugs or treatments of metabolic syndrome. Managing the disorders clustered in this syndrome is of great relevance to prevent and to reduce the risk of all of these pathologies. In animal models, diets high in fructose have specifically been

**Yazışma Adresi /Correspondence:** Prof. Dr. Nevin İlhan

Firat University Medical Faculty, Department of Medical Biochemistry, Elazığ, Turkey Email: drnilhan@yahoo.com

Geliş Tarihi / Received: 20.10.2011, Kabul Tarihi / Accepted: 29.11.2011

Copyright © Klinik ve Deneysel Arařtırmalar Dergisi 2011, Her hakkı saklıdır / All rights reserved

shown to contribute to a metabolic disturbance leading to insulin resistance.<sup>3</sup>

Obesity and insulin resistance have recently been linked to a low-grade chronic inflammatory response characterized by increased macrophage infiltration, altered cytokine production, and activation of the inflammatory signaling pathway in adipose tissue.<sup>4</sup> Several studies have demonstrated that chronic inflammation is an important pathogenic factor in the development of insulin resistance.<sup>5</sup> An excess energy intake leads to obesity and hyperglycemia, which can cause oxidative stress and inflammatory changes (increased levels of tumor necrosis factor [TNF] alpha and interleukin [IL]).<sup>6</sup> These inflammatory changes inhibit insulin signaling and can lead to insulin resistance. Moreover, the inflammatory state induces beta cells dysfunction, which in combination with insulin resistance leads to type 2 diabetes.<sup>7,8</sup> Thus, pharmacological agents and natural products able to reduce inflammatory activity possess anti-diabetic properties. Free fatty acids (FFAs), the product of adipose tissue lipolysis, are elevated in abdominal obesity and artificial elevations of FFA can induce insulin resistance in non-obese persons, it is natural to link enlarged intra-abdominal fat stores with FFA-induced insulin resistance.<sup>9</sup>

Resveratrol (RSV; *trans*-3,5,4'-trihydroxystilbene), a naturally occurring phytoalexin found in juice, peanuts, groundnuts, Itadori tee, grapevines and red wines, has been reported to exert a variety of biological and pharmacological activities, such as anticarcinogenesis, cardiovascular protection, and antiinflammatory properties including an inhibitory effect on the production of various cytokines. Recently, studies have shown resveratrol to protect against the metabolic changes associated with hypercaloric diets in mice with induced insulin resistance, hyperglycemia, and dyslipidemia.<sup>10-12</sup> In obese Zucker rats, administration of resveratrol resulted in a significant reduction in triglycerides, free fatty acids, cholesterol and liver triglycerides.<sup>4</sup> Apart from natural sources, this compound is recently available in tablets and is recommended as a dietary supplement. In the last years, the interest in resveratrol substantially increased and its broad biological activity at the cellular level has been demonstrated.<sup>10</sup> The most recent data indicated that resveratrol play a crucial role in cardiovascular protection provided by grapes and wines.<sup>13</sup> Although it is known that in humans resveratrol is rapidly absorbed after its oral administration and is detected in both plasma and urine, data concerning the potential beneficial effects of the pure compound in humans are still very

limited.<sup>14</sup> However, the most recent data derived from animal studies open a new, promising perspective of the potential use of resveratrol in preventing and/or treating serious metabolic disorders such as obesity and diabetes. The prevalence of both these diseases is very high, especially in Western countries, and tends to be increased.<sup>15</sup>

Resveratrol has been demonstrated to suppress macrophage activation, which would account for the anti-inflammatory effect of the compound.<sup>16</sup> The fructose fed-rat represents a model for the metabolic syndrome, including insulin resistance, hypertension and dyslipidemia, and growing evidence suggests a role for inflammation and oxidative stress in this model. Therefore, in this study, we used a metabolic syndrome animal model to examine the hipolipidemic and anti-inflammatory effects of resveratrol in fructose induced insulin resistance. The main aim of the present study was to examine the effects of daily administration of resveratrol on the disturbances present in metabolic syndrome, was allow to use this group of rats as an integrative experimental model to analyze the effects of fructose diets on free fatty acids, inflammatory cytokine and beneficial effects of resveratrol on these parameters of metabolic syndrome and to analyze the mechanisms involved in its effects. The dose of resveratrol used is equivalent to that used by humans as a diet supplement.

## MATERIALS AND METHODS

### Experimental procedure

Adult Male Wistar albino rats (8 weeks old) weighing 150-180 g maintained in a 12 h light/dark, temperature- and humidity-controlled environment, were used in this study. All rats were fed a standard rat chow before beginning the study and continued to consume standard rat chow composed of 21% protein, 4% fat, 50% carbohydrate (vegetable starch), and 4.5% cellulose for the entire study duration. Both diets contained a standard mineral and vitamin mixture. All protocols described were reviewed and approved by the Local Institutional Committee for the Ethical Use of Animals.

After a habituation period, rats were divided into three groups of 5 animals each. The first group served as control received the control diet containing starch and tap water during 40 day. At the end of this period was given a daily intraperitoneally injection of ethanol (3 g/kg body weight) prepared as a 35% (v/v) solution in 0.9% (w/v) NaCl once a day for 20 days. The second group (10 rats) was given fructose-fed animals received the high fructose

diet and water ad libitum. 10% fructose (prepared every 2 days) was given in their drinking water for 40 days.<sup>17</sup> The animals given the high-fructose diet were then divided randomly into two groups of five animals. One of them (the fructose plus RSV group) received a daily intraperitoneally injection of ethanol (3 g/kg body weight) prepared as a 35% (v/v) solution in 0.9% (w/v) NaCl, while the other one received a daily dose of RSV once a day for 20 days. Resveratrol was given intraperitoneally at a dose of 10 mg /kg day, which is reported to cause a marked antioxidative effect.<sup>18</sup> The fructose plus RSV group received 10 mg/kg RSV (99% purity; Sigma Chemical Co., St. Louis, MO, USA) intraperitoneally once a day throughout 20 days. To eliminate complications arising from the diurnal effects, all rats were sacrificed under anesthesia at the same time of day. Body weights of animals were recorded at the baseline and after the treatment. At the end of the experimental period, rats were fasted overnight, animals were sacrificed by exsanguination and blood was collected in routine biochemical test tubes and centrifuged, and serum was frozen at -70°C in aliquots until biochemical analyses were performed.

### Biochemical analyses

Serum samples analyzed for glucose, TG, total cholesterol (TC) and insulin. Glucose and lipid profile measurement: TC and TG were determined using enzymatic assay on an Auto analyzer (Olympus AU 600, Hamburg, Germany). Quantitative determination of serum insulin concentration was performed with rat/mouse ELISA kit (Linco research, USA). Insulin levels were expressed as ng/mL in ELISA kit and they were converted to mU/ml. Homeostatic model assessment as a measure of insulin resistance (HOMA-IR) was calculated by the formula: insulin (mU/ml) X [glucose (mmol/l/22.5)].<sup>19</sup>

For determination of FFA in serum, GC analysis was carried out on a Shimadzu model GC 2010 gas chromatography (Shimadzu, Milan, Italy). Prior to injection, all samples were transesterified and then diluted with n-heptane. Helium was used as the carrier gas 20 ZIVAK® FA commercial kit used for sample preparation of FFA from serum and for determination of 20 fatty acid by GC, according to kit procedure.

Serum levels of Eotaxin, MCP1, RANTES, IL-10 and TNF- $\alpha$  were analyzed with ELISA kits. All of kits were purchased from Biosource International, Inc, USA.

### Statistical analysis

Results were expressed as the arithmetical mean for each group with its standard deviation (mean  $\pm$  SD). Statistical significance was evaluated by analysis of variance (ANOVA). Spearman's correlation coefficients were used to analyze associations between chemokine concentrations and metabolic variables. A value of  $P < 0.05$  was considered statistically significant.

## RESULTS

### Effect of fructose and resveratrol on the blood glucose and insulin

The mean final body weights of experimental animal groups were as follows - bodyweight: group 1 (Control group, CON), 311.00 g $\pm$ 34.35; group 2 (Fructose group, FRU), 323.00 $\pm$  23.34 g and group 3 (Resveratrol group, RSV), 302.00  $\pm$  20.49 g. Body weight gain was observed in the experimental animals. Body weights were higher in fructose-fed group compared with controls and treatment group but not statistically significant.

In fructose-fed rats group, hyperinsulinaemia and hyperglycemia was developed. Resveratrol treatment restored the blood parameters of glucose and insulin. Glucose and insulin levels were significantly higher in fructose-fed rats compared with controls ( $p < 0.001$ ,  $p < 0.05$  respectively). Insulin level was significantly lower in resveratrol-treatment group compared with only fructose-fed group (group 2) ( $p < 0.001$ ). Although glucose level was lower in resveratrol treatment group compared with fructose-fed group, this was statistically not significant. HOMA-IR values were significantly different between three groups (Table 1).

### Effect of fructose and resveratrol on the blood free fatty acids

All serum free fatty acids were higher in fructose-fed group compared with controls (Table 2). But, in decanoic acid, myristic acid, pentadecanoic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid and eruric acid, these higher levels were statistically significant ( $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$  respectively). In resveratrol treatment rats, there was a significantly reduce in decanoic acid, pentadecanoic acid, stearic acid, oleic acid, cis-11-eicosenoic acid and eruric acid levels compared with only fructose fed rats ( $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.05$  respectively).

**Table 1.** Metabolic parameters characteristics of groups.

	Group 1 (CON)	Group 2 (FRU)	Group 3 (FRU+RSV)
Insulin (mU/ml)	47.1±3.6	72.2±1.4 <sup>a</sup>	52.9±4.1 <sup>b,c</sup>
Glucose (mg/dL)	75.4±7.5	130.4±36.8 <sup>b</sup>	103.4±33.9
HOMA	8.7±0.7	23.2±6.6 <sup>a</sup>	13.6±4.8 <sup>d</sup>
Triglyceride (mg/dL)	118.6±12.1	175.2±13.7 <sup>a</sup>	139.4±15.9 <sup>b,d</sup>
Cholesterol (mg/dL)	75.0±4.5	114.8±10.4 <sup>a</sup>	84.4±4.7 <sup>c</sup>

<sup>a</sup>P<0.0001, compared with group 1; <sup>b</sup>P<0.05, compared with group 1; <sup>c</sup>P<0.0001, compared with group 2; <sup>d</sup>P<0.01, compared with group 2

**Table 2.** Levels of serum fatty free acids in groups (nmol/mL)

	Group 1 (CON)	Group 2 (FRU)	Group 3 (FRU+RSV)
Decanoic Acid	197±40	256±18 <sup>a</sup>	207±17 <sup>b</sup>
Undecanoic Acid	732±203	774±108	746±72
Lauric Acid	179±63	196±26	178±50
Tridecanoic Acid	577±90	546±80	473±115
Myristic Acid	254±18	210±21 <sup>c</sup>	178±42 <sup>d</sup>
Pentadecanoic Acid	446±41	576±74 <sup>c</sup>	436±113 <sup>b</sup>
Palmitic Acid	353±66	396±48	344±72.2
Palmitoleic Acid	84±45	110±39	97±28
Stearic Acid	494±49	585±62 <sup>c</sup>	493±55 <sup>b</sup>
Oleic Acid	262±24	333±23 <sup>a</sup>	270±43 <sup>e</sup>
Linoleic Acid	895±55	1169±112 <sup>c</sup>	1034±248
Arachidic Acid	1429±373	1816±158	1716±349
Cis-11-Eicosenoic Acid	1045±178	1182±97	938±209 <sup>b</sup>
Cis-5,8,11,14,17-EA	1403±307	1516±543	1656±626
Arachidonic Acid	1271±127	1603±124 <sup>c</sup>	1416±348
Heneicosanoic Acid	1706±242	1760±123	1635±404
Eruric Acid	3052±649	3974±567 <sup>c</sup>	2910±617 <sup>b</sup>
Cis-4,7,10,13,16,19-DA	6971±1159	7404±576	6823±1513

Results are means± SD, n = 5. EA:Eicosapentaenoic Acid, DA: Docosahexaenoic Acid; <sup>a</sup>P<0.005, compared with group 1; <sup>b</sup>P<0.05, compared with group 2; <sup>c</sup>P<0.05, compared with group 1; <sup>d</sup>P<0.001, compared with group 2; <sup>e</sup>P<0.01, compared with group 2

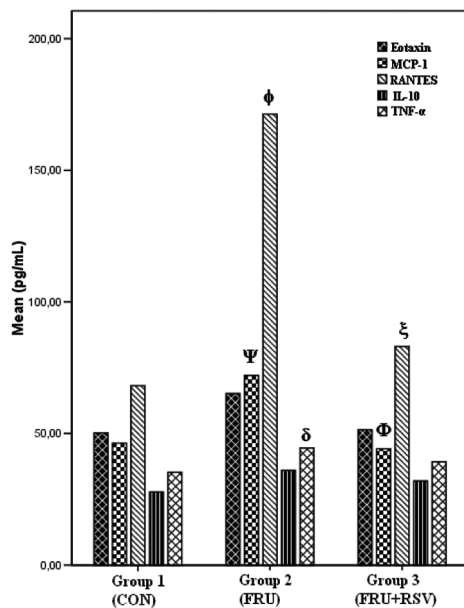
**Table 3.** Correlation of cytokines and chemokines levels with metabolic parameters

	Eotaxin		MCP-1		RANTES		IL-10		TNF-α	
	r	P	r	P	r	P	r	P	r	P
Insulin	0.45	0.09	0.75	0.001	0.68	0.005	0.37	0.17	0.55	0.035
Glucose	0.48	0.07	0.37	0.17	0.35	0.17	0.11	0.68	0.24	0.38
HOMA	0.55	0.035	0.56	0.029	0.50	0.05	0.21	0.06	0.38	0.15
Triglyceride	0.41	0.12	0.68	0.005	0.62	0.015	0.48	0.07	0.49	0.085
Cholesterol	0.38	0.16	0.68	0.005	0.73	0.002	0.25	0.37	0.47	0.076



## Effect of fructose and resveratrol on the blood chemokines

Serum levels of Eotaxin is; CON (pg/mL):  $50.23 \pm 6.55$ , FRU (pg/mL):  $65.19 \pm 30.33$  and FRU+RSV (pg/mL):  $51.44 \pm 6.68$ . Although there were statistically no significant difference among groups, serum eotaxin level was higher in group 2 compared with groups 1 and 3. Serum levels of MCP-1 is; CON (pg/mL):  $46.34 \pm 6.83$ , FRU (pg/mL):  $72.13 \pm 5.52$  and FRU+RSV (pg/mL):  $44.23 \pm 14.32$ . MCP-1 level was significantly higher in group 2 compared with group 1 and group 3 ( $p < 0.001$ ). RANTES levels in groups were: CON (pg/mL):  $681.86 \pm 354.48$ , FRU (pg/mL):  $1713.58 \pm 697.71$  and FRU+RSV (pg/mL):  $831.10 \pm 90.63$ . RANTES level was significantly higher in group 2 compared with group 1 and group 3 ( $p < 0.005$ ,  $p < 0.01$  respectively). Serum levels of IL-10 is; CON (pg/mL):  $27.80 \pm 8.67$ , FRU (pg/mL):  $36.00 \pm 8.06$  and FRU+RSV (pg/mL):  $32.00 \pm 2.91$ . There was statistically no significant difference among groups. Serum levels of TNF- $\alpha$  is; CON (pg/mL):  $35.27 \pm 6.46$ , FRU (pg/mL):  $44.49 \pm 3.45$  and FRU+RSV (pg/mL):  $39.30 \pm 6.87$ . TNF- $\alpha$  level was significantly higher in group 2 compared with group 1 ( $p < 0.05$ ) (Figure 1).



**Figure 1.** Serum levels of cytokines and chemokines [Eotaxin, MCP1, RANTES (Rantes levels divided to 10), IL-10, TNF- $\alpha$ ]

$\psi p < 0.001$ , compared with group 1;  $\phi p < 0.001$ , compared with group 2;  $\phi p < 0.005$ , compared with group 1;  $\xi p < 0.01$ , compared with group 2;  $\delta p < 0.05$ , compared with group 1

## Correlations

By Spearman's analysis, we performed to define the independent relationship between eotaxin, MCP-1, RANTES, IL-10, TNF- $\alpha$  and various metabolic markers (Table 3). As shown, eotaxin was related to insulin ( $p = 0.09$ ), glucose ( $p = 0.07$ ) and HOMA-IR index ( $p = 0.002$ ) but not to triglyceride and cholesterol. The MCP-1 and RANTES levels were significantly related to insulin ( $p = 0.001$  for MCP-1,  $p = 0.005$  for RANTES), HOMA-IR index ( $p = 0.029$  for MCP-1,  $p = 0.05$  for RANTES), triglyceride ( $p = 0.005$  for MCP-1,  $p = 0.015$  for RANTES) and cholesterol ( $p = 0.005$  for MCP-1,  $p = 0.002$  for RANTES) but not with glucose level. The IL-10 level was not correlated with metabolic markers. There was a significant positive correlation between TNF- $\alpha$  and insulin level ( $p = 0.035$ ).

## DISCUSSION

The rat model described in this article mimics a typical unhealthy Western diet as it featured high fructose intake, an insult that induced dyslipidemia and insulin resistance. Fructose, found naturally in many fruits, is now consumed by humans in large quantities due to the popularity of convenient, prepackaged foods and the consumption of soft drinks and juice beverages containing sucrose or high-fructose corn syrup. Mice allowed ad libitum access to water containing fructose or to a soft drink showed increased adiposity and specifically increased hepatic lipid storage. High-fructose diets have been shown to induce insulin resistance, weight gain, hyperlipidemia, and hypertension in several animal models including rats, hamsters, dogs, and certain mice species.<sup>21</sup>

Raised levels of plasma FFAs may provide a mechanistic link between increased fat mass and insulin resistance, and ultimately metabolic syndrome. Acute elevations of plasma FFA levels raise plasma insulin levels by stimulating insulin release or by decreasing insulin clearance.<sup>22,23</sup> In this study serum levels of insulin and glucose were enhanced and HOMA-IR index, indicating insulin resistance, was higher in fructose fed rats. Serum fatty acids also were higher in fructose fed rats compared with controls. A key contributor to insulin resistance and the metabolic syndrome appears to be the abundance of TG and fatty acids that occurs in obesity (perhaps in part due to high fructose intake), exceeding the storage capacity of adipose tissue and impairing adipocyte signaling. The end result is ectopic fat storage, accompanied by modified secretion of hormones and cytokines by adipose tis-

sue and an inflammatory state, all of which cause damaging abnormalities in signaling within insulin-sensitive tissues.

Increased exposure of FFAs is thought to be a primary cause of insulin resistance, at least in skeletal muscle, but other mechanisms may also contribute. In addition to FFAs, adipokines are produced by adipose tissue. Some of these inflammatory cytokines (e.g. TNF- $\alpha$ ), may activate components of the inflammatory pathway such as NF $\kappa$ B, and thereby inhibit insulin signaling. In this study serum MCP-1, RANTES and TNF- $\alpha$  levels were significantly higher in fructose fed rats but there were statistically no significant in levels of serum IL-10 and eotaxin among groups. Free fatty acids seem to be linked to inflammatory activity in overweight subjects and in subjects with insulin resistance. In rats, FFAs appear to be important in the early development of insulin resistance, while TNF- $\alpha$  accelerates insulin resistance together with FFA in the later stages.<sup>24</sup> Experimental studies in humans and animals have shown that treatment with pro-inflammatory cytokines induces hypertriglyceridemia and insulin resistance.<sup>25,26</sup> TNF- $\alpha$  downregulates the tyrosine kinase activity of the insulin receptor, thereby increases insulin resistance.<sup>26-28</sup> In addition, it has been reported that insulin promotes numerous deleterious vascular effects by stimulating the actions of various growth factors acting through the MAPK signaling pathway.<sup>29</sup> Therefore, further investigation is needed to clarify the expression of MCP-1 through the MAPK pathway in the insulin-resistant state.

We have found a few studies about the effects of cytokines and chemokines such as MCP-1, IL-10, RANTES and eotaxin on fructose fed rats. But it's known that pro-inflammatory cytokines have earlier been associated with the development of the metabolic syndrome and type 2 diabetes. In this study there were no correlation between IL-10 and HOMA-IR index. Exel et al.<sup>30</sup> showed that an association between low IL-10 production capacity and high serum glucose, high HbA1c, type 2 diabetes, and dyslipidemia. Dancan and Schmidt<sup>31</sup> suggested that chronic activation of the innate immune system can be related to metabolic syndrome. Forsythe et al.<sup>32</sup> showed that low carbohydrate diet decreased greatly TNF- $\alpha$ , and MCP1. RANTES appears to have a causal role in atherosclerosis; since inactivation of RANTES receptors in a hypercholesterolemic mouse model prevented the progression of disease.<sup>33</sup> Herder et al.<sup>34</sup> analyzed of subjects with IGT and revealed that RANTES is strongly associated with IGT. Vasudevan et al.<sup>35</sup> showed that circulating levels of eotaxin in serum/plasma are increased

in diet-induced obesity in both mice and humans. Later studies will be needed to further evaluate the relevance of RANTES, eotaxin, IL10 and MCP-1 in the disease process and to clarify whether they can be considered as an early risk factor for metabolic syndrome.

The reduction of inflammation is an important target in the treatment of metabolic syndrome. With regard to inflammatory mechanisms, polyphenols have been shown to inhibit pro-inflammatory enzymes including cyclooxygenase, lipoxygenase and inducible nitric oxide synthase via activation of peroxisome proliferators-activated receptor gamma. In addition, phenolic compounds have been shown to inhibit phosphoinositide 3-kinases, tyrosine kinases, nuclear factor-kappa B, the expression endothelin-1, and the activation of sirtuin-1.<sup>36,37</sup>

In the present study, we showed that RSV possesses hypoglycemic, hypolipidemic and anti-inflammatory activities in fructose fed rats. The fructose fed rats in our study were characterized by hyperglycemia, hyperlipidemia, high plasma insulin levels and high HOMA-IR index as a predictor for insulin resistance. Administration of RSV at 10 mg/kg is associated with a moderate, yet persistent, improvement of the hyperglycemic and hyperlipidemic conditions over an experimental period of 20 days. The administration of RSV was also associated with a prominent improvement of the cytokines and chemokines such as MCP-1, RANTES and TNF an exhibited fructose fed rats. Administration of RSV to fructose fed rats resulted in a decline in the blood glucose levels. It was of interest to note that RSV administration also caused a reduction in the plasma insulin level. The reduced plasma insulin level is probably secondary to the reduced plasma glucose level caused by RSV. However, it is also possible that insulin sensitivity is improved by RSV. HOMA-IR index as a measure of insulin resistance was reduced in resveratrol treatment rats. Olholm et al showed that anti-inflammatory effects of RSV on adipokine expression and secretion in human adipose tissue in vitro through the SIRT1 pathway. Thus, RSV was hypothesized to possess beneficial effects and might improve the metabolic profile in human obesity.<sup>38</sup> The RSV effects observed in the present study are mediated through its anti-inflammatory properties. The present study also shows that at the maximal dose of RSV is a reduced MCP-1, RANTES and TNF- $\alpha$  level.

Resveratrol inhibits the activation of the pro-inflammatory transcription factor, NF- $\kappa$ B, resulting in exhibits immunomodulatory effect by suppressing overproduction of inflammatory cytokines

like TNF- $\alpha$ , IL-1 $\beta$ , and IL-6.<sup>39</sup> NF- $\kappa$ B activation in macrophages may be of fundamental importance in severity of inflammation and a target in stress inflammation for resveratrol. It is possible that resveratrol will be considered as an agent for reducing the severity of inflammation.

Hypertriglyceridemia is also associated with the metabolic consequences of hyperinsulinemia, insulin resistance, and glucose intolerance.<sup>40</sup> In our study, administration of RSV to fructose fed rats significantly improved some of these parameters. The observed hypolipidemic effect by RSV may be due to increased plasma lipid uptake or by decreased fatty acid synthesis. Our study showed that there was a moderate reduction in FFAs, especially in decanoic acid, pentadecanoic acid, stearic acid, oleic acid, cis-11-eicosenoic acid and erucic acid levels.

In conclusion, RSV has an insulin-like effect in fructose-induced metabolic syndrome rats. In our fructose fed rats, plasma insulin, glucose as well as HOMA-IR index were reduced via this dose resveratrol, suggesting that the RSV effect was due to reduction of insulin resistance and/or improved insulin sensitivity. Also there was improving on serum FFAs levels by RSV administration. The exact mechanism underlying the activity of the RSV awaits further investigation.

#### Acknowledgements

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors would like to thanks for supply of resveratrol to Dr. Aysun Bay Karabulut.

#### REFERENCES

- Zavaroni I, Sander S, Scott S, Reaven GM. Effect of fructose feeding on insulin secretion and insulin action in the rat. *Metabolism* 1980; 29 (10): 970-3.
- Reaven GM. Syndrome X: 6 years later. *J Intern Med* 1994; 736: 13-22.
- Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab* 2005; 2 (1): 5-8.
- Rivera L, Morón R, Zarzuelo A, Galisteo M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol* 2009; 77 (6): 1053-63.
- Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; 25 (1): 4-7.
- Mazur A, Maier JAM, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the inflammatory response: potential physiopathological implications. *Arch Biochem Biophys* 2007; 458(1):48-56.
- Sjöholm A, Nyström T. Endothelial inflammation in insulin resistance. *Lancet* 2005; 365(9459):610-2.
- Sjöholm A, Nyström T. Inflammation and the etiology of type 2 diabetes. *Diabetes Metab Res Rev* 2006; 22(1):4-10.
- Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. Splanchnic lipolysis in human obesity. *J Clin Invest* 2004; 113(11):1582-8.
- Bhat KPL, Kosmeder JW 2nd, Pezzuto JM. Biological effects of resveratrol. *Antioxid Redox Signal* 2001; 3(6):1041-64
- Robb EL, Page MM, Wiens BE, Stuart JA. Molecular mechanisms of oxidative stress resistance induced by resveratrol: Specific and progressive induction of MnSOD. *Biochem Biophys Res Commun* 2008;367(2):406-12.
- Szkudelska K, Szkudelski T. Resveratrol, obesity and diabetes. *Eur J Pharmacol* 2010;635(1-3):1-8.
- Bertelli AA, Das DK. Grapes, wines, resveratrol and heart health. *J Cardiovasc Pharmacol* 2009;54(6):468-76.
- Bishayee A. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. *Cancer Prev Res* 2009;2(5):409-18.
- Ashcroft F, Rorsman P. Type 2 diabetes mellitus: not quite exciting enough? *Hum Mol Genet* 2004;13 (1):R21-31
- Tsai SH, Lin-Shiau SY, Lin JK. Suppression of nitric oxide synthase and the down-regulation of the activation of NF $\kappa$ B in macrophages by resveratrol. *Br J Pharmacol* 1999;126(3):673-80.
- Sánchez-Lozada LG, Tapia E, Jiménez A, Bautista P, et al. Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *Am J Physiol Renal Physiol*. 2007;292(1):F423-9.
- Hascalik S, Celik O, Turkoz Y, et al. Resveratrol, a red wine constituent polyphenol, protects from ischemia-reperfusion damage of the ovaries. *Gynecol Obstet Invest* 2004; 2004;57(4):218-23.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412-9.
- Meng Y, Wang G, Yang N, et al. Two-step synthesis of fatty acid ethyl ester from soybean oil catalyzed by *Yarrowia lipolytica* lipase. *Biotechnol Biofuels* 2011;4(1):6-9.
- Rutledge AC, Adeli K. Fructose and the Metabolic Syndrome: Pathophysiology and Molecular Mechanisms. *Nutr Rev* 2007;65(6 Pt 2):S13-23.
- Crespin SR, Greenough WB III, Steinberg D. Stimulation of insulin secretion by long-chain free fatty acids. A direct pancreatic effect. *J Clin Invest* 1973; 52(8):1979-84.
- Peiris AN, Mueller RA, Smith GA, Struve MF, Kissebah AH. Splanchnic insulin metabolism in obesity.

- Influence of body fat distribution. *J Clin Invest* 1986; 78(6):1648-57.
24. Liu RH, Mizuta M, Kurose T, Matsukura S. Early events involved in the development of insulin resistance in Zucker fatty rat. *Int J Obes Relat Metab Disord* 2002; 26(3):318-26.
  25. Fernández-Veledo S, Nieto-Vazquez I, Vila-Bedmar R, et al. Molecular mechanisms involved in obesity-associated insulin resistance: therapeutical approach. *Arch Physiol Biochem* 2009; 115(4):227-39.
  26. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes: central role of tumor necrosis factor- $\alpha$ . *J Clin Invest* 1994; 94(4):1543-9.
  27. Hopkins PN, Hunt SC, Wu LL, Williams GH, Williams RR. Hypertension, dyslipidemia and insulin resistance: links in a chain or spokes on a wheel? *Curr Opin Lipidol* 1996; 7(4):241-53.
  28. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- $\alpha$  and obesity-induced insulin resistance. *Science* 1996; 271(5249):665-8.
  29. Hsueh WA, Law RE. Insulin signaling in the arterial wall. *Am J Cardiol* 1999;84(1A):21J-24J.
  30. van Exel E, Gussekloo J, de Craen AJ, Frölich M, Bootsma-Van Der Wiel A, Westendorp RG. Low Production Capacity of Interleukin-10 Associates with the Metabolic Syndrome and Type 2 Diabetes: the Leiden 85-Plus Study. *Diabetes* 2002; 51(4):1088-92.
  31. Duncan BB, Schmidt MI. Chronic activation of the innate immune system may underlie the metabolic syndrome. *Sao Paulo Med J* 2001; 119(3):122-7.
  32. Forsythe CE, Phinney SD, Fernandez ML, Quann EE, Wood RJ, Bibus DM, Kraemer WJ, Feinman RD, Volek JS. Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. *Lipids* 2008; 43(1):65-77.
  33. Veillard NR, Kwak B, Pelli G, et al. Antagonism of RANTES receptors reduces atherosclerotic plaque formation in mice. *Circ Res* 2004; 94(2):253-61.
  34. Herder C, Haastert B, Müller-Scholze S, et al. Association of Systemic Chemokine Concentrations with Impaired Glucose Tolerance and Type 2 Diabetes: Results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). *Diabetes* 2005 Dec;54 Suppl 2:S11-7.
  35. Vasudevan AR, Wu H, Xydakis AM, et al. Eotaxin and obesity. *J Clin Endocrinol Metab* 2006;91(1):256-61.
  36. Dell'Agli M, Busciala A, Bosisio E. Vascular effects of wine polyphenols. *Cardiovasc Res* 2004; 63(4):593-602.
  37. Milne JC, Lambert PD, Schenk S, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* 2007; 450(7170):712-6.
  38. Olholm J, Paulsen SK, Cullberg KB, Richelsen B, Pedersen SB. Anti-inflammatory effect of resveratrol on adipokine expression and secretion in human adipose tissue explants. *Int J Obesity* 2010;34(10):1546-53.
  39. Marier JF, Chen K, Prince P, Scott G, del Castillo JR, Vachon P. Production of ex vivo lipopolysaccharide-induced tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and interleukin-6 is suppressed by trans resveratrol in a concentration-dependent manner. *Can J Vet Res* 2005; 69(2):151-4.
  40. Yki-Järvinen H. Liver fat in the pathogenesis of insulin resistance and type 2 diabetes. *Dig Dis* 2010;28(1):203-9.