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The Effects of Resveratrol and Pioglitazone Treatment on Serum Resistin and Apelin Levels and Histochemical Parameters in the Metabolic Syndrome Rat Model: Animal Experiment

Rat Metabolik Sendrom Modelinde Resveratrol ve Pioglitazon Tedavisinin Serum Resistin ve Apelin Düzeyleri ile Histokimyasal Parametreler Üzerine Etkileri: Hayvan Deneyi

Onur GÜRSU[a], Sevgi ALTAŞ[b], Emir DÖNDER[c], Mehmet Ferit GÜRSU[b], Muhammed Emre KARAMAN[d]

[a]Clinic of Anesthesiology and Reanimation, Ümraniye Training and Research Hospital, İstanbul, Türkiye
[b]Department of Medical Biochemistry, Fırat University Faculty of Medicine, Elazığ, Türkiye
[c]Department of Internal Medicine, Fırat University Faculty of Medicine, Elazığ, Türkiye
[d]Department of Coach Training, Fırat University Faculty of Sport Sciences, Elazığ, Türkiye

ABSTRACT Objecvtive: The current research was aimed to determine the serum resistin and apelin levels in rats with metabolic syndrome and to examine the impact of resveratrol and pioglitazone intervention on serum resistin, apelin levels and metabolic parameters. **Material and Methods:** Fourty male Wistar albino rats were divided into 4 groups. Group 1 [n=10, control group; without (metabolic syndrome "MetS")]; Group 2 (n=10, MetS group without treatment); Group 3 (n=10, MetS and treatment with resveratrol); Group 4 (n=10, MetS and treatment with pioglitazone). The rats were decapitated and resistin, apelin and biochemical parameters were studied in blood samples. **Results:** Apelin levels did not decreased in MetS+pioglitazone treatment group. Resistin level significantly decreased in treatment groups. Myxoid degeneration and edema in aortic vessel wall were less severe in MetS+resveratrol group. Periportal sinusoidal enlargement, congestion and lymphocyte infiltration in rat liver tissue with metabolic syndrome were absent in the group treated with resveratrol, and regressed in the group treated with pioglitazone treatment. **Conclusion:** Resistin and apelin concentrations have found to be increased in MetS and associated with insulin re- sistance. Although pioglitazone and resveratrol treat- ment decreased resistin levels, it was ineffective on apelin levels. Although pioglitazone treatment pro- vided improvement in histopathological changes, resveratrol treatment had more benefits on histopathological changes. In the treatment of metabolic syndrome, resveratrol may be useful in support therapy. This study is the first study which shows that pioglitazone does not effects the apelin levels in metabolic syndrome treatment.

Keywords: Metabolic syndrome; resistin; apelin; resveratrol; pioglitazone

ÖZET Amaç: Sunulan araştırmada, metabolik sendrom oluşturulan ratların serum rezistin ve apelin seviyelerinin incelenmesi ve resveratrol ve pioglitazon tedavisinin serum resistin, apelin düzeyleri ve metabolik parametreleri nasıl etkilediğinin araştırılması amaçlandı. **Gereç ve Yöntemler:** Kırk erkek Wistar albino rat 4 gruba ayrıldı. Grup 1 [n=10, kontrol grubu; (metabolik sendrom "MetS") olmadan]; Grup 2 (n=10, tedavisiz MetS grubu); Grup 3 (n=10, MetS ve resveratrol ile tedavi); Grup 4 (n=10, MetS ve pioglitazon tedavisi). Ratlar dekapite edilerek, kan örneklerinde rezistin, apelin ve biyokimyasal parametreler çalışıldı. **Bulgular:** MetS+pioglitazon tedavi grubunda apelin seviyeleri düşmedi. Rezistin düzeyi tedavi gruplarında önemli ölçüde azaldı. Aort damar duvarında miksoid dejenerasyon ve ödem MetS+resveratrol grubunda daha az şiddetliydi. MetS'li sıçan karaciğer dokusunda periportal sinüzoidal genişleme, tıkanıklık ve lenfosit infiltrasyonu resveratrol ile tedavi edilen grupta yokken, pioglitazon tedavisi alan grupta geriledi. **Sonuç:** Resistin ve apelin konsantrasyonları MetS'de artmış ve insülin direnci ile ilişkili olduğu bulunmuştur. Pioglitazon ve resveratrol tedavisinin resistin düzeylerini düşürmesine karşın, apelin düzeyleri üzerine etkisiz olduğu gösterilmiştir. Pioglitazon tedavisi histopatolojik değişikliklerde düzelme sağlasa da, resveratrol tedavisi histopatolojik değişiklikler üzerinde daha fazla fayda sağladığı gösterilmiştir. MetS tedavisinde resveratrolün destek tedavisinde faydalı olabiliceği düşünülmektedir. Bu çalışma, MetS tedavisinde pioglitazonun apelin düzeylerini etkilemediğini gösteren ilk çalışmadır.

Anahtar Kelimeler: Metabolik sendrom; resistin; apelin; resveratrol; pioglitazon

Correspondence: Muhammed Emre KARAMAN
Department of Coach Training, Fırat University Faculty of Sport Sciences, Elazığ, Türkiye
E-mail: mekaraman@firat.edu.tr

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Metabolic syndrome (MetS) is a common health problem associated with lifestyle changes and increased obesity. MetS causes an increase in cardiovascular diseases due to hypertension, hypertriglyceridemia, Type 2 diabetes mellitus (T2DM), abdominal obesity, high serum glucose levels and low serum high density lipoprotein cholesterol (HDL-C) concentrations.[1] The estimated prevalence of metabolic syndrome in the adult population worldwide is 20-25%.[2]

Visceral adipose tissue, which produces adipokine and is considered an endocrine organ today and has a key role in the pathogenesis of MetS.[3] Studies have emphasized that adipokines have a key role in various biological processes which determine cardiovascular risks such as inflammation, lipid metabolism, oxidative stress and adipogenesis.[4] Currently, resistin and apelin are the most important adipokines emphasizing their relation with MetS. Resistin is so named because it causes insulin resistance. It has been reported that thiazolidone group drugs used in the treatment of T2DM reduce insulin resistance by inhibiting the production of adipocyte-derived resistin level.[4] Apelin is known to have a key role in obesity-related mechanisms and insulin regulation. In both the human and rat adipocytes study, apelin was reported to be released from adipose tissue and up-regulated by insulin. Plasma apelin levels have been reported to increase in obesity, due to hyperinsulinemia and insulin resistance, and the plasma concentration of apelin is higher in obese individuals than in lean ones.[5]

In the treatment of MetS, in addition to drugs such as pioglitazone, herbal antioxidants, which are recently shown as alternative medicine methods, are also used. Resveratrol is usually found in red wine, grapes, strawberries and peanuts. And it is rich of natural polyphenol with anti-inflammatory and anti-cancer properties and antioxidants. It has been reported that resveratrol can be used in anti-obesity treatment due to its effect of modulating lipid and lipoprotein metabolism and increasing metabolic rate.[6] Resveratrol has been reported to prevent the negative changes caused by a high-calorie diet.[7]

In the present study it was aimed to investigate that the relationship between resveratrol and pioglitazone interventions with serum resistin and apelin levels and the effect of this intervention on metabolic syndrome-induced biochemical and pathological changes in rats with metabolic syndrome.

MATERIAL AND METHODS

The present study was confirmed by Fırat University Animal Experiments Local Ethics Committee (date: March 26, 2009, no: 2009/20-32). Animal rights are protected by making it in line with the principles of the Guide for the Care and Use of the Laboratory Animals. This study was conducted in accordance with the Declaration of Helsinki. The type of this research is Experimental Studies (Animal experiments) type.

EXPERIMENTAL DESIGN

The current study was carried out in Fırat University Experimental Research Unit. 40 male and five-week-old adult Wistar albino rats (weighing 180±10), were participated in the research. Rats were provided from Fırat University Experimental Research Center and sheltered in private cages at constant temperature and ventilated rooms under standard conditions (22-24°C) before and during the experiment. Tap water and 8 mm rat pellet feed supplied from Elazığ feed factory were given daily without any restrictions. The study was planned as a total of 12 weeks. Serum glucose, HDL-C, and triglyceride levels were evaluated to diagnose the metabolic syndrome as a result of the presence of at least 3 of the National Cholesterol Education Programme Adult Treatment Panel III metabolic syndrome diagnostic criteria.

Rats were divided into 4 equal number of groups, and study and control groups were formed. The weights of the rats were measured using a sensitive electronic scale device. Of the 40 rats included, 30 were given 10% fructose to drinking water for 8 weeks to induce metabolic syndrome, while the remaining 10 rats were assigned to the control group.

Group 1 (Control group; n=10); in order to compensate for the injection stress that occurred in the other groups, 1 mL/kg saline was administered intraperitoneal for four weeks between 8 and 12. week of study.

Group 2 (MetS Group; n=10); MetS was induces by adding 10% fructose to drinking water for eight weeks and 1 mL/kg of saline was administered intraperitoneal for 4 weeks.

Group 3 (pioglitazone group; n=10); after the MetS was induced, pioglitazone was administered orally at a dose of 10 mg/kg/day for 4 weeks with diet.

Group 4 (resveratrol group; n=10); after the MetS was induced, resveratrol was administered intraperitoneal at a dose of 10 mg/kg/day with diet for 4 weeks.

Preparation of resveratrol: Resveratrol (Sigma Chemicals, St Louis, MO, USA) was dissolved in freshly prepared 50% ethanol and diluted with 2% saline and made ready for injection.

COLLECTION AND PREPARATION OF BLOOD SAMPLES

After 12-weeks of follow-up, rats were decapitated and plasma and serum samples were taken into biochemistry tubes with ethylene diamine tetra acetic acid (EDTA) to be suitable for analysis. The blood taken was centrifuged at 3,000 rpm for 10 minutes, and serum and plasma were separated. Since many parameters will be examined in the study, the obtained serum and plasmas were placed in polypropylene tubes in small portions and stored at -80°C until analysis.

MEASUREMENT OF SERUM RESISTIN LEVELS

Serum resistin levels were studied using rat resistin enzyme-linked immunosorbent assay (ELISA) kit (BioVendor, catalog number: RD391016200R, USA) in accordance with the kit procedure. Plate absorbance were measured spectrophotometrically at 450 nm using the ELISA reader Bio-tek ELX800. Plates were washed using auto washer (Bio-tek ELX50). The last measurement results were multiplied by 20 due to the 1:20 dilution and expressed as ng/mL (Kit sensitivity: <0.05 ng/mL, measuring range: 0.25-20 ng/mL, intra-assay CV: 4.9-5.2% and inter-assay CV: 4.9-9.3%).

MEASUREMENT OF SERUM APELIN LEVELS

Serum apelin levels were studied using the rat apelin ELISA kit (Phoenix, catalog number: EK 057-23, USA) and in accordance with the kit user manual. Plate absorbance were measured spectrophotometrically at 450 nm using the ELISA reader Bio-tek ELX800. Plates were washed using auto washer (Bio-tek ELX50). The last measurement results are reported in ng/mL. The measuring range was accepted as 0-100 ng/mL.

MEASUREMENT OF SERUM INSULIN LEVELS

Serum insulin levels were studied using the rat insulin ELISA kit (Millipore, catalog no: EZRMI-13K, USA) and in accordance with the kit manual. Absorbances were read spectrophotometrically at 450 nm in the ELX800 ELISA reader. An automatic plate washer used for plate washing. Test results are reported in ng/mL (Kit sensitivity: <0.2 ng/mL, measuring range: 0.2-10 ng/mL, intra-assay CV: 1.17-3.22% and inter-assay CV: 6.95-9.23%).

MEASUREMENT OF HEMOGLOBIN A1C LEVELS

To determine the hemoglobin A1c (HbA1c) levels the Olympus AU 2700 (Olympus Optical Co. Ltd, Tokyo-Japan) auto analyzer were used and Olympus branded commercial kits using whole blood samples taken into EDTA tubes. According to this method, normal % HbA1c value was accepted as a range of 4.0-6.2%.

MEASUREMENT OF BIOCHEMICAL PARAMETERS

The serum glucose, triglyceride, total cholesterol, HDL-C, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and uric acid concentrations have analyzed in with an auto analyzer (Olympus Optical Co. Ltd, Tokyo-Japan) using Olympus branded commercial kits.

The Homeostatic model assessment (HOMA) test were used to determine the insulin resistance (IR). HOMA-IR=[fasting serum insulin (μU/mL) xfasting serum glucose (mmol/L)]/22.5.

MEASUREMENT OF SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 LEVELS

Serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were studied using the rat sVCAM-1 ELISA kit (Cusabio Biotech Co, catalog no: CSB-E07990r, USA) in accordance with the kit procedure. The absorbances were measured spec-

trophotometrically at 450 nm in the ELX800 ELISA reader. Bio-tek ELX50 was used as an automatic washer for plate washings. Test results were expressed in ng/mL. Measuring range accepted as: 15.6-1,000 ng/mL.

IMMUNOHISTOCHEMICAL MEASUREMENTS

Immunohistochemical staining was performed on all tissue. Four-micrometer-thick section as cut and placed on charged slides. The slides were deparaffinized and rehydrated, and endogenous peroxidases were blocked by incubation with 3% H_2O_2. Antigen retrieval was accomplished by incubating the slides with 10 mmol citrate buffer at pH 6.0 and microwaving for 20 minutes. The slides were counterstained with Mayer's hematoxylin.

STATISTICAL EVALUATIONS

Kruskal Wallis variance analysis test was used to compare the parameters between groups. In pairwise comparisons between groups, Mann-Whitney U test was used. Pearson correlation test was used in the analysis of relationship in the parameters of groups with each other. p values <0.05 were accepted as the lowest level of significance.

RESULTS

Body weight levels increased statistically significantly in the MetS, MetS+pioglitazone and MetS+resveratrol groups. Glucose, total cholesterol (T. cholesterol), triglyceride, uric acid, HOMA-IR, VLDL, LDL, HbA1c and insulin levels were significantly increase in MetS rats. Resistin level significantly increase in MetS than control group. Resistin level significantly decreased in treatment group (MetS+pioglitazone and MetS+resveratrol) compared to MetS group. Apelin level significantly increased in MetS compared to control group. MetS+resveratrol group's apelin level was higher than other groups (Table 1).

Resistin level was significantly positively correlated with insulin and HOMA-IR level in all groups. Resistin level was significantly negative correlated with apelin level in MetS group and treatment groups (Table 2).

When the aortic vessel wall of rats with MetS were compared with the control group, rats with un treated MetS and MetS+pioglitazone groups had perivascular lymphocytic infiltration, mild Myxoid

TABLE 1: General characteristics of the experimental groups.

	Group 1 (n=10) (Control)	Group 2 (n=10) (MetS)	Group 3 (n=10) (MetS+pioglitazone)	Group 4 (n=10) (MetS+resveratrol)
Weight (g)	308.2±36.6	349.1±36.2^{c}	337.1±26.4^{c}	332.7±30.1^{c}
Glucose (mg/dL)	95.90±9.46	149.10±16.67^{a}	132.10±9.49$^{a\,z}$	124.60±10.23$^{a\,y}$
T. cholesterol (mg/dL)	68.50±7.91	98.30±13.74^{a}	81.90±11.33^{z}	73.30±9.95^{x}
Triglyceride (mg/dL)	86.20±11.79	178.70±11.31^{a}	128.90±10.23$^{a\,x}$	113.80±20.08$^{x\,b\,*}$
Uric acid (mg/dL)	1.21±0.11	2.31±0.29^{a}	1.62±0.12$^{a\,x}$	1.58±0.10$^{a\,x}$
HDL-C (mg/dL)	36.70±5.1	29.60±2.6^{b}	33.10±4.31^{x}	40.20±3.32$^{a\,x\,*}$
HOMA-IR	2.76±0.53	7.74±0.91^{a}	5.54±0.55$^{a\,x}$	4.84±0.44$^{a\,x}$
VLDL (mg/dL)	17.24±3.88	35.98±8.23^{a}	25.78±6.18$^{b\,y}$	22.76±4.37$^{c\,z}$
LDL-C (mg/dL)	26.40±2.55	52.80±4.87^{a}	37.4±2.79$^{b\,x}$	30.90±2.39$^{x\,*}$
HbA1c (%)	4.28±0.52	5.27±0.29^{a}	4.45±0.40^{x}	4.56±0.40^{y}
Resistin (ng/mL)	19.92±3.14	39.01±4.54^{a}	27.77±3.03$^{a\,x}$	25.32±2.41$^{b\,x}$
Apelin (ng/mL)	1.43±0.53	2.07±0.46^{c}	2.24±0.42^{b}	2.60±0.46$^{a\,y\,x}$
Insulin (ng/mL)	0.48±0.12	0.87±0.11^{a}	0.71±0.08$^{a\,x}$	0.65±0.08$^{a\,x}$
sVCAM-1 (ng/mL)	151.98±32.61	229.21±41.92	206.84±36.31	189.95±33.04

^{a}p<0.001 Compared to the control group; ^{b}p<0.01 compared to the control group; ^{c}p<0.05 compared to the control group; ^{x}p<0.001 compared to the MetS group; ^{y}p<0.01 compared to the MetS group; zp<0.05 compared to the MetS group; *p<0.05: Compared to MetS+pioglitazone and MetS+resveratrol; MetS: Metabolic syndrome; HDL-C: High density lipoprotein cholesterol; HOMA-IR: Homeostatic model assessment for insulin resistance; VLDL: Very low density lipoprotein; LDL-C: Low density lipoprotein cholesterol; HbA1c: Hemoglobin A1c; sVCAM-1: Serum soluble vascular cell adhesion molecule-1.

TABLE 2: Correlation between resistin level and apelin and biochemical parameters.

	Group 1 (n=10) (Control)	Group 2 (n=10) (MetS)	Group 3 (n=10) (MetS+pioglitazone)	Group 4 (n=10) (MetS+resveratrol)
Glucose (mg/dL)	r=0.685 p<0.05	r=0.342 p>0.05	r=0.279 p>0.05	r=0.394 p<0.05
HOMA-IR	r=0.963 p<0.001	r=0.547 p<0.05	r=0.455 p<0.05	r=0.796 p<0.01
HbA1c (%)	r=0.606 p<0.05	r=0.279 p>0.05	r=0.350 p>0.05	r=0.312 p>0.05
Insulin (ng/mL)	r=0.937 p<0.001	r=0.780 p<0.01	r=0.848 p<0.01	r=0.790 p<0.01
Apelin (ng/mL)	r=-0.358 p>0.05	r=0.451 p<0.05	r=-0.459 p<0.05	r=-0.627 p<0.05

MetS: Metabolic syndrome; HOMA-IR: Homeostatic model assessment for insulin resistance; HbA1c: Hemoglobin A1c.

degeneration and edema in the aortic vessel walls. Perivascular lymphocytic infiltration has significantly reduced in the MetS+resveratrol group. Compared with untreated metabolic syndrome and MetS+pioglitazone group, Myxoid degeneration and edema were less severe in MetS+resveratrol group (Figure 1).

Sinusoidal enlargement, congestion and lymphocytic infiltration in the periportal area were detected in the histopathological examination of the liver tissue of the group with untreated metabolic syndrome compared to control group. There was no lymphocytic infiltration and sinusoidal enlargement in the liver tissue of the resveratrol group, but very mild congestion was detected. Sinusoidal enlargement and congestion were decreased in the liver tissue of the group treated with pioglitazone compared to the liver tissue of rats with untreated MetS, but it was found to continue mildly (Figure 2).

DISCUSSION

This current study investigated the relationship between apelin and resistin levels and MetS, as well as the effect of pioglitazone and resveratrol treatment on biochemical parameters and histopathological changes.

The current study has shown that resistin level increased in Mets group. Increased resistin concentrations have been reported in MetS in both animal and human studies in the literature.[8,9] The literature

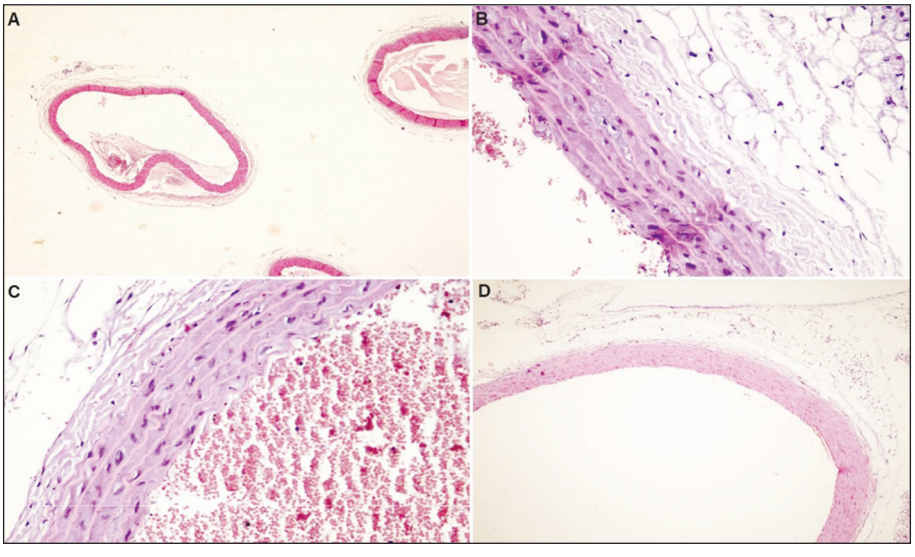

FIGURE 1: Histopathological features of aortic vascular structure. **A)** Control group (without MetS), **B)** Untreatment MetS group, **C)** MetS+pioglitazone treatment, **D)** MetS+resveratrol treatment. A, B and C panels x 400, D panel x100.
MetS: Metabolic syndrome.

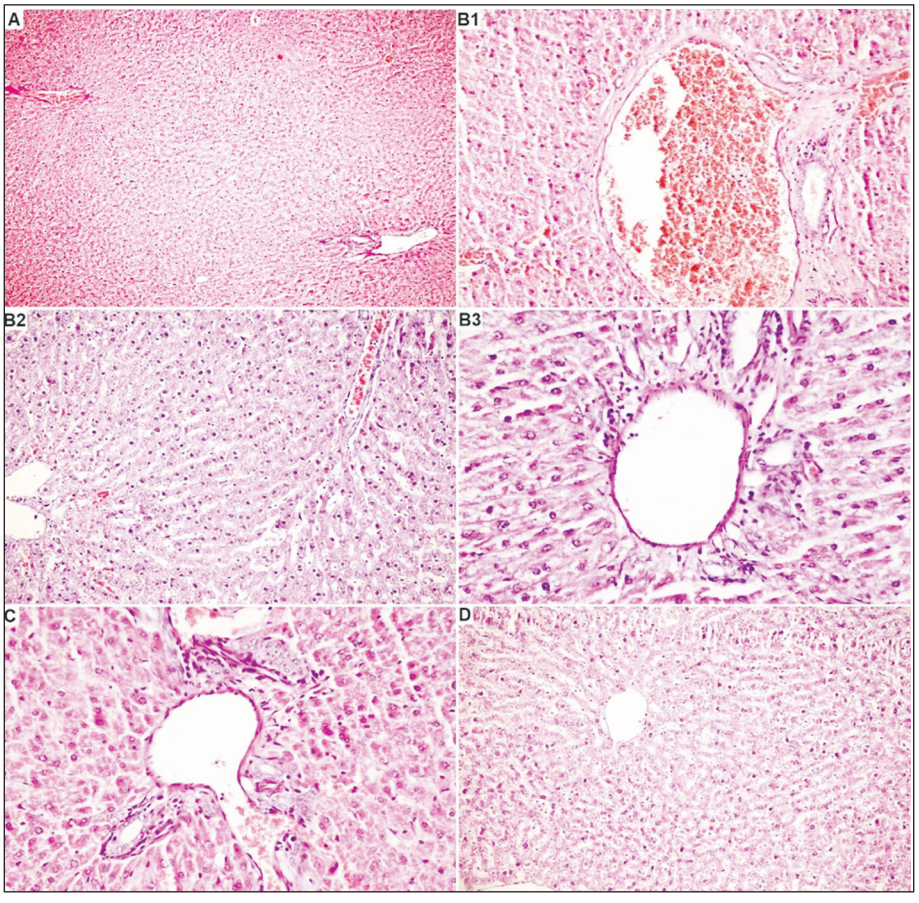

FIGURE 2: Histopathological features of liver tissue. **A)** Control group (without MetS) Panel x100, **B1)** Untreatment MetS group (congestion) panel x200, **B2)** Untreatment MetS group (sinusoidal enlargement) panel x200, **B3)** Untreatment MetS group (periportal lymphocytic infiltration) panel x200, **C)** MetS+pioglitazone treatment panel x400, **D)** MetS+resveratrol treatment. Panel x200.
MetS: Metabolic syndrome.

data and current study data once again show that resistin levels increase in MetS. Resistin is named as such because it causes insulin resistance. Therefore, it is an expected finding that the increase in resistin level related with increased insulin resistance and biochemical parameters. In the current study, the resistin level was positively correlated with the HOMA-IR score which is an indicator of IR, total cholesterol, LDL, triglyceride and glucose levels. Most of the studies have emphasized that plasma resistin levels are positively associated with obesity, IR and MetS on the other hand, there are studies reporting no correlation between plasma resistin levels, other metabolic biomarkers, and difference in plasma resistin levels in participants with MetS.[9-13]

In the current study, it was found that the level of apelin, which is an another adipokine associated with insulin resistance, increased in MetS compared to the control group. Clinical studies have reported that insulin resistant and obese individuals with T2DM have increased apelin levels compared to control groups and it is correlated positively with HbA1c.[9-14] Contrary to the general literature data, a few studies have reported that plasma apelin levels are decreased in individuals with newly diagnosed T2DM.[15] It is thought that new studies on large populations are needed to explain the correlations in apelin level, IR and MetS.

The present study has concentrated on the impacts of interventions that reduce resistin and apelin levels in MetS treatment. Current research has focused on the effects of treatments to reduce resistin and apelin levels in MetS treatment. In the present study, it was found that pioglitazone and resveratrol

treatment provided a decrease in resistin levels compared to untreatment MetS group. In addition, pioglitazone and resveratrol treatment was found to have no effect on apelin levels in our study. Al-Muzafar et al. have reported that pioglitazone reduces increased resistin levels as a result of high fat-caloric diet.[16] Sharma et al. showed that pioglitazone used in the treatment of MetS and diabetes has an agonistic effect on the peroxisome proliferator-activated receptor (PPAR) gamma receptor and PPAR gamma reduces the release of resistin.[17] There is not sufficient data in the literature regarding the effect of resveratrol on apelin and resistin. Kjær et al have reported that no beneficial effects of resveratrol on the MetS.[18] In a meta-analysis study conducted by Akbari et al., resveratrol treatment did not provide sufficient benefit in MetS, but resveratrol may have a potential cardio-protective effect in patients with MetS and related disorders.[19] The current study has shown that both pioglitazone and resveratrol therapy are associated with a decrease in resistin levels in MetS, as well as a decrease in insulin resistance and lipid parameters but could not explain its effect on apelin levels.

In the current study, the effect of pioglitazone and resveratrol treatment on histopathological changes was another subject of investigation. According the current study, although pioglitazone treatment provided a decrease in pathological findings secondary to MetS in liver and aortic tissue, resveratrol treatment was found to be more beneficial. Resveratrol's positive effects on health have been demonstrated in several studies. In all these studies, the anti-oxidant and anti-inflammatory effects of resveratrol have been emphasized and it has been reported that it may be protective against anti-carcinogenic and cardiovascular diseases.[20-22]

CONCLUSION

In the current study, resveratrol treatment caused regression in lymphocytic infiltration, congestion and sinusoidal enlargement in aortic vessel wall and liver tissue, suggesting that it has an anti-inflammatory effect and may be useful in the treatment of MetS when supported by the literature data.

Dissolution of resveratrol in 50% ethanol was considered as an important limitation due to its effect on other biochemical and pathological parameters.

Resistin and apelin concentrations have found to be increased in MetS and associated with insulin resistance. Although pioglitazone and resveratrol treatment decreased resistin levels, it was ineffective on apelin levels. Although pioglitazone treatment provided improvement in histopathological changes, resveratrol treatment had more benefits on histopathological changes. Increase in resistin and apelin levels may be associated with MetS. In the treatment of MetS, resveratrol may be useful in support therapy.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Sevgi Altaş, Mehmet Ferit Gürsu, Emir Dönder, Onur Gürsu;* ***Design:*** *Sevgi Altaş, Mehmet Ferit Gürsu;* ***Control/Supervision:*** *Mehmet Ferit Gürsu, Emir Dönder;* ***Data Collection and/or Processing:*** *Sevgi Altaş, Muhammed Emre Karaman;* ***Analysis and/or Interpretation:*** *Sevgi Altaş, Onur Gürsu, Mehmet Ferit Gürsu;* ***Literature Review:*** *Sevgi Altaş, Onur Gürsu, Muhammed Emre Karaman;* ***Writing the Article:*** *Sevgi Altaş, Onur Gürsu, Muhammed Emre Karaman, Mehmet Ferit Gürsu, Emir Dönder;* ***Critical Review:*** *Mehmet Ferit Gürsu, Emir Dönder;* ***Materials:*** *Sevgi Altaş, Onur Gürsu.*

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