Istanbul J Pharm 52 (1): 20-30
DOI: 10.26650/IstanbulJPharm.2022.979195
Original Article

Sex-dependent effects of resveratrol and regular exercise on markers related to cellular stress in the hearts and kidneys of rats

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Cite this article as: Bal, N.B., Han, S., Uslanmaz, S.E., Uludag, M.O., & Demirel Yilmaz, E. (2022). Sex-dependent effects of resveratrol and regular exercise on markers related to cellular stress in the hearts and kidneys of rats. *İstanbul Journal of Pharmacy, 52*(1), 20-30. DOI: 10.26650/IstanbulJPharm.2022.979195

ABSTRACT

Background and Aims: Regular exercise and several food supplements are recommended for a healthy lifestyle. In this study, the sex-dependent effects of resveratrol and regular exercise training on cellular stress response were investigated in the hearts and kidneys of rats.

Methods: Male and female Wistar rats (3-month-old) were used. Resveratrol (7.5 mg/kg, in the drinking water) and exercise training (for 40 minutes, three days per week) were applied for 6 weeks. The markers related to cellular stress were measured in left ventricle and kidney tissues.

Results: Cardiac total antioxidant capacity (TAC) levels were greater in males than females, but no difference was observed in kidney tissue. Exercise training increased both TAC and nitrite levels in the heart in females but not in males. Even cardiac nitrite levels were decreased in males by exercise. Cardiac phosphorylated-protein kinase RNA-like endoplasmic reticulum kinase (p-PERK) expression and renal glucose-regulated protein-78 (GRP78) expression were higher in the control male group compared to the female group. Cardiac tumor necrosis factor-α (TNF-α) expression was higher in females than males but not in kidneys. However, matrix metalloproteinase-2 (MMP-2) expression was higher in males than females in both kidney and cardiac tissues. The phospho-inhibitor κB-α (p-IκB-α) did not change between gender in cardiac tissues but had a decreased level in male kidneys compared to the female control group. Expression of phospho-extracellular signal-regulated kinase (p-ERK) in females and phospho-protein kinase B (p-Akt) in males were lower in kidney tissue. Resveratrol and exercise treatments markedly decreased Akt protein expression in male kidney tissue.

Conclusion: Our findings indicate that the markers involved in cellular stress response were affected differently by the resveratrol and exercise treatments considering sex and tissue dependencies.

Keywords: Cellular stress, Sex, Resveratrol, Exercise, Heart, Kidney

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Submitted: 09.08.2021
Revision Requested: 13.12.2021
Last Revision Received: 31.01.2022
Accepted: 15.02.2022
Published Online: 28.04.2022

INTRODUCTION

Sex is a serious determinant of life span. Biological differences between males and females resulting from genetic, epigenetic, and hormonal factors are known as sex differences. Sex differences are implicated as a crucial factor in the biology and pathophysiology of various disorders as well as outcomes of treatments (EUGenMed Cardiovascular Clinical Study Group et al., 2016; Campesi et al., 2017; Bairey Merz et al., 2019). However, little is known about the sex differences in physiological and pathophysiological processes, and the information about the effect of sex in these processes is not sufficient, either.

Epidemiological studies suggest that non-pharmacological approaches, such as dietary intervention and regular physical activity, can prevent or delay the progression of diseases. Physical activity is associated with better health and recent research has reported that regular physical activities can protect body organs against several acute and chronic diseases (Chen et al., 2019, Zhang et al., 2019). Regular exercise training has been shown to improve total antioxidant capacity (TAC), increase endothelial nitric oxide (NO) production, re-establish impaired endoplasmic reticulum (ER) homeostasis and calcium cycling, alleviate inflammation and fibrosis to protect many organs, including the heart, vessels, and kidney (Rolim et al., 2007; Tung et al., 2015; Han et al., 2018; Kosaki et al., 2019). Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a polyphenolic phytoalexin found in grapes, berries, peanuts and wines. Resveratrol has been reported to exert antioxidant, anti-inflammatory, anti-fibrotic and anti-apoptotic effects by affecting different signaling pathways (Han et al., 2018, Singh et al., 2019; Cheng et al., 2020). There are many studies examining the beneficial effects of orally administered resveratrol in the range of 3-22 mg/kg (Soylemez, Gurdal, Sepici & Akar, 2008; Soylemez, Sepici & Akar, 2009, Han et al., 2018; Cheng et al., 2020). Importantly, resveratrol has been shown to display hormetic actions, being protective at lower doses and detrimental at higher doses in both humans and animals (Juhasz, Mukherjee, & Das, 2010; Singh et al., 2019; Cheng et al., 2020). In our previous study, it has been shown that resveratrol intake and regular exercise training have beneficial effects on vessel functions and the vascular expression of mRNA of inflammation and oxidative stress-related molecules in an age- and sex-dependent manner (Han et al., 2018). Although there is growing evidence suggesting that resveratrol and regular exercise have beneficial effects on heart and kidney, sex differentiation of the effects and exact mechanisms of their action have not been clarified yet. Considering that gender difference affects many physiological/pathological processes and is even organ tissue-specific, this issue becomes more important.

Therefore, in the current study, the sex-dependent effects of resveratrol intake and regular exercise training on the NO and TAC levels and the expressions of some proteins related to cellular stress response, which play a role in the pathogenesis of cardiovascular and renal diseases, were investigated in the heart and kidney tissue of rats.

MATERIAL AND METHODS

Animal care and experimental protocol

The experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The research protocols were approved by the Local Ethics Committee for Animal Experiments of Ankara University.

Wistar male and female rats (n:8 rats per group, total 48 rats) were taken from The Laboratory Animal Service of the University of Ankara and maintained under standard conditions (24±1°C room with a 12:12 hour light/dark cycle) for the duration of the experiment. The ages of the rats were adjusted to be 3 months-old at the time of sacrification. Water and standard rat chow were provided *ad libitum* during the experimental period. The rats were randomly divided into six groups: Control male group (n=8), Control female group (n=8), Resveratrol male group (n=8), Resveratrol female group (n=8), Exercise male group (n=8) and Exercise female group (n=8).

The rats in the control groups were given tap water (without resveratrol) and the exercise training was not performed. All rats in the exercise groups were familiarized with a horizontal rodent treadmill (May Tme 0804 Animal Treadmill, Turkey) for one week (with a speed of 20 m/min at 0° incline, for 15 min/day, 3 days a week). After the one-week acclimatization period, the animals underwent a 40-min running session on a horizontal treadmill at 20 m/min (0° incline), 3 days per week, for 6 weeks. In the resveratrol group, resveratrol was dissolved in absolute ethanol and diluted with drinking water to a concentration of 50 mg/L, at a level sufficient to provide the appropriate dose (7.5 mg/kg) based on the consumption (bottles were protected from light). The concentration and administration route of resveratrol were chosen from previous *in vivo* studies (Soylemez et al. 2008; Soylemez et al. 2009). At the end of the study, the rats were anesthetized with 40 mg/kg i.p. sodium pentobarbital. The left ventricle and kidney tissues were quickly removed and frozen in liquid nitrogen after washing with phosphate-buffered saline (PBS) and stored at -80°C.

Biochemical measurements

The left ventricle and kidney tissues were homogenized in PBS (approximately 1g tissue/10 mL PBS) on ice. The homogenates were centrifuged at 4°C and 10000 rpm for 30 minutes. Then, the supernatants were carefully collected for biochemical measurements. The protein concentration of the supernatants was evaluated using the Bradford method. The tissue nitrite level as an indicator of nitric oxide (NO) production was measured spectrophotometrically by the method based on the Griess reaction. TAC levels of the tissue homogenates were measured by the method based on the reduction of Cu^{+2} to Cu^{+1} by the antioxidants (Usanmaz & Demirel-Yılmaz, 2008).

Tissue homogenization and Western Blot experiments

The total protein was extracted from left ventricle and kidney tissue via homogenization in homogenization buffer (approximately 50 mg tissue/1mL buffer) (including 50 mM Tris pH: 8.8, 1% NP40, 1 mM PMSF, 2 mM disodyum-EDTA, 10% Sucrose and protease-phosphatase inhibitor) with a homogenizer. The homogenate was centrifuged at 1000 g for 15 min at 4°C, and then the supernatant was collected. The protein concentration of the supernatants was evaluated using the Bradford method. Equal amounts (50 µg *per* lane) of proteins from different groups were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 4 % stacking gel and 10 % separating gel with a Mini Protean Tetra electrophoresis apparatus (Bio-Rad Laboratories) and transferred onto PVDF membranes using a semi-dry electro-blotting apparatus (TransBlot Turbo, BioRad, Puchheim, Germany) (Towbin, Staehelin & Gordon, 1979; Bal et al., 2020). After the transfer period, the membranes were incubated for 1-1.5 h in blocking solution containing 5% non-fat dried milk or 3% BSA (for phospho proteins) in Tris-buffered saline (TBS). Then, the membranes were incubated overnight at 4 °C with one of the following antibodies: glucose-regulated protein-78 (GRP78), phosphorylated-protein kinase RNA-like endoplasmic reticulum kinase (p-PERK), sarco/endoplasmic reticulum Ca^{2+}-ATPase2 (SERCA2), inhibitor κB-α (IκB-α), p-IκB-α, matrix metalloproteinase-2 (MMP-2), Tumor necrosis factor-alpha (TNF-α), extracellular signal-regulated kinase (ERK), protein kinase B (Akt), p-ERK and p-Akt. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Then, the membranes were incubated in chemiluminescence substrate solution (Clarity TM Western ECL solution, Bio-Rad). Images of the blots were taken with the chemiluminescence detection system (Bio-Rad). GAPDH was used as the loading control protein. The relative expression of proteins with respect to GAPDH was calculated using the Image Lab 4.1 software. The results of protein expression demonstrate the average of all rats used in the experiment (n= 5-8), and the best image representing the result has been used.

Statistical analysis

The data were expressed as means±SEM. Statistical analyses were performed using GraphPad Prism Instat 5.01 (GraphPad Software, Inc., La Jolla, USA). Results were analyzed using Student's t-test (for comparison of sex differences) or One-way ANOVA followed by post hoc Dunnet's test (for inter-group comparisons). P-values ≤0.05 were considered statistically significant.

RESULTS

In heart tissue, TAC levels of the female control and resveratrol groups were lower than those of the male groups (Fig. 1A). Exercise training significantly increased the cardiac TAC levels only in female animals. In heart and kidney tissue, the TAC levels of female exercise group were higher than those of the female resveratrol group (Fig. 1A and B). The cardiac TAC levels were lower than the renal TAC levels in female animals (Fig. 1C).

Exercise training significantly alleviated the cardiac nitrite levels in the male rats (Fig 2A). The nitrite levels of heart tissue were higher in female resveratrol and exercise groups compared to those of the male groups (Fig. 2A). In kidney tissue, nitrite levels were similar in all groups (Fig. 2B). The cardiac nitrite levels of female resveratrol and exercise groups were greater than the renal nitrite levels (Fig. 2C).

GRP78 expression, which is an important marker of endoplasmic reticulum stress (ERS), was similar in the hearts of male and female

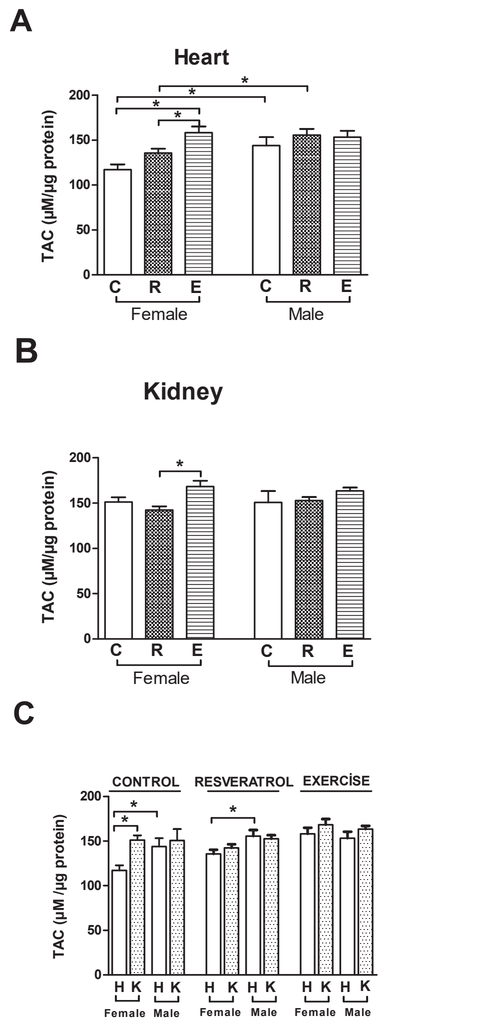

Figure 1. Cardiac and renal TAC levels in control, resveratrol, and exercise training rats. Cardiac TAC levels were higher in males than in females (A). While exercise only increased the cardiac TAC levels in females, resveratrol did not affect the TAC levels in both tissues (B). Renal TAC levels are greater than cardiac TAC levels in control female rats (C). *p < 0.05. Values are expressed as mean ± SEM. H: Heart, K: Kidney.

rats. In male resveratrol and exercise-trained groups, GRP78 expression was higher than that of females (Fig 3A and B). The PERK pathway is one of the unfolded protein responses (UPR) arms that is active during ERS. The p-PERK expression of the male rats was higher than that of females, while resveratrol and exercise did not affect p-PERK expression in both sexes (Fig 3A and C). SERCA2 is an important influx pump in the storage of Ca^{2+} into the ER. The expression of SERCA2 was similar in all groups (Fig 3A and D).

In the kidneys, the GRP78 expression of female rats was lower than that of male rats, but resveratrol consumption and exercise training did not alter the GRP78 expression in both sexes (Fig. 4A and B). Resveratrol significantly increased the p-PERK expression in females. In the female resveratrol and exercise groups, the p-PERK expression was markedly greater than that of male groups (Fig 4A and C).

TNF-α is an important proinflammatory cytokine, and cardiac TNF-α protein levels were higher in the female group. On the

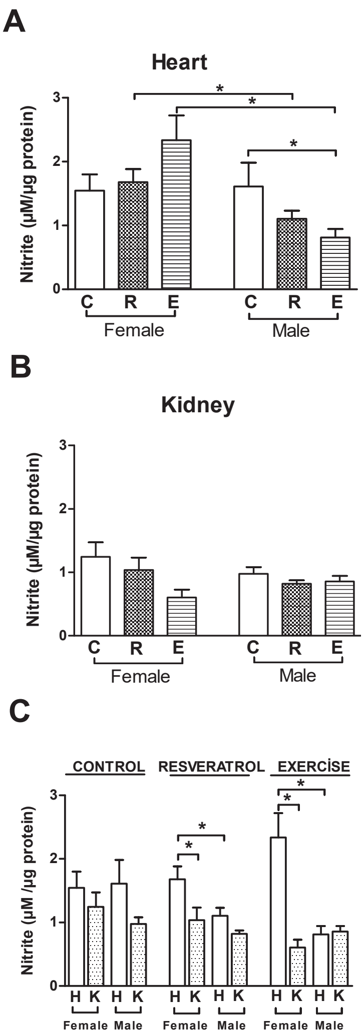

Figure 2. Cardiac and renal nitrite levels in control, resveratrol, and exercise training rats. The cardiac nitrite levels of female exercise and resveratrol groups were higher than those of males. The exercise reduced the cardiac nitrite levels in male rats (A). The renal nitrite levels were similar in all groups (B). Cardiac nitrite levels are higher than renal nitrite levels in resveratrol and exercise-treated female rats (C). *p < 0.05. Values are expressed as mean ± SEM. H: Heart, K: Kidney.

other hand, profibrotic marker MMP-2 expression was lower in the heart of the female group compared to that of the male group (Fig. 5 A, B and E). Resveratrol treatment and exercise training did not affect TNF-α, IκB-α, p-IκB-α, and MMP-2 protein levels in the heart tissue of both sexes (Fig 5).

In the female resveratrol group, renal TNF-α expression was markedly greater than that of the male group (Fig 6A and B). While p-IκB-α protein levels were higher (Fig 6A and C), MMP-2 expression was lower in female rats compared to male rats (Fig. 6A and D). Exercise training did not alter renal TNF-α, p-IκB-α, and MMP-2 protein expressions in both sexes (Fig. 6).

Akt, p-Akt, ERK, and p-ERK protein levels were similar in the heart tissue of female and male rats. Resveratrol intake did not change these protein levels in both sexes, but exercise training significantly decreased the ERK expression only in male group (Fig 7).

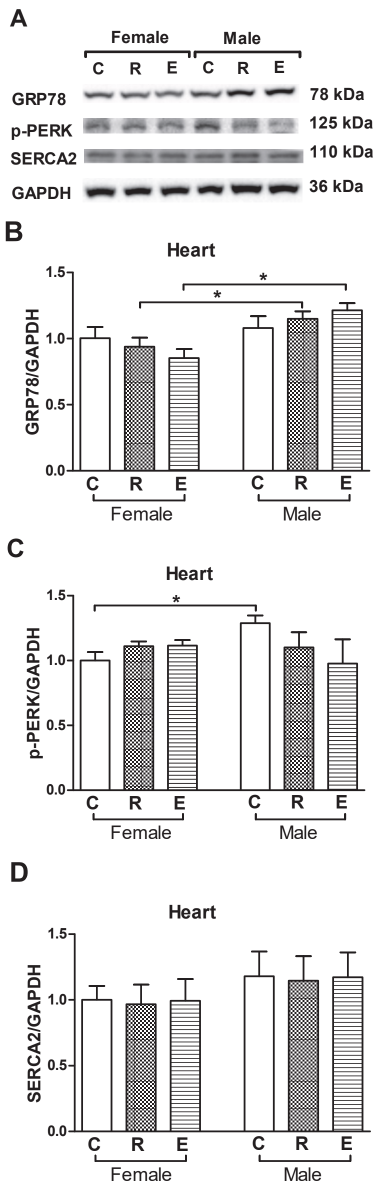

Figure 3. Sex-dependent effects of resveratrol treatment and exercise training on cardiac GRP78, p-PERK, and SERCA2 expression. In male resveratrol and exercise groups, the cardiac GRP78 expression was higher than that of female groups (A and B). In female rats, cardiac p-PERK expression was lower than that of males (A and C). The SERCA2 expression was similar in all groups (A and D). *p < 0.05. Values are expressed as mean ± SEM.

In the kidneys, resveratrol intake and exercise training significantly decreased the Akt expression in males (Fig 8A and B). p-Akt levels of the female control and resveratrol groups were greater than those of the male groups (Fig 8A and C). Similarly, the ERK expression of the female resveratrol group was higher than that of the male resveratrol group. The resveratrol treatment markedly reduced ERK expression in male rats (Fig 8A and D). Unlike p-Akt expression, p-ERK expression was lower in the female rats compared to the male rats (Fig 8A and E).

In this study, SERCA2 and IκB-α expressions were also examined in kidney tissue isolated from all groups. However, since the expres-

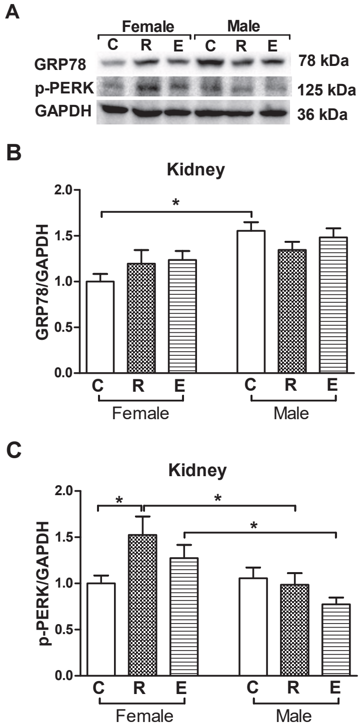

Figure 4. Sex-dependent effects of resveratrol treatment and exercise training on renal GRP78 and p-PERK expression. In females, the renal GRP78 expression was smaller than that of males (A and B). The resveratrol intake increased the renal p-PERK expression in female animals. The expression of p-PERK in the female resveratrol and exercise groups is higher than that of males (A and C). *p < 0.05. Values are expressed as mean ± SEM.

sions of SERCA2 and IκB-α were not good enough to be quantified, these findings were not included in the present study.

All sex-dependent alterations in cardiac and renal protein expressions are summarized in Table-1 and Table2, respectively.

DISCUSSION

In the current study, the effects of resveratrol consumption and exercise-training on various factors related to cellular stress were examined in a tissue- and sex-dependent manner. The differences in TAC levels and p-PERK, TNF-α, and MMP-2 protein expressions in the heart and GRP78, p-IκB-α, MMP-2, p-Akt, and p-ERK expressions in kidney tissue were observed between sexes. Tissue TAC and NO levels and some protein expressions were separately changed by resveratrol intake and regular exercise training in a sex-dependent manner.

The effect of sex in control animals on biomarkers

It has been known that males and females have different responses in both basal and stressful conditions. Revealing sex-specific molecular differences in cellular stress responses in the adult healthy heart and kidney may contribute to a better understanding of the sex-specific aspects of human cardiovascular and kidney disease. In this part, sex- and tissue-dependent differences of the NO and TAC levels and some proteins related to cellular stress response, which play a role in the biology and pathophysiology of cardiovascular and renal diseases, are discussed.

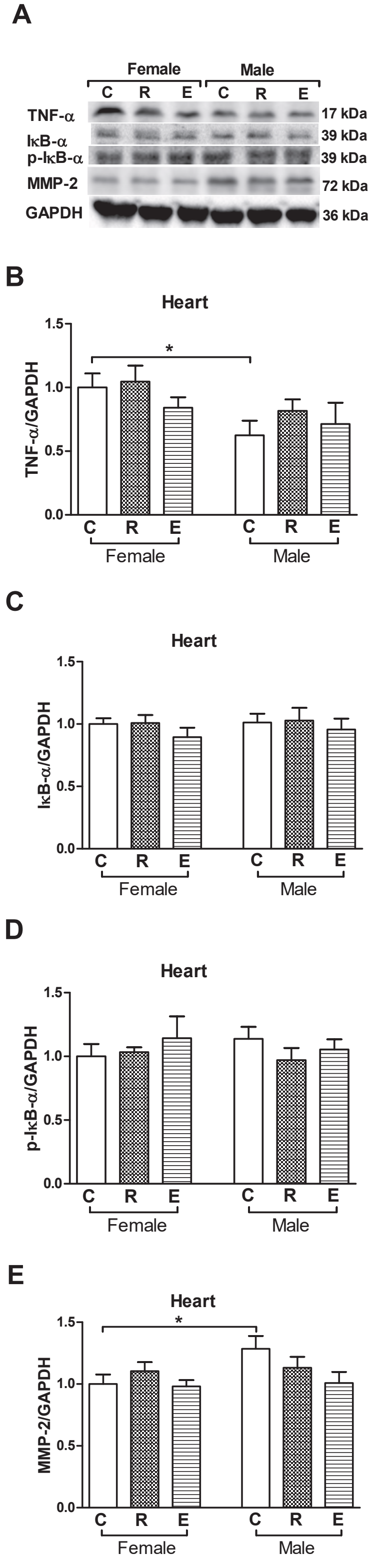

Figure 5. Sex-dependent effects of resveratrol treatment and exercise training on cardiac TNF-α, IκB-α, p- IκB-α, and MMP-2 expression. The cardiac TNF-a (A and B) expression is higher,and the MMP-2 (A and E) expression is lower in the female group compared to those inthe male group. IκB-α and p- IκB-α levels were similar in all groups (A, C and D) *p < 0.05. Values are expressed as mean ± SEM.

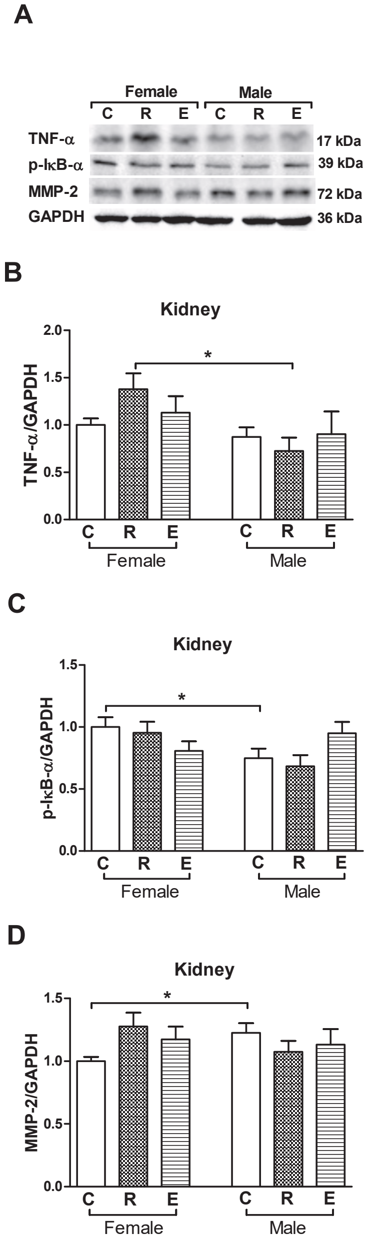

Figure 6. Sex-dependent effects of resveratrol treatment and exercise training on renal TNF-α, p- IκB-α, and MMP-2 expression. Renal TNF-α levels were greater in resveratrol-treated female rats compared to resveratrol-treated male rats (A and B). The renal p-IκB-α expression (A and C) was higher, while the MMP-2 expression (A and D) was lower in female rats compared to those of male rats. *$p < 0.05$. Values are expressed as mean ± SEM.

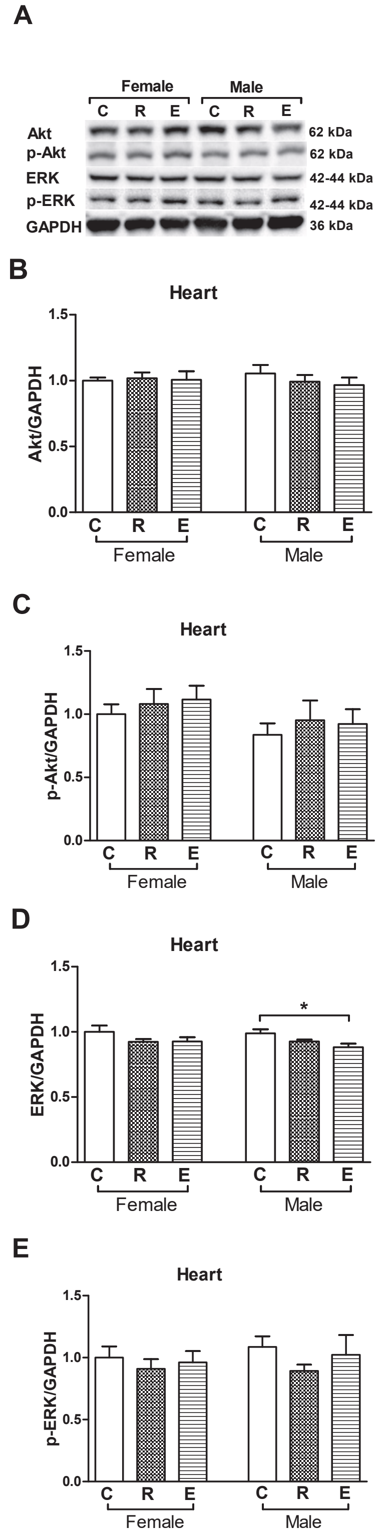

Figure 7. Sex-dependent effects of resveratrol treatment and exercise training on cardiac Akt, p-Akt, ERK, and p-ERK expression. The cardiac Akt, p-Akt, ERK, and p-ERK expressions were not different in females and males (A-E). The exercise only reduced the ERK expression in male rats (A and D). *$p < 0.05$. Values are expressed as mean ± SEM.

Increased production of reactive oxygen species or loss of antioxidant defense, defined as oxidative stress, disrupts many physiological processes, leading to detrimental changes. Due to the many different antioxidant components in the body and the relative difficulty of measuring each known antioxidant separately, the total antioxidant capacity was measured as a general indicator of oxidative stress in tissue homogenates. In the current study, cardiac TAC levels were higher in males than in females, but renal TAC levels were similar in both sexes. This finding shows that the sex-related differences in TAC level may

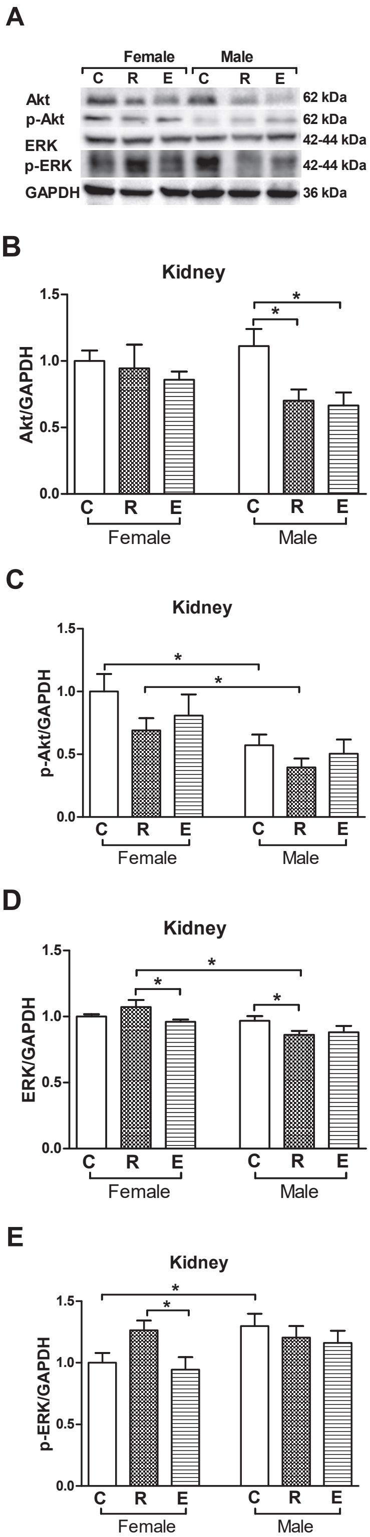

Figure 8. Sex-dependent effects of resveratrol treatment and exercise training on renal Akt, p-Akt, ERK, and p-ERK expression. In females, the renal p-Akt expression (A and C) was higher while the p-ERK expression (A and E) was lower compared to those of males. The resveratrol treatment reduced the Akt and ERK expressions in male animals (A, B and D). *$p < 0.05$. Values are expressed as mean ± SEM.

vary depending on the tissue. Contrary to our results, cardiac and renal TAC levels have been reported to be lower in 15-16-week-old male Wistar rats than in females (Katalinic, Modun, Music, & Boban, 2005). In another study, the cardiac TAC levels in 8-week-old Wistar rats were found to be similar in both sexes (Saghebjoo et al., 2019). These different results in TAC levels may be due to the different measurement methods (FRAP, ABST, etc.) or the use of animals of different ages.

Recent studies have demonstrated the deficiency of NO, which plays a pivotal role as a key secondary messenger that has a role in pathological activities, is both a cause and a consequence of many diseases and organ damages (Baylis, 2012). In the current study, cardiac and renal NO levels were not different in female and male rats. Consistent with our findings, it was reported that cardiac NO levels and renal eNOS expression levels were similar in male and female rats (Erdely, Greenfeld, Wagner & Baylis, 2003; Balcı & Pepe, 2012). In light of these data, it can be predicted that NO levels do not change depending on sex or tissue.

The ER regulates protein folding, maturation, quality control, transport, and intracellular Ca^{2+} levels. Disruption of these ER functions leads to the retention of newly synthesized unfolded proteins in the ER, and this condition is referred as ER stress. When ER stress occurs, GRP78 dissociates from the transmembrane sensors (PERK, ATF6 and IRE1), and these signal transduction pathways are activated for the restoration of ER homeostasis (Hong, Kim K, Kim JH & Park, 2017). In the current study, we observed that renal GRP78 expression and cardiac p-PERK expression were higher in males than those of females. These results show that basal expressions of ER stress proteins may vary depending on sex and tissue. Also, it can be argued that the ER stress burden is higher in males than females. SERCA2 is an important pump in the storage of Ca^{2+} back into the ER. The reduction in SERCA2 activity and/or expression leads to the decrease in the ER Ca^{2+} levels and triggers ER stress (Chemaly, Troncone & Lebeche, 2018). In the present study, cardiac SERCA2 expression was similar in both sexes. In light of this result, it can be said that the change in SERCA-mediated calcium regulation in the heart does not contribute to the sex differences in ER stress burden.

It is known that inflammatory response plays a role in the pathogenesis of various acute and chronic diseases and is associated with organ damage. The activation of transcription factor NF-κB is one of the important inflammatory pathways. Under basal conditions, NF-κB are bound to IκB in the cytoplasm and are inactive. During the activation, IκB is phosphorylated by IKB kinases and separated from NF-κB, which leads to the activation of NF-κB signaling. The activation of this pathway coordinates several gene expression involved in inflammation, fibrosis, and apoptosis (Fiordelisi et al. 2019). However, sex and tissue-dependent alterations of protein expressions related to inflammation are still not studied in detail. In our previous study, it was reported that the mRNA expressions of IL-6, NF-κB, and TNF-α in thoracic aorta were similar in both sexes. In the current study, the cardiac TNF-α expression was greater in females while the cardiac MMP-2 expression was higher in

Table 1. Sex-dependent effects of resveratrol treatment and exercise training on cardiac protein expression.

HEART						
	Female			Male		
Protein	**Control**	**Resveratrol**	**Exercise**	**Control**	**Resveratrol**	**Exercise**
GRP78	-	F<M	F<M	-	F<M	F<M
p-PERK	F<M	-	-	F<M	-	-
SERCA2	-	-	-	-	-	-
TNF-α	F>M	-	-	F>M	-	-
IκB-α	-	-	-	-	-	-
p-IκB-α	-	-	-	-	-	-
MMP-2	F<M	-	-	F<M	-	-
Akt	-	-	-	-	-	-
p-Akt	-	-	-	-	-	-
ERK	-	-	-	C>E	-	C>E
p-ERK	-	-	-	-	-	-

F: Female, M: Male, C: Control, R: Resveratrol, E: Exercise

Table 2. Sex-dependent effects of resveratrol treatment and exercise training on renal protein expression.

KIDNEY						
	Female			Male		
Protein	**Control**	**Resveratrol**	**Exercise**	**Control**	**Resveratrol**	**Exercise**
GRP78	F<M	-	-	F<M	-	-
p-PERK	C<R	F>M, C<R	F>M	-	F>M	F>M
TNF-α	-	F>M	-	-	F>M	-
p-IκB-α	F>M	-	-	F>M	-	-
MMP-2	F<M	-	-	F<M	-	-
Akt	-	-	-	C>R, C>E	C>R	C>E
p-Akt	F>M	F>M	-	F>M	F>M	-
ERK	-	F>M, R>E	R>E	C>R	F>M, C>R	-
p-ERK	F<M	R>E	R>E	F<M	-	-

F: Female, M: Male, C: Control, R: Resveratrol, E: Exercise

males. On the other hand, in kidney tissue the p-IκB-α expression was higher; the MMP-2 expression was lower in female rats compared to those of male rats. These findings suggest expressions of some inflammatory and fibrotic proteins may differ depending on tissue and sex. Considering the above results, it can be argued that the inflammatory response in females and the fibrotic response in males are predominant in the basal state.

Akt and ERK-mediated signal transduction have been implicated in many cellular activities ranging from gene expression to proliferation, stress signaling, inflammatory-fibrotic responses, and apoptosis (Zhang et al., 2016; Liu et al., 2017). In the present study, the cardiac expressions of Akt, ERK, and their phosphorylated forms were similar in both sexes, in parallel with the findings of the previous study on twelve-week-old male and female C57Bl/6 mice (Dworatzek et al., 2014). It was observed that p-Akt expression in females and p-ERK expression in males were greater in kidney tissue. It can be speculated that the Akt signaling pathway in females and the ERK signaling pathway in males is mostly active. In addition, the renal inflammatory state may be mediated by the Akt pathway in females and the renal fibrotic state by the ERK pathway in males, but more evidence is required to confirm this speculation. Also, there was no sex-related difference in these signaling pathways in heart tissue.

The sex specific effect of resveratrol treatment

Resveratrol consumption has beneficial effects in many different pathologies by regulating the oxidant/antioxidant balance, promoting nitric oxide production, and modulating inflammatory, fibrotic, and mitogenic pathways (Cheng et al., 2020). It has been suggested that resveratrol intake increases antioxidant capacity by modulating antioxidant defense systems. In a recent study, it has been shown that resveratrol administration (10 mg/kg resveratrol per day via gavage, for 12 week) increased plasma TAC levels in 6-week-old male Wistar rats (Nasiri et al., 2021). In the present study, resveratrol intake did not affect the tissue TAC levels in both sexes. On the other hand, cardiac TAC levels were higher in resveratrol-treated males than in the females. This may be because control males also have greater cardiac TAC levels than females.

Promoting nitric oxide production by different mechanisms plays an important role in the cardiovascular and renal protective effects of dietary resveratrol. It has been previously reported that resveratrol can increase NO levels through estrogen receptors (Li, Xia & Förstermann, 2012; Cheng et al., 2020). Also, it has been demonstrated that resveratrol intake increased the blood nitrate/nitrite levels and aortic NO production, eNOS mRNA, and protein levels in rats of both sexes (Soylemez et al., 2008; Soylemez et al., 2009; Pektaş, Sadi & Akar, 2015; Han et al., 2018). In contrast to the previous studies, resveratrol intake did not alter the tissue NO levels in any group in the current study. It can be speculated that this may be due to the differences in the protocol of resveratrol treatment (dose, duration, intensity etc.), age, sex, or tissue of the rats. However, in resveratrol-treated groups, cardiac NO levels were significantly higher in females than in the males. This result is attributable to sex differences in hormonal and/or metabolic status and may be due to the fact that resveratrol increases NO production (mediated by eNOS or iNOS) via cardiac estrogen receptors.

It was reported that resveratrol consumption inhibited ER stress induced by different pathologies (Lin et al., 2016). In contrast to previous studies, it was observed in the current study that resveratrol intake did not affect the expression of ER stress markers GRP78 and p-PERK in both tissues of male and female rats. Also, cardiac SERCA2 expression was similar in resveratrol-treated groups. However, in resveratrol-treated male groups, GRP78 expression was higher in the heart, while p-PERK expression was lower in kidney tissue, compared to females. These findings could be interpreted as the sex- and tissue-dependent variable effects of resveratrol on these stress markers. To the best of our knowledge, the tissue- and sex-dependent or independent effects of resveratrol intake on protein expressions related to ER stress have been demonstrated for the first time in this study.

Although previous studies have reported that resveratrol intake has anti-inflammatory and anti-fibrotic effects (Li et al., 2012; Olesen et al., 2013; Gliemann, Nyberg & Hellsten, 2016), in the present study, resveratrol supplementation did not affect the expression of inflammatory and fibrotic proteins in the heart and kidney tissue of both sexes. In females, only renal expression of TNF-α tended to increase with resveratrol treatment (but was not statistically significant) and probably therefore the renal TNF-α level was higher in resveratrol-treated females than in the males. Based on this finding, it can be said that the dose/duration of resveratrol treatment may not be enough to affect inflammatory and fibrotic markers in both male and female rats.

In addition, the sex and tissue-related effects of resveratrol on Akt and ERK signaling pathways, which have an important role in the regulation of many cellular processes and stress response, were investigated. Resveratrol administration reduced Akt and ERK expressions only in the kidney tissue of males, but it did not affect the cardiac protein expressions. On the other hand, renal p-Akt expression was lower in resveratrol-treated males than in the females. This may be because control males also have smaller renal p-Akt expression than females. Further studies are needed to elucidate the exact mechanisms and signaling pathways of the resveratrol in a sex- and tissue-dependent manner.

The sex specific effect of regular exercise

Like resveratrol supplementation, regular exercise is a lifestyle change that has beneficial effects on many pathologies by regulating various cellular stress responses (Gliemann et al., 2016). In a recent study, it has been shown that exercise training (5 times *per* week at the speed of 10 m/min for 10 min; the speed gradually increased to 30 m/min for 60 min. for 12 weeks) decreased plasma TAC levels in 6-week-old male Wistar rats (Nasiri et al., 2021). Also, it has been reported that 8-week-exercise training increased plasma TAC levels (TRAP method) but did not alter cardiac TAC levels in male Wistar rats (Farah et al., 2016). In the present study, regular exercise increased the cardiac TAC levels only in female animals. It has been reported that the sex-specific favorable cardiac effects of exercise are modulated by estrogen receptor-β (Dworatzek et al., 2014). Considering these results, it can be suggested that the effects of regular exercise on cardiac TAC levels may be mediated by estrogen receptors.

In our previous study, it was reported that exercise training augmented the mRNA levels of eNOS in the aortas of 3-months old female rats (Han et al., 2018). In this study, regular exercise tended to increase cardiac NO levels in females (but was not statistically significant), while markedly decreasing it in males. Therefore, cardiac NO levels were different between the exercise-treated female and male groups. As with TAC levels, the sex-specific effect of regular exercise on cardiac NO levels may be attributed to hormonal status and cardiac estrogen receptors. In addition, regular exercise did not alter renal NO levels in both sexes. The sex-dependent effects of exercise training on cardiac and renal NO levels have been presented for the first time in the current study. These findings suggest that the effect of regular exercise training on NO levels may be tissue and sex-dependent.

It was reported that regular exercise training inhibited ER stress, inflammation, and fibrosis induced by different pathologies (Dworatzek et al., 2014; Farah et al., 2016; Chengji & Xianjin, 2019, Kosaki et al., 2019). In contrast to previous studies, it was observed in the current study that regular exercise did not alter the protein levels related to ER stress in heart and kidney tissues. Although there is no sex difference between control groups, in exercise-treated male groups, GRP78 expression was higher in the heart, while p-PERK expression was lower in kidney tissue, compared to females. Also, regular exercise did not affect the expression of inflammatory and fibrotic proteins in both tissue of male and female rats. It can be said that the exercise program that was performed (exercise training intensity, duration, and frequency) may not have been enough to affect the examined inflammatory and fibrotic biomarkers in both sexes. All these findings suggest that the effects of regular exercise on cellular stress response may vary depending on gender and tissue.

Exercise training decreased the cardiac ERK expression and renal Akt expression of male animals. In addition, there was no sex-related difference in Akt, p-Akt, ERK, and p-ERK expressions in both tissues of the exercise-treated groups. Further studies are needed to elucidate the exact mechanisms and signaling pathways of the exercise applications in a sex- and tissue-dependent manner.

Considering above all results, it can be said that regular exercise acts by increasing cardiac TAC and NO levels in females, and decreasing cardiac NO level (unwanted effect), cardiac ERK expression, and renal Akt expression in males.

CONCLUSION

In summary, our results demonstrated that both resveratrol intake and regular exercise altered the markers involved in several pathologies such as tissue TAC/NO levels, GRP78, p-PERK, TNF-α, p-IκB-α, MMP-2, Akt, p-Akt, ERK, and p-ERK protein expressions, regarding sex and tissue dependencies. These findings suggest that the both physiological and pathophysiological process may not show the same pattern in males and females, therefore the effects of non-pharmacological approaches such as resveratrol and regular exercise also may vary with sex.

Acknowledgement: The authors are thankful to Prof. Dr. H. Gurdal and B. Dalkiliç for supporting this research with the Western Blot experiments.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- B.N.B., S.H., E.D.Y.; Data Acquisition- N.B.B., S.H., S.E.U.; Data Analysis/Interpretation- N.B.B., S.H., S.E.U., M.O.U., E.D.Y.; Drafting Manuscript- N.B.B., E.D.Y.; Critical Revision of Manuscript- N.B.B., S.H., S.E.U., M.O.U., E.D.Y.; Final Approval and Accountability- N.B.B., S.H., S.E.U., M.O.U., E.D.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This research was funded by the Ankara University Research Faundation(Grant number: 12B3330001)

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