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The effects of resveratrol and exercise on age and gender-dependent alterations of vascular functions and biomarkers

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ABSTRACT

The purpose of this study was to determine the effects of resveratrol and regular aerobic exercise on vascular functions and biomarkers related to vessel responsiveness in an age and gender-dependent manner.

The study used young (3 months) and old (12 months) male and female Wistar albino rats. Resveratrol was given in the drinking water (0.05 mg/ml; approximately 7.5 mg/kg) for 6 weeks. In the exercise group, all rats performed treadmill running at 20 m/min on a 0° incline, 40 min/day, 3 times a week, for 6 weeks.

Acetylcholine-induced, endothelium-dependent and sodium nitroprusside-mediated, endothelium-independent relaxations of rat thoracic aorta and blood levels of biomarkers were separately changed by resveratrol intake and exercise-training in an age and gender-dependent manner. Antioxidant enzymes and eNOS expressions in vessels were elevated by resveratrol and exercise. Resveratrol and exercise enhanced gene expressions of non-selective PDE1, 2, 3 and cAMP selective PDE4 but not cGMP selective PDE5 in the aorta. In addition, the aortic mRNA expression of inflammation markers were altered by resveratrol and exercise-training.

The results of the study demonstrated that vessel responsiveness and biomarkers related to vascular functions were altered by resveratrol consumption and exercise-training in an age and gender-dependent manner.

1. Introduction

Aging is the main risk factor for cardiovascular diseases (Hayflick, 2007). The effect of aging on cardiovascular risk is partly attributed to the development of vascular endothelial dysfunction. Endothelial cells play a pivotal role in the regulation of vascular tone by secreting various active substances (Sader and Celermajer, 2002). However, endothelial dysfunction has been correlated with decreased synthesis, release or the effect of nitric oxide (NO). NO is involved in various physiological and pathological responses, including vascular smooth muscle relaxation, platelet agregation and immune function. It has been shown that endothelium-dependent, NO-mediated relaxations are decreased by aging in different species (Kim et al., 2009; Aggarwal et al., 2008; Taddei et al., 1995).

Another important feature of endothelial dysfunction is attributed to the failure of endothelium-derived NO bioavailability. Excessive generation of reactive oxygen species (ROS), such as superoxide radicals, inactivates NO and inhibits NO synthase (NOS) (Brandes et al., 2005; van der Loo et al., 2000). Thus, endothelial NO bioavailability is decreased and endothelial function is impaired. Increased ROS production in oxidative stress has been determined during the aging process in plasma (Kalani et al., 2006; Iciek et al., 2004) and isolated rat aorta (Li et al., 2010; Marmol et al., 2007; Ungvari et al., 2011; Lund et al., 2009). Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS, is increased with advancing age and plays an important role in endothelial dysfunction (Tanahashi et al., 2013). Vascular inflammation is enhanced with age (Li et al., 2010) and increased inflammation has been reported to be involved in the mechanism of aging-induced endothelial dysfunction (Brandes et al., 2005).

In the target cell, NO activates soluble guanylate cyclase (sGC) and then increases the cyclic nucleotide second messenger cGMP level. The relaxant effect of NO on the vessels is mediated by cGMP (Omori and Kotera, 2007). Cyclic nucleotide phosphodiesterases (PDEs) regulate relaxation of vascular smooth muscle by degrading the cyclic nucleotides (Bobin et al., 2016). It has been suggested that there is a relationship between PDE expression and vascular abnormalities in aging (Yan, 2015).

While aging may impair endothelial function, some nutritional

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supplements produce beneficial effects on endothelial function. For example, resveratrol, a natural polyphenol, reverses organ pathologies associated with aging and cardiovascular diseases (Buluc and Demirel-Yilmaz, 2002, Buluc and Demirel-Yilmaz, 2006, Buluc et al., 2007, Han et al., 2015). Recent studies have shown evidence that resveratrol treatment exerts vasoprotective effects in aged mice (Pearson et al., 2008) and rats (Ungvari et al., 2007), alleviating oxidative stress, improving endothelial function, inhibiting vascular inflammation, and decreasing endothelial apoptosis. Regular aerobic exercise training has similar biological benefits to those of some nutritional supplements, and has been shown to prevent and restore age-related alterations in endothelial function (DeSouza et al., 2000), possibly by reducing oxidative stress and increasing NO bioavailability in the endothelium of aging humans (Taddei et al., 2000; Eskurza et al., 2004) and animals (Spier et al., 2004; Durrant et al., 2009).

Although there is abundant evidence suggesting that resveratrol and regular aerobic exercise have beneficial effects on age-related changes, sex differentiation of the effects and detailed mechanisms of action have not yet been fully determined. In the present study, the effects of resveratrol and regular aerobic exercise on vessel functions, the expression of aortic PDEs, oxidative stress and inflammation related genes, and blood levels of biomarkers related to endothelial function (NO, ADMA and TAC) was examined in age and gender-dependent manner in rats.

2. Materials and methods

2.1. Ethical approval

Animal care and research protocols were approved by the Local Ethics Committee of Ankara University, Ankara, Turkey.

2.2. Animals and experimental design

Young (3 months) and old (12 months) female and male Wistar rats (n = 9–10 rats per group) obtained from The Laboratory Animal Service of the University of Ankara, were used in the present study. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. The rats were housed at constant room temperature (24 ± 1 °C), humidity (50–60%) and light cycle (12:12 h light-dark) with free access to standard rat chow and tap water. The young and old rats were randomly separated into 3 groups as the control group, resveratrol group and exercise group. The rats were weighed weekly.

The control group rats were given tap water and did not perform treadmill running. The control group (C) were assigned as control-young female (C-YF), control-young male (C-YM), control-old female (C-OF) and control-old male (C-OM).

In the resveratrol (R) group, the young and old rats were assigned as young female (R-YF), young male (R-YM), old female (R-OF) and old male (R-OM). Resveratrol was administered in the drinking water (0.05 mg/ml) at a level sufficient to provide the appropriate milligrams per kilogram bodyweight dose (7.5 mg/kg) based on the consumption.

In the exercise (E) group, young and old rats were assigned as young female (E-YF), old female (E-OF), young male (E-YM) and old male (E-OM). All the rats in this group were habituated to moderate treadmill exercise for one week using a motor-driven treadmill (May Tme 0804 Animal Treadmill, Turkey) at 20 m/min (0° incline), for 15 min/day, 3 days per week. At the end of the one-week habituation period, the exercise-trained rats performed treadmill running at 20 m/min on a 0° incline, 40 min/day, 3 days per week, for 6 weeks.

2.3. Measurement of vascular reactivity in the thoracic aorta

At the end of the experiments, the animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Blood was collected from the heart and then centrifuged. Plasma was separated and immediately frozen for biochemical measurements. Then, the thoracic aortas of the rats were isolated and immediately placed into cold Krebs buffer solution (composition in mM: 112 NaCl, 5 KCl, 1.2 NaH_2PO_4, 11.5 dextrose, 0.5 $MgCl_2$, 2.5 $CaCl_2$, and 25 $NaHCO_3$; pH 7.4). The aorta was cleaned of adherent fat and connective tissue. Four ring segments of 3–4 mm length from each rat aorta were cut and mounted in the organ bath containing Krebs solution at 37 °C and aerated with 95% O_2 and 5% CO_2. Each ring was mounted between stainless-steel hooks and connected to a Grass Model (FT 03) force displacement transducer under an initial tension of 2 g. The rings were allowed to equilibrate for 40 min at resting tension, then potassium chloride (KCl) (90 mM) stimulated contractions were recorded. The relaxant effects of Acetylcholine (ACh, 10^{-8}–10^{-5} M) and sodium nitroprusside (SNP, 10^{-11}–10^{-5} M) were studied in the arterial rings pre-contracted with phenylephrine (10^{-6} M). Isometric contractions were recorded by force displacement transducer (FT03) and polygraph (Grass 79D). Vasorelaxation was expressed as a percentage of phenylephrine (10^{-6} M) stimulated contraction.

2.4. Biochemical measurements

In this study, plasma nitrite level was used as a marker to evaluate NO production. It was measured spectrophotometrically using the Navarro-González method based on Griess reaction, involving a shortened incubation period of nitrate with cadmium (Navarro-Gonzalvez et al., 1998). This method was modified in our laboratories for 96-well plates. The TAC of the plasma was measured using a previously described method (Usanmaz and Demirel Yilmaz, 2008), based on the reduction of Cu^{+2} to Cu^{+1} by the antioxidants of plasma. Neocuproine (Nc) was used as a chromogenic agent and the color of the formed colored complex (Nc–Cu^{+1}) was detected spectrophotometrically at 455 nm. For the measurements of ADMA levels, ELISA kits (Immundiagnostic A.G., Bensheim Germany) were used according to the manufacturer's instructions.

2.5. Quantitative real time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from each sample using TRIzol reagent (Invitrogen), according to the manufacturer's instructions. A total of 8 randomly chosen thoracic aorta samples from each group were used for the qRT-PCR analysis. Total RNA was isolated from thoracic aorta samples using RNeasy total RNA isolation kit (Qiagen), according to the manufacturer's instructions. After isolation, the amount and quality of total RNA were determined using spectrophotometry at 260/280 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA). Then, 500 ng of total RNA in each sample was used for cDNA synthesis using cDNA synthesis kit (Thermo Fisher Scientific, USA). The expression levels of target genes were evaluated with qRT-PCR. The PCR mixture consisted of 2.5 µl 2× SYBR Green Master Mix (Roche FastStart Universal SYBR Green Master mix), 1 µl of forward primer, 1 µl of reverse primer (2 µM each) and 0.5 µl of cDNA. Reactions were performed using the LightCycler480 II (Roche, Basel, Switzerland) as follows: initial denaturation at 95 °C for 10 min, denaturation at 95 °C for 15 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s for 45 cycles. Green fluorescence was measured at the end of each extension step. The PCR reactions were performed in triplicate and the specificity of PCR products was confirmed using melt analysis. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference gene was run for each cDNA sample and the relative expression of genes was calculated with the efficiency corrected advance relative quantification (LightCycler 480 SW 1.5.1 software). Primer and gene details are summarized in Table 1.

2.6. Data analysis

Statistical analysis of all study data was performed using GraphPad

Table 1
Primer sequences used for qRT-PCR.

Gene symbol	Forward primer (5′®3′)	Reverse primer (5′®3′)
PDE1A	CCACTTTGTGATCGGAAGTC	TTCTGCTGAATGATGTCCACC
PDE1B	CAGGGTGACAAGGAGGCAGAG	GACATCTGGTTGGTGATGCC
PDE2A	CCTCCTGTGACCTCTCTGACC	TGAACTTGTGGGACACCTTGG
PDE3A	TCACAGGGCCTTAACTTACAC	GGAGCAAGAATTGGTTTGTCC
PDE3B	ACAAATGCACCTCAGGCAGT	GATCTTTTGCTGGGCCGTTG
PDE4A	GTGGAGAAGTCTCAGGTGGG	TGGAACTTGTCAGGCAGGG
PDE4B	TAGAAGATAACAGGAACTGG	GCAATGTCTATGTCAGTCTC
PDE4D	TCGAAACCAGTCGAGTCTGC	TCTCGGAGCAAAAGTGCCTC
PDE5A	CCCTGGCCTATTCAACAACGG	GTGGGTCAGGGCCTCATACAG
SOD-1	TAGCAGGACAGCAGATGAGT	GCAGAAGGCAAGCGGTGAAC
SOD-2	GCACATTAACGCGCAGATCA	AGCCTCCAGCAACTCTCCTT
CAT	GCGAATGGAGAGGCAGTGTAC	GAGTGACGTTGTCTTCATTAGCACTG
GPx	CCACCACCGGGTCGGACATAC	CTCTCCGCGGTGGCACAGT
GST-Mu	AGAAGCAGAAGCCAGAGTTC	GGGGTGAGGTTGAGGAGATG
eNOS	TGCACCCTTCCGGGGATTCT	GGATCCCTGGAAAAGGCGGT
Nrf2	GATTCGTGCACAGCAGCA	GCCAGCTGAACTCCTTAGAC
NFκB	GGGTCAGAGGCCAATAGAGA	CCTAGCTTTCTCTGAACTGCAAA
IL-6	CCAGTTGCCTTCTTGGGACT	GCCATTGCACAACTCTTTTCTCA
TNF-α	ATGGGCTCCCTCTCATCAGT	GCTTGGTGGTTTGCTACGAC
GAPDH	TCCTTGGAGGCCATGTGGGCCAT	TGATGACATCAAGAAGGTGGTGAAG

Instat 5.01 (GraphPad Software, Inc., La Jolla, USA). Repeated-measures of two-way ANOVA and post hoc Bonferroni (for ACh and SNP dose-response curves) and the Student's *t*-test were used for statistical analysis. Data were presented as mean ± SEM. For all comparisons, differences were considered statistically significant at a value of $p < 0.05$.

3. Results

The average bodyweight of the old animals was significantly more than that of the young animals ($p < 0.05$) and the average bodyweight of the male animals was significantly higher than that of the female animals ($p < 0.05$). Exercise only caused an increase in the bodyweight of the young male group, while resveratrol consumption did not affect the bodyweight of any animals (Fig. 1).

KCl-induced (90 mM) contractions in old male rats were greater than in young male and old female groups ($p < 0.05$) (Fig. 2). These contractions in young male rats were elevated by resveratrol treatment ($p < 0.05$). Exercise training significantly increased KCl-mediated contractions in young female animals and decreased these responses in old female rats ($p < 0.05$) (Fig. 2).

SNP-induced endothelium-independent relaxations were lower in the control group young animals compared with the old animals ($p < 0.05$) (Fig. 3A). Resveratrol significantly reduced endothelium-independent relaxation in old animals, but increased this response in young animals (Fig. 3B). Exercise training significantly enhanced SNP-induced relaxation in young groups ($p < 0.05$) but had no effect on old groups (Fig. 3B). SNP (10^{-5} M)-induced maximum relaxation responses were not changed with age in both groups. (Fig. 3C). Resveratrol increased endothelium-independent maximum relaxation only in the young female group ($p < 0.05$). Exercise training significantly increased maximum relaxation in both young and old female rats ($p < 0.05$) (Fig. 3C). EC_{50} values for SNP in the older groups were lower than in the younger groups ($p < 0.05$) (Fig. 3D). Resveratrol treatment significantly decreased EC_{50} values of SNP in young animals, but increased it in old animals ($p < 0.05$). Similarly, exercise caused a decrease in EC_{50} values of SNP in young animals ($p < 0.05$), but did not affect this value in old animals (Fig. 3D).

ACh-induced endothelium-dependent and SNP-induced endothelium-independent relaxations were obtained against phenylephrine-induced (10^{-6} M) submaximal contraction in the isolated aortic rings. ACh-induced relaxations in the old groups were significantly smaller than in the young animals ($p < 0.05$) (Fig. 4A). Resveratrol intake significantly enhanced ACh-induced endothelium-dependent relaxations in the young groups, these were diminished in the old animals ($p < 0.05$) (Fig. 4B). ACh (10^{-5} M) induced maximum relaxations of all the female groups were significantly decreased with age ($p < 0.05$) but did not change in the male groups (Fig. 4C). Resveratrol and exercise training did not affect endothelium-dependent maximum relaxations within the groups. EC_{50} values were calculated from the ACh concentration-relaxation response curves. EC_{50} values for ACh in older control animals were markedly lower than in the younger control group rats ($p < 0.05$) (Fig. 4D). The consumption of resveratrol caused a decrease in the EC_{50} values of young female and male rats, and

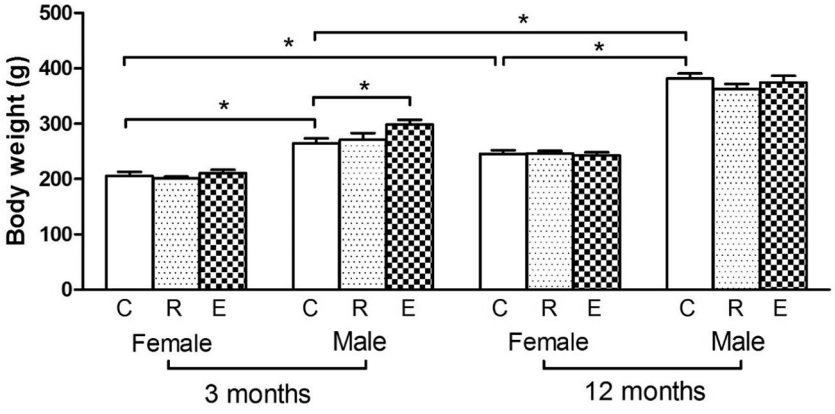

Fig. 1. Age and gender-dependent effects of resveratrol treatment and exercise training on the bodyweight of rats. The average bodyweight was lower in the young groups than in the old groups. The bodyweight of the male rats was markedly greater than that of female rats. Exercise only increased the bodyweight of young male rats, while resveratrol intake did not alter the bodyweight of any animals. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0.05$. Values are expressed as mean ± SEM.

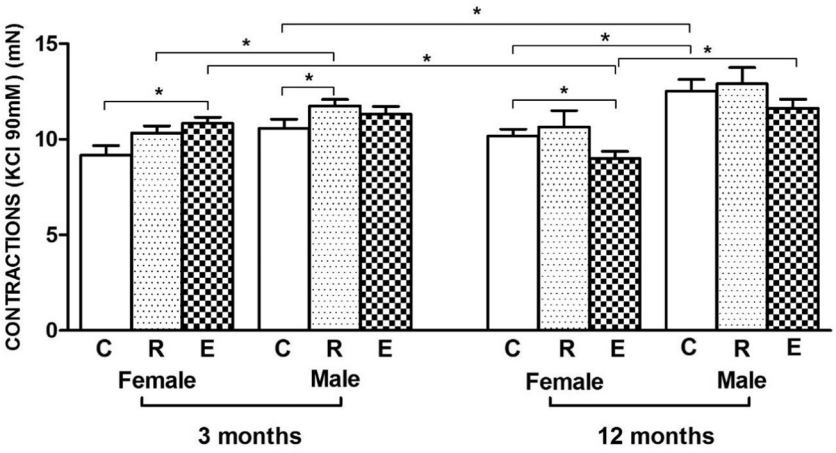

Fig. 2. KCl (90 mM)-induced contractions of thoracic aorta isolated from control, resveratrol and exercise-training rats. The KCl-induced contraction of old male animals was higher than that of young males and old females. Resveratrol treatment augmented these contractions in young male rats. Exercise training enhanced KCl-mediated contractions in young female rats and reduced these responses in old female animals. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0.05$. Values are expressed as mean ± SEM.

an increase in old males.

Biomarkers related to endothelial function were examined by measuring plasma levels of NO, ADMA and TAC and vascular expression of eNOS mRNA. Plasma NO levels significantly decreased with age in all male groups ($p < 0.05$) but did not change in female groups (Fig. 5A). The expression of eNOS was decreased with age in male rats (Fig. 5B). Gene expressions of eNOS were reduced in old male groups when compared with old female rats ($p < 0.05$). Resveratrol enhanced the mRNA levels of eNOS in young and old rats of both genders ($p < 0.05$). Physical exercise augmented the mRNA levels of eNOS in young female and old male rats ($p < 0.05$). The level of endogenous NO synthase (NOS) inhibitor ADMA significantly decreased with age in male and female animals ($p < 0.05$) (Fig. 5C). Plasma ADMA levels of old resveratrol groups were smaller than those of the young resveratrol groups ($p < 0.05$). Resveratrol decreased plasma ADMA levels in the young male group compared to the control group ($p < 0.05$). Exercise training significantly alleviated plasma ADMA levels in the young groups compared to the control groups ($p < 0.05$). Total antioxidant capacity (TAC) is used as a marker of oxidative stress. Plasma TAC levels increased with age in male and female groups ($p < 0.05$) (Fig. 5D). Plasma TAC levels were higher in old female group compared to old male group ($p < 0.05$) but there was no difference between younger groups. Resveratrol significantly increased plasma TAC levels only in the young male group ($p < 0.05$).

In old male rats, expression of SOD1 was decreased when compared to the young male group ($p < 0.05$) (Fig. 6A). Gene expression of SOD1 was decreased in the old male group when compared to old female rats. SOD1-2 gene expressions were increased by resveratrol in young female rats ($p < 0.05$) (Fig. 6B). Resveratrol enhanced mRNA levels of CAT in young and old rats of both genders ($p < 0.05$) (Fig. 6C). Physical exercise increased mRNA levels of CAT in young female and old male rats. GPx expressions were similar in all groups (Fig. 6D). GST-mu and Nrf2 expressions were higher in old female animals ($p < 0.05$) (Fig. 6E, F). GST-mu expression was higher in young male rats than in young female rats ($p < 0.05$). Resveratrol decreased mRNA level of GST-mu in young and old rats of both genders ($p < 0.05$). Nrf2 expressions were increased in young rats by resveratrol ($p < 0.05$). Resveratrol treatment decreased GST-mu gene expression in all groups ($p < 0.05$). Physical exercise increased mRNA level of GST-mu in young female and old male rats ($p < 0.05$).

Gene expressions of PDE 1A, 1B, 2A, 3B and 4A were reduced with age in male rats ($p < 0.05$) (Fig. 7). However, PDEs mRNA expressions were similar in young and old female rats. No significant difference was determined between young female and young male rats in respect of PDEs gene expressions. In the old rat groups, PDE1B and 4A expression were lower in male rats when compared to female rats ($p < 0.05$). Resveratrol treatment enhanced gene expression of PDE 1A, 1B, 2A, 3A, 3B, 4A and 4D in both female and male young rats, and PDE 1A, 2A and 4D in old rats ($p < 0.05$). Treadmill exercise augmented PDE2A, 3B, 4A and 4D gene expression in young female rats, and PDE 1B and PDE2A expression in young male rats ($p < 0.05$). In the old male group, exercise enhanced PDE4A and 4D expressions ($p < 0.05$).

In old male rats, expressions of IL-6, NFκB and TNFα were decreased when compared to the young male group ($p < 0.05$) (Fig. 8A, B, C). Gene expression of IL-6 was decreased in old male rats compared to old female rats ($p < 0.05$). Resveratrol treatment augmented TNFα gene expression in all groups ($p < 0.05$). Treadmill exercise augmented IL-6 and NFκB expressions in young female rats and reduced TNFα gene expression in young male rats ($p < 0.05$).

4. Discussion

The results of the present study indicated the mechanisms of the beneficial effects of resveratrol consumption and exercise-training on the age-dependent failure of vascular functions in a gender-dependent manner. Antioxidant enzymes and eNOS expressions in vessels and plasma NO levels were improved by resveratrol and exercise. In addition, resveratrol and exercise elevated gene expressions of non-selective PDE1, 2, 3 and cAMP selective PDE4 but not cGMP selective PDE5 in the aorta.

Aging, which is a major risk factor for cardiovascular diseases, is associated with changes in vascular structure and function (Hayflick, 2007). Vascular aging involves the activation of complex pathways, most of which are not yet completely understood (Kim et al., 2009; Aggarwal et al., 2008; Taddei et al., 1995). Age and sex can induce changes in vascular wall, which may result in different passive/active contractile forces in blood vessels (Blough et al., 2007) In this regard, the ability was evaluated of aortic rings to contract to depolarization to KCl (90 mM) without any agonist stimulation. The contractile response of KCl in the old male group was observed to be greater than in the young male and old female rats. Similar to these findings, it has been reported that KCl-mediated coronary vascular resistance was higher in adult (12–18 months) male rats than young (3–4 months) and immature (1–2 months) male rats (Hinschen et al., 2001). The current study results are also consistent with the findings of a study by Costa et al. of 3 and 12-month old male and female CD1 mice (Costa et al., 2016). These results demonstrated that agonist-independent contraction of blood vessels may alter depending on age and sex.

In addition, it has been observed that relaxations of blood vessels are affected by age and sex in different species. Recent studies have reported no significance in the differences between young and old groups in respect of SNP-induced endothelium-independent relaxations (Sun et al., 2016; Luttrell et al., 2013). In the present study, although maximum relaxations of SNP were not changed by aging, the

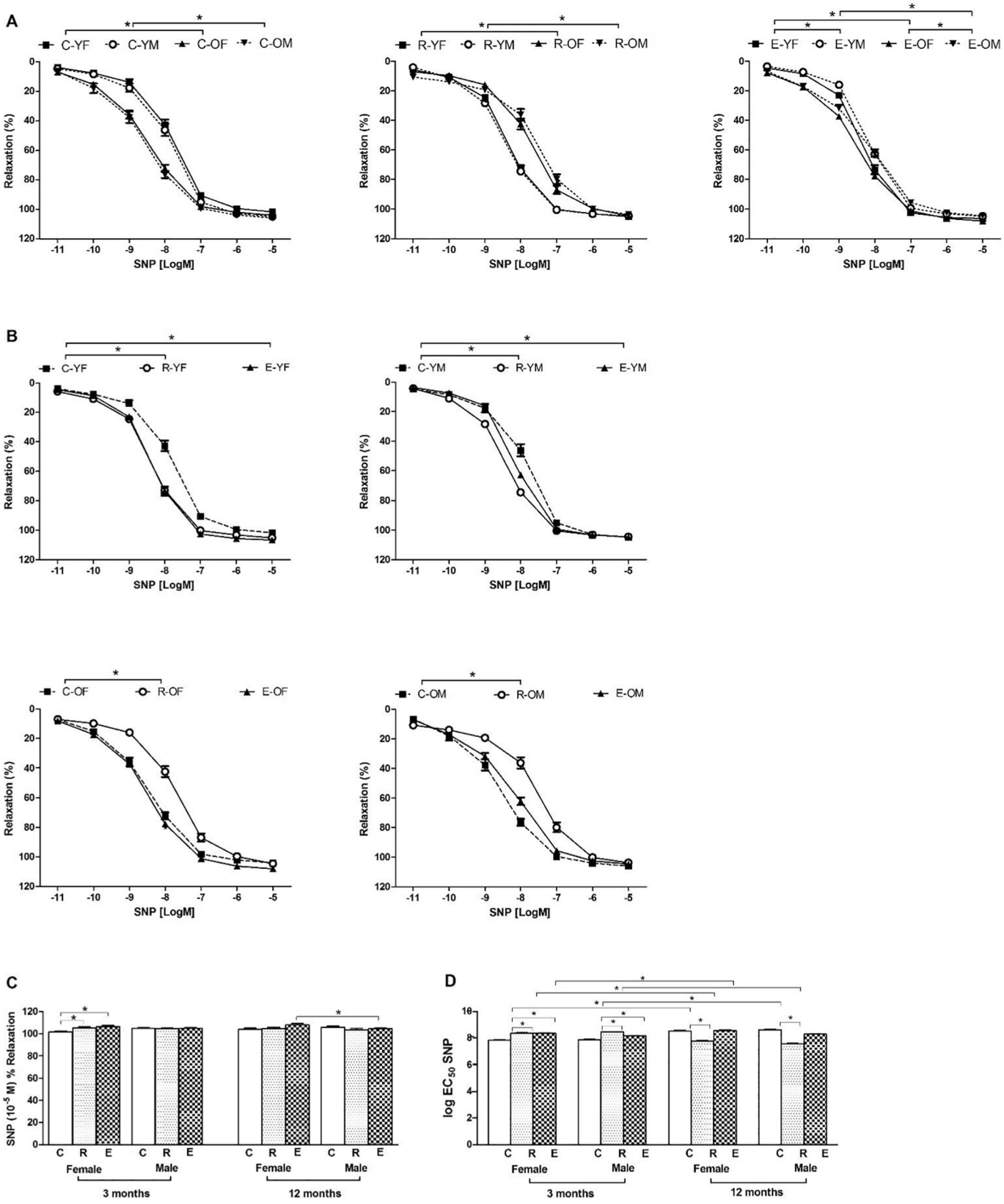

Fig. 3. Effect of resveratrol and exercise on SNP-induced endothelium-independent relaxations of thoracic aorta. (A and B) SNP-induced relaxations of control young animals were lower than control old animals. Resveratrol intake reduced endothelium-independent relaxation in old groups, but increased this response in young groups. Also, exercise training augmented SNP-induced relaxation in young animals. (C) Exercise training enhanced maximum relaxation in young female animals. (D) EC_{50} values for SNP in older animals were smaller than younger animals. Resveratrol intake and exercise reduced EC_{50} values for SNP in young rats. Vasorelaxant responses are expressed as a percentage of submaximal contraction of phenylephyrine (10^{-6} M). The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0,05$. Values are expressed as mean ± SEM (n = 27–36).

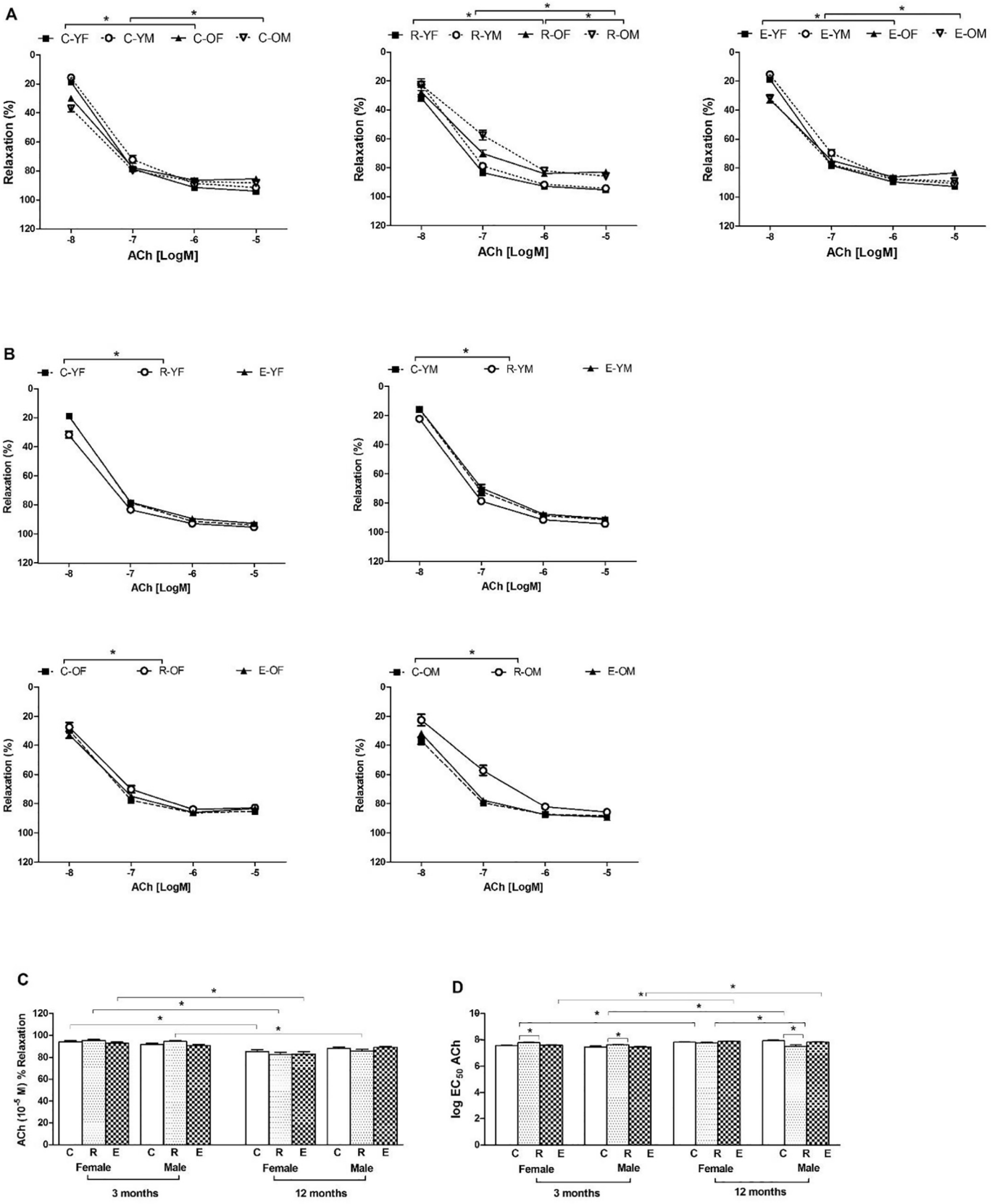

Fig. 4. Effect of resveratrol and exercise on ACh-induced endothelium-dependent relaxations of thoracic aorta. (A and B) ACh-induced relaxations in the old groups were lower than those of the young animals. Resveratrol consumption markedly increased ACh-induced endothelium-dependent relaxations in young rats, while it was alleviated in old rats. (C) In all the female animals, ACh-induced maximum relaxations were decreased with aging but there was no change in male animals. (D) EC_{50} values for ACh in older control rats were lower than those of the younger control animals. Resveratrol intake decreased the EC_{50} values of young female and male rats and increased the values of old males. Vasorelaxant responses are expressed as a percentage of submaximal contraction of phenylephyrine (10^{-6} M). The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0.05$. Values are expressed as mean ± SEM.

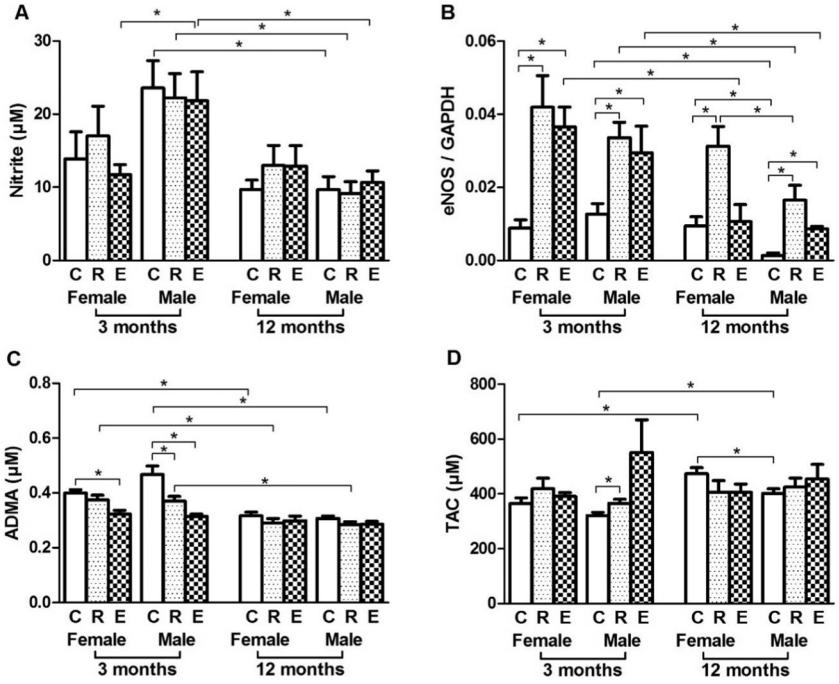

Fig. 5. Plasma NO levels, vascular eNOS gene expression, plasma ADMA and TAC levels in control, resveratrol and exercise training rats. (A and B) Plasma NO and aortic eNOS expression levels were decreased with age in male groups. Resveratrol treatment enhanced eNOS expression in all groups. Exercise increased eNOS expression in young female rats, and young and old male rats. (C and D) TAC levels were augmented and ADMA levels reduced with age in both female and male rats. Exercise decreased ADMA levels in the young groups. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. * $p < 0.05$. Values are expressed as mean ± SEM.

concentration-response curve of SNP was shifted left in the vessel isolated from the older rats. Consistent with the results of the young groups in the current study, Soylemez et al. showed that the relaxations to SNP were similar in young male and female Wistar rats (Soylemez et al., 2009). To the best of our knowledge, the present study is the first to have demonstrated that SNP-induced endothelium-independent relaxations change with aging but do not differ between the genders. The study results suggested that endothelium-independent relaxations may alter in an age-dependent manner.

In the current study, resveratrol intake significantly increased SNP-induced endothelium-independent relaxations in the young groups but these responses were reduced with resveratrol in the old groups. But, it

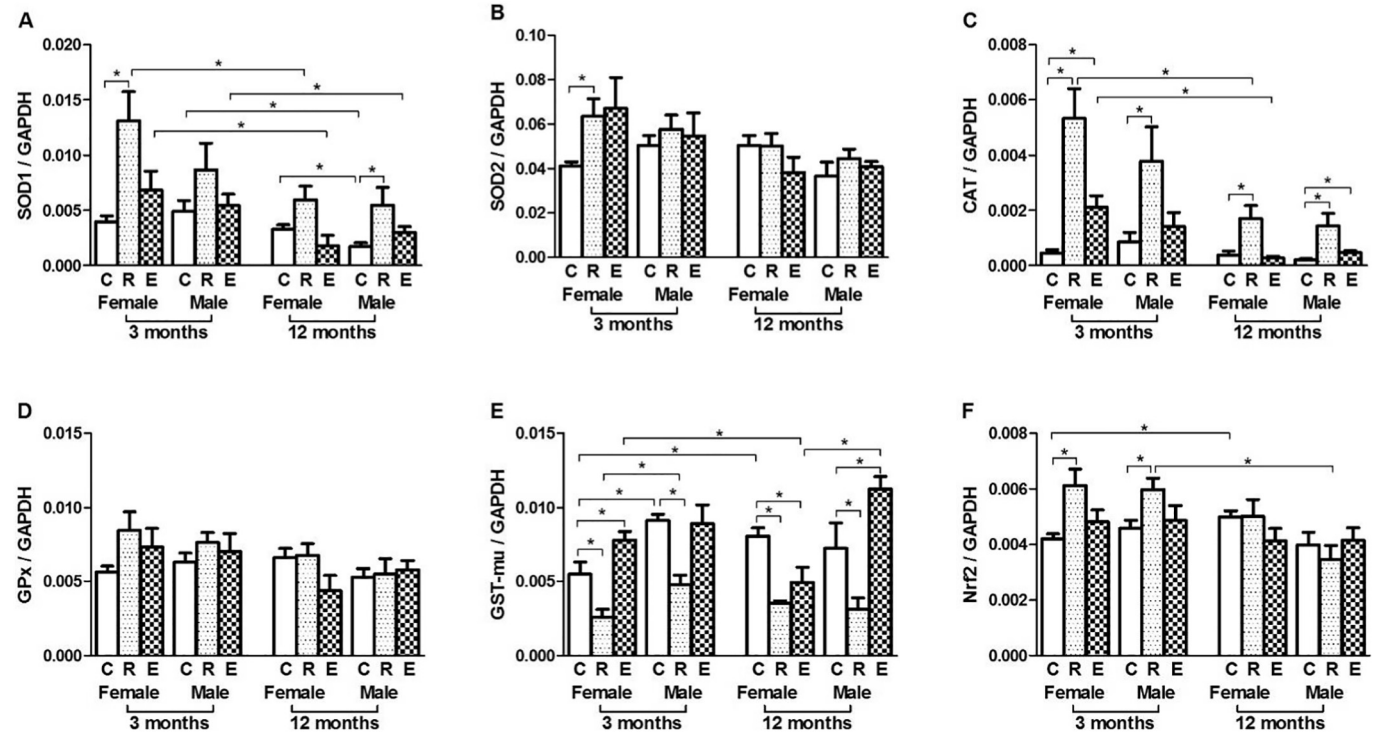

Fig. 6. Age and gender-dependent effects of resveratrol treatment and exercise training on the gene expression of redox status. Gene expressions of SOD1 were reduced with age in male rats (A). SOD1 expression was lower in the old male group than in old female rats. SOD1-2 gene expressions were augmented by resveratrol in young female rats (A, B). Resveratrol increased gene expression of CAT in young and old rats of both genders (C). GST-mu and Nrf2 expressions were enhanced with age in female animals (E, F). GST-mu gene expressions were decreased by resveratrol in all groups. Resveratrol treatment increased Nrf2 expressions in young rats. Physical exercise enhanced mRNA levels of CAT and GST-mu in young female and old male rats. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0.05$. Values are expressed as mean ± SEM.

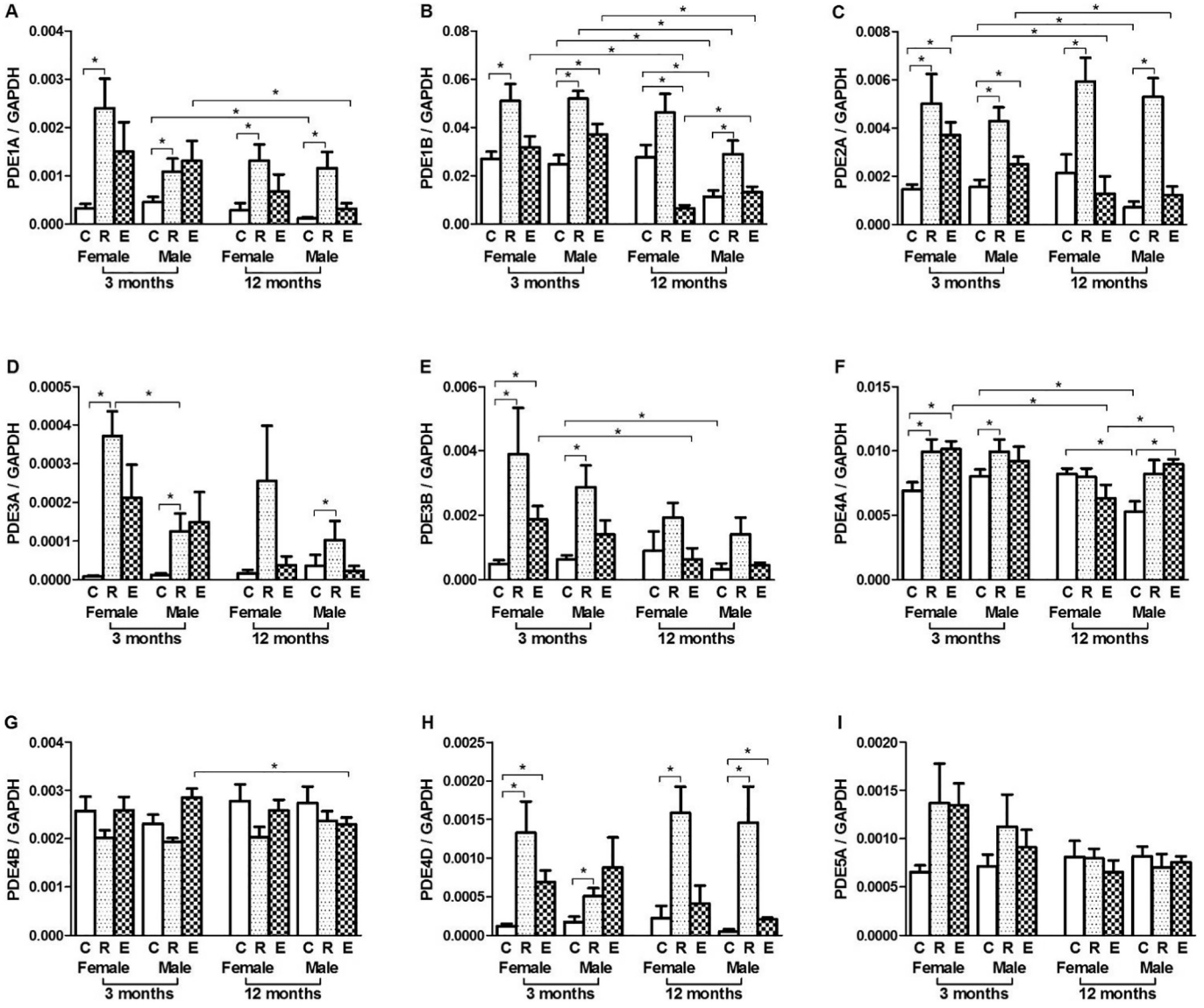

Fig. 7. Age and gender-dependent effects of resveratrol treatment and exercise training on the gene expression of PDEs. Gene expressions of PDE 1A, 1B, 2A, 3B and 4A were reduced with age in male rats. PDE1B and 4A expression were lower in old male rats than in old female rats. Resveratrol treatment increased gene expression of PDE 1A, 1B, 2A, 3A, 3B, 4A and 4D in both female and male young rats and PDE 1A, 2A and 4D in old rats. Treadmill exercise enhanced PDE2A, 3B, 4A and 4D gene expression in young female rats, PDE 1B and PDE2A expression in young male rats, and PDE4A and 4D expressions in old male rats. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0.05$. Values are expressed as mean ± SEM.

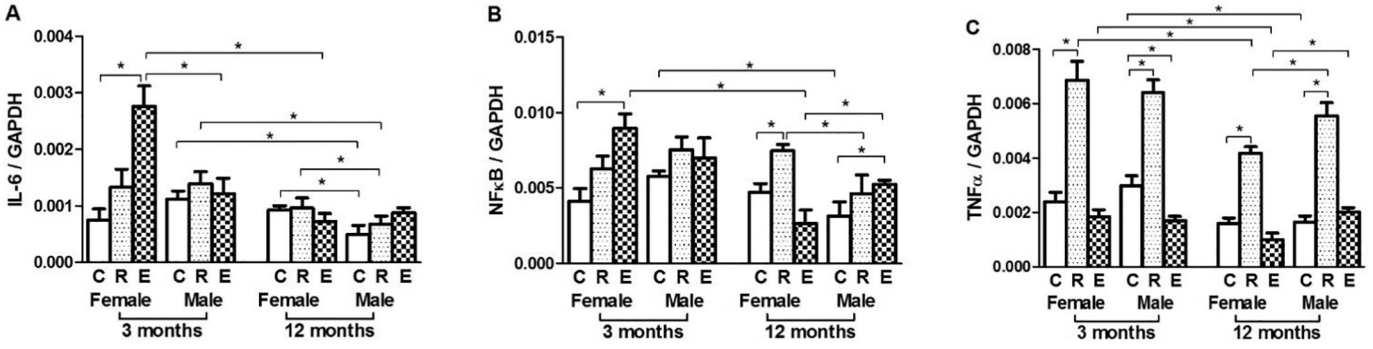

Fig. 8. Age and gender-dependent effects of resveratrol treatment and exercise training on the gene expression of inflammation markers. Gene expressions of IL-6 (A), NFκB (B) and TNFα (C) were decreased with age in old male rats. IL-6 expressions were lower in old male rats than in old female rats. In young females, treadmill exercise augmented IL-6 and NFκB expressions. Resveratrol treatment enhanced TNFα expressions in all groups. The ages of the animals were adjusted to be 3 and 12 months after 6 weeks of application. *$p < 0.05$. Values are expressed as mean ± SEM.

has been reported that resveratrol treatment did not change SNP-induced relaxations in the aortas of Wistar rats (Soylemez et al., 2009). On the other hand, exercise training significantly augmented SNP-induced relaxation in young animal but had no effect on old animals in this study. Previous studies have showed that exercise did not affect the endothelium-independent relaxations in both young and older men (DeSouza et al., 2000) and rats (Luttrell et al., 2013). Dose or duration of resveratrol intake and exercise treatment protocol may be responsible for these discrepancies.

Previous studies have demonstrated an age-related decline in endothelium-dependent vasorelaxation in different species (Kim et al., 2009; Aggarwal et al., 2008; Taddei et al., 1995; Smith et al., 2006; Delp et al., 1995). Age-associated endothelial dysfunction is attributed to reduced nitric oxide (NO) bioavailability, secondary to oxidative stress. Previous studies have shown that increased reactive oxygen species (ROS) production and oxidative stress have been determined during the aging process in plasma (Kalani et al., 2006; Iciek et al., 2004) and isolated rat aorta from male Wistar or Fischer 334 rats of different ages (1.5 months to 31 months) (Li et al., 2010; Marmol et al., 2007; Ungvari et al., 2011). It has been also reported that impairments in aging-related endothelial function preceded females in males (Luttrell et al., 2013; Celermajer et al., 1994). In consistent with previous studies, ACh-induced endothelium-dependent relaxations significantly were decreased with age in both female and male groups in the current study. Additionally, the plasma NO level and eNOS mRNA expression of the aorta were significantly decreased with age in male groups but did not change in female groups. This result may be attributed to hormonal status. These results suggested that the endothelial function may change in sex and age dependent manner.

Resveratrol consumption and exercise training are known to have beneficial effects on vascular functions. However, the effects of resveratrol and exercise on age and gender-dependent changes in vascular functions have not been documented in detail. It has been clearly shown that resveratrol, a natural polyphenol, exerts vasoprotective effects on male 12–18-month old mice (Pearson et al., 2008) and 20–24-month old male WKY rats (Rajapakse et al., 2011). Soylemez et al. showed that resveratrol consumption significantly increased ACh-induced relaxations in young female and male Wistar rats (Soylemez et al., 2009). In the current study, it was shown for the first time that consumption of resveratrol increases endothelium-dependent relaxation in young rats, whereas it decreases this response in old rats. These results could be interpreted as a variable age-dependent effect of resveratrol on endothelial function.

Exercise training has been shown to reverse age-related reductions in NO bioavailability (Taddei et al., 2000; Eskurza et al., 2004; Spier et al., 2004; Durrant et al., 2009) and improve endothelium-dependent vasodilation (DeSouza et al., 2000; Luttrell et al., 2013; DeVan et al., 2013). However, based on previous studies it is reasonable to suggest that at least 10 weeks of exercise training is necessary to stimulate improvements in endothelial function (DeSouza et al., 2000; Clarkson et al., 1999). In the current study, the short exercise training did not affect ACh-induced relaxations of the aorta in young or old groups.

It has been reported that resveratrol treatment increased blood nitrite/nitrate levels and basal NO production in aorta taken from young Wistar rats of both genders (Soylemez et al., 2008, 2009). In contrast to previous studies, it was observed in the current study that resveratrol intake did not significanltly affect plasma NO levels. In addition, exercise training did not alter plasma NO levels in the current study. To the best of our knowledge, the age and gender-dependent effects of resveratrol intake and exercise training on plasma NO levels are shown for the first time in this study. Resveratrol has been demonstrated to improve eNOS mRNA and protein levels in both female and male rat aortas (Pektaş et al., 2015). Accordingly, resveratrol enhanced mRNA levels of eNOS in young and old rats of both genders in the present study. The mRNA expression of eNOS in the aorta has been found to be higher in a training-aged group than in a sedentary-aged group (Tanabe et al., 2003). Similarly, it was reported that physical exercise increased mRNA levels of eNOS in young female and old male rats in the current study. These data suggest that the effect of resveratrol on eNOS expression is independent of age and gender, although the effect of exercise training on eNOS gene expression may be age and gender-dependent.

The synthesis of NO is blocked by the inhibition of the NOS L-arginine analogs such as ADMA, which is a naturally occurring amino acid found in plasma (Yamagishi and Matsui, 2011). Previous studies have indicated that plasma ADMA levels increase with age (Tanahashi et al., 2013; Schulze et al., 2005). However, other authors could not find any relationship between ADMA and the age-related impairment of endothelial function (Gates et al., 2007). In the current study, plasma ADMA levels significantly decreased with age and it was similar in male and female groups. It has been reported that resveratrol treatment in different pathologies significantly reduced plasma ADMA levels in male rats (Han et al., 2015; Develi-Is et al., 2014). In the current study, resveratrol consumption decreased plasma ADMA levels only in the young male group. Recent studies have shown that aerobic exercise training significantly decreased plasma ADMA concentrations in postmenopausal women (van der Loo et al., 2000; Tanahashi et al., 2014). Exercise training significantly reduced plasma ADMA levels in young groups in the current study. Resveratrol intake and exercise training did not affect plasma ADMA levels in old groups. These results suggest that resveratrol and exercise may produce the beneficial effects through the reduced level of ADMA in the younger age groups.

Age-associated endothelial dysfunction is attributed to reduced nitric oxide (NO) bioavailability, secondary to oxidative stress. Total antioxidant capacity (TAC) is used as a marker of oxidative stress. While some studies have demonstrated that total antioxidant capacity of plasma decreased in 26 months male Wistar rats (Sivonová et al., 2007), others found it to be unchanged in 15-month old Sprague Dawley rats (Nakamura and Omaye, 2004). This discrepancy in TAC level may depend on the stage of aging. In the present study, plasma TAC levels increased with age in male and female groups and plasma TAC levels were higher in old female groups compared to old male groups. In this study, the increment of plasma TAC levels with age might be a compensatory (or defensive) mechanism, despite an increase in ROS levels. It is known that resveratrol has an antioxidant effect (Martinez and Moreno, 2000). Chronic exercise training enhances antioxidant defense systems, effectively lowering the concentration of ROS and increasing resistance to oxidative damage (Mankowski et al., 2015). In the present study, resveratrol significantly increased plasma TAC levels only in the young male group, but exercise training did not affect plasma TAC levels. Numerous factors are involved in the metabolism of blood biomarkers. Dose or duration of resveratrol application and exercise protocol may not have the ability to affect them in all age and gender groups.

It is known that increased oxidative stress contributes to the aging of vessels (Camici et al., 2015). Antioxidant enzyme SOD expression is decreased with aging in male rats (Wu et al., 2017) and aortic SOD activity has been found to be lower in old male rats than in young rats (Li et al., 2010). Similarly, the current study results showed that SOD1 gene expression was reduced with age in male rats, but not in female rats. In old groups, SOD1 expression was lower in male rats compared to female rats. These results showed that aging may have dissimilar effects on the expressions of antioxidant enzyme SOD in females and males. Although, resveratrol produces antioxidant and anti-aging effects (Baur and Sinclair, 2006), it is unclear how the effect of resveratrol on oxidative stress changes in an age and gender-dependent manner. In this study, resveratrol increased SOD1-2 gene expressions in young female rats. Resveratrol enhanced mRNA levels of CAT in young and old rats of both genders while conversely decreasing GST-mu levels. Nrf2 expressions were increased in young rats by resveratrol. This increase in antioxidant enzymes may be involved in reducing oxidative stress in the vessel. It has been suggested that regular exercise promotes endothelial

function through a reduction in oxidative stress (Ross et al., 2016). It was observed in the current study that physical exercise increased mRNA levels of CAT and GST-mu in young female and old male rats. In the light of the above findings, it can be said that resveratrol and regular exercise may possess beneficial effects on antioxidant status in the vessels through various antioxidant enzymes, and that these effects may vary depending on age and gender.

Cyclic nucleotide PDEs play a critical role in vascular muscle function through degradation of the second messengers, cAMP and cGMP (Bobin et al., 2016). Alterations in cyclic nucleotide homoeostasis have been shown to be involved in various cardiovascular diseases (Bobin et al., 2016). A strong relationship between aging-induced vascular abnormalities and PDE1A expression was suggested by Yan (2015). It has been shown that gene expressions of PDE1A, PDE1C and PDE5 are up-regulated in cultured senescent human vascular smooth muscle cells (Bautista Niño et al., 2015). On the other hand, the function of PDE3 in vascular smooth muscle has been seen to be preserved in healthy human aging (Elvebak et al., 2010). Gender- and age-dependent alterations of PDEs expression and function still remain to be investigated. In the present study, gene expressions of PDEs did not change with age in female rats but PDE1A, 1B, 2A, 3B and 4A expressions were decreased in old male rats. PDE1B and PDE4A expressions were lower in old male rats when compared to old female rats. From these results it may be said that PDE expression levels possess diversity in gender which may be involved in different age-related cardiovascular modifications in female and male rats.

Studies have shown that resveratrol reduced PDE4A mRNA expression in brain tissue (Wan et al., 2016) and PDE3B, PDE8A and PDE10A gene expressions in pancreatic β-cells (Rouse et al., 2014). However, no studies have examined the gender- and age-dependent effect of resveratrol on the aortic expression of PDE genes. In the current study, resveratrol treatment increased the mRNA expressions of PDE 1A, 1B, 2A, 3A, 3B, 4A and 4D in young female rats; PDE 1A, 2A and 4D in old females; PDE 1A, 1B, 2A, 3A, 3B, 4A and 4D in young male rats; and PDE1A, 1B, 2A, 3A and 4D in male old rats. The expression of PDE4B and PDE5A was not changed by resveratrol in any of the groups. These results firstly showed that the effect of resveratol on PDE gene expression was altered at different ages and gender of the rat aorta. It has been reported that exercise produces an increase in cardiac PDE activities (Palmer, 1988; Dunbar and Kalinski, 1994), but it is still unknown how exercise affects the aortic gene expression of PDEs in different gender and age groups. In the present study, it was first observed that treadmill exercise enhanced PDE2A, 3B, 4A and 4D gene expression in young female rats, and PDE 1B and PDE2A expression in young male rats. In the old male group, exercise increased PDE4A and 4D expressions. It was therefore posited that the effect of exercise on PDE gene expression is age and gender-dependent.

Inflammation is another characteristic process in the development of vascular aging. There has been shown to be an association between aging and vascular inflammation in experimental models (El Assar et al., 2013). Enhanced activity of NFκB has been reported in the aorta of 22–24-month old male rats (Li et al., 2010) and increased IL-6 and TNFα expressions in the vessels of 25-month old male rats (Csiszar et al., 2003). In the present study, inflammatory factors IL-6, NFκB and TNFα gene expression were higher in the 12-month old male rats than in the 3-month old male rats. IL-6 expression was lower in male rats compared to female rats in the old groups. These findings suggest that the effect of aging on vascular inflammation may be different in females and males. Further studies are needed to elucidate the inflammatory status of vessels during the lifespan. Many studies have suggested that resveratrol produces an anti-inflammatory effect (Baur and Sinclair, 2006; Gliemann et al., 2016). In contrast to previous reports, Feng et al. (2002) reported that a low dose of resveratrol had the capability to enhance the function of lymphocytes and could promote immune response. In the current study, resveratrol treatment augmented TNFα gene expression in all groups and NFκB expression in old female rats. This discrepancy may be explained by the different doses of resveratrol used. It has been previously stated that exercise induces a reduction in inflammation (Gliemann et al., 2016). In the present study, treadmill exercise augmented IL-6 and NFκB expressions in young females and reduced TNFα gene expression in young male rats. Further studies are needed to examine the protein expressions and activation of these proteins to give a reliable explanation.

In summary, the results of this study demonstrated that endothelium-dependent and independent relaxations of rat thoracic aorta, blood levels of biomarkers, aortic gene expression of eNOS, PDEs and oxidative stress-related genes were separately altered by resveratrol intake and exercise-training in an age and gender-dependent manner. These findings suggest that vascular aging in females does not show the same pattern as in males, therefore the effect of anti-aging approaches on vessels may change with gender.

5. Limitations

This study is subject to the following limitations. First, 3 and 12 month old rats have been used to evaluate the age dependent differences. It would be better adding an older age group in terms of the difference being more apparent. Second, because of limited tissue content, only mRNA expressions were measured in the vessel.

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