Experimental Gerontology 110 (2018) 191–201

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

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

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ARTICLE INFO

Section Editor: Christiaan Leeuwenburgh

Keywords:
Aging
Resveratrol
Regular exercise
Oxidative stress
Phosphodiesterases
Inflammation

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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https://doi.org/10.1016/j.exger.2018.06.009
Received 6 April 2018; Received in revised form 25 May 2018; Accepted 11 June 2018
Available online 13 June 2018
0531-5565/

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