Experimental Gerontology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

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

Chronic aerobic exercise training attenuates aortic stiffening and endothelial dysfunction through preserving aortic mitochondrial function in aged rats

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ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 22 February 2014
Accepted 24 February 2014
Available online xxxx

Section Editor: Natascia Ventura

Keywords:
Chronic aerobic exercise training
Aging
Vascular dysfunction
Mitochondrial dysfunction
Oxidative stress

ABSTRACT

Aging leads to large vessel arterial stiffening and endothelial dysfunction, which are important determinants of cardiovascular risk. The aim of present work was to assess the effects of chronic aerobic exercise training on aortic stiffening and endothelial dysfunction in aged rats and investigate the underlying mechanism about mitochondrial function. Chronic aerobic exercise training attenuated aortic stiffening with age marked by reduced collagen concentration, increased elastin concentration and reduced pulse wave velocity (PWV), and prevented aging-related endothelial dysfunction marked by improved endothelium-mediated vascular relaxation of aortas in response to acetylcholine. Chronic aerobic exercise training abated oxidative stress and nitrosative stress in aortas of aged rats. More importantly, we found that chronic aerobic exercise training in old rats preserved aortic mitochondrial function marked by reduced reactive oxygen species (ROS) formation and mitochondrial swelling, increased ATP formation and mitochondrial DNA content, and restored activities of complexes I and III and electron-coupling capacity between complexes I and III and between complexes II and III. In addition, it was found that chronic aerobic exercise training in old rats enhanced protein expression of uncoupling protein 2 (UCP-2), peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), manganese superoxide dismutase (Mn-SOD), aldehyde dehydrogenase 2 (ALDH-2), prohibitin (PHB) and AMP-activated kinase (AMPK) phosphorylation in aortas. In conclusion, chronic aerobic exercise training preserved mitochondrial function in aortas, which, at least in part, explained the aorta-protecting effects of exercise training in aging.

1. Introduction

Despite reductions in death rates from cardiovascular diseases over the last four decades, cardiovascular diseases remain the leading cause of morbidity and mortality in modern societies (Lloyd-Jones et al., 2010). Aging is the major risk factor for cardiovascular diseases (Lakatta, 2002; Lakatta and Levy, 2003), which is largely due to dysfunctional arteries. Vascular dysfunction in aging includes large artery stiffness, as indicated by structural changes in the arterial wall such as the development of fibrosis and degeneration of the elastin matrix and increased aortic pulse wave velocity, and vascular endothelial dysfunction, as indicated by reduced endothelium-dependent dilation in response to chemical (typically acetylcholine) or mechanical (intravascular shear) stimuli (Fleenor, 2012; Seals et al., 2011).

Aerobic exercise reduces the risk for cardiovascular diseases, and attenuates age-related arterial stiffening (Steppan et al., 2012; Vaitkevicius et al., 1993) and vascular endothelial dysfunction (DeVan et al., 2013; Luttrell et al., 2013) in older adults and aged rodents. Although the mechanisms underlying this protective effect probably include favorable changes in blood pressure, plasma lipids and lipoproteins, and glucose–insulin metabolism (Shephard and Balady, 1999), little is known about the cellular and molecular mechanisms by which aerobic exercise exerts these beneficial vascular effects with aging.

Abbreviations: CS, citrate synthase; PWV, Pulse wave velocity; ACh, acetylcholine; SNP, sodium nitroprusside; MDA, malondialdehyde; ROS, reactive oxygen species; O_2^-, superoxide; $OONO^-$, peroxynitrite; SCCR, succinate cytochrome c reductase; NCCR, nicotinamide–adenine dinucleotide cytochrome c reductase; PGC-1α, peroxisome proliferator-activated receptor γ co-activator 1α; UCP-2, uncoupling protein 2; AMPK, AMP-activated kinase; Mn-SOD, manganese superoxide dismutase; ALDH-2, aldehyde dehydrogenase 2; OCR, oxygen consumption rates; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; PHB, prohibitin.

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http://dx.doi.org/10.1016/j.exger.2014.02.014
0531-5565/

Oxidative stress has been shown to be present in arteries of older rodents (Fleenor et al., 2012; Sindler et al., 2011), and plasma markers of oxidative stress have been demonstrated to be the independent predictors of arterial stiffness in healthy humans (Patel et al., 2011). Oxidative stress describes an imbalance between antioxidant defenses and the production of reactive oxygen species (ROS), which at high levels cause cell damage (Seddon et al., 2007). Mitochondria and NADPH oxidases have been suggested to be the key sources of these ROS. Jessica et al. demonstrated that voluntary wheel running abated oxidative stress in conduit arteries of old mice by downregulating NADPH oxidase (Durrant et al., 2009). Mitochondria have a critical function to regulate redox state, energy metabolism, apoptosis and intracellular signaling (Picard et al., 2011; Ryan and Hoogenraad, 2007). Over the last decade, accumulating evidence has suggested a causative link between mitochondrial dysfunction and major phenotypes associated with aging (Bratic and Larsson, 2013) and age-related diseases such as neurodegenerative diseases, cancer and diabetes (Wallace, 2005). Fleenor et al. found that superoxide-lowering therapy with TEMPOL reversed arterial dysfunction with aging in mice (Fleenor et al., 2012); Wenzel et al. found that manganese superoxide dismutase deficiency increased mitochondrial oxidative stress and aggravated age-dependent vascular dysfunction, which revealed that mitochondrial radical formation significantly contributed to age-dependent vascular dysfunction (Wenzel et al., 2008).

This study was designed to elucidate whether chronic aerobic exercise training improved mitochondrial function in aortas from aged rats, which might explain the aorta-protecting effects of chronic aerobic exercise training in aging.

2. Materials and methods

2.1. Animals

The Young (3-month old) and Old (23-month old) male Fisher 344 rats were provided by Vital River Laboratory Animal Technology Company (Beijing, China). All the animals were entrained to controlled temperature (24 ± 1 °C), 12-h light and 12-h dark cycles (light, 08:00–20:00 h; darkness, 20:00–08:00 h), and free access to food and tap water.

All the animals used in this work received humane care in compliance with institutional animal care guidelines, and were approved by the Local Institutional Committee. All the surgical and experimental procedures were in accordance with institutional animal care guidelines.

2.2. Study design

Animals were divided into three groups (n = 60 in each group) as follows: (1) sedentary young group (Young); (2) sedentary old group (Old); and (3) exercised-trained old group (Old + EX). Chronic aerobic exercise training on treadmill (Table 1) was performed as indicated in the published protocol (Husain, 2004).

Table 1
Exercise training protocol for rats on treadmill.

Week	Belt speed (m/min)	Inclination (degrees)	Total time (min/day)
1	8	10	30
2	12	10	30
3	12	10	45
4	16	10	45
5	16	10	60
6	20	10	60
7	20	10	60
8	20	10	60
9	20	10	60
10	20	10	60
11	20	10	60
12	20	10	60

2.3. Measurement of collagen and elastin contents in aorta

Thoracic aortas of three groups were removed. Total soluble collagens were extracted overnight by using 5 mg/ml pepsin in 0.5 mol/l acetic acid followed the instruction. The soluble collagens of aortas were measured by using the Sircol collagen assay kit (Biocolor, UK) followed the manufacturer's instructions.

The aortas were dissected and added by 800 μl of 0.25 mol/l oxalic acid. The samples were placed into a metal heating block with the thermostat set at 100 °C for an hour. Then the aortic elastin content was measured by using the Fastin elastin assay kit (Biocolor, UK) followed the manufacturer's instructions.

2.4. Assessment of efficacy of the exercise protocol

Citrate synthase (a respiratory enzyme which underwent adaptive increases due to exercise in skeletal muscle fibers) was used as a marker of training efficacy. Soleus muscles and gastrocnemius muscles from each rat were collected for determination of citrate synthase (CS) activity to determine the efficacy of the training protocol (Ogihara et al., 2010). CS activity was measured from whole muscle homogenate by using a citrate synthase activity assay kit (Sigma, St. Louis, MO, USA). The CS activity was expressed as nanomoles per minute per milligram of protein. Protein content of muscle homogenate was determined as described by Bradford using bovine serum albumin as a standard.

2.5. Aortic relaxation in response to acetylcholine (ACh) and sodium nitroprusside (SNP)

Thoracic aortas of three groups were removed, cleared of adhering connecting tissue, cut into rings 2 mm in length and placed in Krebs buffer. Protocols were performed on rings beginning at their optimum resting tone, previously determined to be 3 g for rat aorta. This resting tone was reached by stretching rings in 500 mg increments separated by 10-min intervals. Data were collected using a MacLab system and analyzed using Dose Response Software (AD Instruments, Colorado Springs, CO, USA). Vessel rings were preconstricted with phenylephrine (1 μmol/l) (Sigma, St. Louis, MO, USA), and their vasorelaxant dose responses to acetylcholine (1 nmol/l to 10 μmol/l, Sigma) and sodium nitroprusside (1 nmol/l to 10 μmol/l, Sigma) were recorded. Relaxation to ACh and SNP was expressed as a percent relaxation to phenylephrine-induced contraction.

2.6. Pulse wave velocity (PWV) measurement

Aortic pulse wave velocity is the gold standard clinical measure of large elastic artery stiffness (Vlachopoulos et al., 2010). For PWV measurement, the foot-to-foot method was used to determine the time delay between the proximal and the distal aorta (Mitchell et al., 1997). This method has been shown to be highly reproducible and to cause minimal variability compared with the method that uses transfer function (Mitchell et al., 1997).

2.7. Measurement of malondialdehyde (MDA)

Aortic homogenates and plasma were used for the determination of MDA (a presumptive marker of oxidant-mediated lipid peroxidation) using a kit (Cayman, Ann Arbor, USA). Final results of aortic MDA were normalized to protein concentration.

Please cite this article as: Gu, Q., et al., Chronic aerobic exercise training attenuates aortic stiffening and endothelial dysfunction through preserving aortic mitochondrial..., Exp. Gerontol. (2014), http://dx.doi.org/10.1016/j.exger.2014.02.014