Interaction of regular exercise and chronic nitroglycerin treatment on blood pressure and rat aortic antioxidants

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Abstract

Many cardiac patients undergo exercise conditioning with or without medication. Therefore, we investigated the interaction of exercise training and chronic nitroglycerin treatment on blood pressure (BP), aortic nitric oxide (NO), oxidants and antioxidants in rats. Fisher 344 rats were divided into four groups and treated as follows: (1) sedentary control, (2) exercise training (ET) for 8 weeks, (3) nitroglycerin (15 mg/kg, s.c. for 8 weeks) and (4) ET + nitroglycerin. BP was monitored with tail-cuff method. The animals were sacrificed 24 h after the last treatments and thoracic aorta was isolated and analyzed. Exercise training on treadmill for 8 weeks significantly increased respiratory exchange ratio (RER), aortic NO levels, and endothelial nitric oxide synthase (eNOS) protein expression. Training significantly enhanced aortic glutathione (GSH), reduced to oxidized glutathione (GSH/GSSG) ratio, copper/zinc-superoxide dismutase (CuZn-SOD), Mn-SOD, catalase (CAT), glutathione peroxidase (GSH-Px) glutathione disulfide reductase (GR) activities and protein expressions. Training significantly depleted aortic malondialdehyde (MDA) and protein carbonyls without change in BP. Nitroglycerin administration for 8 weeks significantly increased aortic NO levels and eNOS protein expression. Nitroglycerin significantly enhanced aortic Mn-SOD, CAT, GR and glutathione-S-transferase (GST) activities and protein expressions with decreased MDA levels, protein carbonyls and BP. Interaction of training and nitroglycerin treatment significantly increased aortic NO levels, eNOS protein expression, GSH/GSSG ratio, antioxidant enzymes and normalized BP. The data suggest that the interaction of training and nitroglycerin maintained BP by up-regulating the aortic NO and antioxidants and reducing the oxidative stress in rats.
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Keywords: Aorta; Antioxidant enzyme; Blood pressure; Exercise training; Lipid peroxidation; Nitric oxide; Nitroglycerin; Rat

1. Introduction

Many patients with cardiovascular diseases such as angina pectoris, chronic heart failure and hypertension participate in organized group exercise rehabilitation programs or pursue individual exercise with or without medication. Reactive oxygen species (ROS)/free radicals and NO play an important role in the modulation of cardiovascular diseases as well as in the regulation of cardiovascular functions [1–3]. However, the cardiovascular system has an elaborate antioxidant defense system including antioxidant enzymes—superoxide dismutase (SOD), CAT, GSH-Px, GR and other endogenous antioxidants, alpha-tocopherol, ascorbic acid and glutathione to protect the cell against

harmful ROS [4,5]. Exercise increases the utilization of oxygen in the body, and seems to enhance the free radical formation in various tissues of the rat [6]. Exercise-generated ROS seem to increase the activity of antioxidant enzymes in the cardiovascular system [7,8]. Exercise training also generates NO in the cardiovascular system by induction of endothelial nitric oxide synthase (eNOS) [9,10]. In the cardiovascular tissues, NO reacts with superoxide anion to form toxic peroxynitrite radical [11]. Therefore, NO may be considered as a superoxide scavenger [12].

Nitroglycerin (glyceryl trinitrate) is most commonly used in the clinic for the treatment of angina pectoris, acute congestive heart failure, myocardial ischemia or infarction and also in the management of hypertension. The pharmacological action of nitroglycerin is mediated through release of nitric oxide during its metabolism by glutathione-S-transferase (GST) [13]. Nitroglycerin-derived NO relaxes smooth muscle cells in the vascular system by stimulating the formation of cyclic GMP through activation of guany-

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late cyclase leading to lowering of the blood pressure [14]. Many cardiovascular patients undergo exercise-induced preconditioning along with nitroglycerin medication. There are scanty reports on alterations in vascular oxidants/antioxidants and NO systems following physical training or nitroglycerin treatment [13,15,16]. However, no report is available on the interactive effects of regular physical activity and chronic nitroglycerin treatment specifically on the vascular oxidant/antioxidant and NO systems as well as BP in rats. Therefore, this study was designed in order to investigate the interaction of exercise training and chronic nitroglycerin treatment on blood pressure and alterations in NO, antioxidant system and lipid peroxidation in the aorta of the rat.

2. Materials and methods

2.1. Chemicals

Chemicals such as GSH, oxidized glutathione (GSSG), NADPH, γ-glutamyl glutamate; enzymes (SOD, CAT, GSH-Px, GR, GST), 1-chloro-2,4-dinitrobenzene (CDNB), 1,1,1,1-tetraethoxy-propane were purchased from Sigma Chemical Co. (St. Louis, MO). Nitroglycerin was purchased from (American Regent Laboratory, CA). Coomassie protein assay reagent was purchased from Pierce Company (Rockford, IL).

2.2. Animals

Male Fisher 344 rats (Harlan Industries, Indianapolis, IN) were used throughout the study. The rats were fed ad libitum with Rodent Laboratory Chow (Ralston Purina Company, Indianapolis, IN). Feed consisted of protein (23.4%), fat (4.5%) and balanced with carbohydrates, fibers, vitamins and minerals. The rats were maintained on a 12:12 h light/dark photoperiod. They were randomly divided into four groups and treated as follows: Group I (sedentary control (SC)). Rats were administered normal saline vehicle (1 ml/kg, subcutaneously (s.c.)) daily for 8 weeks and were put on the treadmill (Omnitech Instruments, Columbus, OH) belt for 5 min for equivalent handling ($n = 8$). Group II (exercise training (ET)). Rats conducted training on treadmill daily for 8 weeks as indicated in the published protocols [5–8] and were given normal saline 1 ml/kg, s.c. ($n = 8$) (Table 1). Group III: (nitroglycerin (NG)). Rats were administered nitroglycerin (15 mg/kg, s.c.) daily in the morning for 8 weeks and put on treadmill belt daily for 5 min ($n = 8$). Group IV: (nitroglycerin (NG) plus ET). Rats were administered nitroglycerin (15 mg/kg, s.c.) daily in the morning for 8 weeks and given exercise training on treadmill for 8 weeks ($n = 6$).

The dose of the drug treatment in rats has been adapted to previous reports [17,18]. The animals were monitored for

Table 1
Exercise training protocol for rats on treadmill

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

Rats were exercised on treadmill at a belt speed of 8 m/min at 10° angle of inclination for 30 min during the first week, at 12 m/min and the same angle of inclination for 30 min during the second week, at 16 m/min and the same angle of inclination for 45 min during the third week, and at 20 m/min and the same angle of inclination for 45 min during the fourth week. During the fifth to eighth weeks, rats were exercised at a belt speed of 20 m/min and the same angle of inclination for 60 min daily.

exercise parameters such as oxygen consumption, and respiratory exchange ratio (RER) throughout the study using computerized Oxyscan system (Omnitech Instruments). The blood pressure was monitored every week using tail-cuff method with manometer–tachometer model #KN-201-1 (Nastume Instruments, Japan). The animals were sacrificed by decapitation 24 h after the last treatments. Thoracic aorta were isolated and immersed in liquid nitrogen and stored at − 80 °C until further analysis could be completed.

2.3. Determination of glutathione (GSH) and its disulfide (GSSG)

The concentrations of GSH and GSSG were determined in the frozen aortic tissue by a modified high-performance liquid chromatography (HPLC) method of Fariss and Reed [19] using UV detector. Frozen tissues were homogenized in a cold solution containing 10% perchloric acid and 1 mM bathophenanthroline disulfonic acid. The cold homogenate was centrifuged at − 5 °C for 10 min at 1000 rpm. Two-hundred and fifty microliters of this acidic supernatant extract containing internal standard (gamma-glutamyl glutamate) was mixed with 100 μl of 100 mM iodoacetic acid in a 0.2 mM *m*-cresol purple solution. This acidic solution was brought to basic conditions (pH 8.9) by the addition of approximately 400 μl of 2 M KOH–2.4 M KHCO$_3$. The sample was placed in the dark at room temperature for 1 h. Rapid S-carboxymethyl derivatization of GSH, GSSG and gamma-glutamyl glutamate occurred soon after the change in pH. *N*-dinitrophenyl derivatization of the samples was obtained by incubation for 12 h at 4 °C in the presence of 1% 1-fluoro-dinitrobenzene. Multiple samples were analyzed using the ISCO auto sampler controlled by ISCO Chemical research program. The sensitivity of the HPLC for GSH was 50 pmol/injection volume and 25 pmol/injection volume for GSSG. The concentrations of GSH and GSSG were expressed as nmol per milligram of protein ($n = 6-8$).

2.4. Enzyme assays

SOD activity was determined at room temperature according to the method of Misra and Fridovich [20]. SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. Mn-SOD activity was determined by adding 100 μl of 20 mM NaCN to inhibit CuZn-SOD activity. CuZn-SOD activity was determined by subtracting Mn-SOD activity from total SOD activity ($n = 6-8$).

CAT activity was determined at room temperature by a slight modification of a method of Aebi [21]. The molar extinction coefficient of 43.6 mM cm^{-1} was used to determine CAT activity. One unit of CAT activity was defined as the mmol of H$_2$O$_2$ degraded/min/mg protein ($n = 6-8$).

GSH-Px activity was determined by a method of Flohe and Gunzler [22] at 37 °C. The millimolar extinction coefficient of 6.22 mM cm^{-1} was used to determine the activity of GSH-Px. One unit of activity was equal to the millimoles of NADPH oxidized/min/mg protein ($n = 6-8$).

GR activity was determined by the method of Carlberg and Mannervik [23] at 37 °C. The millimolar extinction coefficient of 6.22 cm^{-1} was used to determine the activity of GR. One unit of GR activity was equal to the millimoles of NADPH oxidized/min/mg protein ($n = 6-8$).

GST activity was assayed by the method of Habig et al. [24] using 10 mM CDNB as a substrate. The enzyme activity was calculated using the extinction coefficient 9.6 mM cm^{-1} and expressed as μmol of CDNB utilized/min/mg protein ($n = 6-8$).

2.5. Antioxidant enzyme protein levels by ELISA

The antioxidant enzyme protein expressions were determined using enzyme linked immunosorbent assay (ELISA) technique developed in our laboratory [7,8]. Tissue extracts (0.05 ml) prepared in phosphate buffered saline (PBS) (10 mM phosphate buffer, pH 7.4, 150 mM NaCl and 0.1% sodium azide) were pipetted into each well of polyvinyl microtiter plate and incubated overnight at 4 °C. Coating solution was removed and washed three times with washing buffer (10 mM phosphate buffer, pH 7.4, 150 mM NaCl, 0.05% Tween 20) and distilled water. One hundred microliters of monoclonal antibody (CuZn-SOD) (Sigma) diluted in PBS (1:300) or other diluted (1:300) antibodies viz. anti-Mn-SOD (Biodesign International, Kennebunk, ME), anti-catalase (CN Biosciences, San Diego, CA), anti-glutathione peroxidase (Biodesign International, Saco, ME), and anti-alpha-GST (Biodesign International), respectively, were added to each well, incubated at room temperature for 2 h, and washed three times as before. These monoclonal antibodies were specifically recognized pure protein antigens as per our initial Western blot analysis. Only GSH-Px antibody was cross-reacted with GSH-Px from mouse tissues. One hundred microliters

of peroxidase conjugated secondary antibody diluted in PBS (1:100) was added to each well, incubated for 2 h and washed three times as before. One hundred microliters of substrate (1% H$_2$O$_2$ and 1 mg/ml 5-amino salicylic acid) in reaction buffer (0.02 M phosphate buffer, pH 6.8) was added to each well and incubated for 30 min. The reaction was stopped by adding 0.1 ml of 3 N NaOH and absorption of the microtiter wells read at 450 nm using an ELISA reader (Automated Microplate Reader, Model EL311, Bio-Tek Instruments, Inc., Winooski, VT). The enzyme protein concentrations were expressed as microgram per milligram of protein ($n = 6-8$).

2.6. Endothelial (eNOS) and inducible (iNOS) protein expression assay

The enzyme protein expressions of eNOS and iNOS were analyzed using ELISA technique as described earlier [7,8]. Tissue extracts (0.05 ml) prepared in PBS (10 mM phosphate buffer, pH 7.4, 150 mM NaCl and 0.1% sodium azide) was pipetted into each well of polyvinyl microtiter plate and incubated overnight at 4 °C. Coating solution was removed and washed three times with washing buffer (10 mM phosphate buffer, pH 7.4, 150 mM NaCl, 0.05% Tween 20) and distilled water. Fifty microliters of monoclonal antibody (eNOS) (R&D Systems, Inc., Minneapolis, MN) and (iNOS) (Upstate Biotechnology, Lake Placid, NY) diluted in PBS (1:300) was added to each well, incubated at room temperature for 2 h, and washed three times as before. These monoclonal antibodies were specifically recognized pure protein antigens as per our initial Western blot analysis. No cross-reactivity was observed using rat monoclonal antibodies. Fifty microliters of peroxidase-conjugated secondary antibody (Sigma) diluted in PBS (1:100) was added to each well, incubated for 2 h and washed three times as before. One hundred microliters of substrate (1% H$_2$O$_2$ and 1 mg/ml 5-amino salicylic acid) in reaction buffer (0.02 M phosphate buffer, pH 6.8) was added to each well and incubated for 30 min. The reaction is stopped by adding 0.1 ml of 3 N NaOH and absorption of the microtiter wells read at 450 nm using an ELISA reader (Universal Microplate Reader, Model ExL800, Bio-Tek Instruments). The enzyme protein concentrations were expressed as microgram per milligram protein ($n = 6-8$).

2.7. Lipid peroxidation assay

The end product of lipid peroxidation (malondialdehyde (MDA)) was estimated by the method of Ohkawa et al. [25]. MDA concentration was expressed as nmol/mg protein ($n = 6-8$).

2.8. Nitric oxide assay

The NO levels in the aortic tissues were determined by NO assay kit (Cayman Chemical, Ann Arbor, MI) as

Table 2
Effect of exercise training, chronic nitroglycerin treatment and combination of both for 8 weeks on RER and BP in rats

Groups	RER	BP
(1) Sedentary control ($n = 10$)	0.62 ± 0.03	115 ± 9
(2) Exercise training ($n = 8$)	1.09 ± 0.05**	109 ± 7
(3) Nitroglycerin ($n = 8$)	0.67 ± 0.04	80 ± 9*
(4) Training + nitroglycerin ($n = 8$)	0.99 ± 0.05**	112 ± 8

All the values are expressed as mean $\pm$ S.E.
Rats were exercised on a treadmill and given nitroglycerin at a dose of 15 mg/kg, s.c. daily for 8 weeks. RER and BP are expressed as VCO_2/VO_2 and mm Hg, respectively.
   * Significant ($P < 0.05$) as compared to groups 1, 2 and 4.
   ** Significant ($P < 0.001$) as compared to groups 1, 3 and 4.

described previously [7,8]. The concentration was expressed as nmol per mg protein ($n = 6–8$).

2.9. Protein assay

Protein concentration was estimated according to the method of Read and Northcole [26] using Coomassie protein assay dye and bovine serum albumin as a standard.

2.10. Statistical analysis

The data were expressed as mean $\pm$ S.E. The data for biochemical and physiological parameters were analyzed statistically using two-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SAS statistical software package (SAS Institute, Cary, NC) for comparison of groups with each other. The 0.05 level of probability was used as criterion for statistical significance.

3. Results

The RER significantly ($P < 0.001$) increased in exercise-trained rats compared to sedentary control. However, no significant change in RER was observed in nitroglycerin-treated group. A significant ($P < 0.001$) increase in

Table 3
Effect of exercise training, chronic nitroglycerin treatment and combination of both for 8 weeks on aortic NO levels, eNOS and iNOS enzyme protein expressions in rats

Groups	NO	eNOS	iNOS
(1) Sedentary control ($n = 10$)	151.8 ± 6.0	3.52 ± 0.22	2.30 ± 0.20
(2) Exercise training ($n = 8$)	185.9 ± 6.4**	3.99 ± 0.26*	2.43 ± 0.21
(3) Nitroglycerin ($n = 8$)	176.8 ± 4.8**	3.01 ± 0.23#	1.48 ± 0.16##
(4) Training + nitroglycerin ($n = 8$)	171.9 ± 5.5**	3.49 ± 0.21+	2.28 ± 0.18++

All the values are expressed as mean $\pm$ S.E.
Rats were exercised on a treadmill and given nitroglycerin at a dose of 15 mg/kg, s.c. daily for 8 weeks. NO concentration is expressed as nmol/mg protein and endothelial eNOS and inducible iNOS enzyme protein levels are expressed as µg/mg protein.
   * Significant ($P < 0.05$) as compared to group 1, 3 and 4.
   ** Significant ($P < 0.001$) as compared to group 1.
   # Significant ($P < 0.001$) as compared to group 1, 2 and 4.
   ## Significant ($P < 0.001$) as compared to group 1, 2 and 4.
   + Significant ($P < 0.05$) as compared to group 3.
   ++ Significant ($P < 0.001$) as compared to group 3.

RER observed in nitroglycerin plus exercise training group compared to control group (Table 2). Exercise training maintained the blood pressure up to 8 weeks compared to sedentary control. Chronic nitroglycerin administration to rats for 8 weeks significantly ($P < 0.05$) decreased BP. The combination of both nitroglycerin and training resulted in normalization of BP after 8 weeks (Table 2).

The effects of training, chronic nitroglycerin administration and the combination of both on aortic NO levels, eNOS, and iNOS enzyme protein expressions are depicted in Table 3. Training for 8 weeks significantly elevated aortic NO levels ($P < 0.001$), eNOS protein expression ($P < 0.05$). Chronic nitroglycerin administration for 8 weeks resulted in a significant elevation of aortic NO levels ($P < 0.001$) but a significant depression of aortic eNOS protein expression ($P < 0.05$) and iNOS protein expression ($P < 0.001$). The combination of nitroglycerin and training resulted in enhanced aortic NO levels ($P < 0.001$) and normalization of

Table 4
Effect of exercise training, chronic nitroglycerin treatment and combination of both for 8 weeks on aortic GSH, GSSG, GSH/GSSG ratio, MDA, and protein carbonyl content in rats

Groups	GSH	GSSG	GSH/GSSG	MDA	Carbonyl
(1) Sedentary control ($n = 10$)	10.64 ± 0.60	3.03 ± 0.22	3.52 ± 0.28	1.8 ± 0.15	60.8 ± 2.20
(2) Exercise training ($n = 8$)	13.50 ± 0.56++	2.33 ± 0.27*	5.70 ± 0.55**	1.1 ± 0.10**	55.4 ± 2.04*
(3) Nitroglycerin ($n = 8$)	10.93 ± 0.83	2.22 ± 0.32*	4.92 ± 0.49*	1.4 ± 0.14*,+	56.0 ± 2.40*
(4) Training + nitroglycerin ($n = 8$)	11.54 ± 1.10	2.46 ± 0.19*	4.70 ± 0.42*	1.4 ± 0.13*,+	54.6 ± 2.60*

All the values are expressed as mean $\pm$ S.E.
Rats were exercised on a treadmill and given nitroglycerin at a dose of 15 mg/kg, s.c. daily for 8 weeks. MDA, GSH, GSSG, NO and protein carbonyl concentrations are expressed as nmol/mg protein.
   * Significant ($P < 0.05$) as compared to group 1.
   ** Significant ($P < 0.001$) as compared to group 1.
   + Significant ($P < 0.05$) as compared to group 2.
   ++ Significant ($P < 0.001$) compared to groups 1, 3 and 4.

Table 5
Effect of exercise training, chronic nitroglycerin treatment and combination of both for 8 weeks on aortic antioxidant enzyme activities in rats

Groups	CuZn-SOD	Mn-SOD	CAT	GSH-Px	GR	GST
(1) Sedentary control ($n = 10$)	15.20 ± 1.86	4.10 ± 0.33	19.25 ± 2.10	50.38 ± 6.80	28.10 ± 2.75	3.24 ± 0.29
(2) Exercise training ($n = 8$)	20.88 ± 1.25*	5.62 ± 0.48**	24.70 ± 1.60*	62.87 ± 6.80*	35.75 ± 1.99*	3.21 ± 0.16
(3) Nitroglycerin ($n = 8$)	14.92 ± 1.21	4.82 ± 0.29*	29.00 ± 2.40**	67.70 ± 4.88*	37.50 ± 2.70*	4.70 ± 0.24**
(4) Training + nitroglycerin ($n = 8$)	16.33 ± 1.69	6.48 ± 0.83**,+	33.20 ± 2.20**,#	69.83 ± 8.96*	45.90 ± 2.99**,+,#	3.90 ± 0.20**,+ +

All the values are expressed as mean ± S.E.
Rats were exercised on a treadmill and given nitroglycerin at a dose of 15 mg/kg, s.c. daily for 8 weeks. CuZn-SOD and Mn-SOD activities are expressed as units/mg protein; CAT activity is expressed as mmol hydrogen peroxide degraded/min/mg protein; GSH-Px and GR activities are expressed as μmol of NADPH oxidized/min/mg protein; GST activity is expressed as μmol of CDNB utilized/min/mg protein.
* Significant ($P < 0.05$) as compared to group 1.
** Significant ($P < 0.001$) as compared to group 1.
# Significant ($P < 0.05$) compared to group 2.
+ Significant ($P < 0.05$) as compared to group 3.
++ Significant ($P < 0.01$) as compared to group 3.

aortic iNOS, and eNOS protein expressions indicating beneficial role of medication and exercise on aortic NO levels.

The effects of training, chronic nitroglycerin administration and the combination of both on aortic GSH, GSSG, GSH/GSSG ratio, protein carbonyls and MDA levels are depicted in Table 4. Training significantly increased aortic GSH levels ($P < 0.001$), GSH/GSSG ratio ($P < 0.001$) and significantly decreased GSSG levels ($P < 0.05$), MDA levels ($P < 0.001$) and protein carbonyls ($P < 0.05$). Chronic nitroglycerin administration for 8 weeks significantly increased aortic GSH/GSSG ratio ($P < 0.05$) and significantly decreased GSSG levels ($P < 0.05$), MDA levels ($P < 0.05$) and protein carbonyls ($P < 0.05$). The combination of nitroglycerin and training resulted in a significant increase in aortic GSH/GSSG ratio ($P < 0.05$) and depletion of GSSG ($P < 0.05$), MDA levels ($P < 0.05$) and protein carbonyls ($P < 0.05$) indicating the beneficial role of the medication and exercise against oxidative injury to aorta.

The effects of training, chronic nitroglycerin administration and the combination of both on aortic antioxidant enzyme activities and protein expressions are depicted in Tables 5 and 6. Training for 8 weeks significantly enhanced aortic Mn-SOD activity ($P < 0.001$) and protein expression ($P < 0.05$), CuZn-SOD activity ($P < 0.05$) and protein expression ($P < 0.05$), CAT activity ($P < 0.05$) and protein expression ($P < 0.05$), GSH-Px activity ($P < 0.05$) and protein expression ($P < 0.05$), and GR activity ($P < 0.05$). The rat aortic antioxidant enzyme activities and protein levels in the present study are consistent with the data reported by other reports [27–31]. The data suggest the up-regulation of antioxidant enzymes due to training-induced adaptation. Chronic nitroglycerin administration for 8 weeks significantly increased aortic Mn-SOD activity ($P < 0.05$) and protein expression ($P < 0.05$), CAT activity ($P < 0.001$) and protein expression ($P < 0.05$), GSH-Px activity ($P < 0.05$) and protein expression ($P < 0.05$), GR activity ($P < 0.05$) and GST activity ($P < 0.001$) and protein expression ($P < 0.05$). The combination of nitroglycerin and training resulted in a significant increase of aortic Mn-SOD activity ($P < 0.001$) and protein expression ($P < 0.001$), CAT activity ($P < 0.001$) and protein expression ($P < 0.05$), GSH-Px activity ($P < 0.05$) and protein expression ($P < 0.05$), GR activity ($P < 0.001$), GST activity ($P < 0.001$) and protein expression ($P < 0.001$). Interestingly, alterations in antioxidant enzyme activities were also related with enzyme protein expressions in the aorta indicating influence of exercise and nitroglycerin on gene regulation of these enzymes.

Table 6
Effect of exercise training, chronic nitroglycerin treatment and combination of both for 8 weeks on aortic antioxidant enzyme protein levels in rats

Groups	CuZn-SOD	Mn-SOD	CAT	GSH-Px	Alpha-GST
(1) Sedentary control ($n = 10$)	3.18 ± 0.60	0.50 ± 0.14	1.31 ± 0.12	3.48 ± 0.37	2.14 ± 0.15
(2) Exercise training ($n = 8$)	4.67 ± 0.71*	0.91 ± 0.16*	1.74 ± 0.18*	4.06 ± 0.21*	2.45 ± 0.16
(3) Nitroglycerin ($n = 8$)	3.32 ± 0.51	0.88 ± 0.13*	1.76 ± 0.15*	3.97 ± 0.20*	2.59 ± 0.13*
(4) Training + nitroglycerin ($n = 8$)	3.20 ± 0.70	0.99 ± 0.15**	1.56 ± 0.11*	4.42 ± 0.39*,+	3.49 ± 0.36**,+,#

All the values are expressed as mean ± S.E.
Rats were exercised on a treadmill and given nitroglycerin at a dose of 15 mg/kg, s.c. daily for 8 weeks. The enzyme protein levels were expressed as μg/mg protein.
* Significant ($P < 0.05$) as compared to group 1.
** Significant ($P < 0.001$) as compared to group 1.
+ Significant ($P < 0.05$) as compared to group 3.
# Significant ($P < 0.05$) compared to group 2.

4. Discussion

The present study addresses the influence of exercise training and chronic nitroglycerin treatment alone and the combination of both on RER, BP and associated biochemical alterations in the aorta of rats. The RER is the reflection of adaptation due to aerobic exercise training. Increased RER following 8 weeks of exercise training on the treadmill is indicative of the conditioning of the cardiorespiratory system. Similar adaptive responses of the training have been reported in humans [32,33]. This cardiovascular conditioning also maintained the BP in the normal range. Our earlier studies have demonstrated that physical conditioning attenuates the NO-deficient hypertension in rats [7,8]. The antihypertensive effect of the training was related to the preservation of the endothelial NO levels [9,10]. The present study demonstrates the elevated level of NO corresponding to the induction of eNOS enzyme protein expression in the aorta of exercise-trained rats. The enhanced production of aortic NO through eNOS activation may also be related to vascular endothelial growth factor (VEGF) secretion in exercise conditioning [34,35]. The adaptive response due to exercise training resulted in the up-regulation of aortic GSH/GSSG ratio and antioxidant enzymes. Earlier reports also demonstrated an increased GSH/GSSG ratio in the cardiovascular system of exercise-trained animals [5–8]. The enhanced aortic GR activity that converts GSSG to GSH after training is also indicative of enhanced GSH/GSSG ratio and supports an earlier finding [7]. The depletion of aortic MDA levels (an end product of lipid peroxidation) and protein carbonyl (end product of protein oxidation) is indicative of adaptive response of the training against oxidative injuries. The antioxidant enzymes are the first line of defense against oxidative injuries. The present study shows the up-regulation of the aortic antioxidant enzyme activities and protein levels (CuZn-SOD, Mn-SOD, CAT and GSH-PX) in exercise-trained rats. The CuZn-SOD activity is known to be induced on account of adaptation due to exercise training. Even this enzyme has been shown to increase in other body tissues such as heart and muscle following exercise training [5–8]. These findings indicate the activation/induction of antioxidant enzymes due to training-induced adaptation. Other reports show antioxidant enzyme gene and protein expressions in the cardiovascular system of exercise-trained rats [4–8]. However, it is important to note that the degree of activation/induction of antioxidant enzymes in the cardiovascular system is related to intensity, duration and mode of exercise training.

Chronic nitroglycerin treatment resulted in a fall of BP without a change in RER, indicating that chronic nitroglycerin treatment is devoid of respiratory changes in rats. The BP is regulated by change in cardiac output and peripheral vascular resistance. These processes involve several factors such as vasoconstrictors and vasodilators and their receptors [34,35]. It is likely that chronic nitroglycerin treatment might have decreased the peripheral vascular resistance as a result of vasodilatation through adenosine receptors, because we did not observe very high NO production in the aorta compared to control. The small increase in aortic NO by chronic nitroglycerin treatment compared to training may be due to tolerance after chronic doses of nitroglycerin in the rat [17,18,36,37]. There are several mechanisms proposed in the tolerance of nitroglycerin. These include superoxide and peroxynitrite formation [36,38], antioxidant depletion [38], NO/cGMP-mediated desensitization [39], and the up-regulation of phosphodiesterase 1A1 [40]. There was no significant increase in aortic superoxide and nitrotyrosine contents after nitroglycerin treatment (unpublished observations), indicating that free radicals are not involved in nitroglycerin tolerance [41,42]. The antioxidant response of the nitroglycerin is indicated by a significant increase in aortic GSH/GSSG ratio, antioxidant enzyme activities as well as enzyme protein expressions. A significant decrease in aortic MDA and protein carbonyl levels further support the antioxidant effects of the nitroglycerin [43]. The enzyme GST catalyzes the conjugation of nitroglycerin to GSH leading to yield of NO as well as detoxification of the other end products of lipid peroxidation such as 4-hydroxy alkenals [13,44]. The induction of GST activity and protein expression in the aorta of rats after chronic nitroglycerin treatment is indicative of enhanced metabolism of nitroglycerin to yield NO and detoxification of harmful aldehydes.

The interaction of exercise training and chronic nitroglycerine treatment restored the depression in BP and aortic eNOS and iNOS protein expressions caused by nitroglycerin alone. A lower decrease in BP in the combination group may be due to exercise-induced changes in the pharmacokinetics and pharmacodynamics of the nitroglycerin, hence, blood pressure. The beneficial role of exercise conditioning in maintaining the BP and enhanced RER is also due to suppression of nitroglycerin tolerance in rats. These effects of the exercise training are known to be related to increased capillary blood flow, tissue oxygen utilization, and the up-regulation of the antioxidants and NO systems in cardiovascular tissues [7,9,10]. The enhanced iNOS protein expression in the aorta of rats in the combination group compared to exercise and nitroglycerin group alone may be due to release of inflammatory factors, and thereby induction of nuclear factor kappa B (NF-κB). These factors are known to enhance the induction of iNOS in the aorta [2,34,35]. The present study demonstrates that interaction of training and chronic nitroglycerin administration resulted in the up-regulation of aortic antioxidant enzymes, GSH/GSSG ratio and depressed aortic MDA and protein carbonyl contents. Interestingly, the mitochondrial Mn-SOD activity is profoundly induced as compared to cytosolic CuZn-SOD activity in the aorta. Mn-SOD is an inducible enzyme, which is sensitive to tissue oxygenation, and endogenous factors such as cytokines, adenosine, and corticosteroids [5,7]. Thus, it is likely that the above factors might have induced the enzyme activity in the aorta of rats treated with

nitroglycerin and exercised. The cytosolic CuZn-SOD activity is very susceptible to inactivation by free radicals and ROS. It is suggested that nitroglycerin in the presence of exercise might have been able to induce oxidative inactivation of this enzyme. Exercise training is known to influence the kinetics of antioxidant enzymes in tissues of the body [6]. A lesser effect on GST enzyme in the nitroglycerin plus exercise group may be due to exercise-induced changes in the pharmacokinetics of nitroglycerin leading to lower metabolism through GST enzyme on account of changes in enzyme kinetics. These results indicate the adaptive response of the combination in preserving the endogenous antioxidants in the aorta of rats.

It is concluded that the interaction of aerobic exercise training and chronic nitroglycerin treatment resulted in the maintenance of BP and RER by up-regulating the aortic NO levels, GSH/GSSG ratio, antioxidant enzyme activity and protein expressions, and by down-regulating the lipid peroxidation and protein oxidation processes in rats.

Acknowledgements

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