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SHORT COMMUNICATION

Effect of high sustained +Gz stress on myocardial mitochondrial ultrastructure, respiratory function, and antioxidant capacity in rats

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Abstract Exposure to high sustained positive acceleration (+Gz) is known to have a pathophysiological effect on the heart of the rat. As critical regulators of cardiac myocyte survival and death, mitochondria may be crucially involved in +Gz-induced pathogenesis. It was, therefore, of interest to investigate myocardial mitochondrial ultrastructure, respiratory function, and antioxidant capacity in rats after exposure to +10 Gz for 5 min. The results showed that high +Gz stress could damage mitochondrial ultrastructure; this was apparent from swollen, degenerated, and reduced mitochondria, and mitochondrial cristae broken or disappeared. This resulted in significant changes of quantitative indicators of mitochondria morphometry, for example increased surface density, volume density, average volume, and average surface area, and reduced numerical density. The studies also revealed that exposure to +Gz stress induced dysfunction of the mitochondrial respiratory chain, reduced the activity of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase), and increased malondialdehyde content. We thus conclude that high +Gz stress not only damaged mitochondrial ultrastructure but also impaired respiratory function and antioxidant capacity.

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Introduction

Modern high performance fighters (F15, F16) are capable of producing acceleration as high as +9 Gz sustained for 15–45 s, which may exceed human physiological tolerance [1]. High +Gz stress may challenge the pilot's physical ability, and the effect of high sustained +Gz stress on the heart has long been a concern [2, 3]. Whinnery et al. [4] reported that high sustained +Gz stress could cause cardiac dysfunction, for example shortened QT interval, in healthy male subjects. Martin et al. found a statistically significant association between pulmonic insufficiency and exposure to high +Gz stress in pilots versus non-pilots. They also reported a greater incidence of tricuspid regurgitation and concurrent pulmonic insufficiency and tricuspid regurgitation in the pilot group [5]. Because it is unethical to modify heart pathophysiology in humans, most studies have used animal centrifuge models to examine the pathophysiological effects of high sustained +Gz stress. Burton and Mackenzie showed that high sustained +Gz stress could cause cardiac subendocardial hemorrhage and minor cardiomyopathy in miniature swine [6]. Increased harm to the heart under conditions of high sustained positive acceleration had also been identified in studies using rat models. It can affect cardiac function, reducing early diastolic myocardial velocity and systolic myocardial velocity in both ventricles and left ventricular systolic pressure [7, 8]; it also induced metabolic disorder of catecholamines and energy in the hearts of rats [9, 10]. It can also damage myocardial structure. Zhang et al. and Zheng et al. reported

that high sustained +Gz exposure caused obvious edema of myocardial and endothelial cells, myofibril disorder, injuries of mitochondria and nucleus, and changes of several myocardial enzymes in rats [11, 12]. Although the harmful effect of high sustained positive acceleration on the heart is well known, there is limited knowledge of how high +Gz stress is related to cardiac injury. Previous studies have suggested that cardiac injury may be related to redundant reactive oxygen species (ROS) produced as a result of ischemia and/or hypoxia during high +Gz stress [2, 13].

Mitochondria are the major source of intracellular ROS [14]; they can, however, also be the primary target of ROS that initiate lipid peroxidation and eventually lead to cell death by apoptosis and necrosis. Under physiological conditions, low levels of ROS are required for normal cell function, for example cell signaling, and redundant ROS are, in vivo, reduced by a network of antioxidant defense mechanisms [15]. Under pathological conditions, however, because of elevated ROS production, these mechanisms may fail to eliminate the large amount of ROS generated, which might therefore cause oxidative stress and cellular damage [16]. Thus, mitochondria are important both as an ROS producer and as a sensitive ROS target. Abundant ROS inside the cells will eventually result in mitochondria dysfunction and activate the mitochondria-dependent apoptotic pathway [17].

In the work discussed in this paper we used the animal centrifuge model to investigate the effect of exposure to +Gz on myocardial mitochondrial ultrastructure, respiratory function, and antioxidant capacity in the heart of the rat, to elucidate the mechanisms of cardiac injury.

Materials and methods

Animals

Thirty male Sprague–Dawley rats, weighing 280 ± 20 g, obtained from Weitong Lihua Experimental Animal Technology (Beijing, China), were randomly assigned to three groups: blank control group, stress control group, and high +Gz stress group. Rats were housed individually in an animal room for 3 days, and were starved for 12 h before the experiment to reduce the effects of endogenous substrates, for example myocardial tissue glycogen and fat, on the experimental results. All experiments and procedures performed in this study were reviewed and approved by the Animal Care and Use Committee of the Institute of Aviation Medicine, Air Force.

Exposure of animals to acceleration

The animal centrifuge was 2 m in radius and was capable of producing a range of gravity from +1 to +15 Gz, with an onset rate of 0.1–6 Gz/s. Each rat was put into a 15 cm × 5 cm × 3 cm plexiglass box which was clamped to the centrifuge arm with the head of the rat facing the axis of the centrifuge for +Gz orientation. Rats in the high +Gz stress group were exposed to +10 Gz for 5 min as reported elsewhere [18]. The onset/offset rate was +1 G/s. Stress control rats were placed on the arms of centrifuge and underwent a process similar to that described above, but were not exposed to acceleration. No experiments were conducted on the blank control group.

Sample preparation for transmission electron microscopy and quantitative analysis of myocardial mitochondria morphology

After exposure to acceleration for 4 h, rats were anesthetized and the hearts were quickly removed. Part of the left ventricle tissue was cut into small pieces (approx. 1 mm^3).These were then rapidly fixed with 5 % glutaraldehyde and then fixed again with 1 % osmic acid. The fixed tissue were dehydrated with acetone and embedded in epoxy resin 812. Ultrathin sections, approximately 30–50 nm, were cut with ultramicrotome (PowerTome-PC, RMC, USA), mounted on copper grids, and stained with uranyl acetate and lead citrate. The grids were examined by transmission electron microscopy (model H-75000-2; Hitachi, Japan). One piece of section was prepared for each animal, and five photographs magnified 12,500 times were obtained randomly between the microvilli and nucleus for each section.

The photographs were used as input for a CM2000B pathology image-analysis system (Beijing University of Aeronautics and Astronautics, Beijing, China) for quantitative analysis of myocardial mitochondria morphology, and measurement of volume density, surface density, average volume, average surface area, and numerical density of the mitochondria.

Isolation of myocardial mitochondria

Myocardial mitochondria were isolated by differential centrifugation as described elsewhere [19], with slight modifications. Briefly, 1 g fresh left ventricle tissue was placed in ice-cold isolation medium (50 mM sucrose, 200 mM mannitol, 5 mM KH_2PO_4, 1 mM EGTA, 5 mM Mops, and 0.2 % BSA, pH 7.3), minced to small pieces (approx. 1 mm^3), then homogenized for 10 s by use of a homogenizer (Pro200; ProScientific, USA) in 5 ml isolation buffer. The samples were then diluted to 15 ml with isolation buffer and centrifuged at 480*g* for 10 min. The supernatant, containing the mitochondrial fraction, was further centrifuged at 10,000*g* for 10 min at 4 °C. Mitochondria were washed and resuspended in isolation

medium. Protein concentration was determined by use of a BCA Protein Assay Kit (Bio-Rad, USA) then adjusted to 10–20 mg/ml.

Measurement of myocardial mitochondrial respiratory function

Mitochondrial respiration was measured by determining oxygen consumption by use of a biological oxygen monitor (Model 5300; YSI, USA) equipped with a Clark oxygen electrode. The oxygen consumption studies were conducted at 30 °C in respiration medium (25 mM sucrose, 75 mM mannitol, 95 mM KCl, 5 mM KH_2PO_4, 20 mM Tris–HCl, 1 mM EGTA, pH 7.4), containing 5 mmol/L sodium succinate as respiratory substrate. Approximately 0.5 mg mitochondrial protein was pre-incubated in the oxygen electrode in a total volume of 1 ml, with substrates, for 2 min. State 3 respiration (ST3) was induced by the addition of adenosine diphosphate (ADP) (1 mM). Approximately 3 min later, ST3 was terminated and state 4 respiration (ST4) was detected. The rates of oxygen consumption during State 4 and State 3 were expressed as nanomoles of oxygen consumed per milligram mitochondrial protein per minute. The ratio of State 3 to State 4 mitochondrial respiration was used to calculate the respiratory control ratio (RCR = ST3/ST4).

Determination of antioxidant levels and lipid peroxidation products in myocardial mitochondria

Isolated mitochondria were disrupted by ultrasonication and protein concentration was determined by use of a BCA Protein Assay Kit (Bio-Rad). The activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), and the concentration of malondialdehyde (MDA) in myocardial mitochondria were measured by use of commercial assay kits (Nangjin Jiancheng Bioengineering Institute, China) in accordance with the manufacturer's instructions. The nitrite method was used to determine SOD activity; absorbance was measured at 550 nm. CAT activity was measured at 405 nm, in accordance with Góth [20], by use of an assay kit. GSH-Px activity was measured at 412 nm by quantifying the rate of oxidation of reduced GSH to oxidized glutathione. The thiobarbituric acid coloration method was used to determine MDA concentration; absorbance was measured at 532 nm.

Statistical analysis

All results are expressed as mean ± standard deviation (SD). Comparisons among groups were made by one-way analysis of variance (ANOVA) followed by the least-significant-difference (LSD) post-hoc multiple comparisons

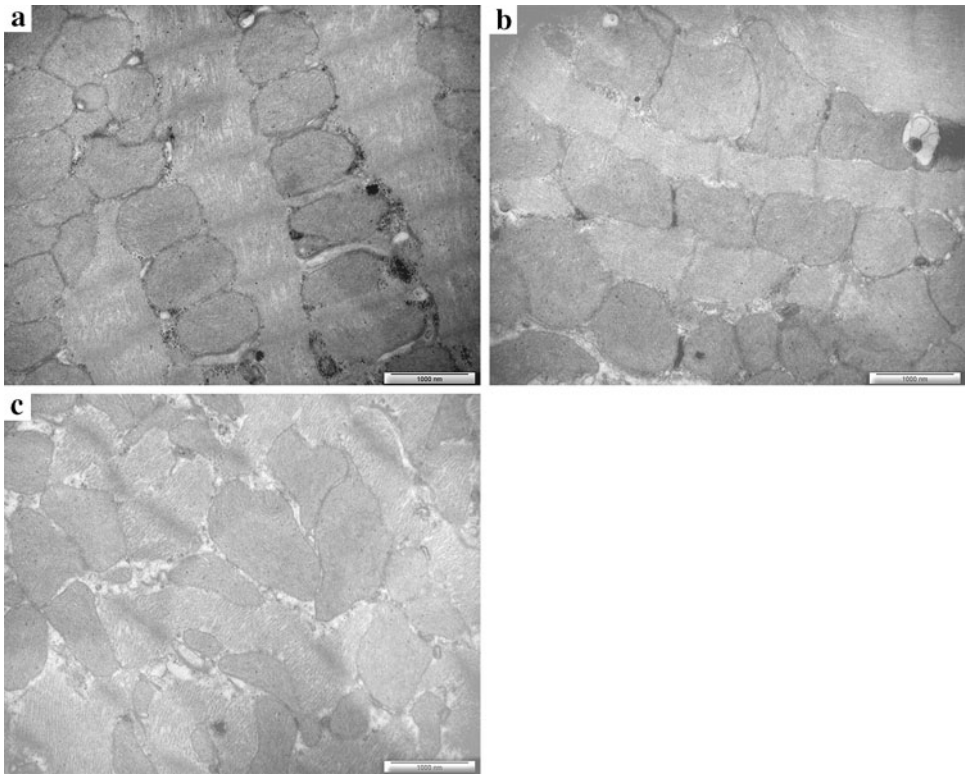

Fig. 1 High +Gz stress impairs the ultrastructure of myocardial mitochondria in rats (*bar* = 1,000 nm). **a** Representative transmission electron microscopy photograph from blank control group; **b** representative transmission electron microscopy photograph from stress control group; **c** representative transmission electron microscopy photograph from high +Gz stress group. Original magnification ×12,500

test. The statistical software package SPSS 16.0 for Windows (SPSS, Chicago, IL, USA) was used. Statistical differences of $P < 0.05$ were considered to be significant.

Results

Observation of ultrastructure, and quantitative analysis of myocardial mitochondria morphology

Electron microscopy photographs of mitochondria from myocardiocytes of each group are shown in Fig. 1. The ultrastructure of the cardiac muscle from rats of the blank control and stress control groups was identical. Mitochondria of normal size and with well-delineated cristae were distributed among the cardiac muscle. However, myocardial mitochondria from the high +Gz stress group had suffered substantial structural damage; they were substantially swollen, with broken or disrupted cristae or incomplete membranes, and some mitochondria had a condensed or polymorphic matrix. Moreover, deformation of some degenerated mitochondria was apparent as monstrous shapes containing myelin-like figures. This damage was confirmed by quantitative analysis of morphology; the high +Gz stress group was associated with higher surface density, volume density, average volume, and average surface area, and lower numeral density of the mitochondria compared with the blank control and stress control groups ($P < 0.01$) (Fig. 2).

Myocardial mitochondrial respiratory function

Results from measurement of the myocardial mitochondrial respiratory function of the rats are shown in Fig. 3, which includes ST3, ST4, and RCR of mitochondria from all groups. High +Gz exposure impaired the myocardial

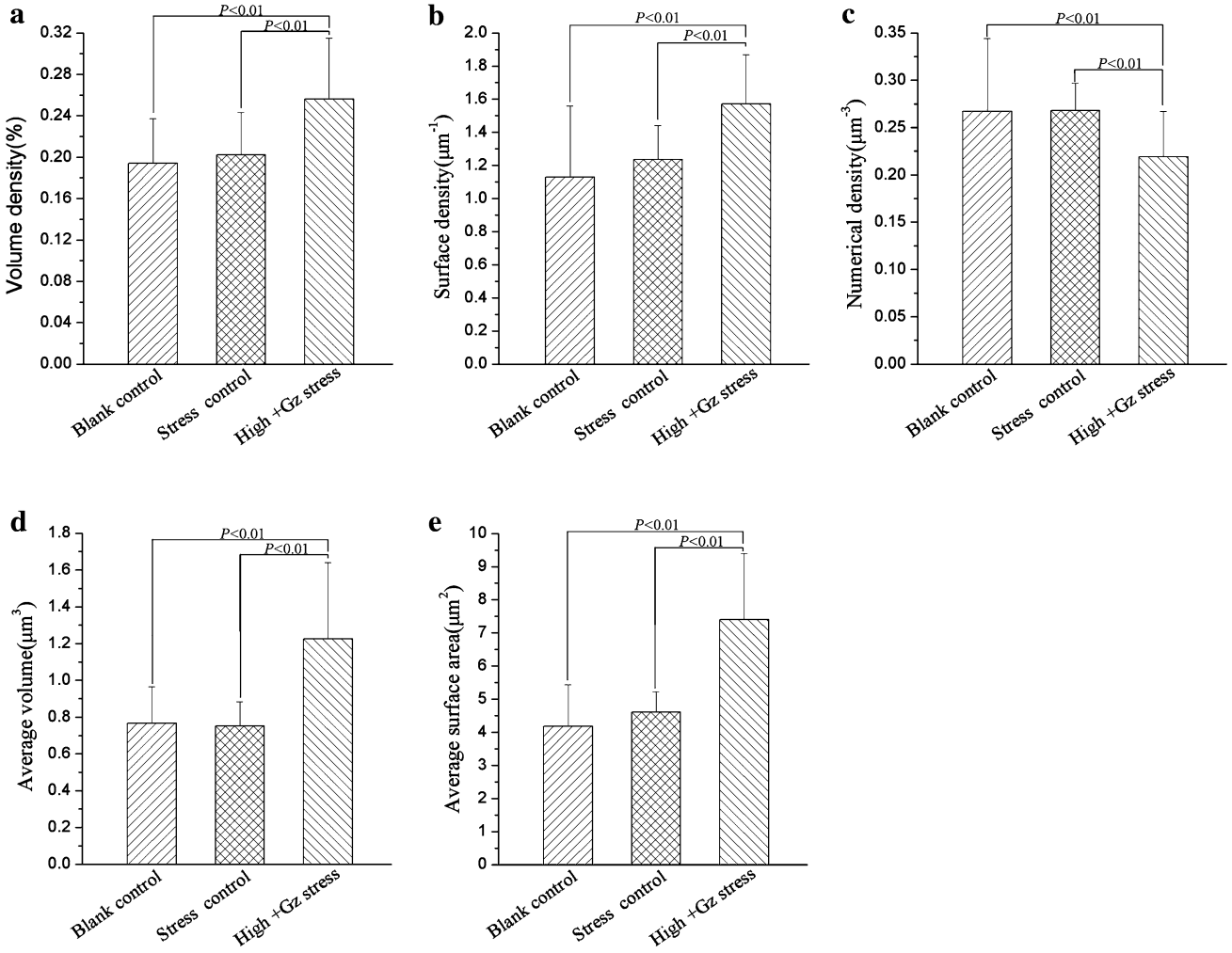

Fig. 2 Effects of high +Gz stress on quantitative indicators of the morphology of myocardial mitochondria from rats: **a** volume density, **b** surface density, **c** numerical density, **d** average volume, **e** average surface area. *Columns* and *vertical bars* represent mean ± SD from 50 different photographs

mitochondrial respiratory function of rats. In the high +Gz stress group, ST3 was significantly lower and ST4 was significantly higher than in the blank control group or the stress control group ($P < 0.01$ or $P < 0.05$). RCR calculated from ST3 and ST4 respiration rates was also substantially lower in high +Gz-stressed rats ($P < 0.01$).

Activity of myocardial mitochondrial antioxidant enzymes, and MDA content

Figure 4 illustrates the effect of high +Gz stress on the activity of antioxidant enzymes and on the level of MDA. In comparison with the blank and stress control group, high +Gz stress induced significant down-regulation of SOD, CAT, and GSH-Px activity ($P < 0.05$) but increased MDA production ($P < 0.01$). These results suggest that high +Gz stress can reduce the activity of antioxidant enzymes and increase lipid peroxidation.

Discussion

It has been known for decades that high sustained +Gz can damage the hearts of experimental animals [6, 7]. G-related cardiomyopathy occurs as two general forms of cardiac cell damage: myofibrillar degeneration and frank myocardial necrosis, and both forms of damage can be caused by a great number of humoral, ischemic, mechanical, and stressful stimuli [2, 21–23]. The main harmful factors related to +Gz injury have not been completely clarified. Laughlin [2] proposed it is strongly related to the effects on the heart of sympathetic nervous and hormonal catecholamines. However, most other studies regarded ischemia or hypoxia as the most important aspects of this heart lesion [24, 25]. Because upregulation of the concentration of blood catecholamines or ischemia and/or hypoxia augment ROS production and lead to oxidative stress, oxidative stress was of concern in this study. Interestingly, food and

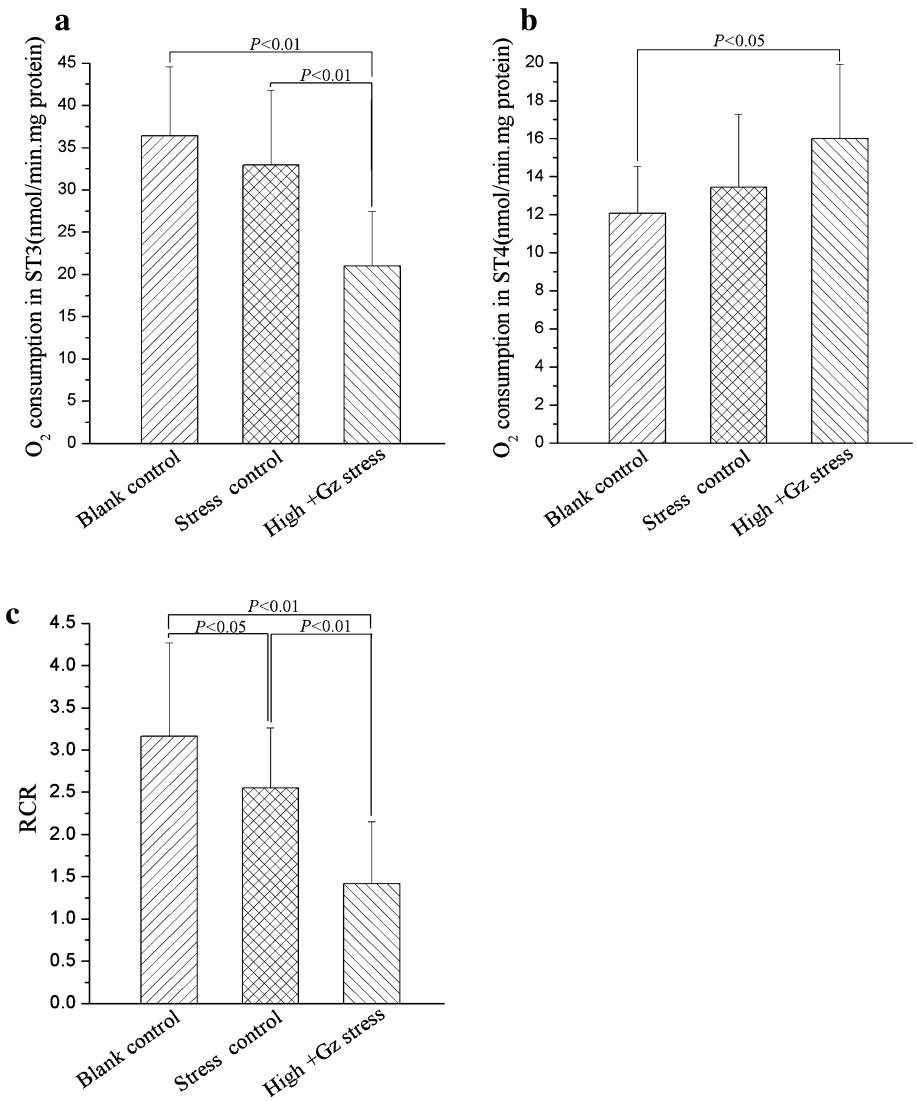

Fig. 3 Effects of high +Gz stress on respiratory function of myocardial mitochondria in rats. **a** State 3 respiration (*ST3*), **b** state 4 respiration (*ST4*), **c** respiratory control ratio (*RCR*). *Columns* and *vertical bars* represent mean ± SD from 10 rats

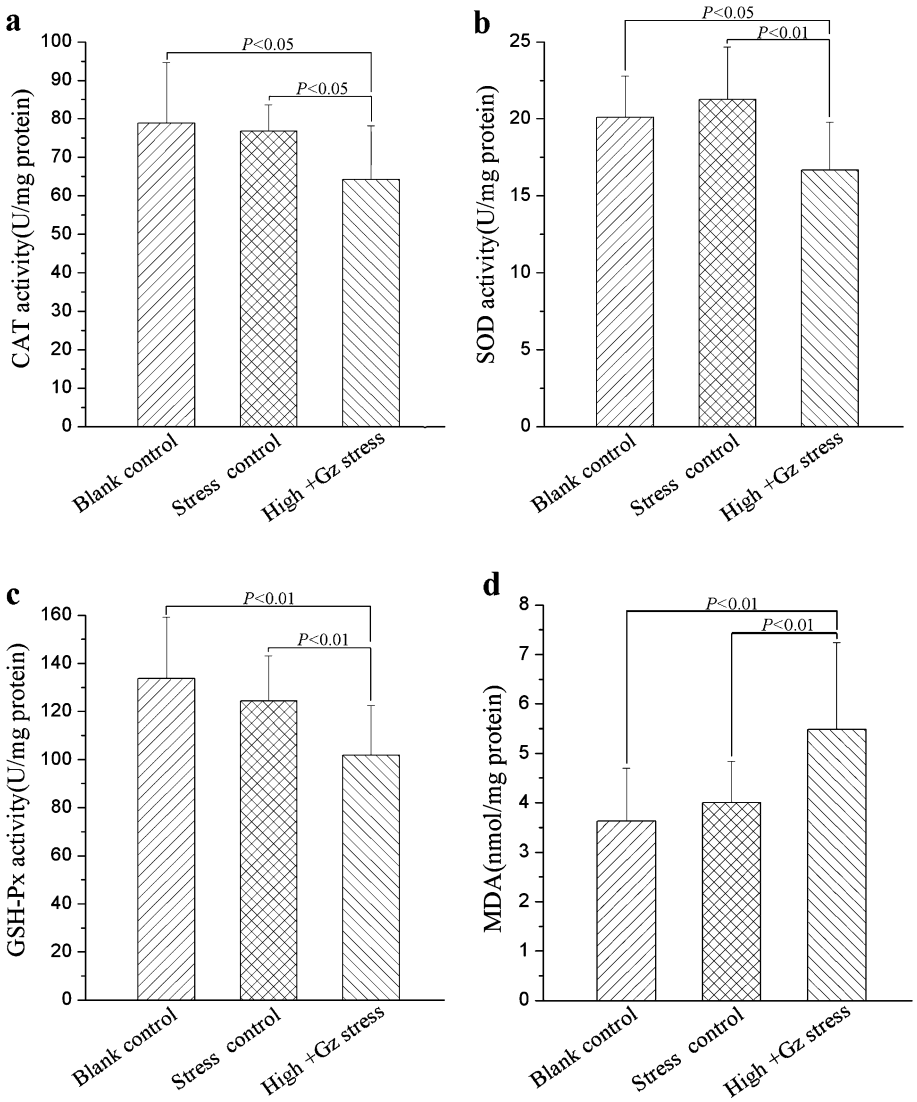

Fig. 4 Effects of high +Gz stress on the activity of antioxidant enzymes and on MDA production by myocardial mitochondria in rats: **a** catalase (*CAT*), **b** superoxide dismutase (*SOD*), **c** glutathione peroxidase (*GSH-Px*), **d** malondialdehyde (*MDA*). *Columns* and *vertical bars* represent mean ± SD from 10 rats

drugs known to reduce oxidative stress also reduce G-related injury of experimental animals [8].

Because mitochondria are the major source of intracellular ROS and can also be the primary organelle damaged by ROS, we focused on alteration of mitochondrial ultrastructure, respiratory function, and antioxidant capacity in the animals after high sustained +Gz exposed. The results showed the mitochondria of +Gz exposed animals swelled, degenerated, and decreased in number, and their cristae broke or disappeared, as reported elsewhere [12]. Swelling of the mitochondria of rats in the high +Gz stress group led to an increase in surface density, volume density, average volume, and average surface area, and to a decrease in numerical density (reduction of the number of mitochondria per unit visual field in electron microscopy). Changes of quantitative indicators of morphology were therefore consistent with electron microscopy observation.

Besides reported ischemia and/or hypoxia [24, 25], cardiac workload also increased among centrifuged rats [10]. Both may lead to depletion of ATP and ADP and impair the respiratory chain in high sustained +Gz exposed animals [26]. Thus, electrons are transferred directly to molecular oxygen to generate more oxygen radicals [27, 28]. Although mitochondrial respiration can be a major source of oxygen radicals, production of these oxygen radicals can also interrupt oxygen delivery to myocardial cells and aggravate depression of mitochondrial respiratory function [29]. Among the five states during the process of mitochondrial respiration, state 3 and state 4 are most important. State 3 is the fast oxidation period in the presence of ADP and respiratory substrate [30]. When ADP is exhausted, mitochondrial respiration enters state 4. Respiration in state 4 can be used to reflect mitochondrial permeability [31]. The ratio of state 3 to state 4 respiratory rates is the RCR. The

RCR is a measure of the coupling between substrate oxidation and phosphorylation and basically informs the researcher how intact or how coupled mitochondria are [32]. Alteration of the properties of myocardial mitochondria isolated from high sustained +Gz exposure animals was indicative of functional impairment of oxidative phosphorylation and less efficient utilization of oxygen by high +Gz stress-treated rats. ROS damages the process of oxidative phosphorylation of mitochondria and may reduce, even abolish ATP production. As a consequence, Na^+ concentration was increased in mitochondria, which caused mitochondria to absorb much water; this finally resulted in swelling. Mitochondrial respiratory dysfunction may therefore contribute to disruption of its ultrastructure.

In this study, the concentration of MDA, a marker of lipid peroxidation, was shown to be increased in mitochondria from rats from the high +Gz stress group; this is a consequence of increased generation of oxygen radicals. CAT, SOD, and GSH-Px are regarded as the first line of defense of the antioxidant enzyme system against ROS [33]. SOD catalyzes the reaction of superoxide radicals into oxygen and hydrogen peroxide (H_2O_2). GSH-Px is responsible for removal of a wide range of hydroperoxides, from complex organic hydroperoxides to H_2O_2. Thus, it may protect membrane lipids, proteins, and nucleic acids from oxidation. The main function of CAT is to degrade H_2O_2 into H_2O and O_2 [34]. In our study, the activity of the enzymes SOD, CAT and GSH-Px was reduced in rats from the high +Gz stress group. It is possible the reduced activity of these antioxidant enzymes was because of consumption by ROS, and other reasons. Irrespective of the reason, high +Gz stress-induced damage is a result not only of overproduction of ROS but also reduction of antioxidant enzyme activity. The changes of antioxidant enzymes and MDA in this study were similar to those for ischemia and/or hypoxia rats [35], which could also confirm ischemia or hypoxia as the main cause of high +Gz stress-induced heart lesion.

In conclusion, in this study we examined the integrity of mitochondria and measured a variety of indicators of mitochondrial function in rats suffering high +Gz stress. We showed that high +Gz stress could damage mitochondrial ultrastructure, compromise mitochondrial respiratory function, reduce the activity of antioxidant enzymes, and enhance lipid peroxidation. This research may aid discovery of more potentially harmful effects of high +Gz stress on the human heart, and, subsequently, help to prevent heart injury.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments conducted in this study comply with the current laws of China.

References

1. Jones DR (1991) A review of central nervous system effects of G-induced loss of consciousness on volunteer subjects. Aviat Space Environ Med 62:624–627
2. Laughlin MH (1982) An analysis of the risk of human cardiac damage during +Gz stress: a review. Aviat Space Environ Med 53:423–431
3. Öztürk C, İlbasmış MS, Akın A (2012) Cardiac responses to long duration and high magnitude +Gz exposure in pilots: an observational study. Anadolu Kardiyol Derg 12:668–674
4. Whinnery CC, Whinnery JE (1988) Acceleration-induced electrocardiographic interval changes. Aviat Space Environ Med 59:102–106
5. Martin DS, D'Aunno DS, Wood ML, South DA (1999) Repetitive high G exposure is associated with increased occurrence of cardiac valvular regurgitation. Aviat Space Environ Med 70:1197–1200
6. Burton RR, Mackenzie WF (1976) Cardiac pathology associated with high sustained +Gz: I. Subendocardial hemorrhage. Aviat Space Environ Med 47:711–717
7. Lu WH, Hsieh KS, Li MH, Ho CW, Wu YC, Ger LP, Wang JS, Chu H (2008) Heart status following high G exposure in rats and the effect of brief preconditioning. Aviat Space Environ Med 79:1086–1090
8. Zhan H, Dong HJ, Xin YM, Tang GX (1999) Effects of tea polyphenols on cardiac function and myocardial ultrastructure in rats after repeated +Gz stress. Space Med Med Eng (Beijing) 12:79–83
9. Zhan H, Zhang Z, Xin YM, Li T, Wei SH (2003) Changes of cardiac catecholamines in rats after repeated +Gz exposures and protective effects of low-G preconditioning and tea polyphenols. Space Med Med Eng (Beijing) 16:239–242
10. Tran CC, Aussedat J, Ray A, Lortet S, Rossi A, Quandieu P (1996) Bioenergetic effects of repeated +Gz acceleration on rat heart: a 31P-NMR study on isolated hearts. Aviat Space Environ Med 67:146–152
11. Zhang Z, Zhan H, Lu JY, Xin YM, Li T, Zhang QJ (2001) Effects of repeated high +Gz exposure on several enzyme activities in cardiomyocytes in rats and some protective measures. Space Med Med Eng (Beijing) 14:410–413
12. Zheng J, Liu CG, Ren L, Xiao XG, Xu SX, Wang P, Ji GY (2004) Myocardial ultrastructural changes in rats following different levels of acute +Gz exposure. Space Med Med Eng (Beijing) 17:162–165
13. McCord JM (1985) Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312:159–163
14. Ross CM (2005) Folate, mitochondria, ROS, and the aging brain. Am J Med 118:1174–1175
15. Pi J, Zhang Q, Fu J, Woods CG, Hou Y, Corkey BE, Collins S, Andersen ME (2010) ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function. Toxicol Appl Pharmacol 244:77–783
16. Ivanović-Matić S, Mihailović M, Dinić S, Martinović V, Bogojević D, Grigorov I, Poznanović G (2010) The absence of cardiomyopathy is accompanied by increased activities of CAT,

MnSOD and GST in long-term diabetes in rats. J Physiol Sci 60:259–266
17. Gottlieb RA (2000) Role of mitochondria in apoptosis. Crit Rev Eukaryot Gene Expr 10:231–239
18. Feng S, Wang Q, Wang H, Peng Y, Wang L, Lu Y, Shi T, Xiong L (2010) Electroacupuncture pretreatment ameliorates hypergravity-induced impairment of learning and memory and apoptosis of hippocampal neurons in rats. Neurosci Lett 478:150–155
19. Holmuhamedov EL, Ozcan C, Jahangir A, Terzic A (2001) Restoration of Ca2+ -inhibited oxidative phosphorylation in cardiac mitochondria by mitochondrial Ca2+ unloading. Mol Cell Biochem 220:135–140
20. Góth L (1991) A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 196:143–151
21. Matsubara BB, Matsubara LS, Franco M, Padovani JC, Janicki JS (1999) The effect of non-antihypertensive doses of angiotensin converting enzyme inhibitor on myocardial necrosis and hypertrophy in young rats with renovascular hypertension. Int J Exp Pathol 80:97–104
22. Riezzo I, De Carlo D, Neri M, Nieddu A, Turillazzi E, Fineschi V (2011) Heart disease induced by AAS abuse, using experimental mice/rats models and the role of exercise-induced cardiotoxicity. Mini Rev Med Chem 11:409–424
23. Manni L, Di Fausto V, Fiore M, Aloe L (2008) Repeated restraint and nerve growth factor administration in male and female mice: effect on sympathetic and cardiovascular mediators of the stress response. Curr Neurovasc Res 5:1–12
24. Laughlin MH (1986) The effects of +Gz on the coronary circulation: a review. Aviat Space Environ Med 57:5–16
25. Shubrooks SJ Jr, Burns JW, Erickson HH (1975) Coronary hemodynamics during positive (+G-z) acceleration. Aviat Space Environ Med 46:413–418
26. Hermann R, Marina Prendes MG, Torresin ME, Vélez D, Savino EA, Varela A (2012) Effects of the AMP-activated protein kinase inhibitor compound C on the postconditioned rat heart. J Physiol Sci 62:333–341
27. Cadenas E, Boveris A, Ragan CI, Stoppani AO (1977) Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. Arch Biochem Biophys 180:248–257
28. Turrens JF, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421–427
29. Vanden Hoek TL, Shao Z, Li C, Schumacker PT, Becker LB (1977) Mitochondrial electron transport can become a significant source of oxidative injury in cardiomyocytes. J Mol Cell Cardiol 29:2441–2450
30. Gao C, Xu L, Chai W, Sun X, Zhang H, Zhang G (2005) Amelioration of intestinal ischemia-reperfusion injury with intraluminal hyperoxygenated solution: studies on structural and functional changes of enterocyte mitochondria. J Surg Res 129:298–305
31. Xu DZ, Lu Q, Kubicka R, Deitch EA (1999) The effect of hypoxia/reoxygenation on the cellular function of intestinal epithelial cells. J Trauma 46:280–285
32. Sarandol A, Sarandol E, Eker SS, Erdinc S, Vatansever E, Kirli S (2007) Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. Hum Psychopharmacol 22:67–73
33. Lenaz G (1998) Role of mitochondria in oxidative stress and ageing. Biochim Biophys Acta 1366:53–67
34. Gomes EC, Silva AN, de Oliveira MR (2012) Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. Oxid Med Cell Longev 1–12
35. Bayrak O, Bavbek N, Karatas OF, Bayrak R, Catal F, Cimentepe E, Akbas A, Yildirim E, Unal D, Akcay A (2008) Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys. Nephrol Dial Transpl 23:2206–2212