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Effect of N-2-mercaptopropionyl glycine (MPG) on exercise-induced cardiac adaptations.

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Abstract

The purpose of this study was to test the hypothesis that exercise-induced cardiac adaptations would be attenuated by the free radical scavenger N-2-mercaptopropionyl glycine (MPG). Male Sprague-Dawley rats were divided into four groups (9-13/group) for 3-4 weeks: sedentary (S), S+MPG (100 mg/kg IP daily), exercised on a treadmill (E) (60 min/day, 5 days/wk, at a speed of 20 m/min up a 6° grade in a 6° C room), or E+MPG given 10 min prior to exercise. Additional rats (N=55) were used to determine acute exercise effects on myocardial redox state (nonprotein nonglutathione sulfhydryls (NPNGSH)) and PI3K/Akt signaling pathway activation. Compared to S, NPNGSH levels were 48% lower in E (P<0.05) and unchanged in E+MPG (P>0.05). MPG also attenuated exercise-induced activation of the signaling proteins Akt and S6. Hearts from the 4-wk groups were weighed and cardiac function evaluated using an isolated perfused working heart preparation. Similar increases (P<0.05) in both exercised groups were observed for heart wt and heart wt to body wt ratio. Cardiac function improved in E vs S as indicated by greater (P<0.05) external work performed (Cardiac output x Systolic pressure) and efficiency of external work (work/VO₂). MPG prevented these exerciseinduced functional improvements. Skeletal muscle mitochondria content increased to similar levels in E and E+MPG. This study provides evidence that free radicals do not play an essential role in the development of exercise-induced cardiac hypertrophy; however, they appear to be involved in functional cardiac adaptations, which may be mediated through the PI3K/Akt pathway.

Keywords: Exercise Training, Oxidative Stress, Hypertrophy, Hemodynamics, Heart, Rat, Akt

Introduction

Pathological and physiological cardiac hypertrophy are two distinct forms of hypertrophy resulting from different types of stresses. Pathological hypertrophy that accompanies various disease states is characterized by interstitial fibrosis, induction of fetal genes, LV dilatation, and contractile dysfunction; whereas exercise-induced cardiac hypertrophy (EICH) is a form of physiological hypertrophy resulting in enlargement of the heart with maintained or enhanced function, lack of interstitial fibrosis, and no induction of fetal cardiac genes (12, 36 for reviews). Interestingly, the phosphatidylinositol-3-kinase (PI3K)-Akt pathway, that regulates multiple aspects of cellular functions, participates in the development of both of these distinctly different forms of cardiac hypertrophy (36). In response to stimulation of membrane-bound PI3K, Akt is recruited to the plasma membrane where it is sequentially phosphorylated at its serine/threonine regulatory sites, thr308 and ser473. Activated Akt then translocates to various sites within the cell to exert its biological effects by phosphorylating a variety of downstream substrates. The form of hypertrophy resulting from Akt activation appears to be related to the level and duration of activation; short-term Akt activation has been shown to induce physiological hypertrophy while prolonged Akt activation leads to pathological hypertrophy (36). The molecular signals that initiate either form of cardiac hypertrophy are not completely understood.

Reactive oxygen and nitrogen species (ROS and RNS) are normal byproducts of cellular function and metabolism. They are usually eliminated by the antioxidant system before causing significant oxidation of intracellular molecules, thus maintaining redox homeostasis. Evidence has been accumulating that pathological hypertrophy is the result of increased production of ROS resulting in a chronic imbalance in the redox status (8,

32, 43). Angiotensin II (ANG II), which plays a vital role in pathological cardiac hypertrophy and remodeling, is known to activate membrane NAD(P)H oxidases to produce ROS and thereby stimulate PI3K and activate Akt (25). Also, direct exposure of cardiac myocytes to H_2O_2 has been shown to rapidly activate Akt in a concentration-dependent fashion (29).

There is also increasing evidence that ROS generated during exercise activate redox-sensitive transcription pathways that lead to a variety of performance-enhancing adaptations (1, 18, 28). Exercise causes brief perturbations and cellular stress that quickly stabilize post-exercise whereas most diseases, including hypertension, are associated with continual ROS production. Differences in the timing or magnitude of the disruption in redox balance appear to result in differences in signaling pathway activation (18, 28).

Evidence that intermittent ROS elevation during exercise can lead to positive adaptations to the heart comes from studies using N-2-mercaptopropionyl glycine (MPG), a potent cell permeable scavenger of hydroxyl radicals and peroxynitrite (3). When this antioxidant is administered a few minutes prior to an exercise stimulus it will scavenge free radicals during the stimulus, but will gradually lose its ineffective afterwards. Papers by Yamishita et al. (44) and Akita et al. (1) reported that administering this drug during exercise prevented the subsequent adaptations in the heart that protects it against ischemia-reperfusion injury. Whether ROS generation and alteration of redox balance during exercise also plays a role in EICH or other adaptations that improve myocardial performance has not been investigated. Thus, the purpose of the current study is to test the hypothesis that free radicals generated during endurance

exercise are involved in exercise induced cardiac adaptations. Disruption of redox homeostasis during treadmill exercise was attenuated by administration of MPG as used by several investigators to test whether free radicals trigger a variety of pathological or physiological adaptations (1, 2, 8, 39, 43, 44), including cardiac hypertrophy induced by aortic banding (8) and by downregulation of the thioredoxin antioxidant system (43).

Methods

Animals and training. Male, 10-wk-old, Sprague-Dawley rats were obtained from the breeding colony maintained by the University of Texas Animal Resource Center. This investigation was approved by the University's Institutional Animal Care and Use Committee and conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Animals were kept on a 12:12-h light-dark cycle and fed ad libitum. Rats were randomly assigned to one of four groups (N = 9-13/group) for four weeks: sedentary control (S), sedentary+MPG (S+MPG), exercise (E), or exercise+MPG (E+MPG). MPG was administered during the last three weeks. It was dissolved in PBS and injected IP (100mg/kg) 10 min prior to exercise as described by Yamashita *et al.* (44). An additional 55 animals were used to evaluate acute exercise effects to be described later.

The exercise protocol using a motor driven treadmill has been described previously (14, 26). Briefly, rats were habituated to the treadmill by gradually increasing running time and speed each day so that at the end of one week they were running for 30 minutes at 20 m/min up a 6° grade, which corresponds to an intensity of ~70% maximum oxygen consumption in the untrained state (5). Following habituation animals began a three-week running program in a refrigerated room (6°C) with their fur dampened to help

dissipate heat produced by exercise. The speed was maintained at 20 m/min and the running time gradually increased to one hour per day by the end of the week. Training continued at this intensity and duration for an additional two weeks. All exercised animals were housed at 23°C when not running, and immediately toweled dry following exercise. Sedentary animals (with dry fur) were placed in the cold environment during the exercise sessions. We previously reported that for these rats, running in normal room temperature at this intensity raised core temperature at least 2.4°C above basal within 20 min, whereas the same exercise in the colder conditions described above raised core temperature only about 0.6°C above basal (14). We also found this exercise program to result in a 13% increase in heart mass and 20% increase in heart wt/body wt ratio after just three weeks and a 23% increase in heart mass and 17% increase in heart wt/body wt ratio after nine weeks in rats identical to those used herein, whereas the same exercise program carried out at normal room temperature did not result in cardiac hypertrophy at any time point (14). Furthermore, these hypertrophied hearts displayed enhanced function and gene expression consistent with physiological hypertrophy.

Isolated heart perfusions. Myocardial function was evaluated 24 hours after the last treatment using an isolated, working heart preparation. Rats were anesthetized with an intraperitoneal injection (IP) of 40 mg/kg pentobarbital sodium and hearts were excised, weighed, and mounted on the perfusion apparatus as previously described (37, 38). The perfusate was a modified Krebs-Henseleit buffer containing (in mM): 10 glucose, 3.0 CaCl₂, 118.5 NaCl, 4.7 KCl, 1.2 MgSO₄, 24.7 NaHCO₃, 0.5 EDTA, and 12 IU/l insulin. It was gassed with 95% O₂-5% CO₂ and maintained at 37°C. Myocardial function was evaluated while working against a low afterload set by an 80 cm high aortic

column (ID 3.18 mm) and against a high afterload created by inserting a 1.5 inch 23gauge needle into the aortic column. Atrial filling pressure was maintained at 12.5 mmHg throughout. Hearts were electrically paced at 300 beats/min with an electrode placed at or near the SA node. Coronary flow (CF) and aortic flow (AF) were determined by timed collection of the effluent dripping off the heart and aortic column overflow, respectively, and cardiac output (CO) was calculated as the sum of CF and AF. Cardiac external work was defined as the product of CO and peak aortic systolic pressure. Aortic pressure and heart rate were monitored using a Gould DTX pressure transducer (Gould cardiovascular products, Oxnard, CA) interfaced to a Dell Dimension Desktop computer (Dell, Round Rock, TX) equipped with Bio Bench data acquisition software (National Instruments, Austin, TX). Myocardial oxygen consumption (VO₂) was calculated from the arteriovenous difference in oxygen concentration multiplied by the coronary flow. Arterial oxygen concentration was maintained at 956 µM and venous oxygen concentration was continuously monitored by immediately diverting 5 ml/min of coronary effluent through an in-line Clark-type oxygen electrode as previously described (37, 38). Myocardial work efficiency (EFF) was calculated by dividing external work by VO₂ (37). After approximately 15 minutes of equilibration on the perfusion apparatus, hearts were evaluated for 10 minutes at the low workload, 10 minutes at the high workload, and returned to the low workload to verify the stability of the perfusion system. The beating hearts were then freeze-clamped and stored at -80°C until analysis.

Antioxidant enzymes and cytochrome oxidase. For all enzyme assays, tissues were homogenized in 20 volumes of 50 mM KH₂PO₄, 0.1 mM EDTA, 0.1% (v/v) Triton X-100, pH 7.4, centrifuged at 1,500xg for 10 minutes, and the supernatant analyzed.

Catalase activity in the left ventricle was determined polarographically using a Clark-type oxygen electrode according to del Rio et al. (9). Left ventricular manganese superoxide dismutase (MnSOD) was determined by spectophotometric analysis at 550 nm according to McCord & Fridovitch (22). Cytochrome oxidase activity in the plantaris muscle was determined polarographically using a Clark-type oxygen electrode as described previously (30). This enzyme is a marker of exercise-induced changes in mitochondria content. Plantaris was used for this purpose because we reported previously that the exercise program used herein does not increase myocardial cytochrome oxidase activity (14). Catalase and cytochrome oxidase were determined on fresh supernatants shortly after homogenization and the remaining supernatant stored at -80°C for later determination of SOD. Preliminary experiments indicated that catalase and cytochrome oxidase activites were decreased after freeze-thawing the supernatant, but SOD activity was unaffected. All tissue preparation steps were carried out at ice-cold temperatures and enzyme assays run at 25°C.

Acute effects of MPG administration. Additional animals from S, E, and E+MPG were used to monitor myocardial redox status and activation of Akt and S6, two proteins within the PI3K/Akt signaling pathway. Exercised animals were habituated to the treadmill for one week and then subjected to a single one-hr exercise bout in the refrigerated room as described above. Exercised animals that were administered MPG, received the antioxidant 10 min prior to the one-hr bout.

For an estimation of redox status, animals were anesthetized with 40 mg/kg pentobarbital sodium IP immediately after the one-hr exercise bout and hearts were excised. Nonprotein nonglutathione sulfhydryls (NPNGSHs) were measured as an

buffer protein sulfhydryl groups and is composed of cysteine, derivatives of cysteine, and other low-molecular-weight molecules containing sulfhydryl groups (20). It is derived by subtracting GSH values from total acid soluble thiols (TST). Ventricular GSH was measured enzymatically using a reversal of the glutathione transferase method as modified by Bowles and Starnes (4) and TST were measured according to Lesnefsky et al. (20). In our previous study we found that less sensitive measures were not significantly changed in the rat heart after an exhaustive exercise bout lasting an average of 84 minutes (35). Similarly, Lesnefsky et al. (20) reported that a brief period of myocardial ischemia lowered NPNGSHs, but that longer periods were required before changes in other redox indicators were apparent.

Exercise-induced activation of myocardial Akt and S6 was measured at selected times post exercise using Western blotting. A portion of the left ventricle (~100 mg) was homogenized in nine volumes of EIL buffer containing (in mM) 20 Hepes, 2 EGTA, 40 sodium flouride, 100 KCl, 0.2 EDTA, 50 β-glycerophosphate, 1 DL-dithiothreitol, 0.1 benzamidine hydrochloride hydrate, 0.5 sodium orthovanadate and adjusted to pH 7.4. An aliquot of the homogenate was further diluted 1:1 with Laemmli (19) sample buffer and 15 μl of the sample was subjected to SDS-PAGE. A standard consisting of an aliquot from a single homogenate was loaded onto every gel. This method assures that the same amount of the protein being probed is loaded as a standard on each gel. Protein concentration of homogenates was determined by the Bradford method. Following electrophoresis, samples were transferred to polyvinylidene difluoride membranes (Bio-Rad) and immunoblotted with one of the following antibodies: anti-Akt rabbit

polycolonal IgG (no. 9272, Cell Signaling), anti-phospho-Akt1 rabbit monoclonal IgG (no. 05-736, Upstate), or anti-phospho-S6 ribosomal protein rabbit polyclonal (no. 2211, Cell Signalling). Membranes were then blotted with anti-rabbit IgG, HRP (no. 7074 Cell Signaling) and detected with SuperSignal West Pico chemiluminescent substrate on Kodak Biomax ML imaging film. The densities of resulting bands were quantified using NIH image software then corrected for the amount of protein loaded in each lane.

Statistics. All results are expressed as the mean \pm S.E. Statistical analysis to determine interactions between chronic exercise and MPG was performed using a 2x2 ANOVA. To determine acute effects of MPG, a 1x3 ANOVA was used. When appropriate, Tukey's HSD Test was used for post-hoc analysis. Differences were considered significant when P values were less than 0.05.

Results

Acute MPG effects As displayed in Figure 1, a change in the redox state occurred during the one-hr exercise bout as indicated by the 48% decrease in NPNGSH. However, the redox status in exercised animals treated with MPG was similar to sedentary animals as indicated by similar NPNGSH levels in E+MPG and S. Exercise-induced activation of Akt and S6 was attenuated by MPG administration for at least 30 min following the exercise bout (Fig. 2 and 3). Total Akt level was not changed up to 6 hrs following acute exercise (data not shown).

Body and heart weights. Body weights, heart weights and heart weight to body weight ratios are summarized in Table 1. There was no interaction between the exercise and MPG treatment for any of these variables. Administration of MPG caused a significant main effect for both heart weight and body weight resulting in a decrease of

approximately 10% in both variables with no change in the heart weight to body weight ratio. Exercise treatment did not change body weight; however, it resulted in an 8.5% increase (P<0.05) in heart weight in both exercised groups. As a result of this there was also a significant increase in the heart weight to body weight in the exercise only condition.

Enzyme activities. Administration of MPG did not alter cytochrome oxidase activity in plantaris of the sedentary animals nor did it attenuate the exercise-induced increase (Table 1). Furthermore there was no interaction between exercise and MPG administration. Left ventricular activities of two antioxidant enzymes, catalase and MnSOD, are displayed in Table 2. The exercise program did not affect either enzyme. However, MPG administration was associated with increased catalase activity with no interaction between the treatments.

Cardiac function. Cardiac external work during an initial low afterload, a high afterload, and then another low afterload period is displayed in Figure 4. As indicated in the figure cardiac function was stable throughout. Statistical analysis by 2x2 ANOVA revealed significant exercise effects at all times except during the initial low workload measurement. Further analysis revealed that the performance increases only occurred in the untreated exercise group (E vs S). Cardiac function for the MPG-treated exercised animals was no different from that of the sedentary animals. Improved performance in E was still apparent when the values were normalized for heart weight; for example, the normalized external work performed by E at the first high afterload measure was 24% higher (P<0.05) than S (9,182 ± 654 and 7,240 ± 358 mmHg x ml/min/g, respectively).

Statistical analysis by 2x2 ANOVA also revealed a significant exercise effect for efficiency of external work (Fig. 5). This parameter is not affected by heart weight because both numerator (external work) and denominator (energy required to accomplish external work, i.e., VO₂) are affected the same by heart size. E+MPG demonstrated improved efficiency during part of the initial low work period, but this was lost when put under a high work challenge. Furthermore, E+MPG did not demonstrate improved efficiency compared to its control (S+MPG) when returned to the low workload at the end of the overall perfusion period. On the other hand, E was higher than S throughout the high workload period and the second low workload period. It should be mentioned that although the statistical analysis revealed that E+MPG was not different from its control group during the high workload, the mean values for E and E+MPG were similar. The similarity between E and E+MPG and the finding that E+MPG was different from its control during part of the initial low work period makes the results for efficiency less clear cut than those for cardiac external work displayed in Figure 4.

Discussion

The hypothesis tested in the present study was that free radicals generated during exercise play a prominent role in exercise-induced cardiac adaptations. This hypothesis was based on observations by others that antioxidant administration attenuated cardiac hypertrophy induced by other means (8, 43) and that quenching free radicals during short-term exercise prevented exercise-induced cardioprotection against ischemia-reperfusion injury (1, 44). The results of the present study indicate that our hypothesis was only partially correct. In disagreement with our hypothesis, we found that administration of the antioxidant MPG during a chronic endurance exercise program did

not attenuate exercise-induced cardiac hypertrophy when expressed in absolute or relative terms (Table 1). However, we also found that administration of MPG prevented the exercise-induced improvement in functional performance (Fig. 4 and 5). Reasons why free radical generation during exercise is more crucial to improved performance than to increased heart mass will be discussed below.

Like many previous studies investigating whether free radicals generated during an imposed stress are important in the development of a subsequent adaptation, we administered the antioxidant MPG prior to each exercise bout. The dose and mode of administration we used is the same as that used by others that successfully blocked the cardioprotective effects of exercise (1, 44) and heat stress (2). As mentioned previously, the disruption in redox balance in the healthy heart is modest during exercise. Even when it is carried out to exhaustion there is no change in biomarkers for lipid peroxidation, protein oxidation, or oxidative DNA damage (Liu et al, 2000). Accordingly, we carried out quality control measurements verifying that the redox balance was altered by our exercise bout and that the drug as administered prevented a redox imbalance beyond that in the normal sedentary rat (Fig. 1). These results are consistent with those of Akita et al. (1) who exercised mice for one hr with or without MPG pretreatment. We also found that MPG was effective in attenuating exercise-induced activation of the PI3K/Akt pathway proteins, Akt and S6 (Fig. 2 and 3), which is consistent with the results of Joo et al. (15) who reported that MPG administration prevented Akt activation following renal ischemia-reperfusion.

Downstream of phospho-Akt is mTOR which ultimately activates S6 and eIF-4e through separate pathways. Once activated S6 and eIF-4e increase translation in the cell.

We only followed E+MPG for 30 min post exercise because that is the time point where we observed peak activation in the untreated exercise groups. Our observations that S6 activation is relatively short lived in the untreated exercised animals and may not occur in the MPG-treated animals is consistent with the finding by McMullen et al. (24) that S6 activation is not needed for physiological cardiac hypertrophy. We did not measure the activation of eIF-4e which has been shown to be activated by aerobic exercise (16). As previously mentioned Akt activation was attenuated, but not blocked. At 30 min post exercise Akt activation in E+MPG was two-fold greater than S and would be expected to remain upregulated for at least a few hrs based on the activation pattern of E. The Akt activation observed in E+MPG was likely due to increased insulin-like growth factor (IGF-1), which is considered to play a major role in physiological hypertrophy by activating PI3K/Akt via the IGF-1 receptor (36). Cardiac IGF-I levels are increased with short- and long-term swimming training in rats (33) and increased cardiac IGF-I production was associated with physiological cardiac hypertrophy in athletes (27). Although the extent of Akt activation is greater when both IGF-1 and ROS are present, the activation without ROS is apparently still sufficient to initiate cardiac hypertrophy.

Exercise-induced hypertrophy in the untreated group was accompanied by improved intrinsic performance. The improvement in pumping ability of the exercise-trained hearts was most noticeable at the high workload where cardiac output and external work (COxSP) were 19% and 35%, respectively, above the sedentary values (Fig. 4). The improvement appeared to be the result of a larger and more hyperdynamic heart as external work performed when normalized for heart weight was still 24% higher in E than S. The exercise-trained hearts without MPG also demonstrated improved

efficiency of external work performed during the high workload and second low workload periods compared to their sedentary counterparts (Fig 5). Overall, the results for cardiac external work and efficiency provide strong evidence of exercise-induced performance adaptations within the myocyte of E. In this regard, recent studies on exercise-induced hypertrophied rat hearts have reported several intrinsic changes that could improve performance and efficiency including greater SERCA content, increased calcium sensitivity, and changes in the force-length relationship (10, 42).

Exercised animals receiving MPG did not demonstrate improvement in the ability to perform increased cardiac work (Fig. 4) and improvement in efficiency was very minor, if it occurred at all (see results). These results indicate that attenuation of exercise-induced redox signaling will attenuate intrinsic adaptations that lead to a more hyperdynamic heart. Kemi et al. (16) reported that enhancement of cardiac performance is another end product of upregulating the PI3K/Akt pathway. They found that the phosphorylation of Akt as well as S6 and elF-4e phosphorylation increased in animals undergoing physiological cardiac hypertrophy due to exercise. Additionally, they found that these proteins were dephosphorylated when pathological cardiac hypertrophy was induced by transverse aortic constriction (TAC). Others (6) have also demonstrated decreased phosphorylation of Akt following TAC while animals that over expressed Akt while undergoing TAC retained more function than animals in the TAC only condition. One potential reason for Akt's apparent ability to increase or maintain function might be that it increases the SERCA-2 content of the heart (7, 17), a known adaption to endurance exercise (42). Thus, prevention or attenuation of exercise-induced S6 phosphorylation by

MPG may at least partially explain why the exercise protocol used herein did not result in enhanced performance in the MPG treated animals.

The exercise protocol did not cause any changes in myocardial MnSOD or catalase activities; however, MPG treatment elevated catalase activity in both sedentary and exercised animals (Table 2). The increased activity is likely due to post-translational modification associated with the MPG-induced improved redox balance. Like most proteins, catalase activity is affected by the status of its thiol groups. For example, modification of Cys438 to serine has been shown to cause a 30% reduction in catalase specific activity (34). In an unpublished experiment from our lab we noticed a 60% increase in catalase activity after just two days of MPG administration in sedentary rats. It is unlikely that such a large increase in this short time period would have been due solely to increased protein synthesis. Consistent with our finding, El-Missiry et al. (11) reported that 7 days of daily MPG administration resulted in an increase in SOD activity and they also speculated that it was due to protecting essential –SH groups. Importantly, the increase catalase activity is unlikely to have had an impact on the results since there were no differences in heart weight or function between the two sedentary groups and the increase in catalase activity was modest (15%) in the MPG exercised animals.

As in many exercise studies, we measured a marker of mitochondria content in an active skeletal muscle to verify the training status of our animals. As in our previous studies using this exercise protocol (14, 26), the exercise program resulted in a large increase in plantaris cytochrome oxidase activity (Table 1). Although it was not the purpose of this study to evaluate the effects of MPG on skeletal muscle mitochondrial biogenesis, it is interesting to note that MPG treatment did not attenuate the exercise-

induced increase. This appears to conflict with the results of Gomez-Cabrera et al. (13) who concluded that antioxidant supplementation with vitamin C during exercise training decreases mitochondrial content. A 2.5-fold increase by the non-supplemented exercise group was found to be statistically different from sedentary controls, but a 2-fold increase by the vitamin C group was not. The large variance in the supplemented group could have been responsible for the conclusions. Also, Wadley and McConell (40) recently reported that the same high-dose vitamin C supplementation used by Gomez-Cabrera et al. does not prevent acute exercise-induced increases in markers of skeletal muscle mitochondrial biogenesis in rats.

The exercise protocol did not cause any changes in body weight; however, we observed that body weights were 10.4% lower in the sedentary animals and 7.6% lower in the exercised animals (Table 1). We do not have an explanation for the small weight differences. There was no evidence of any type of infection or sickness from the IP injections in the MPG groups. If there were, we would have expected a greater weight difference between the exercised groups than between the sedentary groups because the exercised groups are placed under daily physical stress. This was not the case. Other indications of the health of these animals include the following. In the MPG groups there was a low amount of variance in body weight, plantaris cytochrome oxidase activity, and cardiac function data, which would not have been the case if some of the animals were sick. The MPG treated animals did not display any difficulties in carrying out the exercise protocol and had the same increases in cardiac hypertrophy and skeletal muscle mitochondrial content as the non-MPG treated exercised animals. In sedentary animals, cardiac function and heart wt/body wt ratio was not different from the non-MPG treated

controls. In summary, the lower weights of the MPG animals did not influence the results of this study.

Perspectives and Significance

The results of the present study indicate that changes in the redox balance are involved in certain exercise-induced adaptations. Specifically, we found that generation of ROS during exercise can enhance the PI3K/Akt pathway to improve intrinsic cardiac performance. Supplementation with the antioxidant MPG attenuated the amount of PI3K/Akt activation by exercise and resulted in cardiac hypertrophy that was not accompanied by additional improvements in performance. Thus it appears that the activation required for improved performance may be more robust than that needed for hypertrophy, which could explain why some exercise studies have reported hypertrophy without additional improvements in intrinsic performance (23). A broad implication of the study is that dietary antioxidant supplements may have a negative influence on selected exercise-induced adaptations.

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	No MPG	MPG
Body Weight (g)		
S	366 ± 7	328 ± 9 †
E	355 ± 10	326 ± 11 †
Heart Weight (mg)		
S	1218 ± 22	1089 ± 26†
Е	$1322 \pm 43*$	1181 ± 36*†
RATIO (mg/g)		
S	3.33 ± 0.04	3.33 ± 0.06
E	$3.73 \pm 0.06*$	3.60 ± 0.05 *
Plantaris Cytochrome		
Oxidase Activity (µmol		
$O_2/min/g)$		
S	18.31 ± 1.58	14.43 ± 0.56
Е	$27.19 \pm 1.29*$	$27.24 \pm 1.43*$

Table 1 – Animal Characteristics. Values are mean \pm SE for N = 9-13. S, sedentary; E, exercise; MPG, N-2-mercaptopropionyl glycine. *P<0.05 vs. sedentary with same MPG treatment. †P<0.05 vs. No MPG with same exercise treatment.

	No MPG	MPG
Catalase		
S	1101 ± 23	1461 ± 122†
Е	1116 ± 51	1270 ± 77†
MnSOD		
S	2943 ± 57	2956 ± 82
Е	2917 ± 66	3062 ± 54

Table 2. Ventricular antioxidant enzyme activity. Values are mean Units/g wet weight±SE for N = 8-11 per group. S, sedentary; E, exercise; MPG, N-2-mercaptopropionyl glycine; MnSOD, manganese superoxide dismutase; Catalase Unit = $1 \mu mole H_2O_2/minute$; MnSOD Unit = 50% inhibition of cytochrome c reduction. $^{\dagger}P$ <0.05 vs no MPG.

Figure legends

Figure 1. Effect of MPG administration on oxidative stress following exercise.

Values are means ±SE for number of animals indicated. S, sedentary; E, exercise; MPG, N-2-mercaptopropionyl glycine. *P<0.05 vs all groups.

Figure 2. Levels of phosphorylated Akt following exercise. Values are means \pm SE for number of animals indicated. S, sedentary; min, post exercise times for animals without MPG treatment; min MPG, post exercise times for animals with MPG treatment. * P<0.05 vs S. \dagger P<0.05 vs time matched exercise with MPG. Total Akt levels were not changed from sedentary following acute exercise.

Figure 3. Levels of phosphorylated ribosomal S6 following exercise. Values are means \pm SE for number of animals indicated. S, sedentary; min, post exercise times for animals without MPG treatment; min MPG, post exercise times for animals with MPG treatment. *P<0.05 vs S. \dagger P<0.05 vs time matched exercise with MPG.

Figure 4. Cardiac external work over time in response to changing workload. Values are means ± SE for N= 8 per group. S, sedentary; E, exercise; MPG, N-2-mercaptopropionyl glycine; CO, cardiac output; SP systolic pressure. *P<0.05 vs S.

Figure 5. Efficiency of work over time in response to changing workload. Values are means ± SE for N= 8 per group. S, sedentary; E, exercise; MPG, N-2-mercaptopropionyl glycine; CO, cardiac output; SP systolic pressure; VO₂, oxygen consumption. *P<0.05 vs S of same MPG treatment group.

Figure 1.

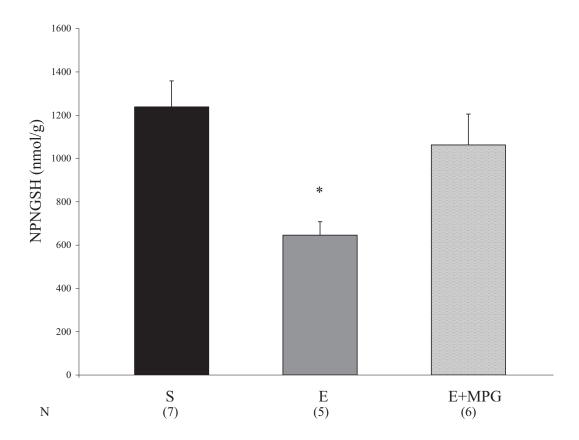


Figure 2.

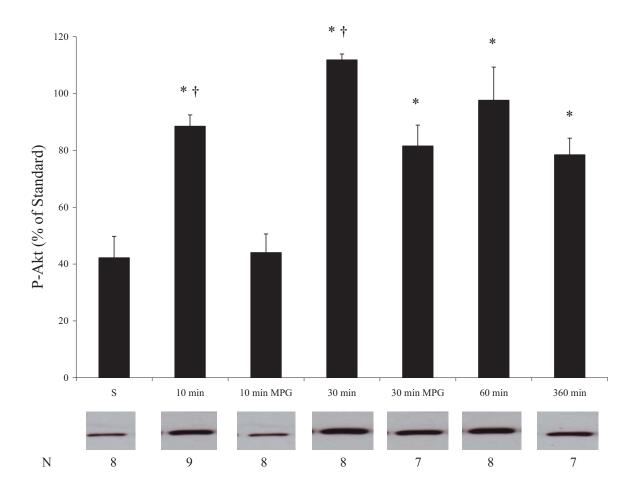


Figure 3.

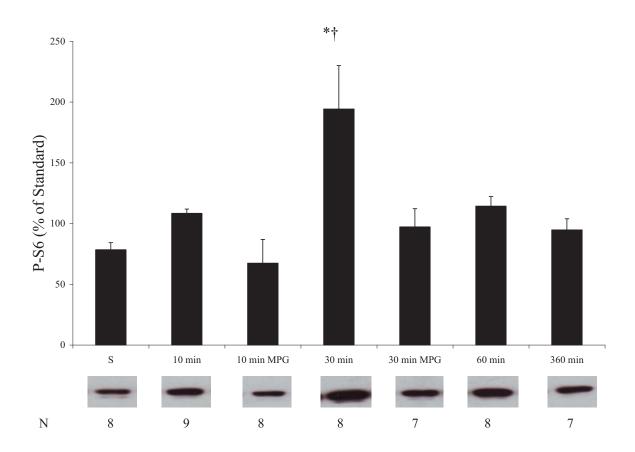


Figure 4.

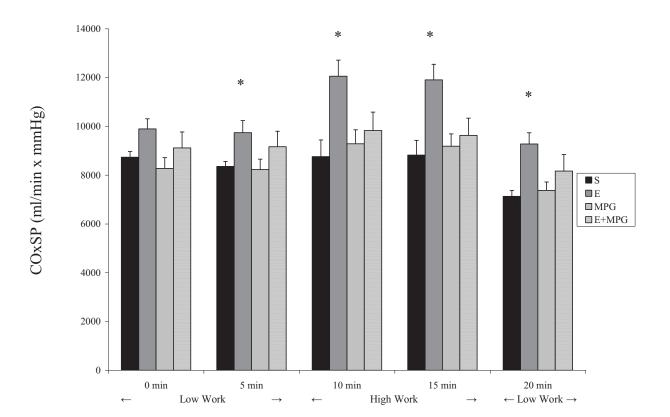


Figure 5.

