Exercise alters the regulation of myocardial Na\(^+\)/H\(^+\) exchanger-1 activity

**Bryan J. Feger and Joseph W. Starnes**

*Department of Kinesiology, University of North Carolina at Greensboro, Greensboro, North Carolina*

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**Feger BJ, Starnes JW.** Exercise alters the regulation of myocardial Na\(^+\)/H\(^+\) exchanger-1 activity. *Am J Physiol Regul Integr Comp Physiol* 305: R1182–R1189, 2013. First published September 18, 2013; doi:10.1152/ajpregu.00228.2013.—The myocardial Na\(^+\)/H\(^+\) exchanger-1 (NHE1) plays a major role in regulation of intracellular pH, and its upregulation has been implicated in increased ischemia-reperfusion injury and other pathologies. Hydrogen peroxide (H\(_2\)O\(_2\)) increases NHE1 activity acutely via ERK1/2 signaling. Chronic strenuous exercise upregulates NHE1 in skeletal muscle, but we hypothesize this will not occur in the heart, because exercise creates a cardioprotective phenotype. NHE1 activity and its regulation by H\(_2\)O\(_2\) remained 185% greater in E. ERK1/2 inhibition abrogated the increases.

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**Address for reprint requests and other correspondence:** J. W. Starnes, Dept. of Kinesiology, Univ. of North Carolina at Greensboro, 1408 Walker Ave., Rm. 250, Greensboro, NC 27412 (e-mail: jwstarn@uncg.edu).
of NHE1, and H2O2-mediated phosphorylation of ERK1/2 in adult rat ventricular myocytes.

METHODS

Animals. Female, 5-mo-old, Sprague-Dawley rats were purchased and housed in the campus animal facility in an isolated room maintained at 22°C with a 12:12-h light-dark cycle and fed ad libitum with Harlan 2018 Teklad Global 18% Protein Rodent Diet. These animals were selected to match those used in our preliminary study on NHE1 expression (12). Animals were randomly divided into either a sedentary control (S; n = 15) or exercise-trained (E; n = 11) group. Twenty-six total animals were used; however, not every animal was used for every experiment due to cell number yielded from the heart cell isolation, which is described below. This investigation was approved by the University’s 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).

Exercise treatment. Exercise was carried out on a motor-driven treadmill using a protocol previously found to result in cardiac hypertrophy and improved cardiac performance in rats matched for sex, age, and strain to those used herein (12). Briefly, during the first week (habituation), animals ran on a 6° grade, gradually increasing the time and speed to 30 min at 25 m/min [70–75% maximal O2 uptake (9)]. Following habituation, animals began running in the cold (8°C) to prevent an excessive increase in core temperature, and running time was gradually increased over the course of 1 wk (5 days/wk) until the animals were running for 1 h/day at 25 m/min. Speed was then gradually increased until it reached 30 m/min and maintained at that speed for the remainder of the 5 wk program. Exercising rats rested for 24 h after the last exercise bout to remove acute training effects, and then cardiomyocytes were isolated as described below. This investigation was published by the National Institutes of Health (NIH Publication no. 85–23, Revised 1996).

Preparation of isolated myocytes. Myocytes were isolated as described by Xu et al. (47). Briefly, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt). Hearts were removed following a transverse thoracotomy and rapidly perfused in a Tyrode solution containing (in mM) 135 NaCl, 5.9 KCl, 1.2 MgCl2, 1.2 KH2PO4, 1.2 MgSO4, 24.7 NaHCO3, and 10 glucose for 5 min to wash out blood. The buffer was bubbled with 95% O2/5% CO2. Then the heart was perfused with Ca2+ and glucose for 5 min to wash out blood. The buffer was bubbled with KCl, 1.25 CaCl2, 1.25 KH2PO4, 1.25 MgSO4, 24.7 NaHCO3, and 10 glucose for 5 min to wash out blood. The buffer was bubbled with 95% O2/5% CO2. Then the heart was perfused with Ca2+-free buffer. After 5 min of perfusion, collagenase (type II) and CaCl2 were added to final concentrations of 0.075% and 25 μM, respectively, and the heart was perfused in a recirculating mode for ~25 min. The heart was removed from the apparatus, and the ventricles placed into a beaker containing the Ca2+-free buffer. The ventricles were agitated in a shaking bath (37°C) for 10 min at a rate of 50 cycles/min, which allowed individual cells to be released. Additional trituration using a wide-tipped transfer pipette assisted in cell dispersion. The released cells were suspended and washed three times in an incubation buffer containing all of the components of the Ca2+-free buffer plus 1% BSA, 30 mM HEPES, 60 mM taurine, 20 mM creatine, and amino acid supplements at 37°C.

Cells for NHE1 activity were filtered through nylon mesh and plated onto laminin-coated coverslips in 35-mm dishes. Cells for ERK1/2 activation were filtered and resuspended in Tyrode solution containing (in mM) 135 NaCl, 5.9 KCl, 1.2 MgCl2, 11.5 glucose, and 10.0 HEPES, pH 7.4. Aliquots of cell suspensions were stored in Tyrode at −80°C for later measurements of activities of antioxidant enzymes and cytochrome-c oxidase (cytox). Experiments were performed on isolated cells within 4 h of isolation.

Estimation of cell volume. Cell volume was determined after fixing cells in phosphate-buffered gluteraldehyde (1% final concentration) and observing the length and width of 100 cells/group (from 3 random hearts/group) under a light microscope. The equation V = l × w × 0.00759, where V is volume, l is length, and w is width, was used to determine cell volume, according to Satoh et al. (38).

Preparation of cell lysates for detection of ERK1/2 phosphorylation. Myocytes in suspension were incubated at 25°C in Tyrode containing H2O2 at concentrations of 0 [control (Con)], 50, 100, and 200 μM for 5 min followed by a 10-min washout, as described by others (37, 40, 45). The ERK1/2 kinase inhibitors PD-98059 [10 μM (40)] and U-0126 [5 μM (45)] were present with a 100 μM H2O2 exposure to block ERK1/2 activation and determine the extent of H2O2 regulation on ERK1/2. Inhibitor or vehicle alone (DMSO) was present 10 min before H2O2 exposure. Following washout, cells were lysed in ice-cold perfusion buffer containing (in mM) 50 NaCl, 50 NaF, 50 sodium pyrophosphate, 5 EDTA, 5 EGTA, 2 Na3VO4, 0.5 phenylmethylsulfonyl fluoride, 10 HEPES, pH 7.4, 0.1% Triton X-100, and 10 μg/ml leupeptin. Lysed cells were stored at −80°C for later determination of protein expression.

Measurement of pHi. The acetoxyl methyl ester of 2’-7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) was used to measure pHi on a Varian Cary Eclipse fluorescence spectrophotometer. BCECF-AM [1.25 μM (37)] was used to incubate cells for 15 min at room temperature in the dark in Tyrode buffer, supplemented with 0.02% pluronic acid. A coverslip with >80% rod-shaped myocytes was inserted into a 4.5-ml optical methacrylate cuvette at a 30° angle to the light beam. All experiments were carried out at 25°C. The solution bathing the cells (pH 7.4) was changed by perfusing fresh solution into the bottom of the cuvette while aspirating continuously from just above the coverslip. The perfusion rate (7.5 ml/min) was kept constant using a peristaltic pump. At the perfusion rate used, the half time of mixing in the cuvette was ~20 s. The cells were excited alternately at 490- and 440-nm light every 0.02 s using a rotating excitation filter wheel, and average fluorescence intensity ratios (490/ 440 nm) were recorded at 1.0-s intervals. This technique allowed measurement of pHi that is independent of cell concentration and loading. Emission was at 535 nm. At the end of each experiment, the fluorescence ratio values were converted to pHi using the nigericin high-K+ technique (44). This clamps the H+ gradient and sets pHi equal to external pH. Cells were perfused with high-K+ solution containing (in mM) 5 NaCl, 135 KCl, and 50 HEPES at varying pH levels (6.8 to 7.5) in the presence of nigericin (4 μg/ml). There was a linear relationship between fluorescence intensity ratios and pH over this range (r2 = 0.982 ± 0.002, n = 35).

Determination of intrinsic βi. Intracellular intrinsic βi (mM/H+ unit) was determined for each group by stepwise removal of extracellular NH4Cl and observing the change of pHi produced by this load. Cells were exposed to Tyrode solution (pH 7.4) containing 20 mM NH4Cl for 90 s, followed by a stepwise reduction of extracellular NH4Cl to 10, 5, 2.5, and 0 mM. At each step, calculated changes in intracellular NH4+ concentration and measured changes in pHi were used to estimate βi, from the equation βi = ΔNH4+/ΔpHi, ΔNH4+ = [NH4+Cl] × 10(pKa – pHi)/[1 + 10(pKa – pHi)], where Δ is change, NH4+Cl is extracellular NH4Cl, pHi is extracellular pH, and pKa is acid dissociation constant. The Henderson-Hasselbach relation using a pKa for NH4+ of 9.21 determined the equilibrium between NH4+ and NH3 and pH in extracellular medium (39).

Calculation of NHE1 activity. Since all experiments were carried out in HCO3−-free medium to eliminate interference from the HCO3−/ H+ exchanger, the rate of proton efflux during recovery from an acid load (jH+), was taken as a direct measure of NHE1 activity (22, 30). Tyrode with ammonium chloride (15 mM) was used for 2 min, followed by washout, to transiently generate an acid load (40). During ammonium chloride perfusion, the pHi initially decreases due to the capture of intracellular protons by NH3. As ammonium chloride is washed out, the excess intracellular NH4+ dissociates into NH3 and H+. NH3 leaves the cell, and protons remain inside the cell, causing acidification. To recover from acidification, the cuvette was perfused with NH4Cl-free Tyrode buffer (pH 7.4). The rate of pH change...
(dpH/dt) during the first 20 s of the recovery period was used to calculate NHE1 activity. The pH, reached during the middle of recovery from the lowest pH, induced by acidosis is considered the “mid pH.” Representative tracings of pH recovery in myocytes from S and E are displayed in Fig. 1. Following 10 min of recovery, a second ammonium chloride-induced acidification occurred in the presence or absence of 10 min of 100 μM H2O2; the first pulse acted as an internal control (31). This concentration of H2O2 is considered to be a physiologically relevant ROS concentration (19) and also was used to determine ERK1/2 phosphorylation (described above). Additionally, the 10-min H2O2 exposure for NHE1 activity has been previously used (40) and was shown to elicit an increase in NHE1 activity in preliminary experiments (data not shown). When studying the effects of ERK1/2 on NHE1 activity, the second pulse was performed in the presence of PD-98059 (10 μM). The inhibitor was present 10 min before the start of the 10-min exposure to 100 μM H2O2; DMSO, the vehicle for the inhibitor, was present during the first pulse. Because exercise-induced hypertrophy resulted in differences in cell volume between groups, a volume correction was used to calculate “true” Δ[H+] from cardiomyocytes (ΔC[H+]) using the equation 

\[ \Delta C[H+] = \frac{d(V_0 \times pH_0 \times V)}{dt} \] (22, 39).

Western blotting. Lysates from ERK1/2 activation experiments described above were thawed, sonicated, and centrifuged at 10,000 g for 30 min at 4°C. Protein concentrations were determined on supernatants using the Lowry method, and samples were processed for immunoblotting. Laemmli’s sample buffer (4×) was added to prepare samples for electrophoresis. Loaded sample volume was based on protein concentration of the sample. All samples were subjected to SDS-PAGE electrophoresis on a resolving gel until the desired molecular weight bands were separated. Thereafter, proteins were transferred onto a polyvinylidene difluoride membrane, incubated at room temperature in 5% evaporated milk in Tris-buffered saline. The membranes were immunoblotted overnight at 4°C with a primary antibody: anti-NHE1 (Millipore, AB3081), β-actin (Cell Signaling, 4967), phospho-p44/42 (ERK1/2, Thr202/Tyr204) (Cell Signaling, 9101), or p44/42 MAPK (ERK1/2) (Cell Signaling, 9102). Membranes were incubated for 1–2 h at room temperature with a horse-radish peroxidase-linked secondary antibody: anti-rabbit IgG (Cell Signaling, 7074). Proteins were detected with SuperSignal West Pico chemiluminescent substrate in a Bio-Rad Chemidoc Station, and densities were measured using Quantity One software. The membrane was stripped with a buffer containing (in mM) 13.3 glycine, 3.5 SDS, and 1% Tween 20, pH 2.2. The membrane was reprobed for another protein: β-actin after NHE1 and total ERK1/2 after phospho-ERK1/2. Densities of the blots were used as a direct indication of amount of protein expression.

Catalase, glutathione peroxidase, and glutathione reductase activity. Because H2O2 was used as the stimulus for the aforementioned experiments, three primary antioxidants involved in quenching H2O2 were examined. Briefly, cells from random hearts were lysed on ice in approximately one volume of 50 mM KH2PO4, 0.1 mM EDTA, 0.1% (vol/vol) Triton X-100, pH 7.4. They were then centrifuged at 10,000 g for 5 min, and the supernatant kept on ice for measurement of the maximum activities of catalase, glutathione peroxidase (GPx), and glutathione reductase (GR) within 1 h. GPx activity was measured by the method described by Flohe and Gunzlger (15), and GR activity was measured as described by Frasier et al. (17). These assays were performed at 37°C in a Varian Cary 50 Bio UV-spectrophotometer at a wavelength of 340 nm and done in triplicate. Catalase activity was measured polarographically with a Clark-type O2 electrode at 37°C, as described by Aeberli et al. (1) and done in duplicate.

Training status. Whole plantaris muscle and cardiomyocyte suspensions were homogenized in ratios of 1:4 wt/vol or ~1:1 vol/vol, respectively, in 50 mM KH2PO4, 0.1 mM EDTA, 0.1% (vol/vol) Triton X-100, pH 7.4 and centrifuged at 10,000 g for 5 min, and the supernatants were analyzed at 37°C. Cytosol activity, a marker of mitochondria content, was determined polarographically using a Clark-type O2 electrode, as described previously (32) and done in duplicate. Heart weight-to-body weight ratio was also used as a measure of training. Protein content for all enzyme assays and Western blotting was determined by the Lowry method using BSA as the standard.

Statistical analysis. Factorial ANOVAs or independent t-tests were used to uncover main effects. All significance values were set at the α = 0.05 level, and, if necessary, Tukey’s honestly significant difference was implemented to dissect main effects. For NHE1 protein expression and intrinsic NHE1 activity characteristics, an unpaired t-test was used to detect differences between groups S and E. For ERK1/2 phosphorylation, a 2 × 4 factorial ANOVA was used; the 2 groups were S and E, while the 4 treatments were H2O2 concentrations of 0 μM (Con), 100 μM, 100 μM + PD-98059, and 100 μM + U-0126. For H2O2-stimulated NHE1 activity, a 2 × 3 factorial ANOVA was used; the 2 groups were S and E, while the 3 treatments were Con, 100 μM H2O2, and 100 μM H2O2 + PD-98059 Unpaired t-tests were used to compare cell volume measurements, catalase activity, GPx activity, GR activity, and cytox activity between S and E.

RESULTS

Exercise training adaptations. The animal characteristics of Table 1 display the differences between S and E rats. The exercise training protocol increased both heart weight and body weight in the E group by 13.3% (P < 0.001) and 6.0% (P < 0.01), respectively. The greater increase in heart weight ex-
plains the significant increase in heart weight-to-body weight ratio of 6.8% ($P < 0.01$). Plantaris cytox activity was 1.9-fold higher in E animals ($P < 0.001$). No exercise-induced change was observed for heart cytox ($P = $nonsignificant), which is typical for this extremely aerobic muscle. Overall, the presence of cardiac hypertrophy and the almost twofold increase in skeletal muscle mitochondria indicate a high state of endurance training.

**NHE1 protein expression.** The cardiomyocyte content of NHE1 was not different ($P = $nonsignificant) between S and E (1.87 ± 0.83 vs. 1.47 ± 0.37 arbitrary units, respectively; $n = 6$ hearts/group). Although the effect of exercise training on NHE1 expression in whole hearts has been reported previously (12, 35), we deemed it important to validate the previous data because generalizations to cardiomyocytes from whole heart tissue may be inaccurate due to the heterogeneous cell population in the heart.

**Cell volume.** The cell volume characteristics of S and E myocytes are presented in Table 2. Cell volume was 55% greater in E myocytes ($P < 0.01$), a result of both greater cell length and width. The estimated volumes of S adult myocytes are comparable to previously reported values (22). The respective cell volume data for S and E were used to calculate the corrected rates of $J_{H^+}$ in the two groups.

**Intrinsic $\beta_i$.** As depicted in Fig. 2, a marked difference existed between the intrinsic $\beta_i$ of S and E myocytes. The best-fit line slopes developed following NH$_4$Cl titration, 55.3 ($R^2 = 0.95$) and 106.9 ($R^2 = 0.84$) for S and E, respectively, indicated a 178% greater $\beta_i$ in E myocytes at physiological pH$_i$ 7.3. As pH$_i$ decreases, the difference in $\beta_i$ becomes less and less until, at pH$_i$ 7.0, $\beta_i$ is virtually identical between groups.

**NHE1 activity.** Starting pH$_i$ was not different between groups [7.33 ± 0.02 for S ($n = 11$), and 7.30 ± 0.02 for E ($n = 10$)]. The degree of NH$_4$Cl-induced alkalosis was not different between groups [7.51 ± 0.02 and 7.46 ± 0.02 ($P = 0.11$), respectively], and average pH$_i$ changes during the 2-min exposure to NH$_4$Cl show the rate of recovery from alkalosis was greater in S compared with E [0.32 ± 0.01 and 0.24 ± 0.01 ($P < 0.05$), respectively]; however, there was no significant difference in the minimum pH$_i$ reached following NH$_4$Cl washout (7.10 ± 0.02 for S and 7.09 ± 0.02 for E). As shown in Table 3, there was no between-group difference regarding the dpH/dt from this acid load, depicted also by the tracings in Fig. 1, whereas $\beta_i$ at the pH$_i$ in the middle of recovery (∼7.17) was determined to be 94% greater ($P < 0.001$) in E compared with S. This increase in $\beta_i$ contributed to a significantly greater rate of $J_{H^+}$ per cell volume in E compared with S ($P < 0.01$). Taking into account cell volume differences, the rate of CJH$_-$ was determined to be 209% greater in E compared with S ($P < 0.001$).

**Influence of H$_2$O$_2$ on NHE1 activity.** Figure 3, left, displays the change in NHE1 activity in 100 µM H$_2$O$_2$ stimulated cells and control cells (vehicle only) relative to their internal control (first pulse acidification in the presence of vehicle only). H$_2$O$_2$ significantly increased NHE1 activity in both groups; however, the increase in E myocytes was significantly less than the increase in S myocytes ($P < 0.05$). The response to 100 µM H$_2$O$_2$ was abolished in the presence of the ERK kinase inhibitor PD-98059, supporting ERKs role as a mediator of the H$_2$O$_2$-induced increase in NHE1 activity in both groups. Although E NHE1 was less responsive to H$_2$O$_2$ stimulation, absolute E NHE1 activity remained ∼185% faster than S as displayed in Fig. 3, right (compare with basal activity in Table 3).

**Effect of H$_2$O$_2$ on ERK1/2 phosphorylation.** Phosphorylated ERK1/2 relative to total ERK1/2 is displayed in Fig. 4. Experiments using a range of H$_2$O$_2$ concentrations from 50 to 200 µM verified that maximum phosphorylation was achieved with 100 µM H$_2$O$_2$ in both groups (quantitative data not shown). The amount of phosphorylated ERK1/2 both in the basal state (Con) and following stimulation by 100 µM H$_2$O$_2$ was not significantly different between groups. Both ERK kinase inhibitors (U-0126 and PD-98059) reduced phosphorylation to values not different from Con values.

**Effect of exercise training on antioxidant enzyme activity.** The results for catalase, GPx, and GR are displayed in Table 4. The between-group means values for all enzymes were similar.
nate pHi fluctuations, it only decreases the magnitude or rate of corrected proton efflux.

Values are mean ± SE n, no. of animal replicates. Pooled data are from the first (internal control) NH4Cl pulses from control and H2O2 groups, dpH/dt, rate of recovery; βi, pH-buffering capacity; JH+, proton efflux; CjH+, volume-corrected proton efflux. *P < 0.01, †P < 0.001 vs. S animals.

(P > 0.05), suggesting that cells from both groups received the same intracellular H2O2 perturbation.

DISCUSSION

The present study is the first to determine the impact of aerobic exercise training on myocardial NHE1 activity. There were two major findings. First, the exercise program used herein resulted in a significant increase compared with S animals in the capacity for JH+, from cardiomyocytes via NHE1 at physiological pH, both in the basal state and following H2O2 stimulation. Second, the exercise program led to a significant alteration to ERK1/2 signaling and resulted in a decrease in the sensitivity of NHE1 activity changes in response to ROS signaling pathways. These findings will now be discussed.

Exercise increases NHE1 activity. NHE1 activity was measured in intact, isolated cardiomyocytes so that the rate of JH+ across the sarcolemma, i.e., activity, could be directly determined. Because our experiments were performed in HCO3−-free medium, any contribution from the Cl-/HCO3− exchanger to recovery from NH4Cl-induced intracellular acidosis was eliminated (22). Cardiomyocyte JH+ per cell volume was 103% greater in E compared with S, and JH+ per cardiomyocyte was 209% greater in E compared with S (Table 3). These differences can be at least partially explained by the increased βi (Fig. 2) and cell volume (Table 2) detected in E cells. Enhanced βi (along with increased NHE1 activity) also is seen in skeletal muscle following exercise training (28) and is likely a reflection of the change in intracellular protein composition in response to training (for example, myofilaments), as intracellular buffers are believed to include H+-titratable groups (5, 7). pHi regulation guidelines posit that raising βi does not eliminate pHi fluctuations, it only decreases the magnitude or rate of a change (6). Consistent with this, the increase in βi in E cardiomyocytes compared with S cardiomyocytes observed herein prevented differences between the two groups in both minimum pHi reached following acid loading and dpH/dt during subsequent recovery (Table 3). Our measurements of NHE1 expression indicate that the elevated JH+ is not due to increased NHE1 content, as there was no significant difference observed. The finding that NHE1 content is not increased by exercise training is consistent with previous studies using whole ventricles and reporting either no change or a decrease (12, 35). Together these points suggest the elevated JH+ may be due to posttranslational modifications. Importantly, the fundamental kinetics of NHE1 do not appear to be affected by exercise training, since steady-state pHi was similar between E and S. The present work opens the door to more closely exploring possible intrinsic changes to NHE1 activity in response to exercise training, such as the phosphorylation or redox status of NHE1.

What is the physiological advantage of increased NHE1 activity? Wisloff et al. (46) exercise trained adult rats on a treadmill for 4 wk and examined pHi changes in isolated cardiomyocytes in response to increasing stimulation frequencies between 2 and 10 Hz. They observed that, at ≥5 Hz, both groups acquired acidic pHi; however, exercised cells had significantly less acidosis compared with S cells. The increased NHE1 activity observed in our E cells can largely explain the attenuated acidosis observed with increasing stimulation frequency. This mechanism also could help explain why the trained myocytes displayed enhanced contractility and shorter systolic relative cell length compared with untrained myocytes at the same frequencies (46); the increased NHE1 activity would result in less H+ being present to interfere with Ca2+ binding to troponin C (4). Furthermore, cardiac function in rats matched to those of the present study for sex, age, strain, and exercise protocol was reported in our previous study using the isolated, perfused working heart model (12). Hearts from the exercised animals displayed cardiac hypertrophy, as well as greater cardiac output, external cardiac work, and efficiency of work compared with the S animals, even when normalized for heart weight. It is tempting to speculate that elevated NHE1 activity played at least a partial role in the improved performance. Although NHE1 is the predominant H+-extruding transporter, Na+-coupled Cl−/HCO3− exchanger and Na+-HCO3− cotransporter warrant investigation to more fully understand how the E heart improves its ability to regulate pHi (33).

The NHE1 activity measured herein was carried out at or near physiological pHi; the extracellular pH was constant at pH 7.4, basal pHi, was on average 7.33 and 7.30 for S and E.
respectively, and the lowest pH reached was ~7.05. Using only a physiological pH range is a limitation of this study, because pH can reach considerably lower levels during pathological situations. As mentioned in the introduction, upregulation of NHE1 activity promotes Ca\(^{2+}\) overload (14), particularly during IR when ATP-dependent sarcolemmal ion pumps are inhibited. In addition, it would speed pH recovery during subsequent reperfusion, which would facilitate mitochondria permeability transition pore opening (21). Thus, if NHE1 activity remains higher in the hearts of E compared with S at the low pH levels associated with IR, it could result in greater injury to the E heart. However, the opposite is actually the case; exercise training is known to create a cardiac phenotype that protects the heart from IR injury (16, 34, 43) and results in less Ca\(^{2+}\) overload following reperfusion (8). There are at least three possible explanations for this. The first possibility is that the overall cardioprotective adaptations induced by exercise training are able to overcome a hyperactive exchanger. Consistent with this possibility is the finding that a detrimental side effect of ischemic preconditioning is an increase in NHE1, and its inhibition provides additive or synergistic cardioprotection above ischemic preconditioning (20). Another possibility is that NHE1 activity in the E heart decreases as pH decreases, so that activity is not greater than that of S animals at pH levels associated with IR. This possibility is supported by our finding that \(\beta_i\) in the E myocyte decreases with decreasing pH (Fig. 2). Further investigation examining dpHi/dt and NHE1 activity in E myocytes at pH levels more comparable to those reached during ischemia would provide more insight into the role NHE1 activity plays in the exercise-induced cardioprotective phenotype. The third possibility is that exercise alters the regulation of NHE1 activity in response to pathological stress. This possibility will be discussed next.

**Exercise decreases sensitivity of NHE1 activation by ROS signaling pathways.** H\(_2\)O\(_2\), which is elevated by many stressful and pathological situations, is known to increase NHE1 activity in heart cells (37, 40). We observed that exercised myocytes had less of an increase in NHE1 activity compared with S upon exposure to 100 \(\mu\)M H\(_2\)O\(_2\). Although the attenuated response was statistically significant, absolute NHE1 activity remained greater in E than in S. It is unlikely that the attenuated response in E was due to greater H\(_2\)O\(_2\) quenching by antioxidants in the present study, since the major antioxidants involved in quenching H\(_2\)O\(_2\), catalase, GPX, and GR were similar between groups. Many, but not all, studies evaluating these antioxidants in the heart following chronic exercise training also have reported no changes (Ref. 16 for review). Thus the attenuated response to H\(_2\)O\(_2\) by the E group observed herein is more likely due to alterations in the signaling pathway responsible for mediating H\(_2\)O\(_2\)-induced stimulation.

![Graph showing effect of 100 \(\mu\)M H\(_2\)O\(_2\) on extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation.](image)

**Fig. 4.** Effect of 100 \(\mu\)M H\(_2\)O\(_2\) on extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation. Ratios are normalized to S Con. Bottom: representative bands. As noted in RESULTS, 100 \(\mu\)M H\(_2\)O\(_2\) afforded maximum ERK1/2 phosphorylation, not different from 50 or 200 \(\mu\)M H\(_2\)O\(_2\). U, U-0126 (5 \(\mu\)M); PD, PD-98059 (10 \(\mu\)M); H\(_2\)O\(_2\), 100 \(\mu\)M H\(_2\)O\(_2\) (in bar charts). Values are means ± SE. *P < 0.05 vs. Con in same group.

**Table 4. Antioxidant levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase, U/mg protein</th>
<th>GSH Peroxidase, mU/mg protein</th>
<th>GSH Reductase, mU/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>16.19 ± 0.92 (10)</td>
<td>17.53 ± 2.47 (10)</td>
<td>39.26 ± 5.58 (10)</td>
</tr>
<tr>
<td>E</td>
<td>19.41 ± 1.56 (9)</td>
<td>23.05 ± 1.92 (9)</td>
<td>41.87 ± 3.42 (8)</td>
</tr>
</tbody>
</table>

P value 0.086 0.101 0.713

Values are means ± SE (no. of animals). Catalase (1 unit = 1 \(\mu\)mol H\(_2\)O\(_2\) consumed/min); GSH peroxidase, glutathione peroxidase; GSH reductase, glutathione reductase (1 mU = 1 \(\mu\)mol NADH·l\(^{-1}\)·min\(^{-1}\)).
ERK1/2 are major regulators of NHE1 activity since they can phosphorylate NHE1 on its cytosolic tail (10). Iemitsu et al. (25) concluded that ERK1/2 become less sensitive to stress activation following exercise training based on their findings that endurance-trained rats had less ERK1/2 activation than S rats following the same exercise bout. However, when we provided identical H2O2 stresses to cells from S and E, we observed similar increases in phosphorylated ERK1/2 relative to baseline. Thus our results clearly indicate that ERK1/2 is not less sensitive to stress activation following exercise training, which is in direct contrast to the conclusions of Iemitsu et al. (25). Their results are likely due to less H2O2 production by the E hearts (27, 42), and to the fact that the acute exercise bout used to induce stress was less stressful to the trained animals because of the animals’ enhanced endurance capacity. The latter possibility was brought up by the authors when discussing possible limitations of their study.

Previous studies focusing on the relationship of ERK1/2 activation and NHE1 activation in rat cardiomyocytes used S animals (40, 45). They reported that H2O2 stimulation increases phosphorylation of ERK1/2 in a manner that corresponds to H2O2-induced NHE1 activation. An important finding in the present study is that the H2O2-stimulated signaling between ERK1/2 and NHE1 is dampened by exercise training. Although we observed that a 100 μM H2O2 stress resulted in similar amounts of phosphorylated ERK1/2 in S and E (Fig. 4), there was less NHE1 activation relative to the basal state in E (Fig. 3). This suggests NHE1 activation becomes less responsive to H2O2-activated ERK1/2 following endurance training. Although we do not have an explanation for this, one possibility is that the exercise-induced posttranslational modification of NHE1 maintains it in a partially activated state, independent of ERK1/2, so that it is closer to “full” activation before H2O2-induced stimulation compared with S animals. The dampening effect clearly deserves further investigation because of the implications of the effect to cardioprotection.

Perspectives and significance. This was the first study to examine the characteristics of sarcolemmal NHE1 activity and signaling in E hearts under basal and H2O2-stimulated conditions. We observed that NHE1 activity is greater in cardiomyocytes of E compared with S at near physiological pHi due partly to enhanced β, and increased cell volume in E myocytes. This may play a role in improved myocardial performance. Additionally, 100 μM H2O2 resulted in similar amounts of phosphorylated ERK1/2 in both groups, but the subsequent activation on NHE1 by ERK1/2 was significantly less in E compared with S. However, absolute NHE1 activity following H2O2 stimulation was still greater in E myocytes. Ordinarily a ROS-induced increase in NHE1 activity would lead to pathological consequences. How exercise-induced cardioprotective adaptations interact with NHE1 to prevent or attenuate such consequences merits investigating.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.J.F. and J.W.S. conception and design of research; B.J.F. and J.W.S. performed experiments; B.J.F. and J.W.S. interpreted results of experiments; B.J.F. prepared figures; B.J.F. drafted manuscript; B.J.F. and J.W.S. edited and revised manuscript; J.W.S. approved final version of manuscript.

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