Exercise training reverses downregulation of HSP70 and antioxidant enzymes in porcine skeletal muscle after chronic coronary artery occlusion

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Lawler, John M., Hyo-Bum Kwak, Wook Song, and Janet L. Parker. Exercise training reverses downregulation of HSP70 and antioxidant enzymes in porcine skeletal muscle after chronic coronary artery occlusion. Am J Physiol Regul Integr Comp Physiol 291: R1756–R1763, 2006.—Oxidative stress is associated with muscle fatigue and weakness in skeletal muscle of ischemic heart disease patients. Recently, it was found that endurance training elevates protective heat shock proteins (HSPs) and antioxidant enzymes in skeletal muscle in healthy subjects and antioxidant enzymes in heart failure patients. However, it is unknown whether coronary ischemia and mild infarct without heart failure contributes to impairment of stress proteins and whether exercise training reverses those effects. We tested the hypothesis that exercise training would reverse alterations in muscle TNF-α, oxidative stress, HSP70, SOD (Mn-SOD, Cu,Zn-SOD), glutathione peroxidase (GPX), and catalase (CAT) due to chronic coronary occlusion of the left circumflex (CCO). Yucatan swine were divided into three groups (n = 6 each): sedentary with CCO (SCO); 12 wk of treadmill exercise training following CCO (ECO); and sham surgery controls (sham). Forelimb muscle mass-to-body mass ratio decreased by 27% with SCO but recovered with ECO. Exercise training reduced muscle TNF-α and oxidative stress (4-hydroxynonenal adducts) caused by CCO. HSP70 levels decreased with CCO (~45%), but were higher with exercise training (+34%). Mn-SOD activity, Mn-SOD protein expression, and Cu,Zn-SOD activity levels were higher in ECO than SCO by 72, 82, and 112%, respectively. GPX activity was 177% greater in ECO than in SCO. CAT trended higher (P = 0.059) in ECO compared with SCO. These data indicate that exercise training following onset of coronary artery occlusion results in recovery of critical stress proteins and reduces oxidative stress.

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respond to endurance exercise training with primarily peripheral adaptations, rather than changes in central (i.e., cardiac) function (20). In healthy adults, exercise training increases protective stress proteins in skeletal muscle including antioxidant enzymes and HSPs (25, 59).

A paucity of evidence exists testing the ability of exercise to increase stress antioxidant enzymes and HSPs in muscle of cardiac patients. A new paper by Linke et al. (40) indicates that exercise results in a partial reversal of the reduction in antioxidant enzyme mRNA, protein expression, and activity in heart failure patients. However, it is unknown whether changes in antioxidant enzymes in skeletal muscle with CHF were due to heart failure, coronary ischemia, and/or humoral factors. In addition, the combined effects of coronary ischemia and subsequent exercise training on HSP70 in skeletal muscle are unknown. Therefore, the purpose of the present study was to use an ameroid coronary artery occlusion (CCO) model in Yucatan swine as a ischemia and infarct model, without atherosclerosis or heart failure, to test the following hypotheses. CCO of the left circumflex artery would reduce skeletal muscle mass, levels of HSP70, and activities of SOD, catalase (CAT), and glutathione peroxidase (G PX), and nonenzymatic antioxidant scavenging capacity (ASC). Subsequently, 12 wk of exercise training would result in significant recovery of the loss of muscle mass, HSP70, and antioxidant enzymes while reducing oxidative stress in skeletal muscle of Yucatan swine following chronic occlusion of the left circumflex coronary artery.

METHODS

Animals. Adult female (9–12 mo of age; 35–55 kg body mass) Yucatan miniature swine were used (Charles River; Wilmington, ME). The porcine model is common for studying the pathophysiology of heart disease with particular relevance toward human pathology. The porcine model for CCO was chosen because the animals are similar to humans in the coronary circulation, heart-to-body weight ratio, cardiac output distribution to skeletal muscle, coronary collateral density, maximal aerobic capacity (VO2 max) and response to exercise (30, 69). All procedures had been approved by the Animal Laboratory Use and Care Committee at Texas A&M University.

Experimental design. Miniature Yucatan swine were divided into three groups (n = 6 each): sedentary controls that underwent sham surgery (sham), sedentary animals with CCO (SCO), and CCO followed by treadmill training (ECO). The coronary occlusion procedure was performed as described in Griffin et al. (21) below, with control animals exposed to sham surgery.

CCO model. We used a porcine preparation with ameroid chronic CCO of the proximal left circumflex as described previously (21). The CCO model employs reproducible occlusion of the proximal left circumflex artery that results in infarction, decreased left ventricular ejection fraction, and myocardial ischemia (26, 41). The ameroid model of CCO allows for the development of a chronically ischemic region of the left ventricular myocardium (41). Coronary artery flow in the left circumflex artery region is indeed reduced with CCO, particularly when dilated with adenosine and is inversely related to elevated left circumflex artery vessel resistance (55, 69). This model and surgery are highly reliable with >95% success rate (18).

Chronic coronary occlusion results in a decreased left ventricular ejection fraction (−25%), decreased left-ventricular wall thickening (index of cardiac contractile function) (17, 27), moderate levels of myocardial infarction (7%) without heart failure or atherosclerosis (18, 26). Previous data showed that hemodynamic variables including resting heart rate, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and mean left atrial pressure are not altered within the first 16 days after CCO of the left circumflex (54, 55). Cardiac function, using myocardial thickening during systole as a marker, is unchanged by CCO at rest (55) or decreases only in the ischemic region of the left circumflex region (−20%), but not the left anterior descending artery region (53, 54). Myocardial thickening during systole is worsened by a bout of acute exercise following CCO, reflective of “myocardial stunning” (53, 69). However, 12 wk of treadmill exercise training attenuates the reduction of ventricular wall thickening during systole (55) and is concomitant with increased collateral circulation in the ischemic region, and decreased HR during exercise (69).

Animals were sedated with ketamine (20 mg/kg im), midazolam (0.5 mg/kg im), and glycopyrrolate (0.004 mg/kg im) and then intubated on isoflurane during aseptic surgery. Sterile surgery was conducted in accordance with the “Position of the American Heart Association on Research Animal Use” (1984). A left thoracotomy was performed through the fifth intercostal space exposing the heart for pericardial incision. A 3-mm ID ameroid constrictor (Research Instruments) was placed around the proximal left circumflex coronary artery. The sham group underwent the same left thoracotomy as CCO groups, except that the ameroid occluder was not placed around the left circumflex coronary artery. Sham swine were housed under identical conditions as the sedentary and exercise CCO groups pre- and postsurgery.

Swine from both sham and coronary occlusion groups displayed a similar amount of activity in their pens, indicating that deconditioning was not a confounding factor in muscle alterations in stress proteins, nonenzymatic antioxidant capacity, and TNF-α with the SCO and ECO groups.

Exercise training protocol. The exercise protocol was similar to that described earlier (21). After 6 wk of stabilization following surgery, all pigs were familiarized with treadmill exercise for a 2-wk period. Then the CCO groups were randomly divided into exercise and sedentary groups. The ECO group performed 12 wk of progressive treadmill exercise training. In brief, Yucatan swine were exercised on a treadmill for 12 wk, 5 days/wk. The first 4 wk included acclimation to treadmill running. By the end of week 4, the exercise regimen consisted of 1) warmup for 5 min at 2 mph, followed by 2) a short speed run lasting 5 min at 5 mph, followed by 3) an endurance run for 40 min at 4 mph, and then 4) a cool-down of 5 min at 2 mph. The speed and length of the runs were gradually increased through week 12, so that the warmup consisted of 5 min of running at 2.5 mph, then a speed-run for 15 min at 6 mph, and the endurance run for 60 min at 4.5–5.5 mph, followed by a cool down for 5 min at 2.5 mph. The intensity levels were dependent on the tolerance levels of each animal. Thus the range during the endurance run represents differing abilities among the swine. This exercise protocol elicits a mean heart rate (HR) of ∼265 beats/min (55) with an intensity level ≥75% VO2 max (32). This intensity and volume of exercise has been used extensively and consistently increases heart/body mass, skeletal muscle citrate synthase levels, and coronary collateral circulation (18, 21, 28).

Tissue preparation. Following CCO and exercise training period, the swine from all groups had their body masses taken and were then euthanized with ketamine (25 mg/kg), xylazine (2.25 mg/kg), and thiopental sodium (20 mg/kg). Then forelimb muscles (brachialis, lateral, medial, and long heads of the triceps brachii) were extracted. During uphill running, the forelimb muscles and particularly the elbow extensors of the swine are heavily utilized, and respond with a large upregulation of citrate synthase (28), and were thus chosen as our muscle response model. Muscle masses were measured, and then muscles were cut into samples. Skeletal muscle samples from the lateral head of the triceps brachii were washed in chilled (4°C) Krebs solution and quickly frozen in liquid nitrogen and stored at −80°C until analysis. Previous work indicates that the triceps brachii muscle responds to exercise training by increasing citrate synthase activity.
levels (18, 21, 28). The muscle samples were prepared for homoge-
nization by being minced into fine pieces and placed in 20:1 dilution
in potassium phosphate buffer (100 mM; pH = 7.40, 5°C) and 0.13
mM butylated hydroxytoluene. Following homogenization, we cen-
trifuged the homogenate for 10 min at 300 g (3°C) to rid it of cellular
debris. Triceps muscle samples were analyzed for the following:
citrate synthase, TNF-α, HSP70, SOD (total, Mn-SOD isofrom,
Cu,Zn-SOD isofrom), GPX, CAT, and nonenzymic ASC.

Antioxidant/oxidative enzymes. Citrate synthase was measured as
described previously (36). It was used as a marker of oxidative
capacity in skeletal muscle and is indicative of mitochondrial density
and function.

Analysis of SOD was conducted using the assay of Vanneste and
Vanneste (65) that utilized the reduction of cytochrome c by super-
oxide anions generated by the xanthine oxidase reaction. Briefly, 0.02
U/ml xanthine oxidase, 6 mM xanthine and 60 µM cytochrome c are
mixed with 300 µl homogenate for a final volume of 1.6 ml. Cu/Zn-
SOD activity was distinguished from Mn-SOD isofrom by the addi-
tion of 1 mM KCN. The change in absorbance at 550 nm was
subtracted from a control using 300 µl phosphate buffer, with 1 unit
of SOD defined as a 50% reduction in Δabsorbance.

Analysis of muscle GPX activity was determined using the tech-
nique of Flohé and Günzler (17). Briefly, 1.6 ml of 0.30U/ml GPX,
1.25 mM reduced glutathione and 0.19 mM NADPH in potassium
phosphate buffer (pH=7.4) were introduced to each cuvette. Then
100 µl of homogenate were added. Finally, 200 µl of t-butyl hy-
droperoxide were added to commence the reaction with absorbance
recorded for 4 min at 340 nm on a spectrophotometer (Beckman
Coulter, Fullerton, CA).

Analysis of muscle CAT activity was accomplished using the tech-
ique of Aebi (5). Briefly, ethanol was added 1:10 vol/vol to
homogenates and incubated for 30 min in an ice bath. Then 1% Triton
X-100 was added, and the homogenates were incubated in ice for 15
min. One milliliter of 100 mM K+ phosphate buffer (pH = 7.4) was
added to 500 µl of the homogenate in a glass cuvette. The reaction
was initiated by the addition of 1 ml of 50 mM H2O2. Absorbance was
read at 240 nm for 1.5 min. Activity units for CAT were calculated
from the rate constant (k) and expressed as U/mg protein.

TNF-α and ASC. TNF-α levels in muscle and serum were quanti-
fied using an ELISA kit designed specifically for porcine use (Pierce
Endogen, Rockford, IL).

The nonenzymatic ASC assay was a modification of our technique
described previously (35). ASC determines the ability of muscle
homogenates to scavenge the radical blue/green chromophore
ABTS+. Briefly, 660 µg/ml of potassium persulfate was added to 7
mM ABTS stock producing the stable radical cation ABTS+.
ABTS+ was diluted to 91 µM and 600 µl added to each cuvette. Then 600 µl
of muscle homogenate or Trolox standard were added to the cuvettes.
Absorbance was determined at 734 nm and recorded for 4 min. ASC
was calculated and equated against a Trolox standard curve.

Western immunoblot analysis. Protein content for HSP70 was
determined by Western immunoblot analysis. Separating gel (375 mM
Tris·HCl, pH = 8.8, 0.4% SDS, 10% acrylamide) and stacking gel
(125 mM Tris·HCl, pH = 6.8, 0.4% SDS, 10% acrylamide monomer)
solutions were made, and polymerization was then initiated by tetra
methyl ethylene diamine and ammonium persulfate. Separating and
stacking gels were then quickly poured into a Protein III gel-box
(Bio-Rad, Hercules, CA). Sixty micrograms of protein from skeletal
muscle homogenates in sample buffer (Tris pH = 6.8 with 2% SDS,
30 mM DTT, 25% glycerol) were then loaded into the wells of the
10% polyacrylamide gels and electrophoresed at 150 V. The gels were
then transferred at 30 V overnight onto a nitrocellulose membrane
(Bio-Rad). Membranes were blocked in 5% nonfat milk in PBS with
0.1% Tween-20 at room temperature for 6 h. After blocking, mem-
branes were incubated at room temperature in PBS and the appro-
priate primary antibodies for 12 h. Primary antibodies included rabbit
polyclonal anti-HSP70 (1:200; Santa Cruz Biotechnology, Santa
Cruz, CA) and 4-hydroxynonenal (4-HNE) adducts (1:1,000; Calbio-
chem, San Diego, CA), marker of oxidative stress. Following three
washings in PBS with 0.4% Tween-20, membranes were incubated
with horseradish peroxidase (HRP)-conjugated secondary antibodies
(Santa Cruz Biotechnology) in PBS at room temperature for 90 min.
Following three washes in PBS with 0.4% Tween-20, an enhanced
chemiluminescence detection system (Amersham, Piscataway, NJ)
was used for visualization. Densitometry (as area times grayscale
relative to background) was performed using a Kodak film cartridge
and function. To ensure equal loading of protein, Ponceau S staining was performed for each membrane, and the lane
background reading was subtracted from each protein blot density.

Statistics. One-way ANOVAs with Student-Newman-Keuls as a
post hoc test were used to assess group mean differences in muscle
mass, muscle mass-to-body mass ratio, TNF-α, 4-HNE, HSP70,
antioxidant enzyme activities, Mn-SOD protein levels, and ASC.

RESULTS

Triceps brachii muscle decreased from 390 ± 34 g for the sham
group to 344 ± 15 g for the SCO group and was higher
(386 ± 25.7 g) in the ECO group. Forelimb muscle mass-to-
body mass ratio was reduced in SCO (7.25 ± 0.04 g/kg)
comparied with sham (10.1 ± 0.95 g/kg) by 27% (Fig. 1A).
However, 12 wk of exercise training elicited significantly
higher muscle mass/body mass (9.85 ± 0.49 g/kg) in ECO than

\[ \text{ Forelimb mass/body mass (g/kg) } \]
\[ \text{ SHAM } \quad \text{ SCO } \quad \text{ ECO } \]

\[ \begin{array}{ccc}
\text{SHAM} & 9.85 & 10.1 \\
\text{SCO} & 9.25 & 7.25 \\
\text{ECO} & 9.85 & 7.25 \\
\end{array} \]

\[ * \text{ ECO significantly different from sham group; } \]
\[ * \text{ ECO significantly different from SCO group. } \]

\[ \text{ Fig. 1. Forelimb muscle mass-to-body mass ratio (A) and citrate synthase activity (B) in the triceps brachii muscle from adult } \]
\[ \text{Yucatan swine from the following groups: sham surgery controls (sham), sedentary pigs with chronic } \]
\[ \text{coronary occlusion of the left circumflex coronary artery (SCO), and pigs with } \]
\[ \text{chronic coronary occlusion who had completed 12 wk of treadmill exercise } \]
\[ \text{training (ECO). } \]

\[ \text{ *SCO significantly different from sham group; } \]
\[ \text{ *ECO significantly different from SCO group. } \]

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SCO. No significant difference in muscle mass/body mass was noted between sham and ECO. Muscle triceps citrate synthase activity was significantly lower (−30%) in SCO than sham (Fig. 1B), indicative of reduced oxidative capacity. Conversely, citrate synthase activity was significantly higher (+75%) in triceps from the exercise group compared with muscle samples from the SCO group, indicating the efficacy of the exercise training program. Citrate synthase activity was not significantly different between sham and ECO plantaris samples.

Muscle levels of the inflammatory cytokine TNF-α were significantly higher (+62%) in muscles of swine that underwent coronary occlusion than sham controls (Fig. 1B). Twelve weeks of exercise training significantly reduced TNF-α levels by 33% compared with SCO. No significant differences between sham and ECO were noted for TNF-α levels. In contrast, neither CCO nor exercise had any effect on serum TNF-α levels (data not shown). In addition, exercise training also attenuated 4-HNE adducts, a marker of oxidative stress, in the triceps brachii muscle of ECO swine compared with the sedentary group with CCO (Fig. 2B). The 25-kDa 4-HNE adduct was significantly higher (+32%) in SCO than sham controls, whereas the ECO group was 51% below sham controls. The 40-kDa adduct was also elevated with CCO in skeletal muscle (+81%), whereas again, exercise attenuated 4-HNE adduct levels (−37%) compared with SCO. In addition, 4-HNE adducts at 34 kDa in the triceps brachii trended lower in ECO compared with SCO. These data indicate that exercise attenuates elevated skeletal muscle levels of TNF-α and oxidative stress.

We then tested the effect of 12 wk of treadmill exercise training on levels of the stress protective protein HSP70. HSP70 protein expression of triceps brachii muscles was 45% lower in SCO than in the sham group (Fig. 3). Exercise training resulted in a marked upregulation (+348%) of HSP70 protein expression in triceps muscle samples compared with those in the SCO group. HSP70 protein levels were also higher in ECO than sham as well.

We then determined whether antioxidant enzyme activities were reduced by CCO in the triceps brachii, and if exercise training attenuated depression of antioxidant enzyme activities, consistent with our hypotheses. Total SOD, Mn-SOD, and Cu,Zn-SOD were all significantly reduced in skeletal muscle by SCO (Fig. 4A). However, exercise training resulted in an elevation of total SOD (+83%), Mn-SOD (+72%), and Cu,Zn-SOD (+112%) in the lateral head of the triceps muscles from the ECO group compared with SCO. Mn-SOD protein expression also decreased (−26%) in the triceps brachii as a result of CCO (Fig. 4B). Twelve weeks of treadmill exercise training following CCO resulted in 82% higher Mn-SOD protein levels compared with the sedentary with CCO.

In contrast, CAT activity was unaltered by CCO in the triceps brachii. Exercise training did result in a trend (P = 0.059) upward (+13%) when compared with the sedentary groups (Fig. 5A). GPX activity exhibited a significant decrease (−64%) as a result of chronic CCO (Fig. 5B). Exercise training also reversed or prevented the occlusion-induced downregulation of GPX. GPX activity was 176% greater in muscle samples from the ECO group than the SCO group.

Nonenzymatic antioxidant enzyme scavenging capacity (ASC) is an integrative marker of antioxidant scavengers (e.g., reduced glutathione, tocopherols, ascorbate, thioredoxin, etc.). ASC was significantly lower with aging, but was significantly elevated in the triceps brachii from exercise-trained swine with CCO, when compared with sedentary pigs with CCO (Fig. 5C).
DISCUSSION

The major findings of our study are as follows. Chronic coronary occlusion of the left circumflex artery resulted in a significant reduction in muscle mass-to-body mass ratio, elevation of muscle TNF-α, increased oxidative stress, and downregulation of stress-protective HSP70, Mn-SOD, and Cu,Zn-SOD. Twelve weeks of treadmill exercise training following CCO resulted in muscle mass-to-body mass ratio, HSP70, SOD, CAT, GPX, and nonenzymatic ASC that were higher than in sedentary swine with occluded left circumflex coronary arteries. Furthermore, exercise training reduced or prevented increases in muscle levels of TNF-α oxidative stress. These data indicate that coronary artery ischemia and mild infarct without deconditioning or heart failure impaired stress proteins, antioxidant capacity, and muscle mass. However, treadmill exercise training following left circumflex occlusion protected against CCO-induced alterations or promoted recovery of porcine muscle mass, HSP70, and antioxidant capacity.

Heart disease, and particularly heart failure, may result in reduction in muscle mass. Here, CCO reduced both muscle mass and muscle mass-to-body mass ratio, indicating that the onset of coronary occlusion can affect skeletal muscle morphology without frank heart failure or atherosclerosis. The ability of exercise to attenuate or reverse changes in muscle mass and muscle mass-to-body mass ratio caused by CCO indicates that peripheral responses of muscle morphology to exercise training are indeed possible following coronary ischemia and mild infarction.

Reduction in muscle mass and increased protein degradation have been linked to increases in elevated TNF-α (1, 3, 11). In the present study, changes in TNF-α mirror alterations in muscle mass. Exercise training significantly decreased TNF-α, while increasing muscle mass (Fig. 2A), indicating an inverse relationship. Adams et al. (3) found that the transcription factor NF-κB is stimulated by inflammatory cytokines and is associated with increased protein degradation. We found that coronary ischemia and exercise affected muscle TNF-α but not serum TNF-α. This indicates that TNF-α produced locally, but

Fig. 4. SOD activity in the triceps brachii muscle for total, Mn-SOD isoform, and Cu,Zn-SOD isoform (A) and Mn-SOD protein expression including a representative blot (B) from adult Yucatan swine. *SCO significantly different from sham group; *ECO significantly different from SCO group.

Fig. 5. Catalase (A), glutathione peroxidase (B), and antioxidant scavenging capacity (ASC; C) in the triceps brachii muscle from adult Yucatan swine. *SCO significantly different from sham group; *ECO significantly different from SCO group.
not humorally, may contribute to muscle dysfunction with our atherosclerotic model of chronic coronary ischemia. Gielen et al. (19) recently reported that exercise training reduced muscle TNF-α levels in heart failure patients. Our data suggest that severe coronary ischemia and mild infarct are a potent stimulus for elevated TNF-α protein expression, even without heart failure.

HSP70 is critical in protection against oxidative stress, ischemia, apoptosis, atrophy, and muscle dysfunction (33, 38, 46, 47). We found that HSP70 protein levels were significantly reduced in skeletal muscle as a result of CCO. To our knowledge, this is the first report of impaired HSP expression in muscle due to coronary ischemia. This finding is consistent with a reduction in muscle mass and impaired stress protection with disuse (38, 46). The marked elevation of muscle HSP70 protein levels by exercise training compared with the sedentary group implies higher levels of stress protection, which may confer resistance against apoptosis and ischemia-reperfusion injury (6, 38, 46, 59) underlying attenuation of reduced muscle mass (Fig. 1A). In addition, upregulation of HSP70 inhibits proinflammatory cytokines such as TNF-α (59), consistent with the current findings. HSP70 may indeed elicit protection against ischemia-reperfusion through its antioxidant ability (24), because addition of antioxidant administration protects against I/R injury while reducing exercise-induced HSP70 upregulation in skeletal muscle and heart (3, 45). Linke et al. (40) reported reduced apoptosis following exercise training in CHF patients.

In addition to reduced HSP70 levels, CCO also downregulated other cell protective proteins in skeletal muscle, specifically the antioxidant enzymes SOD and GPX. SOD catalyzes the removal of superoxide anion ($O_2^-)$ and production of hydrogen peroxide and is coupled with glutathione and CAT to remove hydrogen peroxide. Activities of both the mitochondrial Mn-SOD isoform and the cytosolic Cu,Zn-SOD isoform were reduced with coronary occlusion, indicating impaired ability to remove superoxide anions throughout muscle cells. In addition, failure to remove $O_2^-$ could increase oxidative stress (Fig. 2B) and also react with nitric oxide ($\cdot N\O$), producing peroxynitrite and attenuating the vasodilatory effects of NO (28). Thus impairment of both SOD and GPX would adversely affect antioxidant capacity. Similarly, impairment of SOD and glutathione activities in muscles from heart failure patients were recently reported by Linke et al. (40). Our data suggest that coronary ischemia, and possibly myocardial infarction, may account for a significant portion of the changes in skeletal muscle associated with heart failure.

Importantly, exercise training increased Mn-SOD and Cu,Zn isoforms of SOD, as well as GPX, to levels similar to animals who received sham surgery. Catalase activity also trended toward increased levels. Thus exercise training after application of CCO was able to upregulate antioxidant enzyme protection in skeletal muscle of swine that previously underwent CCO. In addition, nonenzymatic scavenging capacity of skeletal muscle also increased in response to exercise training, indicating broad antioxidant protection of exercise following application of coronary artery ischemia. While exercise has previously been shown to upregulate antioxidant enzymes and protection prior to ischemia (23, 59) and in heart failure patients (35), these are the first data to demonstrate that exercise protects or elevates antioxidant enzymes in an ischemia-infarct model, without heart failure or atherosclerosis as confounding factors.

Our data reinforce the potential for an imbalance of the antioxidant system to contribute to skeletal muscle pathology with heart disease and indicate that coronary ischemia itself can be an important contributor, independent of heart failure or atherosclerosis. The data also demonstrate the ability of exercise training to improve antioxidant protection against oxidative stress to normal levels following CCO. Indeed, 4-HNE adducts increased in skeletal muscle following CCO, whereas exercise training significantly reduced CCO-induced elevation of oxidative stress. This is consistent with additional antioxidant protection provided by exercise-induced upregulation of the stress proteins HSP70, Mn-SOD, Cu,Zn-SOD, GPX, and antioxidant substrate scavengers found in our study (Figs. 3–5).

Exercise effects on skeletal muscle and heart are proposed to be similar to that provided by “preconditioning” before prolonged ischemia-reperfusion (7, 24, 72). Exercise transiently increases production of ROS and cytokines, which, in turn, promote upregulation of protective HSPs and antioxidant enzymes (50, 72). Elevation of cell-protective pathways could be a common mechanism of protection for exercise and preconditioning (72). Preconditioning leads to elevated stress proteins, including HSPs and antioxidant enzymes, and thus results in reduced oxidative stress (50). Therefore, our results are consistent with the notion that exercise training contributes to acquisition of protection against skeletal muscle atrophy, dysfunction, and ischemia-related injury through upregulation of HSP70 and antioxidant capacity, not only before the onset of an ischemic event but also in patients following coronary artery ischemia or a myocardial infarction (39, 60).

Peripheral adaptations in response to exercise training correspond with improved maximal aerobic capacity, work tolerance, and positive clinical outcomes in coronary patients (20, 62, 63). The vast majority of the improvement in maximal aerobic capacity ($V_{O2,\text{max}}$) in cardiac rehabilitation patients is due to peripheral, not central factors (11, 20, 62). Indeed, skeletal muscle wasting, weakness, and fatigue are critical contributors to poor exercise capacity with heart disease and heart failure (14, 20). Our findings suggest that mechanisms by which exercise training may protect skeletal muscle mass and function following CCO would include protection or upregulation of stress proteins (HSP70, Mn-SOD, Cu,Zn-SOD, GPX), concomitant with a reduction in skeletal muscle TNF-α and oxidative stress (Figs. 1–5, Ref. 1).

Cardiac rehabilitation involving exercise for the majority of heart disease patients occurs after symptoms of coronary artery ischemia are expressed (e.g., angina, dyspnea, myocardial infarction) or coronary artery ischemia is detected as a result of screening tests. Thus the ability of exercise to contribute to protection and/or recovery of impairment of HSP70 and antioxidant enzymes, as well as elevation of TNF-α has significant clinical relevance to CAD patients, particularly as exercise-induced alterations were paralleled by changes in forearm limb muscle mass. Our findings illustrate and reinforce the importance of exercise-induced peripheral adaptations of patients suffering from ischemic CAD or myocardial infarction. While cause and effect could not be established by the present study, previous reports indicate that oxidative stress, inflammatory cytokines, and impaired stress proteins, such as HSPs and oxidative stress could contribute to skeletal muscle atrophy and...
impaired function with unloading, cancer, and sepsis (8, 10, 37, 38, 46). In addition, a series of papers in the human literature suggests that oxidative stress and muscle cachexia are alleviated by exercise training and linked to lower cytokine levels and increased antioxidant enzymes (40). However, future studies are needed to establish the causal relationship of HSPs with skeletal muscle atrophy and impaired contractility in the coronary artery ischemia schema.

The link between CCO or ischemia in the heart and changes in skeletal muscle function remains to be fully elucidated. A number of possibilities exist including 1) reduced net skeletal muscle blood flow, 2) disruption of skeletal muscle microcirculation, 3) increased oxidative/nitrosative stress, and 4) humoral factors including inflammatory cytokines (e.g., TNF-α). Cardiac output at rest is not impaired with coronary ischemic patients except for severe CHF (22). There is also a very poor relationship between net muscle blood flow and skeletal muscle dysfunction with cardiac ischemic and failure models (63, 70). However, local vascular reactivity, control, capillarization, and poor heterogeneous matching of blood flow and oxygen uptake are reported to be impaired (16) and could contribute to microischemia, muscle weakness, and fatigue (71). Humoral oxidative stress and cytokine levels increase with ischemic heart disease and heart failure. In our study, plasma TNF-α levels were unchanged, whereas skeletal muscle TNF-α increased as a result of chronic CCO. Skeletal muscle oxidative stress markers increased with CCO. In contrast, exercise training reduced skeletal muscle levels of TNF-α and oxidative stress. It is possible that plasma TNF-α levels could spike shortly after surgery to a greater extent with CCO than in the sham control groups. More research is needed to explain the upstream link between coronary artery ischemia and skeletal muscle dysfunction. Unfortunately, the mechanisms underlying skeletal muscle dysfunction with heart disease and heart failure remain poorly understood (29). In addition, it is not certain whether exercise training diminished TNF-α and oxidative stress while boosting antioxidant enzyme capacity and HSPs via upstream mechanisms occurring within myocytes, in muscle microcirculation, or in the circulating plasma.

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