Effects of endurance training on apoptotic susceptibility in striated muscle

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Vainshtein A, Kazak L, Hood DA. Effects of endurance training on apoptotic susceptibility in striated muscle. J Appl Physiol 110: 1638–1645, 2011. First published April 7, 2011; doi:10.1152/japplphysiol.00020.2011.—An increase in the production of reactive oxygen species occurs with muscle disuse, ischemic cardiomyopathy, and conditions that arise with senescence. The resulting oxidative stress is associated with apoptosis-related myopathies. Recent research has suggested that chronic exercise is protective against mitochondrially mediated programmed cell death. To further investigate this, we compared soleus (Sol) and cardiac muscles of voluntary wheel-trained (T; 10 wk) and untrained (C) animals. Training produced a 52% increase in muscle cytochrome c oxidase (COX) activity. Sol and left ventricle (LV) strips were isolated and incubated in vitro with H2O2 for 4 hr. Strips were then fractionated into cytosolic and mitochondrial fractions. Whole muscle apoptosis-inducing factor (AIF) and Bax/Bcl-2 levels were reduced in both the Sol and LV from T animals. H2O2 treatment induced increases in JNK phosphorylation, coflin-2 localization to the mitochondria, as well as cytosolic AIF in both Sol and LV of T and C animals, respectively. Mitochondrial Bax and cytosolic cytochrome c were augmented under oxidative stress in the LV only. The H2O2-induced increases in P-JNK, mitochondrial Bax, and cytosolic AIF were ablated in the LV of T animals. These data suggest that short-term oxidative stress can induce apoptotic signaling in striated muscles in vitro. In addition, training can attenuate oxidative stress-induced apoptotic signaling in a tissue-specific manner, with an effect that is most prominent in cardiac muscle.

Exercise; cell death; mitochondria; heart; skeletal muscle

Apoptosis has also been implicated in ischemic heart disease immune, and neurodegenerative diseases (5) to name a few. Apoptosis is triggered (40). Apoptosis is a tightly regulated form of programmed cell death, the aberrant regulation of which may lead to pathological conditions including tumorigenesis, auto-immune, and neurodegenerative diseases (5) to name a few. Apoptosis has also been implicated in ischemic heart disease and sarcopenia (29), both of which emerge with advanced age. Thus the increases in ROS observed with aging predispose cardiac and skeletal muscle myofibers to mitochondrially mediated apoptosis. To date, although these apoptotic signaling cascades have been studied extensively, some of the functions and interactions of the major participants in the apoptotic machinery remain poorly characterized. ROS-induced apoptotic events have been linked to the phosphorylation (P) of c-Jun-N-terminal kinase (JNK) and the recruitment of the structurally related Bcl-2 family of proteins to the mitochondria. These proteins function collectively to regulate organelle membrane integrity (4). Bcl-2-associated X protein (Bax) is a proapoptotic member of the Bcl-2 family that exists as a monomer in the cytosol. Upon an apoptotic stimulus, Bax undergoes a conformational change that allows it to homo- or hetero-oligomerize and subsequently translocate and insert into the mitochondrial membrane (2, 8, 10). This step is believed to be critical, but not exclusive, for mitochondrial premeabilization and the formation of the mitochondrial permeability transition pore (mtPTP; Refs. 1, 2, 5, 8). In addition, the actin binding protein coflin-2 was recently found to translocate into the mitochondria and help facilitate membrane permeabilization (21). The pathway by which this takes place, however, remains unknown. The opening of the mtPTP is rapidly followed by an efflux of apoptogenic factors such as cytochrome c and apoptosis inducing factor (AIF). These proteins can either directly or indirectly cause DNA fragmentation (2, 4, 5), resulting in the decay of the myonucleus, and ultimately in the demise of the myonuclear domain that it governs. In contrast to this, endurance-type physical activity has been associated with both the reduced accumulation and production of ROS, as well as with decreased levels of apoptosis (4). However, the mechanisms by which regular exercise mediates the interactions between ROS and apoptosis remain to be elucidated.

Thus the purposes of this study were 1) to utilize a whole muscle preparation for the investigation of apoptotic susceptibility of both skeletal and cardiac muscle in response to acute oxidative stress, and 2) to evaluate the effect of endurance training on apoptotic signaling in both of these muscle types. We hypothesized that training would produce phenotypic adaptations that would confer protection of these striated muscles against oxidative stress.

METHODS

Animals. Male Sprague-Dawley rats (n = 40; Charles River, St. Constant, QC, Canada) weighing between 130 and 160 g were individually housed and given food and water ad libitum. All proce-
dires involving animals were approved by the York University Animal Care Committee in accordance with the Canadian Council on Animal Care.

**Animal training.** The animals were randomly divided into trained (n = 20) or untrained (control, n = 20) groups. The trained animals had access to loaded voluntary running wheels. Animals were trained for 8–10 wk. For the first 2 wk, the wheels remained unloaded. Thereafter, 50 g of resistance was added on a weekly basis until week 5, where a maximal load of 200 g was placed on the wheels (weeks 6–10). This load was maintained until week 10 of training, as done previously (9). This was done to obtain continuous training adaptations as the animals habituated to the running. A training effect was determined by measuring the epididymal fat weight, heart weight-to-body weight ratios, as well as cytochrome c oxidase (COX) activity.

**Tissue extraction.** Animals were taken 24 h past the immobilization of the running wheel to avoid any acute exercise effects. The animals were anesthetized with an intraperitoneal injection of ketamine and xylazine (40 mg/ml ketamine; 5 mg/ml xylazine) at a dose of 0.2 ml/100 g body weight. Both epididymal fat pads, one soleus (Sol) muscle and a portion of the left ventricle (LV), were removed and immediately frozen in liquid nitrogen. The Sol and LV sections were later used to determine COX activity and whole muscle apoptotic protein expression.

**Table 1. Characteristics of control and trained animals**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Trained</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g (n = 19/20)</td>
<td>540.50 ± 16.86</td>
<td>527.60 ± 14.39</td>
</tr>
<tr>
<td>Heart weight-to-body weight ratio, mg/g (n = 19/20)</td>
<td>2.34 ± 0.04</td>
<td>2.60 ± 0.04*</td>
</tr>
<tr>
<td>Soleus weight-to-body weight ratio, mg/g (n = 19/20)</td>
<td>0.42 ± 0.01</td>
<td>0.47 ± 0.02*</td>
</tr>
<tr>
<td>Epididymal fat-to-body weight ratio, mg/g (n = 8)</td>
<td>15.19 ± 1.52</td>
<td>10.40 ± 1.54*</td>
</tr>
<tr>
<td>Food intake, g/day (n = 39/35)</td>
<td>25.89 ± 0.718</td>
<td>30.10 ± 0.84*</td>
</tr>
<tr>
<td>Water intake, ml/day (n = 37/29)</td>
<td>35.32 ± 1.835</td>
<td>60.52 ± 4.65*</td>
</tr>
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All values are means ± SE; n, no. of experiments. *P < 0.05, control vs. trained animals.

**Fig. 1. Running performance and training effects.** Trained animals were exercised with no load for the first 2 wk and then loaded incrementally by 50 g (week 2), 100 g (week 3), and 200 g (weeks 5–10), as indicated by the arrows. Average daily running distance for each week of running (A); work done, average per day at each week of training (B; n = 20); and cytochrome c oxidase (COX) activity (C) in soleus muscle in trained and untrained animals. Values are means ± SE (n = 23, control; n = 24, trained). *P < 0.05, trained vs. control.

**Fig. 2. Effects of training on soleus muscle apoptotic protein expression.** Typical Western blot and quantification of multiple samples of cytochrome c (A; Cyto C), cofilin-2 (B), Bax-to-Bcl-2 ratio (C), and apoptosis-inducing factor (AIF) protein levels (D) between control (C) and trained (T) animals. E: typical Western blot for manganese superoxide dismutase (MnSOD) protein expression. Aciculin was used as a loading control. Values are means ± SE (n = 6–10). *P < 0.05, trained vs. control.
Isolated muscle preparation. Muscle strips (~20 mg each) were removed from the Sol and LV. Strips of this size can be adequately oxygenated and have consistently been shown to remain viable when incubated in vitro (6). The Sol and LV muscles were strategically chosen to represent muscle of varying oxidative capacity. After removal, the strips were incubated with either vehicle (double distilled water) or hydrogen peroxide (H$_2$O$_2$; 3 mM) for a 4-h period. H$_2$O$_2$ is a common ROS used to mimic oxidative stress conditions in muscle. The incubation was performed in vitro using glass scintillation vials containing 5 ml of warmed (30°C), pregressed (~20 min with 95% O$_2$:5% CO$_2$) Krebs-Henseleit buffer (118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, 1.2 mM MgSO$_4$, and 3.4 mM CaCl$_2$ pH 7.4) supplemented with 5 mM n-glucose, 2 mM sodium pyruvate, and 2 mU of insulin. The strips were continuously oxygenated with 95% O$_2$:5% CO$_2$ and kept at a constant temperature of 30°C for the duration of the incubation period.

Tissue fractionation. Following incubation, muscle strips were separated into mitochondrial and cytosolic fractions by way of differential centrifugation (36), with modifications. Briefly, after 4 h of incubation the muscle strips were minced, homogenized using a Teflon pestle and mortar, and suspended in mitochondrial isolation buffer (MIB; 250 mM sucrose, 20 mM HEPES, 10 mM KCl, 1.5 mM MgCl$_2$, 1 mM EDTA, and 1 mM EGTA pH 7.4) supplemented with a cocktail of protease inhibitors (1 mM PMSF, 10 μM leupeptin, 1.5 μM aprotinin, 1.5 μM pepstatin A, and 1 mM sodium orthovanadate). The homogenate was then centrifuged at 1,000 g for 10 min at 4°C to pellet the nuclei. The supernate was carefully removed and centrifuged at 16,000 g for 20 min at 4°C to pellet the mitochondria. The supernate was removed and spun again at 16,000 g for 20 min at 4°C to pellet any residual cytosol; the resulting supernate was considered to be enriched cytosolic fraction. The mitochondrial pellet was resuspended twice in a fourfold dilution of MIB and centrifuged at 16,000 g for 20 min at 4°C after each resuspension. The supernate was finally removed and the mitochondrial pellet was resuspended in a onefold dilution of MIB. Mitochondria were subsequently sonicated to yield the enriched mitochondrial fraction. Based on this separation, we inferred the translocation of various apoptotic proteins from one cellular location to another. Protein concentrations within the samples were determined using the Bradford method. The fractions were frozen at −20°C until further use.

Immunoblotting. Whole muscle protein extracts or isolated mitochondrial and cytosolic fractions were subjected to SDS-PAGE. Proteins were then transferred onto nitrocellulose membranes using a wet electrotransfer technique. Nitrocellulose membranes were blocked in a 5% skim milk solution containing 1% TBST (Tris-buffered saline/Tween-20, 25 mM Tris, 1 mM NaCl, and 0.1% Tween-20, pH 7.5). Membranes were then incubated overnight at 4°C with the appropriate primary antibody (1:500 Bax; 1:5,000 AIF; 1:100 Bcl-2; 1:500 P-JNK; 1:1,000 cofilin-2; 1:750 cytochrome c; 1:4,000 COX IV; 1:1,000 manganese superoxide dismutase (MnSOD); 1:250 acilcin; or 1:20,000 GAPDH). Membranes were subsequently washed in TBST and then incubated with the appropriate secondary antibody coupled to horseradish peroxidase at room temperature for 60 min. Membranes were washed once more and finally visualized using an enhanced chemiluminescence kit. Films were scanned and quantified using SigmaScan Pro (version 5) software (Jandel Scientific, San Rafael, CA).

Mitochondrial content. COX enzyme activity was used as a measure of mitochondrial content. Whole muscle tissue was pulverized into fine powder in the presence of liquid nitrogen. This powder (10–20 mg) was then diluted 20-fold in muscle extraction buffer (0.1 M KH$_2$PO$_4$, 2 mM EDTA pH 7.2) and sonicated on ice (3 cycles of 3 s at 30% intensity each) to disrupt cellular membranes. After centrifugation, the supernatant fractions were recovered as previously described (10). Enzyme activity was determined by the maximal oxidation rate of completely reduced cytochrome c, evaluated as a change in absorbance at 550 nm using a Synergy HT microplate reader. The data were compiled using KC4 software.

Cell death ELISA. DNA fragmentation levels were determined in cytosolic extracts from fractionated muscle strips, using ELISA kit (Cell Death Detection ELISA kit; Roche Diagnostics, Manheim, Germany) modified from the manufacturer’s protocol. Briefly, cytosolic fractions (20 μg protein) were diluted 4-fold (vol/vol) in Immunoreagent as recommended by the manufacturer. The samples were subsequently pipetted on to streptavidin-coated microplates for 2 h at room temperature under vigorous shaking (300 rpm). After being washed, the nonbound antibody was removed and 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) was added to

![Fig. 3. Effects of training on H$_2$O$_2$-induced apoptotic signaling in soleus muscle. Apoptotic signaling was determined by protein expression in soleus muscle treated with either vehicle (V) or 3 mM H$_2$O$_2$ (H). Typical Western blot and quantification of multiple samples of cytosolic P-JNK (A), mitochondrial cofilin-2 (B), mitochondrial Bax (C), cytosolic AIF (D), cytosolic cytochrome c (E) between control (C) and trained (T) animals. Aiculin and GAPDH served as cytosolic fraction loading controls, while COXIV was used as mitochondrial fraction loading control. Values are means ± SE (n = 4–10). *P < 0.05, H$_2$O$_2$ vs. vehicle treated.](http://jap.physiology.org/content/japl.111.091004/f1)
produce a color change, dependent on the amount of fragmented DNA present. The extent of DNA fragmentation was quantified photometrically at a light density of 405 nm, using a Synergy HT microplate reader.

Statistical analyses. Data are expressed as means ± SE. Comparisons between trained and untrained animals in two panel figures were done using unpaired student’s t-test. Comparisons between trained and untrained animals, and between Vehicle and H2O2 treatments in RESULTS (see Figs. 2–5) were made using paired (Veh and H2O2) two-way analyses of variance followed by Bonferroni post hoc test. Statistical differences were considered significant at P < 0.05. All statistical analyses were carried out using Graph Pad Prism 4.0. Number of experiments (n = x) varies from experiment to experiment due to the small amount of protein available per sample.

RESULTS

Animal running behavior, COX activity, and general characteristics. Animals progressively increased their running distance, and work performed, over the first 3 wk of training. Following the third week, the plateaued and then declined, as the loads increased and the distance run decreased (Fig. 1, A and B). This behavior appears to be typical during loaded wheel running, as shown previously (9). A training effect was determined using three indices. First, epididymal fat weight-to-body weight ratio was 32% lower (P < 0.05) in trained animals (Table 1). Second, the heart weight-to-body weight ratio was 11% higher in the trained animals (P < 0.05), typical of a modest training-induced cardiac hypertrophy. Third, COX activity was also measured, to provide an assessment of whole muscle mitochondrial content. Compared with the control animals, COX activity was 52% higher in the Sol muscle of trained animals (P < 0.05; Fig. 1C).

Training resulted in reduced basal apoptotic protein expression but not stress-induced apoptotic signaling in Sol. As expected, training resulted in a 49% (P < 0.05) increase in Sol cytochrome c expression (Fig. 2A), directly mirroring the observed increase in COX activity (Fig. 1C). On the other hand, no change in the expression of the actin-binding protein cofilin-2 was observed (Fig. 2B). Endurance training also resulted in a 40% decrease (P < 0.05) in the Bax-to-Bcl-2 ratio, an indicator of apoptotic susceptibility, as well as a 34% reduction in whole muscle AIF level (P < 0.05; Fig. 2, C and D). The protein expression of the antioxidant enzyme MnSOD was not significantly altered in the Sol muscle of trained, compared with control, animals (Fig. 2E).

In response to H2O2, JNK phosphorylation was elevated by 18–30% (P < 0.05), and an approximate twofold increase in cofilin-2 localization to the mitochondria (P < 0.05) was observed in both control and trained animals (Fig. 3A and B). In contrast, H2O2 treatment did not cause measurable Bax translocation nor did it increase cytosolic cytochrome c expression in either of the two groups (Fig. 3, C and E). However, AIF localization to the cytosol was elevated by 58% (P < 0.05) in controls, but only by 31% (P < 0.05) in trained animals (Fig. 3D) as a result of oxidative stress. Thus, exercise training resulted in some protein-specific adaptations favoring muscle cell survival.

Training resulted in a decrease in both basal apoptotic protein expression and stress-induced apoptotic signaling in cardiac muscle. Endurance training had no effect on cytochrome c or cofilin-2 expression in cardiac muscle (Fig. 4, A and B). However, a 50% decrease (P < 0.05; Fig. 4C) in the Bax-to-Bcl-2 ratio, as well as a 32% decrease in AIF levels (P < 0.05; Fig. 4D) were observed following training. In contrast, no change in the expression of MnSOD was found following exercise training (Fig. 4E). Upon the induction of apoptotic signaling with H2O2 treatment, JNK phosphorylation rose by 76% (P < 0.05) in control animals and only by 58% (P < 0.05) in trained animals (Fig. 5A). JNK phosphorylation was significantly attenuated with exercise training both basally, and in response to oxidative stress (P < 0.05). Moreover, treatment with H2O2 resulted in 128% and 68% increases in cofilin-2 translocation to the mitochondria of control and trained animals, respectively (P < 0.05; Fig. 5B). H2O2 treatment also provoked a 59% increase in mitochondrial Bax and a 39% increase in cytosolic AIF in control animals only (P < 0.05; Fig. 5, C and D). Thus exercise training successfully attenuated both Bax translocation and AIF release in cardiac muscle (Fig. 5, C and D). Interestingly, the H2O2-induced increase in cytosolic cytochrome c was not reduced by training and was 42–50% higher in cardiac muscle from both trained and control animals in response to H2O2 (P < 0.05; Fig. 5E). Thus training
resulted in cardiac muscle that is protected from basal and oxidative stress-induced apoptotic signaling.

**DNA fragmentation.** Given the increases in apoptotic signaling observed with H2O2 treatment, and the attenuation of these events with training, we further investigated whether this protective effect was evident at the level of DNA fragmentation, the most distal step in the apoptotic signaling pathway. Our results revealed that the levels of DNA fragmentation were not different in either heart or Sol muscles in response to training, or in response to 4 h of treatment with H2O2 (Fig. 6, A and B).

**DISCUSSION**

The results of this study suggest that exercise training evokes cardiac and skeletal muscle adaptations that favor cellular survival over apoptotic cell death. Under basal conditions, voluntary wheel running resulted in decreased expression of proapoptotic factors in both muscle types, which is indicative of reduced apoptotic potential. Training also resulted in cardiac and skeletal muscles that were less susceptible to oxidative stress-induced apoptotic signaling in the absence of notable changes in antioxidant enzyme protein levels. This beneficial adaptation was most prominent in cardiac muscle.

There is an increasing body of evidence suggesting that apoptosis may play a vital role in muscular atrophy, as well as in geriatric-related disorders. This type of programmed cell death has been implicated in disuse-related atrophy (3, 11, 36, 39), sarcopenia (7, 10, 13, 14), as well as in ischemic cardiomyopathy (16, 17, 22, 24, 25, 30–32). Moreover, these conditions are often accompanied by increased levels of oxidative stress. On the other hand, regular physical activity has been demonstrated to suppress ROS production (1, 35), increase antioxidant defense systems (20, 38), as well as alleviate some of the asthenia and fatigability that often accompany muscular atrophy (10, 15, 20). With this in mind, we set out to investigate whether exercise training could attenuate H2O2-induced apoptotic susceptibility in cardiac and skeletal muscles.

Our progressively loaded voluntary wheel running protocol produced typical muscle and whole body training effects within 8–10 wk, as is indicated by an increase in mitochondrial content, reduction in epididymal fat stores, as well as a mild
cardiac hypertrophy. Training also resulted in reduced basal proapoptotic protein expression. Accordingly, AIF and Bax-to-Bcl-2 ratios were both reduced in the Sol and cardiac muscles. It is important to note that the execution of apoptosis is dependent on the interplay between pro- and antiapoptotic factors working simultaneously at any given time (27). Thus the ratio of Bax to Bcl-2 is a good indicator of apoptotic potential. This ratio was lower in trained animals, suggesting that training resulted in a muscle phenotype possessing a reduced apoptotic capacity. We then assessed some potential signaling events involved in mediating apoptosis in both skeletal and cardiac muscle.

To test the response of intact striated muscle to a death signal, we incubated muscle strips with supraphysiological level of H$_2$O$_2$ for a period of 4 h to mimic the severe induction of oxidative stress (1, 2, 20, 40). Under basal conditions, both the Bax-to-Bcl-2 ratio as well as total AIF levels were reduced by training. In response to oxidative stress, Bax localization to the mitochondria was increased. This may be due, in part, to the activation of cytosolic JNK, which is phosphorylated in the presence of cellular stressors, subsequently inhibits Bcl-2, and allows for Bax translocation to the mitochondria. Once within the organelle, Bax participates in the opening of the mtPTP, which is promptly followed by release of proapoptotic AIF and cytochrome c into the cytosol (12, 28, 32, 33, 40). Upon release, AIF and cytochrome c proceed to induce DNA fragmentation either directly or via a caspase cascade (12, 28, 32, 33, 40). Accordingly, AIF and cytochrome c localization to the cytosol was evident with H$_2$O$_2$ treatment. In response to training, the same level of oxidative stress resulted in lower JNK phosphorylation in the heart. This coincided with reduced Bax translocation to the mitochondria. In addition, AIF translocation observed with oxidative stress was diminished in the trained heart, further indicating suppressed apoptotic signaling. This reduction in cytosolic AIF may have been mediated by reduced levels of the AIF cleaving protease calpain, which has been found to decrease with chronic contractile activity in previous studies (15). Training-induced reductions in cell death signaling may be occurring as an attempt to spare the cardiomyocyte in response to cellular stressors. This is a favorable adaptation, as apoptotic environments have been strongly implicated in age-related cardiac pathophysiology (20, 23).

In contrast to the adaptations evident in cardiac muscle, endurance training appeared to have little effect on H$_2$O$_2$-induced apoptotic signaling in the Sol muscle. This occurred despite clear evidence of a mitochondrial augmentation to the exercise training regimen, as well as reduced basal proapoptotic protein expression. Although we did observe increases in JNK phosphorylation, cofilin-2 localization to the mitochondria, as well as increased cytosolic AIF with H$_2$O$_2$ treatment, these were unaltered by exercise training in Sol muscle. This may be partially explained by the strength of stimulus provided by the oxidative stress treatment. Perhaps a lower concentration of H$_2$O$_2$, adequate to evoke apoptotic signaling, but insufficient to mask the benefits of exercise observed in whole muscle, could have been employed. Alternatively, since heart and muscle adapt differentially to training, it is possible that adaptations in skeletal muscle would have been evident at an earlier time point within the training protocol. Nonetheless, it is apparent that skeletal muscle is less receptive to exercise training-induced apoptotic adaptations compared with the heart. This is consistent with previous studies (27, 37) where ventricular muscle adapted to a greater extent than skeletal muscle. This discrepancy in adaptation between the two striated muscle types could be due to 1) the differential loading of cardiac vs. skeletal muscle throughout the training period, and 2) the wide variation in inherent tissue mitochondrial content, as mitochondria are prime producers of ROS and contain antioxidant enzymes.

Curiously, cofilin-2, cytochrome c, and MnSOD behaved differently than originally hypothesized. Cofilin-2, an actin binding cytoskeletal protein, was recently associated with oxidative stress-induced apoptosis (21). It resides in the cytosol, but upon oxidation it translocates into the mitochondria where it is thought to help facilitate the opening of the mtPTP. In our study, H$_2$O$_2$ treatment resulted in cofilin-2 translocation to the mitochondria in both cardiac and skeletal muscles, supporting a role for cofilin-2 in apoptosis. However, the level of cofilin-2 was unaltered by training, suggesting that it does not participate in exercise-induced adaptations. In addition, we did not observe an increase in cytosolic cytochrome c with H$_2$O$_2$ treatment in the Sol muscle, but it was elevated in cardiac muscle. The reason for this differential response is not yet clear, but this may be due to an inherently greater mitochondrial content in cardiac muscle, thus leading to more detectable cytochrome c efflux in response to H$_2$O$_2$. Moreover, exercise

Fig. 6. Cell death. Cell death as measured by DNA fragmentation ELISA in the cytosolic fraction of control (C) and trained (T) animals treated with either vehicle (V) or 3 mM H$_2$O$_2$ (H) in soleus (A) and cardiac muscle (B). Values are means ± SE (n = 7–11). OD, optical density.
did not appear to alter cytochrome c localization in either tissue. This may not have been detectable in our model due to the increase in total cytochrome c expression observed with the exercise training. Additionally, no differences in MnSOD levels were found in heart or skeletal muscle following training. MnSOD is an enzyme responsible for catalyzing the dismutation of superoxide radicals into their less reactive form of oxygen and hydrogen peroxide. This enzyme is found in the mitochondria where it plays a key antioxidant role. The activity of this enzyme has previously been shown to increase in the heart following exercise (15), but we were unable to detect a change using immunoblotting techniques with the wheel running training program employed in this study. Our data are in accordance with previous work, where MnSOD protein levels remained constant following 7 days of chronic contractile activity (1, 26). Thus our results suggest that the reduced apoptotic susceptibility observed in trained muscles occurs in the absence of increases in antioxidant enzyme activity, assuming that MnSOD is representative of the expression of other enzymes, such as glutathione peroxidase. However, this remains to be determined.

DNA fragmentation levels were extremely low in both heart and skeletal muscle under steady-state conditions. Thus it was difficult to observe a difference in this measure between trained and control muscle. Furthermore, our incubation time with H2O2 was only 4 h, and while this is sufficient to induce apoptotic signaling, this treatment time was not long enough to elicit DNA fragmentation. Our results are in accordance with current literature, since 24–48 h of treatment with H2O2 are required to induce DNA fragmentation in cell culture (34, 40).

This study has revealed that 1) treatment with a high concentration of H2O2 in a whole muscle ex vivo preparation is sufficient for the activation of programmed cell death cascades, and 2) endurance training can attenuate oxidative stress-induced apoptotic signaling in a tissue-specific manner. Moreover, skeletal muscle appears to be more resistant to change using immunoblotting techniques with the wheel running training program employed in this study. Our data are in accordance with previous work, where MnSOD protein levels remained constant following 7 days of chronic contractile activity (1, 26). Thus our results suggest that the reduced apoptotic susceptibility observed in trained muscles occurs in the absence of increases in antioxidant enzyme activity, assuming that MnSOD is representative of the expression of other enzymes, such as glutathione peroxidase. However, this remains to be determined.

REFERENCES


