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Exercise Training Promotes SIRT1 Activity in Aged Rats

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ABSTRACT

The objective of this study was to determine the effects of aging and exercise training on SIRT1 activity and to identify a pathway linking SIRT1 to antioxidant response and cell cycle regulation in rats. SIRT1 is a NAD^+-dependent deacetylase involved in the oxidative stress response and aging. The effects of aging and of moderate and prolonged exercise training in rats are unknown. We measured SIRT1 activity in heart and adipose tissue of young (6 months old), sedentary old (24 months), and trained old (24 months) rats using an assay kit. Peroxidative damage was determined by measuring levels of thiobarbituric reactive substances (TBARS) and the protein-aldehyde adduct 4-hydroxynonenal (4-HNE). MnSOD, catalase, and FOXO3a levels were evaluated by Western blot, and GADD45a, cyclin D_2, and FOXO3a mRNA by RT-PCR. Aging significantly reduced SIRT1 activity in heart, but not in adipose tissue, increased TBARS and 4-HNE and decreased Mn-SOD and catalase expression in both heart and adipose tissue. Aging did not affect FOXO3a protein expression in the heart or FOXO3a mRNA in adipose tissue. Exercise training significantly increased FOXO3a protein in the heart and FOXO3a mRNA in adipose tissue of aged rats. It also significantly increased Mn-SOD and catalase levels in both heart and adipose tissue. The exercise-induced increase in SIRT1 activity in the heart caused a decrease in cyclin D_2 and an increase in GADD45a mRNA expression. There was a similar decrease in cyclin D_2, and no changes in GADD45a mRNA expression in adipose tissue. We concluded that exercise training, which significantly increases SIRT1 activity, could counteract age-related systems impairment.

INTRODUCTION

THE SIRTUINS (Sir) are a family of NAD^+-dependent histone/protein deacetylases that extend the life span in yeast and worms.[1] The *Saccharomyces cerevisiae* Sir2 protein plays a critical role in transcriptional silencing, genome stability, and longevity. The human homolog of Sir2, SIRT1, is the best characterized of mammalian sirtuins.[2]

SIRT1 is involved in many physiological functions, including control of gene expression, cell cycle regulation, apoptosis, DNA repair, metabolism, oxidative stress response, and aging.[3] SIRT1 also deacetylates non-histone proteins as p53, Forkhead transcription factors

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(FOXO), and nuclear factor κB.[4] FOXO3a has been shown to modulate various genes that control repair of damaged DNA (GADD45a), reactive oxygen species (ROS) detoxification (manganese superoxide dismutase [Mn-SOD] and catalase), cell cycle arrest (Cyclin D2), and apoptosis. In particular, it has been showed that SIRT1 and FOXO3a make a cellular complex in response to oxidative stress, and SIRT1 increases FOXO3a's ability to induce cell cycle arrest and DNA repair.[5]

The dependence of sirtuin activity on NAD^+ prompted the hypothesis that **it** is involved in metabolic signals and in particular in those induced by caloric restriction (CR). CR extends life span in many organisms from yeast[6] to rodents.[7] In mammals, CR delays the onset of age-associated diseases,[8] exerting this effect also by decreasing oxidative damage[9] and improving antioxidant system efficiency.[10]

In addition to CR, exercise training has showed ability to improve antioxidant system efficiency[11] and to be effective in the prevention of several cardiac and metabolic diseases. Clinical studies have shown that regular physical activity decreases cardiovascular morbidity and mortality in adults and elderly,[12] probably delaying the accumulation of cell damage and physiological dysfunction characteristic of the aging process.[13] Because antioxidant system efficiency declines with aging and exercise training effects have not been deeply investigated in old animals, we examined young and sedentary old rats to identify the aging-induced changes, and sedentary old and trained old rats to evaluate the ability of moderately prolonged exercise training to promote a recovery in the antioxidant system efficiency. In particular, in order to determine the molecular mechanisms underlining the beneficial exercise training effects on ROS scavenging protein expression, we examined the possible involvement of SIRT1 in old rats.

MATERIALS AND METHODS

Animals

Ten young (6 months old) and 24 old (24 months) male Wistar rats were divided into three groups: sedentary young (Y, $n = 10$); sedentary old (SO, $n = 12$); and trained old (TO, $n = 12$). Animals were housed at room temperature (22–28°C) with 12:12 h light/dark cycles and free access to food and water. The study protocol conformed to the Position of the American Heart Association on Research Animal Use by the AHA and was approved by the Ethics Committee for the Use of Animals in Research of the Second University of Naples (Italy).

Training protocol

All young and old rats were acclimated to the treadmill by walking at a speed of 10 m/min, 10 min/day, for 2 weeks on a Treadmill Control (Panlab Technology, 2Biological Instruments, Besozzo, Italy). After acclimatization, 12 old rats were randomly selected and assigned to training (45 min/day, 5 days/week, for 6 weeks). Training consisted of running at a speed of 30 m/min. Mild electrical stimulation (<1 mA) was used to make the rats run. The exercise training program (including acclimatization) lasted 8 weeks. Only 10 of the 12 trained old rats completed the 6-week exercise program; one died 2 weeks after training onset, and one was injured. Two of the 12 sedentary old rats died during the study.

Hemodynamic evaluation

Eight weeks after the study started, we assessed *in vivo* cardiac function using catheterization in rats anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg) and instrumented with a procedure described elsewhere for mice[14] that we adapted for rats. After endotracheal intubation, rats were connected to a rodent ventilator and a 2 F high-fidelity micromanometer catheter (Millar Instruments, Houston, TX) inserted into the right carotid artery and retrograde into the left ventricle. Hemodynamic measurements were recorded in 10 young, 10 sedentary, and 10 trained old rats. Ten sequential beats were averaged for each measurement.

Tissue processing and homogenate preparation

After hemodynamic measurements, the rats were decapitated and their hearts and adipose

tissue were immediately removed and rinsed free of blood. The left ventricle was separated, frozen in liquid nitrogen, and stored at −80°C until processing. The tissues were homogenized in a lysis buffer containing 10 mM Tris-HCl (pH 7.4); NP-40 0.5%; 250 mM sucrose; 0.1 mM EGTA; 10 mM NaCl; 15 mM $MgCl_2$; 1 mM PMSF; 1 μg/mL each of aprotinin, leupeptin, and pepstatin; 1 mM Na_3VO_4; and 1 mM NaF. After 1 h, the homogenate was obtained by centrifuging at 1400 rpm for 10 min at 4°C. Protein concentration was determined using a Bio-Rad assay (Milan, Italy).

Biochemical markers of lipid peroxidation

Peroxidative damage to cellular lipid constituents was determined by using the thiobarbituric acid (TBARS) method.[15] Portions were homogenized (10% wt/vol) with a motor-driven glass tissue homogenizer in 1.15% KCl. To 200 μL of homogenate, we added 200 μL of SDS 8.1%, 1.5 mL of acetic acid 20%, and 1.5 mL of tiobarbituric acid 0.8%. After heating at 95°C for 60 min, the absorbance was measured at 550 nm with 1,1,3,3-tetramethoxyprophane as standard, and results were expressed in nmol/μg of protein in tissue homogenate.[16] Protein-aldehyde adducts were determined in homogenates as fluorescence exhibited by interaction between the amino acid residues of protein and 4-hydroxynonenal (4-HNE) at 355/460 nm excitation/emission, respectively, and results were expressed in arbitrary units (AU).

Isolation and extraction of nuclei for SIRT1 deacetylase assay

Aliquots of heart and adipose tissue homogenate (without protease inhibitors) were spun through 4 mL of sucrose 30%, 10 mM Tris HCl (pH 7.5), 10 mM NaCl, and 3 mM $MgCl_2$ at 1,300× *g* for 10 min at 4°C; the pellet was washed with cold 10 mM Tris-HCl (pH 7.5) and 10 mM NaCl. The nuclei were suspended in 50-100 μL of extraction buffer containing 50 mM Hepes KOH (pH 7.5), 420 mM NaCl, 0.5 mM EDTA Na_2, 0.1 mM EGTA, and glycerol 10%, sonicated for 30 s, and stood on ice for 30 min. After centrifugation at 13000 rpm for 10 min, an aliquot of the supernatant (crude extract nuclear) was used to determine protein concentration using a Bio-Rad assay.

SIRT1 deacetylase assay

SIRT1 deacetylase activity was evaluated in crude nuclear extract from heart and adipose tissues of young, sedentary old, and trained old rats. We measured SIRT1 using a deacetylase fluorometric assay kit (Sir2 Assay Kit, CycLex, Ina, Nagano, Japan). The final reaction mixture (100 μL) contained 50 mM Tris-HCl (pH 8.8), 4 mM $MgCl_2$, 0.5 mM DTT, 0.25 mA/mL Lysyl endopeptidase, 1 μM Trichostatin A, 200 μM NAD, and 5 μL of crude extract nuclear sample. The fluorescence intensity at 440 nm (exc. 340 nm) was measured every 30 s for a total of 60 min immediately after the addition of fluorosubstrate peptide (20 μM final concentration) and normalized by protein concentration. All determinations were performed in triplicate on 10 different samples and the results are reported as relative fluorescence/μg of protein (AU).

Western blot analysis

Equal amounts of protein extract, 30 μg for heart homogenate and 100 μg for adipose tissue homogenate, were dissolved in Laemmli sample buffer, boiled for 5 min, subjected to SDS-PAGE (10% polyacrylamide), and then transferred to nitrocellulose membranes. FOXO3a was blocked with 5% non-fat milk dissolved in TBS for 1 h. Membranes were incubated overnight with a polyclonal antibody against FOXO3a protein (1:1000, Upstate, Lake Placid, NY). After further washing in 0.05% TTBS, a conjugated goat anti-rabbit polyclonal IgG HRP was used as a secondary antibody for FOXO3a. For Mn-SOD determination, the blots were blocked for 1 h with 3% non-fat dry milk reagent dissolved in PBS. Membranes were incubated overnight with anti Mn-SOD polyclonal antibody (1:1000, Upstate). After further washing in PBS, a goat anti-rabbit was used as secondary polyclonal antibody. SIRT1 and catalase were blocked with 5% non-fat milk dissolved in TTBS (1 x Tris buffered saline with 0.05% Tween-20) for 1 h. Membranes were incubated with a polyclonal antibody against SIRT1 protein (1:1000, Upstate) or with a monoclonal antibody against catalase protein

(1:1000, Sigma, Milan, Italy) overnight. After further washing in 0.05% TTBS, a conjugated goat anti-rabbit polyclonal IgG HRP and a conjugated goat anti-mouse monoclonal IgG HRP served as secondary antibody for SIRT1 and catalase, respectively. Anti-actin polyclonal antibody (Sigma) was used as internal standard. The blots were visualized with Supersignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL) and autoradiographed. Protein levels were quantified by scanning densitometry (Quantity One software, Gel Doc-2000, Bio-Rad) and the results are shown as AU.

RNA preparation and RT-PCR analysis

RNA was extracted from fresh-frozen heart tissue using Trizol Reagent (Invitrogen Life Technologies, Strathclyde, UK) and reverse transcribed into cDNA using a Super Script First-Strand Kit (Invitrogen). One μL of cDNA was used in a total 50 μL PCR mix containing 10 mM Tris-HCl (pH 8.3), 50 mm KCl, 2 mM $MgCl_2$, 10 pmol of each primer, 200 μM deoxynucleotide triphosphate, and 1 U of Taq polymerase. The primer sets used to amplify Gadd45a cDNA were: forward 5′-catttcaccctcattcgtgc-3′ and reverse 5′-acttaaggcaggatccttcc-3′. The primer sets used to amplify cyclinD_2 cDNA were: forward 5′-tcaagtttgccatgtacccg-3′ and reverse 5′-ctgcttaacagccttcagca-3′. The RT-PCR procedure was standardized by actin primers: forward 5′-gctcttttccagccttcctt-3′ and reverse 5′-gtgctaggagccagagcagt-3′. After initial denaturation for 5 min at 94°C, amplification was carried out for 30 cycles (30 s 94°C, 30 s 58°C, 30 s 72°C). The PCR products were separated on 2% agarose gel and visualized with ethidium bromide staining. Densitometric evaluation of signal strengths in RT-PCR was performed with Quantity One software (Gel Doc-2000, Bio-Rad). The amount of cDNA gave signals that were divided by the corresponding signals of the loading control (actin) as previously described.[17,18]

Statistical analysis

All values are reported as means ± standard deviation (SD). A one-way ANOVA was performed to test separately the main effects of age and exercise training in young and in sedentary old and trained old rats. A p value of <0.05 was considered statistically significant. All data were analyzed with the SPSS 13.0 statistical software (SPSS, Chicago, IL).

RESULTS

Morphological and hemodynamic parameters

Table 1 shows morphological and hemodynamic parameters of the three groups of rats 8 weeks after the study's onset. Body and left ven-

Table 1. Morphological and Hemodynamic Parameters of Young, Sedentary and Trained Old Rats

		Old	
	Young	*Sedentary*	*Trained*
LVW/BW	1.73 ± 0.17	1.35 ± 0.05[a]	1.67 ± 0.27[b]
LVW (g)	0.70 ± 0.07	0.92 ± 0.07[c]	0.85 ± 0.12
BW (g)	407.5 ± 30	675 ± 64.55[d]	513.3 ± 51.5[e]
HR (bpm)	275 ± 34	261 ± 38	196 ± 25[f]
LVSP (mmHg)	124.7 ± 5.6	141 ± 9.1[a]	115.4 ± 8[e]
LV dP/dt max (mmHg/s × 10^3)	4.88 ± 0.46	5.29 ± 0.62	4.68 ± 0.39

Values are means ± SD on 10 animals for each group. BW, body weight; LVW, left ventricular wet weight; HR, heart rate; LVSP, left ventricular systolic pressure; LV dP/dt max, left ventricular dP/dt max.

[a] $p < 0.005$ between sedentary old and young rats.
[b] $p < 0.05$ between trained old and sedentary old rats.
[c] $p < 0.01$ between sedentary old and young rats.
[d] $p < 0.0005$ between sedentary old and young rats.
[e] $p < 0.0005$ between trained old and sedentary old rats.
[f] $p < 0.005$ between trained old and sedentary old rats.

tricular weights were significantly greater in sedentary old animals than in young rats ($p < 0.0005$). Trained aged rats were leaner than untrained aged rats ($p < 0.0005$). Accordingly, the left ventricle-to-body weight ratio was smaller in sedentary than in trained old animals ($p < 0.05$). Heart rate did not differ between young and sedentary old rats, whereas trained old rats had a significantly lower heart rate in sedentary old animals ($p < 0.005$). Left ventricular systolic pressure (LVSP) was significantly higher in sedentary old than in young rats ($p < 0.005$) and exercise training reduced LVSP values in old rats ($p < 0.0005$). There were no differences in left ventricle dP/dt max among the three groups of rats studied.

Biochemical markers of lipid peroxidation

TBARS and 4-HNE-protein adduct levels were significantly higher in the heart's left ventricles of sedentary old versus young rats (3.84 ± 0.68 vs. 1.46 ± 0.36, $p < 0.0005$, and 34.67 ± 3.06 vs. 20.67 ± 1.15, $p < 0.005$, respectively), indicating that the peroxidation reaction was enhanced by aging. Exercise training significantly reduced TBARS and 4-HNE-protein adduct levels (trained old, 2.64 ± 0.3, $p < 0.005$, and 22 ± 2, $p < 0.005$, respectively; Fig. 1A and B).

Similarly, TBARS and 4-HNE-protein adduct levels were significantly higher in the adipose tissue of sedentary old versus young rats (6.09 ± 0.60 vs. 1.75 ± 0.59, $p < 0.0001$, and 171.7 ± 10.4 vs. 94.67 ± 5.13, $p < 0.0005$, respectively). Exercise training significantly reduced TBARS and 4-HNE-protein adduct levels in old rats (4.04 ± 0.52, $p < 0.0005$, and 111.33 ± 3.21, $p < 0.001$) (Fig. 1C and D).

SIRT1 activity

Figure 2 shows the time course of SIRT1 activity and its assay at 45 min in heart and adi-

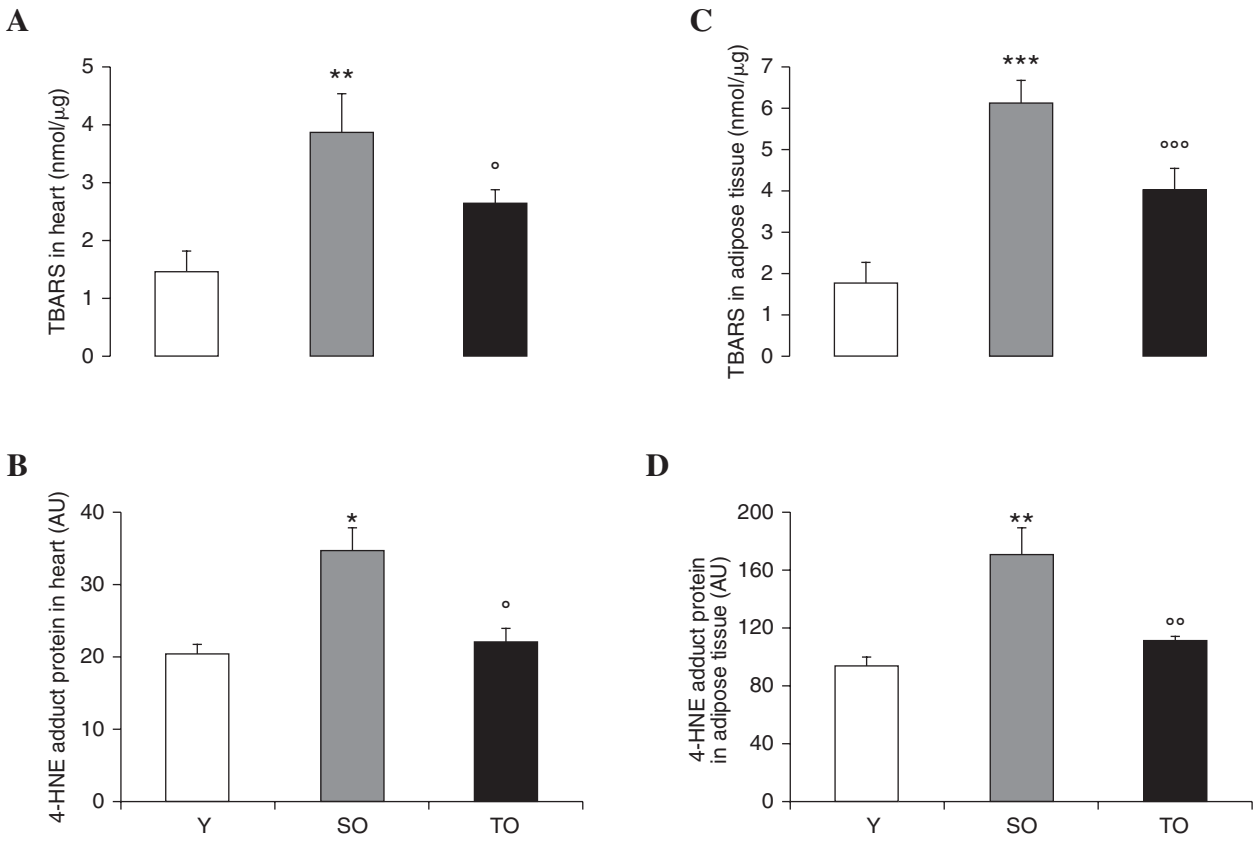

FIG. 1. Effects of age and exercise training on thiobarbituric reactive substances (TBARS) and 4-HNE adduct protein in the heart's left ventricle **(A and B)** and adipose tissue **(C and D).** Bars show mean values ± SD; $n = 10$ animals for each group; Y, young rats; SO, sedentary old rats; TO, trained old rats. *SO vs. Y, $p < 0.005$; **SO vs. Y, $p < 0.0005$; ***SO vs. Y, $p < 0.0001$; °TO vs. SO, $p < 0.005$; °°TO vs. SO, $p < 0.001$; °°°TO vs. SO, $p < 0.0001$.

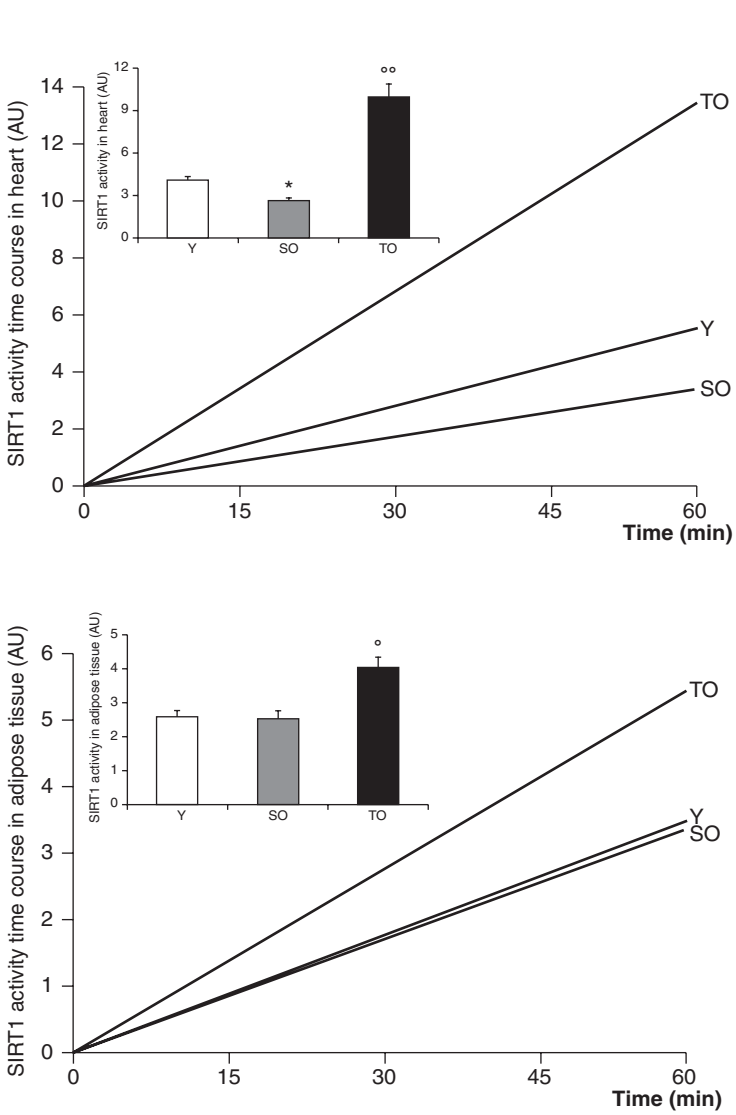

FIG. 2. Time course of SIRT1 deacetylation activity of heart and adipose crude nuclear extracts. Deacetylase activity was evaluated for 60 min with a fluorometric assay of crude nuclear extracts from heart **(A)** and adipose tissue **(B)** of young, sedentary old, and trained old rats. Each value was measured immediately after the fluorophore substrate addition and normalized by protein concentration. The time point of SIRT1 deacetylation assay of heart **(A, insert)** and adipose tissue **(B, insert).** Crude nuclear extracts are reported as mean of deacetylase activity at 45 min reactions, arresting the reaction by the addition of a stop solution. Y, young rats; SO, sedentary old rats; TO, trained old rats. *SO vs. Y, $p < 0.0001$; °TO vs. SO, $p < 0.005$; °°TO vs. SO, $p < 0.0005$.

pose tissue, respectively. Aging induced a decrease in SIRT1 activity in the heart (sedentary old vs. young, 2.5 ± 0.2 vs. 4.1 ± 0.17, $p < 0.0001$), but not in adipose tissue (sedentary old vs. young, 2.5 ± 0.3 vs. 2.6 ± 0.15). Exercise training resulted in a 4-fold increase of SIRT1 activity in both heart (trained old rats, 10 ± 1, $p < 0.0005$) and adipose tissue (trained old rats, 4 ± 0.25, $p < 0.005$).

FOXO3a and its target expression

Aging did not affect FOXO3a protein expression in the heart (sedentary old vs. young, 2.17 ± 0.17 vs. 1.59 ± 0.26) (Fig. 3A) and adipose tissue (sedentary old vs. young, 1.2 ± 0.18 vs. 1 ± 0.25) (Fig. 3B). Exercise training significantly increased FOXO3a expression in the heart (trained old versus sedentary old, 3.57 ± 0.35 vs. 2.17 ± 0.17, $p < 0.02$) (Fig. 3A) and adipose tissue (trained old versus sedentary old, 2.3 ± 0.34 vs. 1.2 ± 0.18, $p < 0.02$) (Fig. 3B).

RT-PCR analysis showed that aging did not affect cyclin D2 or GADD45a mRNA levels in either the heart or adipose tissue (Fig. 3, inserts). Differently, exercise training decreased cyclin D2 transcription in the left ventricle and adipose tissue. GADD45a mRNA levels tended to be higher in the heart's left ventricle of trained old rats compared with sedentary old rats, whereas GADD45a mRNA levels in adipose tissue did not differ between trained old and sedentary old rats (Fig. 3B, insert).

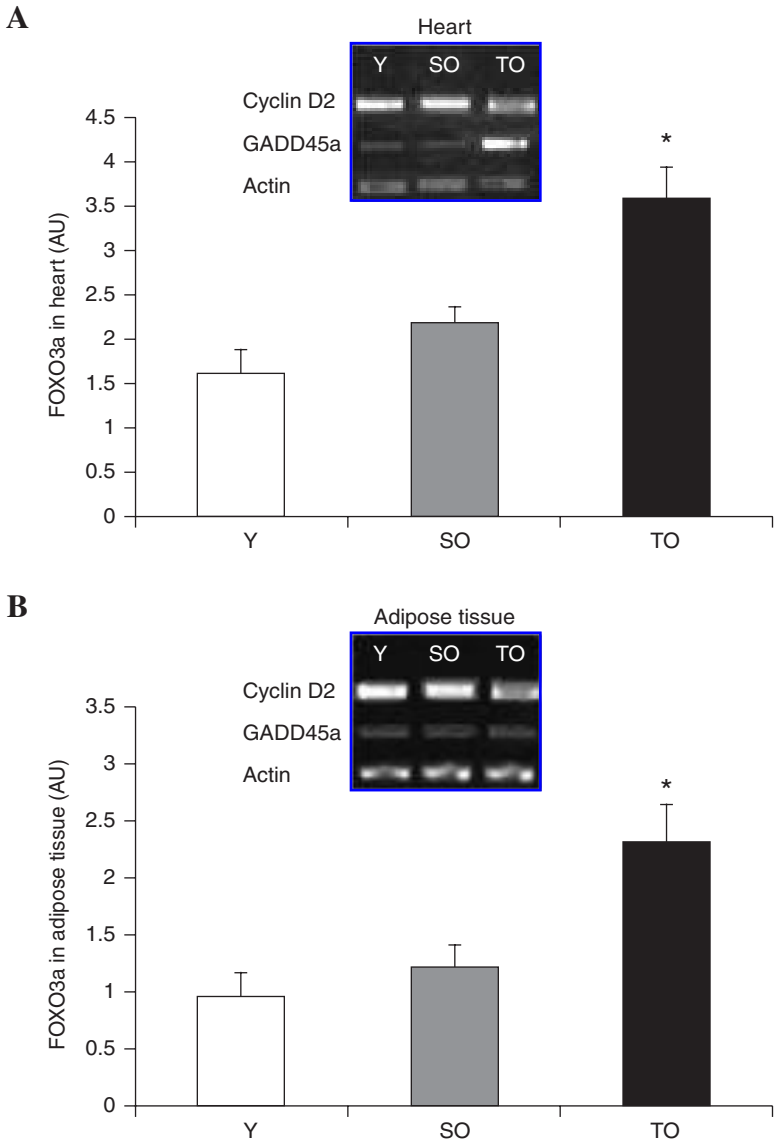

FIG. 3. Effects of age and exercise training for each group of young, sedentary old, and trained old rats on FOXO3a protein expression in heart **(A)** and adipose tissue **(B)** and cyclin D2 and GADD45a mRNA expression in heart (a representative RT-PCR is shown, **A**) and adipose tissue (a representative RT-PCR is shown, **B**). Bars show mean values ± SD; $n = 10$ animals for each group. Y, young rats; SO, sedentary old rats; TO, trained old rats. *TO vs. SO, $p < 0.02$.

Densitometric evaluation by RT-PCR showed that exercise training induced a significant increase in GADD45a mRNA expression in heart (trained old vs. sedentary old, 1.81 ± 0.10 vs. 1.21 ± 0.19, $p < 0.005$) (Fig. 4A) and a significant decrease in Cyclin D2 mRNA expression in heart (trained old vs. sedentary old, 0.31 ± 0.09 vs. 0.75 ± 0.09, $p < 0.005$) (Fig. 4B) and adipose tissue (trained old vs. sedentary old, 0.24 ± 0.1 vs. 0.61 ± 0.09, $p < 0.005$) (Fig. 4D). Exercise training partially increased Mn-SOD expression in the left ventricle in trained old rats in respect to sedentary old rats (3.1 ± 0.05 vs. 2.41 ± 0.03, $p < 0.01$; Fig. 5A), whereas aging did not affect Mn-SOD expression (young rats, 2.30 ± 0.21). Aging significantly affected Mn-SOD expression in adipose tissue as shown by the lower enzyme levels in sedentary old versus young rats (9.46 ± 0.99 vs. 18.38 ± 0.82, $p < 0.02$), whereas MN-SOD expression was significantly increased ($p < 0.005$) in trained old animals (23.19 ± 0.72) versus sedentary animals (Fig. 5B).

Aging significantly decreased catalase expression in the left ventricle (sedentary old vs. young, 3.23 ± 0.21 vs. 10.20 ± 0.70, $p < 0.0001$), whereas exercise training (trained old rats, 8.24 ± 0.63, $p < 0.0001$) induced a partial recovery of protein expression (Fig. 6A). In contrast, aging did not affect enzyme expression in adipose tissue (sedentary old vs. young rats, 12.65 ± 1.60 vs. 15.98 ± 1.06), whereas exercise

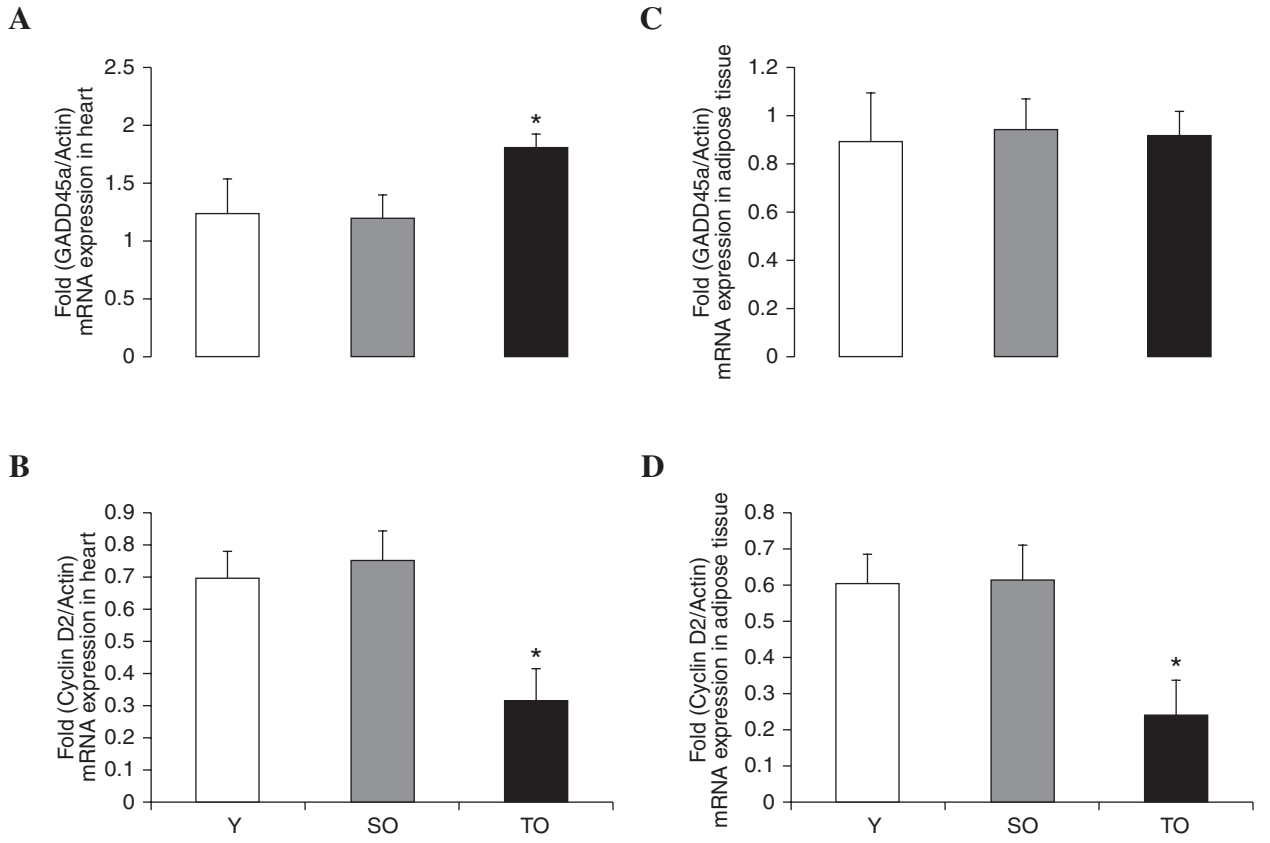

FIG. 4. Densitometric evaluation of GADD45a and Cyclin D2 signals in RT-PCR performed with Quantity One (Gel Doc-2000) in heart **(A and B)** and adipose tissue **(C and D).** Bar graphs show mean values ± SD ($n = 10$ animals for each group) of cDNA signals correct for the loading control (Actin). Y, young rats; SO, sedentary old rats; TO, trained old rats. *TO vs. SO, $p < 0.005$.

training significantly increased catalase expression in trained old vs. sedentary old rats (27.40 ± 1.38 vs. 12.65 ± 1.60, $p = 0.01$) (Fig. 6B).

DISCUSSION

There is a general agreement about the correlation between aging and accumulation of oxidatively damaged proteins, lipids, and nucleic acids.[19,20] The build up of ROS, responsible for oxidative stress generation, plays a crucial role in pathogenesis of several human diseases and in cellular and organism senescence, supporting the concept that protection from oxidative stress could significantly increase life span.[21]

In particular, it is well known that increased oxidative stress affects cardiac structure and function. In fact, the aging heart is characterized by functional changes, such as lengthening of contraction and relaxation times and thus a decrease in heart rate,[22] lessening in fractional shortening, and left ventricle end-systolic pressure.[23] Moreover, aging is also associated with an increasing body weight due to accumulation of adipose tissue.[24,25]

In our study, an 8-week training period was able to reduce body weight and modify left ventricle weight/body weight ratio, heart rate, and left ventricular systolic pressure (Table 1), confirming that the exercise training is able to induce significant structural and functional cardiac changes.

It has been suggested that exercise training and CR have similar effects. In particular, many authors have found relatively small differences in body composition within trained and food-restricted rats.[26] Moreover, both prolonged exercise training and CR have beneficial effects on antioxidant system. CR greatly increases the longevity of rodents, and it is also able to increase stress resistance, decrease age-related

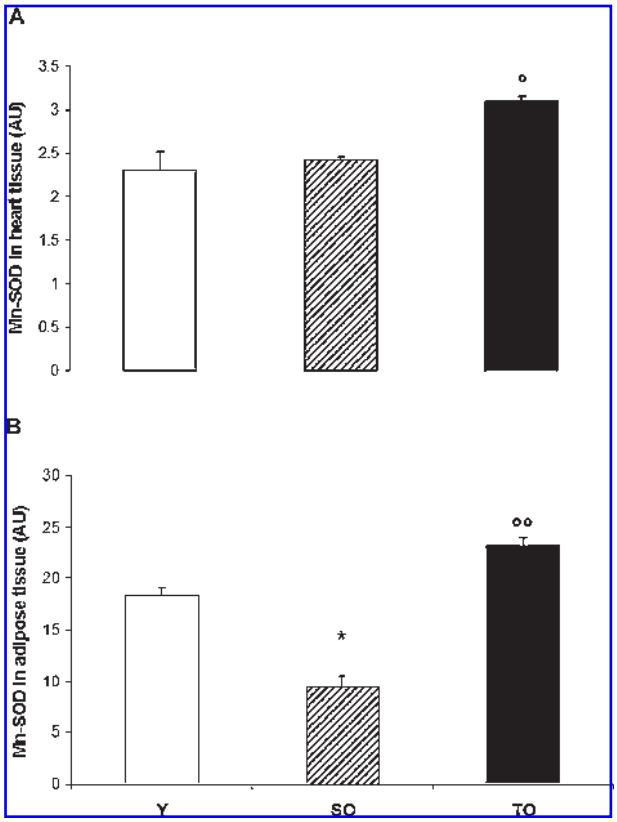

FIG. 5. Effects of age and exercise training on Mn-SOD in heart **(A)** and adipose tissue **(B)** with a representative Western blot of Mn-SOD expression for each group. Bars show mean values ± SD; $n = 10$ animals for each group. Y, young rats; SO, sedentary old rats; TO, trained old rats. *SO vs. Y, $p < 0.02$; °TO vs. SO, $p < 0.01$; °°TO vs. SO, $p < 0.005$.

ROS production, and reduce oxidative damage to lipids, proteins, and nucleic acids.[27]

Some authors have recently linked CR-mediated longevity extension to SIRT1, raising the possibility that this enzyme is a key regulatory factor in the organism's response to CR.[28] Furthermore, SIRT1 could have an important role are age-related metabolic diseases. In fact, it has also been implicated in glucose and fat metabolism, promoting fat mobilization from white adipocytes by repressing PPAR-γ, a gene involved in fat storage.[29] Despite the molecular mechanisms underlying CR's beneficial effects are becoming more clear, little is known about the molecular pathway involved in the exercise training antioxidant effects, especially in aged animals.

In a previous report we demonstrated that prolonged exercise training is able to partially counteract the loss of antioxidant defense in the aged heart.[11]

In the present study, we found an increase in TBARS and 4-HNE adduct protein levels and a decrease of antioxidant efficiency in both the heart and adipose tissue of aged rats, as shown by Mn-SOD and catalase expression levels. TBARS are a naturally occurring product of lipid peroxidation. It reacts with nucleic acid bases to form multiple adducts that are mutagenic and carcinogenic.[30] 4-HNE has been suggested to be a key mediator of oxidative stress-induced cell death[31,32] and also a mutagen, carcinogen, and apoptotic agent in many *in vivo* and *in vitro* systems.[33–36] An increased level of this molecule plays a critical role in the homeostasis of mitochondrial ROS and apoptosis, often resulting in compromised antioxidant defense and enhanced toxicity risk.[37–44]

We found that exercise training reduced TBARS and 4-HNE levels and induced an increase in SIRT1 activity in the heart and adi-

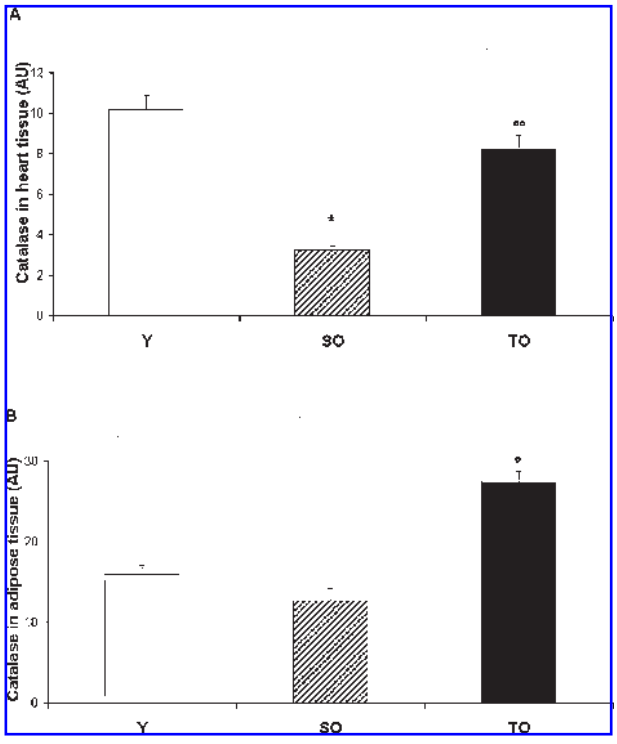

FIG. 6. Effects of age and exercise training on catalase in heart **(A)** and adipose tissue **(B)** with a representative Western blot of catalase expression for each group. Bars show mean values ± SD; $n = 10$ animals for each group. Y, young rats; SO, sedentary old rats; TO, trained old rats. *SO vs. Y, $p < 0.0001$; °TO vs. SO, $p = 0.01$; °°TO vs. SO, $p < 0.0001$.

pose tissue of aged rats, suggesting that chronic exercise, by inducing SIRT1 activity, exerts an antioxidant effect.

Brunet et al. demonstrated that in mammalian cells SIRT1 appears to control the cellular response to stress by regulating FOXO transcription factors,that function as sensors of the insulin signaling pathway and as regulators of longevity.[5] In particular, these authors showed that SIRT1 and FOXO3a form a complex in response to oxidative stress stimulus.[5] Mammalian FOXO factors control several biological functions, such as cell cycle arrest,[45] detoxification of ROS,[46] and repair of damaged DNA.[45] SIRT1 increased FOXO3a ability to induce cell cycle arrest and enhanced expression of a FOXO3a target involved in DNA repair, such as GADD45a.[5] FOXO3a has been demonstrated to mediate the SIRT1 effects during CR.[47] Moreover, Li et al. studied the effects of age in Mn-SOD regulation of vascular smooth muscle cells isolated from 24-month-old versus 6-month-old rats, showing that FOXO3a inhibition led to a reduction in Mn-SOD gene expression.[48]

Because it is known that exercise training exerts its beneficial effects particularly on the cardiovascular system, we tested FOXO3a and its targets involvement in the heart of aged trained rats, showing that exercise training enhanced FOXO3a protein expression. This was associated with a decrease in cyclin D2 and an increase in GADD45a mRNAs in the heart of aged rats.

In adipose tissue we similarly found an increase in FOXO3a protein expression and a decrease in cyclin D2 but no changes in GADD45a mRNAs. This finding could be related to higher oxidative stress in this tissue, as demonstrated by TBARS and 4-HNE levels (Fig. 1) that would induce the adipocytes to choose apoptosis or necrosis rather than repair as mechanism of detoxification.

In conclusion, the current study provides new insight into the potential mechanisms involved in the protective effects of exercise on aging-related diseases, particularly focusing on the role of SIRT1. Prolonged exercise training is a recognized tool for rehabilitation of cardiovascular patients, in particular after myocardial infarction, by improving cardiovascular functions and physical work capacity.[49] It is also effective for prevention and treatment of several conditions related to endothelial dysfunction by decelerating the aging processes on arterial wall and endothelial functions.[50]

Oxidative markers and antioxidants have been reported to decrease, increase, or even remain unchanged in response to exercise.[51] This discrepancy could result from the different method used for assays and from the exercise protocol.

In our study, an 8-week training period was able to increase antioxidant defenses in the aged rat heart and adipose tissue, underling the importance of choosing a specific exercise training protocol in order to obtain these beneficial effects.

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