Oxidative Stress Responses in Older Men during Endurance Training and Detraining

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ABSTRACT

FATOUROS, I. G., A. Z. JAMURTAS, V. VILLIOTOU, S. POUJIPOULOU, P. FOTINAKIS, K. TAXILDARIS, and G. DELICONSTANTINOS. Oxidative Stress Responses in Older Men during Endurance Training and Detraining. Med. Sci. Sports Exerc., Vol. 36, No. 12, pp. 2065–2072, 2004. Purpose: Aging is associated with increased oxidative stress, whereas systematic exercise training has been shown to improve quality of life and functional performance of the aged. This study aimed to evaluate responses of selected markers of oxidative stress and antioxidant status in inactive older men during endurance training and detraining. Methods: Nineteen older men (65–78 yr) were randomly assigned into either a control (C, N = 8) or an endurance-training (ET, N = 11) group. Before, immediately posttraining, and after 4 months of detraining, subjects performed a progressive diagnostic treadmill test to exhaustion (GXT). Plasma samples, collected before and immediately post-GXT, were analyzed for malondialdehyde (MDA) and 3-nitrotyrosine (3-NT) levels, total antioxidant capacity (TAC), and glutathione peroxidase activity (GPX). Results: ET caused a 40% increase in running time and a 20% increase in maximal oxygen consumption (VO2max) (P < 0.05). ET lowered MDA (9% at rest, P < 0.01; and 16% postexercise, P < 0.05) and 3-NT levels (20% postexercise, P < 0.05), whereas it increased TAC (6% at rest, P < 0.01; and 14% postexercise, P < 0.05) and GPX (12% postexercise, P < 0.05). However, detraining abolished these adaptations. Conclusions: ET may attenuate basal and exercise-induced lipid peroxidation and increase protection against oxidative stress by increasing TAC and GPX activity. However, training cessation may reverse these training-induced adaptations. Key Words: OXIDATIVE DAMAGE, ANTIOXIDANT CAPACITY, MDA, 3-NITROTYROSINE, CARDIOVASCULAR EXERCISE, AGING

Reactive oxygen and nitrogen species (ROS/NOS) such as superoxide (O2•−), hydrogen peroxide (H2O2), nitric oxide (NO), and hydroxyl radicals (HO•) generated by either increased oxygen consumption, cytokines, inflammation processes, and alterations in blood flow like ischemia reperfusion or other stressful conditions (trauma, inflammation, chronic diseases, etc.) have been implicated in aging development (21). The free radical theory of aging suggests that an accumulation of oxidative damage to DNA, proteins, and lipids takes place in aged tissues such as skeletal muscle (21). Aging has been associated with increased generation of reactive oxygen- and nitrogen-centered species, limited antioxidant capacity, decreased function of repair systems, and increased macromolecule oxidation (21).

Systematic exercise is believed to extend average life span by reducing the incidence of cardiovascular and other degenerative diseases while increasing functional performance in the aged (7,15). However, the mechanisms by which exercise produces its positive effects are unclear. Whole-body and active muscle oxygen consumption is increased 10–20 times above resting value during acute intense physical exercise (4). Acute endurance exercise causes significant ROS/NOS generation in several tissues (e.g., muscle, heart, and liver (4)), increases oxidative stress biomarkers (i.e., protein carbonyls and MDA) (9), and alters antioxidants’ and antioxidant enzymes’ levels in various tissues (6,9,14). It has been postulated that because endurance exercise causes an augmented generation of oxidants in muscle, systematic exercise training might upregulate muscle’s antioxidant defense system. Chronic exercise training has been suggested to induce positive adaptations to antioxidant defense systems (27). Recent evidence suggests that the amount of physical activity is associated with high antioxidant enzyme activity level, and that this association is intensity-dependent (3). Liu et al. (19) demonstrated that chronic exercise might induce a tissue-specific positive adaptation to oxidative stress development and antioxidant enzyme levels in rats. Radak et al. (24) also suggested that endurance exercise training exerts a beneficial effect in oxidative damage development independent of age. In fact, endurance exercise training has been shown to decrease
DNA damage, increase DNA repair in aged rat skeletal muscle (25), and upregulate erythrocyte antioxidant enzyme activities (15). In addition, 6-month resistance exercise training was shown to attenuate aerobic exercise-induced lipid peroxidation in the elderly (34). In contrast, other studies have shown no change (15) or a decrease in antioxidant enzyme activity in muscle with endurance training (18). Most of the studies conducted in this field used mostly younger individuals and various training protocols. Therefore, it is unclear whether systematic exercise training can alter oxidative stress status induced by ROS/RNOS and antioxidant function in the aged. Although a number of studies examined training-induced adaptations of oxidative stress and antioxidant status markers in young and older adults, there is a lack of information on adaptations caused by prolonged cessation of training stimulus (detraining).

Therefore, the objective of the present study was to investigate: a) whether a long-term endurance exercise training protocol was able to alter oxidative stress biomarkers as well as antioxidant status responses in aged individuals, and b) how cessation of training stimulus affects possible training-induced adaptation of oxidative stress development in the elderly.

METHODS

Subjects. Nineteen apparently healthy older men between 65 and 78 yr volunteered to participate in a 16-wk training study. A written informed consent was signed by all participants regarding their participation after being informed of all risks, discomforts and benefits involved in the study. Procedures were in accordance with the Helsinki Declaration of 1975, and institutional review board approval was received for this study. The physical characteristics of the subjects are shown in Table 1.

A two-group randomized repeated-measures design was followed. Subjects visited the laboratory three times at baseline. During their first visit, subjects were examined by a trained physician for limiting health complications, given a physical activity questionnaire to complete (36), and asked to sign an informed consent. In their second visit, subjects had their body height/weight and skinfolds measured, and underwent a progressive diagnostic treadmill test to exhaustion (GXT) to evaluate their maximal oxygen consumption (VO2max). Blood samples were collected before and immediately post-GXT. Thereafter, subjects were randomly assigned to one of two groups: control group (C, N = 8) and endurance-training group (ET, N = 11). Subjects in ET trained for 16 wk. After training, subjects underwent a 16-wk detraining period in which no training was performed. A GXT and blood sampling was repeated at the end of training and detraining.

Exclusionary criteria. Subjects’ participation was based upon the following criteria: 1) were available to participate in measurements for 32 wk; 2) were completely inactive before the study (VO2max below 20 mL·kg−1·min−1 and a score below 9 in the Modified Baecke Questionnaire for Older Adults were used as indices of physical inactivity) (Table 1) (1,36); 3) were free of musculoskeletal problems and potentially orthopaedic/neuromuscular limitations; 4) had a resting blood pressure below 160/100 mm Hg (subjects on antihypertensive medications (six men) maintained their medication throughout the study); 5) had no signs of cardiovascular/respiratory complications (at rest and during GXT); 6) reported no tobacco use within the 6 months before the study or during the training and detraining period, had normal dietary habits (diet recalls were administered throughout the study), did not consume aspirin (cyclo-oxygenase can affect oxidant/antioxidant status) or alcoholic beverages at least 1 wk before exercise testing, and were not consuming antioxidant compounds including vitamins, minerals, and medications (i.e., probucol, nebivolol, and anti-inflammatory agents).

Measurement of anthropometric variables. Subjects’ body weight was measured while they were wearing underclothes on a balance scale (Seca) calibrated to the nearest 0.1 kg after an 8-h fast. Barefoot standing height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. Subcutaneous skinfold thickness was measured sequentially, in triplicate, at the chest, biceps, triceps, subscapula, abdomen, ilium, calf, and thigh by the same investigator using a skinfold caliper (Harpenden, HSK-BI, British Indicators, UK) and standard technique (1). The average of three measures for each skinfold was used, and the sum of the eight skinfolds was used as an index of body fatness.

Measurement of maximal oxygen consumption (VO2max). VO2max was determined during GXT (walking or jogging) using a modified version of the Bruce protocol (1) before training, immediately posttraining, and after 4 months of detraining. A 12-lead ECG, heart rate, and bra-

### TABLE 1. Physical characteristics, physical activity, treadmill time to exhaustion, and aerobic capacity levels of the subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre (N = 8)</th>
<th>Post (N = 8)</th>
<th>Detraining (N = 5)</th>
<th>Pre (N = 11)</th>
<th>Post (N = 11)</th>
<th>Detraining (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity level*</td>
<td>8.01 ± 1.4 (low)</td>
<td>8.2 ± 1.1 (low)</td>
<td>8.14 ± 1.2 (low)</td>
<td>72.8 ± 4.8</td>
<td>72.1 ± 4.8</td>
<td>72.9 ± 4.2</td>
</tr>
<tr>
<td>(yr)</td>
<td>71.5 ± 6.5</td>
<td>71.0 ± 1.1</td>
<td>71.4 ± 9.8</td>
<td>72.8 ± 4.8</td>
<td>71.1 ± 4.4</td>
<td>73.5 ± 9.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 ± 1.1</td>
<td>70.9 ± 13.0</td>
<td>71.4 ± 9.8</td>
<td>73.5 ± 10.6</td>
<td>106.0 ± 9.2</td>
<td>104.0 ± 13.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.3 ± 11.1</td>
<td>74.2 ± 14.8</td>
<td>74.2 ± 12.3</td>
<td>142.2 ± 13.8</td>
<td>133.5 ± 10.5</td>
<td>140.5 ± 13.1</td>
</tr>
<tr>
<td>Sum of 8 skinfolds (mm)</td>
<td>143.2 ± 14.8</td>
<td>144.1 ± 16.1</td>
<td>142.9 ± 12.3</td>
<td>142.2 ± 13.8</td>
<td>133.5 ± 10.5</td>
<td>140.5 ± 13.1</td>
</tr>
<tr>
<td>Treadmill time (min)</td>
<td>8.6 ± 1.3</td>
<td>8.7 ± 1.4</td>
<td>8.2 ± 1.1</td>
<td>8.2 ± 0.8</td>
<td>11.9 ± 1.3</td>
<td>8.6 ± 0.9</td>
</tr>
<tr>
<td>VO2peak (mL·kg−1·min−1)</td>
<td>19.8 ± 2.9</td>
<td>20.2 ± 2.6</td>
<td>19.2 ± 1.7</td>
<td>20.5 ± 2.1</td>
<td>25.9 ± 2.8</td>
<td>21.1 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SD; C, control group; ET, endurance training group.

*Subjects’ physical activity level according to Baecke Questionnaire for Older Adults, VO2peak, peak oxygen consumption.

* P < 0.05 vs pre values.
chial artery cuff pressure were monitored continuously during the test and the recovery period. Ratings of perceived exertion (1) were determined at the end of each minute of exercise and recovery. A SensorMedics Vmax 29 pulmonary gas exchange system (Yorba Linda, CA) was used to evaluate the participants’ VO_{2max}. Oxygen uptake (VO_2) was measured continuously via breath-by-breath analysis with the use of a computerized system. To ascertain that VO_{2max} had been attained, the following three criteria had to be met: 1) no further increase in O_2 uptake with an increase in work rate (leveling-off criterion), 2) attainment of the age-predicted maximal heart rate, and 3) respiratory exchange ratio greater than 1.10 (7).

**Diet records.** To examine whether dietary changes influenced lipid peroxidation and antioxidant status outcomes (especially on TAC), 5-d diet recalls were completed before and during training and detraining (once every 2 wk). A trained diettian taught the subjects how to complete diet recall questionnaires and determine food serving and sizes. Diet records were analyzed using the computerized nutritional analysis system Science Fit Diet 200A (Sciencefit, Athens, Greece).

**Blood sampling.** Each subject reported to the laboratory at 7:00 a.m. after an overnight fast on three separate occasions (pretraining, postraining, and 4 months after training cessation) for blood sampling. Subjects abstained from alcohol and caffeine consumption for at least 24 h, and did not perform physical exercise for the last 48 h before testing. Peripheral blood samples were drawn with subjects in a seated position. Blood samples were obtained from the antecubital region of the arm by Vacutainers containing either SST Gel and Clot Activator, dipotassium ethylene diamine tetraacetic acid (EDTA), or heparin as anticoagulants to obtain serum and plasma samples and immediately were placed on ice. Blood samples were centrifuged (Beckman centrifuge, Fullerton, CA) at 4°C and 1500 × g for 15 min, and serum or plasma was obtained. These samples were stored frozen in multiple aliquots (~0.25 mL in Eppendorf microtubes) at −80°C until assayed. Samples were thawed only once before analysis.

**MDA measurement.** Plasma MDA was determined with the method developed by Londero et al. (20). MDA was obtained by acid hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) as previously described (20). HPLC separation of MDA-TBA adducts was performed with a spectrofluorometric detector set at 532 nm excitation and 553 nm emission. The mobile phase consisted of 40:60 (v/v) water:acetonitrile at a flow rate of 1 mL·min⁻¹. A photometric detector set at 340 nm was used for the separation (with a detection limit of 0.2 pmol per 30 µL injected). TBA and TMP were purchased from Sigma Chemical Co. (Poole). All samples were determined in duplicate, while inter- and intra-coefficients of variation were 4.8% and 3.8%, respectively.

**Measurement of total antioxidant capacity (TAC).** TAC was measured in duplicate in plasma by chemoluminescence using a luminometer (Burthold, Autoluminat, LB953, U.S.). This assay is based on the ability of antioxidants present in the plasma to inhibit the oxidation of 2,2’-azinobis (3-ethylbenzthiazoline) sulfonic acid (ABTS, Sigma) to the radical cation ABTS⁺ by a peroxidase (5). TAC was evaluated with a trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Aldrich) standard curve, and is expressed as trolox equivalent antioxidant capacity concentration (mmol·L⁻¹). All samples were determined in duplicate, while inter- and intra-coefficients of variation were 5.1% and 5.6%, respectively.

**Determination of glutathione peroxidase (GPX).** Whole-blood GPX activity (U·L⁻¹) was determined as previously described (3). GPX activity was measured in a Hitachi 2001 UV/VIS spectrophotometer (Hitachi Instruments Inc., U.S.) at 37°C. GPX activity was determined using cumene hydroperoxide as the oxidant of glutathione (Ransel RS 505, Randox, Crumlin, UK). All samples were determined in duplicate, while inter- and intra-coefficients of variation were 4.8% and 3.8%, respectively.

**Exercise training.** Subjects in the control group did not train, and participated only in the measurement procedures. Subjects in ET exercised 3× wk⁻¹ for 16 wk. Subjects always completed a 3–5 min warm-up, which consisted of light walking at approximately 40% of their maximal heart rate (attained during the exercise test), before starting their training routine. The maximum duration of each training session never exceeded 60 min, and training sessions were always supervised. The cardiovascular training protocol consisted of walking/jogging on a treadmill (Startrac TR 3500, Tustin, CA) as previously described (7). Briefly, subjects walked/jogged at 50–80% of HR_{max} (0% grade) for 12–42 min each time (duration increased 2 min every week). Blood arterial pressure and heart rate were monitored.

**Detraining.** After the completion of the training period, a number of subjects (N = 7) of the exercise group returned to their pretraining physical activity levels becoming completely inactive (Table 1), whereas four of the subjects in this group continued with their training regimens, and were excluded from detraining measurements.

**Statistical analyses.** Data were analyzed using the SPSS PC program for Windows. Values reported are means ± SD. One-way (grouping was the only factor) ANOVA was conducted initially to examine whether there were differences between groups in the premeasurement values of each dependent variable. MANOVA repeated measures (3 × 2, time by treatment) were performed on each dependent variable.
variable to detect differences in each group for each time point. When $F$-ratios were significant, post hoc comparisons of means were analyzed with Scheffé’s multiple comparison tests. To compare treatments’ effectiveness in changing the status of each dependent variable at each time point of measurement, a one-way ANOVA (with treatment as the independent factor) was applied on the delta differences between different time point measurements. When $F$-ratios were significant, post hoc comparisons of means were analyzed with Scheffé tests. Statistical significance was accepted at $P < 0.05$.

RESULTS

Subjects. Thirty-five men volunteered to participate. Eight men were excluded because they did not meet the selection criteria (two were too frail, four had medical limitations, and two were too fit), whereas three declined participation. During training, three more men (one from C and two from ET) were asked to stop because they had missed more than three training sessions up to that point, whereas two more (one from C and one from ET) stopped because of injury or illness (all stopped within the first 4 wk). There were no differences between individuals who dropped out of the study and those who completed the study for physical activity and VO$_2$max. Participants in exercise groups were required to complete 48 training sessions (100% compliance was achieved). In the detraining measurements, only seven subjects from the exercise group and six subjects from the control group participated. All participants demonstrated a low level of maximal oxygen consumption (VO$_{2\text{max}}$ < 20 mL·kg$^{-1}$·min$^{-1}$), with no differences detected between groups (Table 1).

Subjects’ physical characteristics are shown in Table 1. No significant differences were noted at baseline between groups for age, height, and fitness level at the study and after 4 months of detraining. There were also no differences between groups at any time point relative to dietary intake (antioxidant, nutrient, and fat consumption). Body weight and sum of skinfolds were similar across groups at baseline (Table 1). The endurance-training program resulted in a significant ($P < 0.05$) reduction in body weight (6.6%) and sum of skinfolds (6%) (Table 1) at the end of training, compared with baseline measurements. After 4 months of detraining, body weight and skinfold sum returned to pretraining levels.

Aerobic endurance. Subjects in both groups exhibited a very low initial level of aerobic fitness (VO$_2$ peak < 20 mL·kg$^{-1}$·min$^{-1}$), with no significant differences between groups at baseline (Table 1). ET demonstrated a significant increase (45%) in time to exhaustion, whereas VO$_{2\text{peak}}$ increased by 26.3% by the end of training (Table 1, $P < 0.05$). C group showed no change in these two variables. After 4 months of detraining, VO$_{2\text{max}}$ and time to exhaustion all returned to pretraining levels.

MDA responses. Lipid peroxidation data are shown in Table 2. Pretraining, MDA levels were similar in both groups at rest and after the GXT for both groups. MDA levels were significantly ($P < 0.05$) increased (40–45%) in both groups after exercise, independent of treatment or time of measurement. After training, C demonstrated similar MDA responses both at rest and postexercise. In contrast, ET demonstrated a significant ($P < 0.1$) decrease of MDA at rest (10%) and attenuation of MDA response to exercise (16%) posttraining. MDA levels returned to pretraining levels after 4 months of detraining.

3-Nitrotyrosine (3-NT) responses. Values for 3-NT are shown in Table 3 and were similar in both groups, both at rest and after GXT. Exercise induced a significant ($P < 0.05$) augmentation (>45%) of 3-NT, independent of treatment and time of measurement. However, training elicited a significant ($P < 0.05$) reduction (20%) of 3-NT concentration after GXT, but not at rest. 3-NT levels returned to pretraining levels after 4 months of detraining.

Glutathione peroxidase (GPX) and total antioxidant capacity (TAC). Tables 4 and 5 present GPX and TAC adaptations, respectively. TAC did not differ between groups, either at rest or after exercise at pretraining. Exercise caused a substantial ($P < 0.05$) increase (>9%) of TAC, independent of treatment and time of measurement. Endurance training elicited a significant increase of TAC at rest (6%, $P < 0.1$) and postexercise (13%, $P < 0.05$). However, detraining returned TAC values at baseline. GPX activity was significantly ($P < 0.05$) increased (6%) by exercise, independent of treatment and time of measurement. GPX activity demonstrated a significant ($P < 0.05$) increase (12%) after GXT posttraining. There were no differences between groups for GPX activity at pretraining. Both TAC and GPX activity levels returned to pretraining levels after 4 months of detraining.

DISCUSSION

The present investigation presents evidence that endurance exercise training may attenuate exercise-induced oxidative stress in older individuals while simultaneously enhancing antioxidant defense, both at rest and after exercise. A 16-wk progressive training program increased participants’ fitness level, reduced their body fat content, and enhanced their ability to defend against reactive oxygen and nitrogen species. Results of the present investigation suggest that systematic exercise may offer a protective mechanism against oxidative damage as well as enhancement of functional performance of aged men. However, it is likely that...
cessation of training reverses training-induced adaptations of aerobic fitness as well as oxidative stress and antioxidant status responses.

**Enhanced fitness levels with endurance training.** Subjects in the present investigation demonstrated a significant improvement in VO\textsubscript{2max} (~25%) and exercise time to exhaustion (~45%), indicating a substantial increase of their aerobic capacity. Previous reports have reported endurance-training-induced increases of VO\textsubscript{2max} ranging from 10 to 25%, even in individuals 70–80 yr old who had never exercised before (10). Furthermore, subjects’ body composition was significantly altered because their skinfold sum was reduced (~6%), and this was paralleled by a similar response of their body weight as previously described (16).

**Reduction of lipid peroxidation at rest and post-exercise.** Recent studies employing molecular genetics and pharmacological interventions strongly suggest that ROS may modulate the aging process, and that endogenous production of ROS by normal physiological processes is probably a significant obstacle to lifespan (21). A number of studies have shown an age-related increase of lipid peroxidation in several tissues (18) associated with mitochondrial ROS production, such as hydrogen peroxide (30). The mitochondrial hypothesis of aging suggests that free radical generation in the mitochondria produce free radical reactions that damage specific vital macromolecules in this organelle (11). Therefore, mitochondria are the main source and target of cellular free radicals. An increase in free radical production or a decrease in antioxidant enzyme content leads to oxidative stress and cellular dysfunction. Furthermore, the activity of certain enzymes (NADH-dehydrogenase and cytochrome oxidase) located in the inner membrane of the mitochondria, as well as other mitochondrial proteins (i.e., adenine nucleotide translocase, acyl carnitine transferase, and citrate synthase), are considered antig-owing markers associated with oxidative stress (22). In the present investigation, exhausting exercise increased lipid peroxidation regardless of training. This is in accordance with previous findings indicating that acute aerobic- or resistance-type exercise is capable of inducing an increase of lipid peroxidation in both young and old subjects (34).

Subjects in the present study demonstrated higher basal MDA values compared with previously reported values for older adults (34), but this could be attributed to participants’ greater age and their lower fitness level. Reduced lipid peroxidation levels were observed in the endurance-trained group. Previous research has produced controversial results regarding exercise-training effects on basal lipid peroxidation levels. It was shown that systematic exercise training decreases resting lipid peroxidation levels (18). Others argued that even a long-term, high-intensity aerobic training program (ranging from 60 to 90 min·d\textsuperscript{-1}, with a frequency of 5 d·wk\textsuperscript{-1}) is not associated with lowered basal lipid peroxidation levels (34). Resistance training with weights was unable to decrease basal lipid peroxidation in older adults (33). It is not certain whether exercise training mainly lowers baseline levels of lipid peroxidation or decreases exercise-induced lipid peroxidation. However, in this study unfit older individuals were used instead of animals, whereas endurance training is known to cause far more significant metabolic adaptations in mitochondria and aerobic metabolism than resistance training, which was used in previous studies. Most previous studies trained younger participants, with intensity, duration, and training volume varying among them. It has been suggested that exercise training lowers resting lipid peroxidation by upregulating antioxidant enzyme levels in tissues engaged in systematic exercise (23). However, it must be kept in mind that this study recorded only a tendency toward lower lipid peroxidation, and that additional data are needed in order to be conclusive about this.

Endurance training for 16 wk increased aerobic capacity and decreased exercise-induced lipid peroxidation. Exercise-trained men may be able to produce fewer ROS compared with sedentary controls, because endurance training has been shown to upregulate mitochondrial respiratory chain proteins, leading to a reduced electron flux through each electron transport chain, electron leakage, and the resulting radical formation (4). During aerobic exercise, mitochondria are in state 3. The reduction of the respiratory chain is substantially limited during the state 4 to 3 transi-
tion, while at the same time electron flow through the respiratory chain is increased (13). Lower levels of reactive intermediates produced in state 3 may offer a protective mechanism against oxidative stress by enhancing electron coupling (17). Improved coupling of electron transport and oxygen reduction to water in state 3 may constitute a significant mechanism for controlling free radical production, because skeletal muscle raises its oxygen consumption up to 20-fold during its transition from rest to exercise. However, more work is needed to support the claim that upregulation of electron transport chain complexes reduces oxidant formation. Furthermore, mitochondria may produce other oxidants such as nitric oxide (17). Mitochondrial nitric oxide and superoxide react to form peroxynitrate (a strong oxidant), making mitochondria a significant source of harmful oxidants during aerobic exercise (32). In this work, 3-NT (a marker of NO-mediated tissue damage) was significantly reduced by endurance exercise training. These adaptations are very important to older individuals, as aging seems to increase mitochondria-generated free radicals (23). Findings in this study agree with previous observations that suggest that chronic exercise training may decrease MDA levels in brain mitochondria, indicating a beneficial adaptation of exercise training on oxidative stress development (19). An improvement of antioxidant status by training, as seen in the present investigation, may attenuate ROS production in skeletal muscle mitochondria (4).

3-NT response to training. Nitric oxide and its derivatives are detectable in both intracellular and extracellular muscle compartments, while NO production by NO synthase (NOS) is upregulated (50–200%) during muscular activity (2). NO, its derivatives, and ROS demonstrate similar production and distribution patterns in muscle fiber cytosol and extracellular space (28). NO integrity within muscle is likely to be affected by ROS because NO readily undergoes electron exchange reactions with ROS as they both compete for the same redox-sensitive molecular targets. Endogenously formed NO and O$_2^-$ react rapidly, resulting in peroxynitrite (ONOO$^-$) synthesis (28). Due to the simultaneous generation of NO and O$_2^-$ during rest and exercise, we expect that skeletal muscle will be systemically exposed to ONOO$^-$. 3-NT has been identified as a stable end product formed upon reaction of free or protein-bound tyrosine with NOX$_x$, such as ONOO$^-$ (28). Because of its stability, and because several studies have shown a dose-dependent increase of 3-NT in serum proteins in experimental mammals treated with tetryramethane (a specific protein nitrating agent), this molecule has been proposed as a biological marker of NO-mediated tissue damage, and for the assessment of the exposure of tissue to oxidative stress by NOX in general (5). A recent report further underlines the practical implications of the 3-NT measurement by indicating that nitrotyrosine levels are related to coronary artery disease (CAD) and may be regulated by statin treatment, suggesting a role for nitric oxide oxidants as inflammatory mediators in CAD, with implications in atherosclerosis risk assessment (29). 3-NT could be a potential marker of ONOO$^-$ effect on modified biological tissue, such as muscle, during biological aging.

Aged skeletal muscle’s increased relaxation time has been correlated with a potential age-related dysfunction of the sarcoplasmic reticulum (SR) Ca-ATPase (35). Previous research has shown an in vivo presence of ONOO$^-$ in skeletal muscle and a significantly greater 3-NT content in the SERCA2a slow-twitch isoform of the Ca-ATPase in aged muscle that could serve as an indication of aged-associated increase in susceptibility to oxidation by species such as ONOO$^-$ (35). 3-NT increase in aged skeletal muscle has been observed both during relaxation and contraction of muscle through simultaneous generation of O$_2^-$ and NO (35). Posttranslational modification of proteins by ROS/NOS has been recognized as an important feature of biological oxidative stress (35), as significant levels of such modified proteins may accumulate in aged biological tissue (35), accompanied by modified enzyme activities caused by covalent protein modification. Aged skeletal muscle would be exposed to ONOO$^-$, as indicated by the presence of a significant amount of 3-NT, caused by simultaneous generation of NO and O$_2^-$ and a less effective antioxidant defense system in the elderly.

In the present study, acute intense exercise increased 3-NT, independent of treatment. In a limited number of previous studies, 3-NT has been found to increase in urine, serum, and the liver by acute exhausting exercise (26). This finding may reflect the presence of oxidative stress and augmentation of serum proteins’ nitration and carbonylation during exercise. In contrast with MDA, 3-NT was not reduced at rest after training. Previous studies that used animal subjects revealed either an increase or no change in 3-NT after training (31). However, endurance training was able to decrease exercise-induced 3-NT response after exhausting exercise, indicating a lowering effect of NOS production and protein oxidation. It seems that systematic endurance training offers a protective mechanism against 3-NT accumulation during exercise in inactive older individuals. The biochemical mechanisms underlying this type of adaptation are still unclear.

Effects of endurance training on antioxidant status. It has been postulated that aging causes increased protein breakdown through oxidative damage and selective protein degradation, along with reduced protein synthesis, gradually leading to decreased antioxidant enzyme levels in aging tissue, and especially in skeletal muscles (21). Endurance training can restore the age-associated reduction of muscle protein content, as well as mitochondrial oxidative capacity (8). Results of the present study indicate an increased TAC at rest and enhanced TAC and GPX activity levels postexercise after training. These findings agree with previous reports (14), suggesting a significant increase of GPX activity in rats, although others failed to observe such an adaptation (18). The discrepancy seen in these studies may be largely due to differences in the training threshold employed. Earlier work with younger subjects support the notion that systematic training induces positive adaptations in antioxidant enzyme activities (6,23) associated with in-
creased protein content and mRNA levels in muscle, and this adaptation may be fiber-type specific (23). GPX has been consistently found to increase by training (18,23) in several muscles conducted mostly on younger participants. Because GPX facilitates H2O2 and lipid peroxides removal produced in the mitochondrial inner membrane, its increase with training coincides with the reduction in oxidative stress attenuation seen in this study, both at rest and after exercise. Most of these adaptations were seen in studies using younger or middle-aged individuals, and only a few studies (34) have been conducted with aging populations. Future research should examine the adaptations of the antioxidant system after prolonged training in older humans.

**Detraining responses.** After 4 months of cessation of the training stimulus, all training-induced adaptations were completely reversed, with oxidative stress and antioxidant status markers returning to pretraining values. There are no previous reports on detraining effects in the elderly, and thus it is difficult to be conclusive regarding this aspect. More studies are needed to examine whether detraining attenuates exercise-induced adaptations (and to what extent), and to determine which training protocol is more effective in maintaining these adaptations. This preliminary evidence on detraining suggests that exercise effects in older individuals may be temporary, and that uninterrupted exercise is crucial to maintain any positive adaptations. This study examined only a 4-month detraining period on oxidative stress response. Well-designed studies are needed to investigate shorter and longer periods of detraining.

In summary, 4 months of systematic endurance exercise training lowered oxidative stress levels, whereas it increased antioxidant status in inactive older individuals. Prolonged training cessation abolished these effects. The decrease in oxidative stress capacity, the enhanced total antioxidant capacity, and the improvements in body composition and cardiorespiratory endurance could have important health implications for these individuals.(6)

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**REFERENCES**


