Vitamin C and E supplementation prevents mitochondrial damage of ileum myocytes caused by intense and exhaustive exercise training

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Rosa EF, Ribeiro RF, Pereira FM, Freymüller E, Aboulafia J, Nouailhetas VL. Vitamin C and E supplementation prevents mitochondrial damage of ileum myocytes caused by intense and exhaustive exercise training. *J Appl Physiol* 107: 1532–1538, 2009. First published August 20, 2009; doi:10.1152/japplphysiol.91166.2008.— Intense and exhaustive exercise (IEE) is associated with oxidative stress in skeletal muscle, and we recently reported that intestine is sensitive to IEE. In the present study, we investigated the possible relationship between the effects of IEE on morphology and oxidative markers in the ileum and isolated mitochondria. C57BL/6 mice were ascribed either to a control group comprising two subgroups, one sedentary and another exercised for 10 days (E10), or to a corresponding supplemented control group again comprising two subgroups, one sedentary and another exercised for 10 days (E10-V). The IEE program consisted of a single daily treadmill running session at 85% of V_{max}, until animal exhaustion. Vitamins C (10 mg/kg) and E (10 mg/kg) were concurrently intraperitoneally administered 2 h before the exercise sessions. IEE was shown to cause *1*) impairment of ileum internal membrane mitochondria verified by ultramicrography analysis; *2*) increase in ileum carbonyl content (117%) and reduction in antioxidant capacity (36%); *3*) increase in mitochondria carbonyl content (38%), increase in the percentage of ruptured mitochondria (25.3%), increase in superoxide dismutase activity (186%), and reduction in citrate synthase activity (40.4%) compared with control animals. Observations in the vitamin-supplemented exercised animals (E10-V) were *1*) healthy appearance of myocyte mitochondria; *2*) decrease in ileum carbonyl content (66%) and increase in antioxidant capacity (53%); *3*) decrease in mitochondria carbonyl content (43%), decrease in the percentage of ruptured mitochondria (30%), slight increase in superoxide dismutase activity (7%), and significant increase in citrate synthase activity (121%) compared with E10 animals. Therefore, the present results strongly corroborate the hypothesis that IEE leads to marked disturbances in intestinal mitochondria, mainly in redox status, and affects whole intestinal redox status.

C57BL/6 mice; intestine; mitochondria

SKELETAL AND CARDIAC MUSCLE cells are known to be either positively or negatively sensitive to exercise, depending on intensity and/or volume (1, 18, 22). In contrast to light or moderate exercise, intense exercise is believed to be linked to increased levels of oxidative stress, evident from compromise of membrane lipids, proteins, and DNA molecules (2, 20, 32) and structural and functional damage (23). A large body of evidence shows that acute bouts of strenuous exercise induce oxidative stress in circulating human lymphocytes (33, 37), rodent splenocytes and thymocytes (16), as well as in intestinal lymphocytes (15). However, much less is known about the influence of exercise on intestinal tissue despite the crucial importance of this organ in the control and replacement of cell energy and electrolytes. In addition, frequent complaints among endurance athletes of gastrointestinal disturbances have been associated with strenuous physical exercise (4, 25).

We recently reported that C57Bl/6 murine intestine is particularly sensitive to treadmill running exercise, whereby intestine undergoes structural and/or functional alterations depending on exercise protocol, initial physical conditioning, and age of the animals (8, 28, 29). Short periods of moderate exercise training (55 days, 60-min treadmill running session at 65% of $\dot{V}_{O_{2max}}$, 5 days/wk) have been shown to reduce intestinal lipid peroxidation (8), representing the first evidence of exercise modulation of redox status in intestine. In addition, we also reported that a habitual moderate exercise program protects intestinal cells against age-induced ultrastructure alterations (29). Finally, similar to aging-induced effects, 10 successive days of intense and exhaustive exercise (treadmill running at 85% $\dot{V}_{O_{2max}}$) have been shown to cause significant damage to internal membrane of mitochondria of murine ileum myocytes (28). In both cases, these ultrastructural alterations are associated with enhanced oxidative stress of the tissue (28, 29).

The aim of this study was to ascertain the relationship between mitochondrial degeneration and oxidative stress induced by successive, intense, and exhaustive treadmill running sessions (IEE). We used the same strategy successfully employed in our previously published study, which demonstrated that IEE-induced cognitive impairments in mice can be prevented by enhancing animal antioxidant defenses prior to exercise sessions (30). These novel findings represent unambiguous evidence of the protective effect of vitamin C and E supplementation against oxidative stress in intestinal mitochondria caused by intense and exhaustive exercise.

MATERIAL AND METHODS

Animals. Inbred male C57BL/6 mice (3 mo old, 28 ± 2 g) were obtained from the Center for the Development of Experimental Models for Medicine and Biology of the Universidade Federal de São Paulo animal facility. Mice were housed five animals per cage, with water and food ad libitum. Animals were kept on a 12:12-h light/dark cycle (0600–1800) and maintained at 23°C for at least 5 days before the commencement of experiments. Animals were randomly assigned to four groups: sedentary (CT), submitted to intense and exhaustive exercise (IEE) for 10 days (E10), supplemented-sedentary (CT-V), and supplemented-exercised for 10 days (E10-V). Mice were submitted to an incremental test before the IEE program. Animals were killed by cervical dislocation or by decapitation 24 h after a second

incremental test, performed 24 h after the last exercise session. The ilei were quickly isolated and fixed with the appropriate solutions for the ultrastructure studies, or frozen and stored at $-80°C$ for oxidative stress analysis. Total blood was collected in sterilized tubes with citrate anticoagulant (BD Diagnostics Systems). Animal handling procedures were approved by our University Ethics Committee, in compliance with the International Guiding Principles for Biomedical Research Involving Animals (Geneva, 1985).

Exercise protocol. Animals were submitted to an intense and exhaustive exercise protocol (IEE), described in detail elsewhere, which is effective for increasing plasma lactate and skeletal oxidative stress, and for decreasing animal physical performance (28). In brief, after an adaptation period to a motor-driven treadmill (Exer 3/6 Treadmill, Columbus Instruments, Columbus, OH) environment and after determining animal maximum velocity by means of an incremental test, the exercised animal groups performed daily sessions of intense and exhaustive exercise for 10 consecutive days, according to the following schedule: *1*) 3-min warm-up at 5 m/min; *2*) running exercise at 85% of individual maximum running velocity of each animal until exhaustion; and *3*) 3-min cool-down by running at 5 m/min. Treadmill grade was set at 0% throughout all exercise sessions. Mice were stimulated to run by gentle hand prodding with a soft brush during the final exercise stage.

Supplementation. Experimental animals were concurrently supplemented with 10 mg/kg vitamin E and 10 mg/kg vitamin C (10) once a day, 2 h before the exercise sessions. Vitamin supplementation was administered intraperitoneally in volumes not exceeding 1 ml/kg of body weight. The vitamin treatment was started on the first day of the adaptation period and completed on the last day of the exercise program. Intraperitoneal saline solution was administered to the control animals. The vitamin supplementation protocol was previously used in C57Bl/6 mice to demonstrate successful protective effect of vitamins against IEE-induced memory impairments (30).

Isolation of mice ileum mitochondria. Mitochondria were isolated by standard differential centrifugation (24). The isolated ilei were sliced in ~50 ml of medium containing (in mM): 250 sucrose, 1 EGTA, and 10 HEPES-KOH, pH 7.2, and homogenized in Potter-Elvehjem homogenizer, until all solid parts were liquefied. Homogenates were centrifuged at 770 *g* for 5 min and the resultant supernatant was further centrifuged at 9,800 *g* for 10 min. Pellets were suspended in 10 ml of medium containing (in mM): 250 sucrose, 0.3 EGTA, and 10 HEPES-KOH, pH 7.2, and centrifuged at 4,500 *g* for 15 min (27). The final mitochondrial pellet was suspended in the appropriate buffer for subsequent biochemical analysis.

Ultrastructure studies. Fresh ileum samples were fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate-buffer, pH 7.2 for 60 min at 25°C. Samples were then fixed with 2% osmium tetroxide in cacodylate buffer, pH 7.2 for 1 h at 25°C, dehydrated in graded ethanol, treated with propylene oxide, and embedded in 812 epoxy resin. Ultrathin sections (70 nm) were obtained and stained with uranyl acetate and lead citrate and examined using a Jeol EXII transmission electron microscope (Tokyo, Japan).

Carbonyl assay. Oxidative damage either to intestinal proteins or to isolated intestinal mitochondria was determined by quantifying tissue carbonyl content according to the method described by Reznick and Packer (26). Briefly, 10 mM 2,4-dinitrophenylhydrazine (DNPH), dissolved in 2.5 M HCl, were added to samples from each animal group to generate chromophoric dinitrophenylhydrazones. After the DNPH reaction time, proteins were precipitated in 20% (wt/vol) TCA, followed by successive washing with ethanol/ethyl acetate mixture (1:1) and centrifugation at 6,000 *g*. The resulting pellet was dissolved in 6 M guanidine-HCl solution. The protein carbonyl content was assessed spectrophotometrically (N-200, Hitashi, Tokyo, Japan) at 370 nm, using molar extinction coefficient of DNPH ($\varepsilon = 22{,}000$ $M^{-1} \cdot cm^{-1}$). The total protein content was assessed using a bovine serum albumin standard curve (0.25–2.0 mg/ml), performed at 280 nm (5).

Total antioxidant capacity. Ileum total antioxidant capacity was assessed using the antioxidant assay kit according to manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). In brief, ileum pools from each animal experimental group ($n = 5$) were homogenized in phosphate buffer containing the anti-protease cocktail. The assay relies on the ability of the antioxidants presented in the homogenate to inhibit the oxidation of ABTS to ABTS$\cdot^+$ ($\varepsilon = 22{,}000/M$) promoted by metmyoglobin. The amount of ABTS$\cdot^+$ produced was assessed spectrophotometrically at 750 nm. The antioxidant capacity of the sample was compared with Trolox, a water-soluble tocopherol analog for preventing ABTS oxidation. Results were expressed as nanomoles of antioxidant per milligram of total protein.

Superoxide dismutase assay. Superoxide dismutase (SOD) activity in the whole ileum mitochondria and blood was determined using the superoxide dismutase kit (Cayman Chemical) in accordance with the manufacturer's instructions. Samples of the whole ileum mitochondrial homogenate were suspended in radical detector. The reaction was initiated by addition of xanthine oxidase, followed by 20-min incubation in a shaker at room temperature. SOD activity was evaluated spectrophotometrically (Sunrise, Tecan Group, Seestrasse 103 CH-8708 Männedorf, Switzerland) at 450 nm. Results are expressed as units per milliliter per milligram of total protein. One unit of activity is defined as the amount of enzyme needed to exhibit 50% superoxide radical dismutation.

Blood cells were lysed in four times their volume of water. The radical detector was added to the cell lysate sample. The reaction was initiated by addition of xanthine oxidase followed by a 20-min incubation period in a shaker at room temperature. SOD activity was evaluated spectrophotometrically (Sunrise, Tecan Group) at 450 nm. Results are expressed as units per milliliter per milligram of total protein.

Citrate synthase activity assay. Citrate synthase activity was determined using the citrate synthase assay kit (Sigma-Aldrich, St. Louis, MO) according to manufacturer's instructions. The assay was performed to determine both the ileum total mitochondrial activity and the level of ruptured mitochondria.

Ileum total mitochondrial activity. The final ileum mitochondrial pellet was suspended in CelLytic M cell lysis reagent. Assay buffer, acetyl CoA solution, and DTNB solution were added to samples of the final mitochondrial pellet. Total mitochondrial citrate synthase activity was measured spectrophotometrically (Sunrise, Tecan Group) immediately after the addition of oxalacetic acid by adjusting the kinetic program to nine cycles of 12 s at 412 nm. Results are expressed as micromoles per milliliter per minute per milligram of total protein.

Ruptured mitochondria. The final mitochondrial pellet, suspended in bicine buffer, was submitted to the same procedures as those described for total mitochondrial activity. The percentage of ruptured mitochondria was calculated as the ratio between the citrate synthase activity in bicine buffer and its activity in the same mitochondria preparation treated with CelLytic M cell lysis reagent for each animal experimental group. The final citrate synthase activity is expressed in micromoles per milliliter per minute per milligram of total protein.

Solutions. The following solutions were used for carbonyl assays: homogenizing buffer: 50 mM NaH_2PO_4, 0.1% digitonin, a cocktail of anti-proteases (5 mg/ml leupeptin, 7 mg/ml pepstatin, and 5 mg/ml aprotonin), and 1 mM EDTA.

Chemicals. All chemicals were analytical grade. Salts, D-glucose, ethylic alcohol, sucrose, and xylene were purchased from Merck (Darmstadt, Germany); EDTA, 2,4-dinitrophenylhydrazine, TCA, anti-protease cocktail, digitonin, guanidine and HEPES and EGTA, and citrate synthase assay kit were from Sigma; osmium tetroxide, glutaraldehyde, 812 epoxy resin, formaldehyde, propylene oxide, uranyl acetate, and lead citrate were from EMS (Electron Microscopy Sciences); and antioxidant assay kit and superoxide dismutase kit from Cayman Chemical.

Statistical analysis. Data are presented as means ± SE, with *n* representing the number of experiments. Statistical significance was analyzed by two-way ANOVA followed by Bonferroni's test. $P < 0.05$ was considered statistically significant.

RESULTS

Figure 1 illustrates representative electron micrographs of ileum myocytes isolated from control and exercised animals before (CT and E10, Fig. 1, *left*) and after vitamin supplementation (CT-V and E10-V, Fig. 1, *right*). As illustrated, intestinal myocytes from the control animals, treated with vitamin or otherwise, had central euchromatic nuclei and intact mitochondria of diverse forms and sizes, localized close to the nuclear poles. In contrast, mitochondria from the ileum of E10 animals demonstrated marked signs of internal membrane damage (E10, Fig. 1, *left*), which were not present in those animals supplemented with vitamins before the performance of the exercise program (E10-V, Fig. 1, *right*). These results strongly suggest that the observed mitochondrial damage might have resulted from exercise-induced intestine oxidative stress, since there was a significant protective effect of vitamin C and E supplementation on mitochondrial structure.

To elucidate this possibility, we investigated the effects of an exercise program and/or vitamin supplementation on the redox status of the whole ileum, whole ileum mitochondria, and blood. The whole ileum (E10) protein carbonyl content was 10.2 nmol/mg of protein while in control animals was 4.7 nmol/mg of protein (Fig. 2), representing a 117% increase. Vitamin supplementation significantly reduced protein carbonyl accumulation by 50% in the control group (CT) and by 67% in the E10 group (Fig. 2). Finally, a 39% increase in carbonyl content in the whole ileum of E10-V animals was observed compared with CT-V animals (Fig. 2). The whole ileum antioxidant capacity in E10 animals was 0.25 nmol/μg of protein vs. 0.40 nmol/μg of protein in CT animals (Fig. 3), constituting a 36% reduction. Vitamin supplementation led to a slight but significant 9% increase in ileum total antioxidant capacity in CT animals, and a substantial 53% (Fig. 3) increase in the tissue isolated from the E10-V animals. Last, a slight reduction in whole ileum antioxidant capacity of ~10% was observed in the CT-V compared with E10-V animals. Taken together, these results strongly indicate that vitamin E and C supplementation protects ileum redox status from the oxidative stress caused by intense and exhaustive exercise.

Similar results were obtained for the redox status of the whole ileum mitochondria. The carbonyl content in the ileum mitochondria from E10 animals was 38% higher than CT values (Fig. 4*A*). The vitamin supplementation totally prevented mitochondria protein oxidation in the E10-V animals (1.2 ± 0.2 nmol/mg of protein) and also caused a significant decrease of 43% in protein carbonyl accumulation in E10-V animals (1.15 ± 0.03 nmol/mg of protein) compared with E10 animals (Fig. 4*A*). No difference was observed in carbonyl
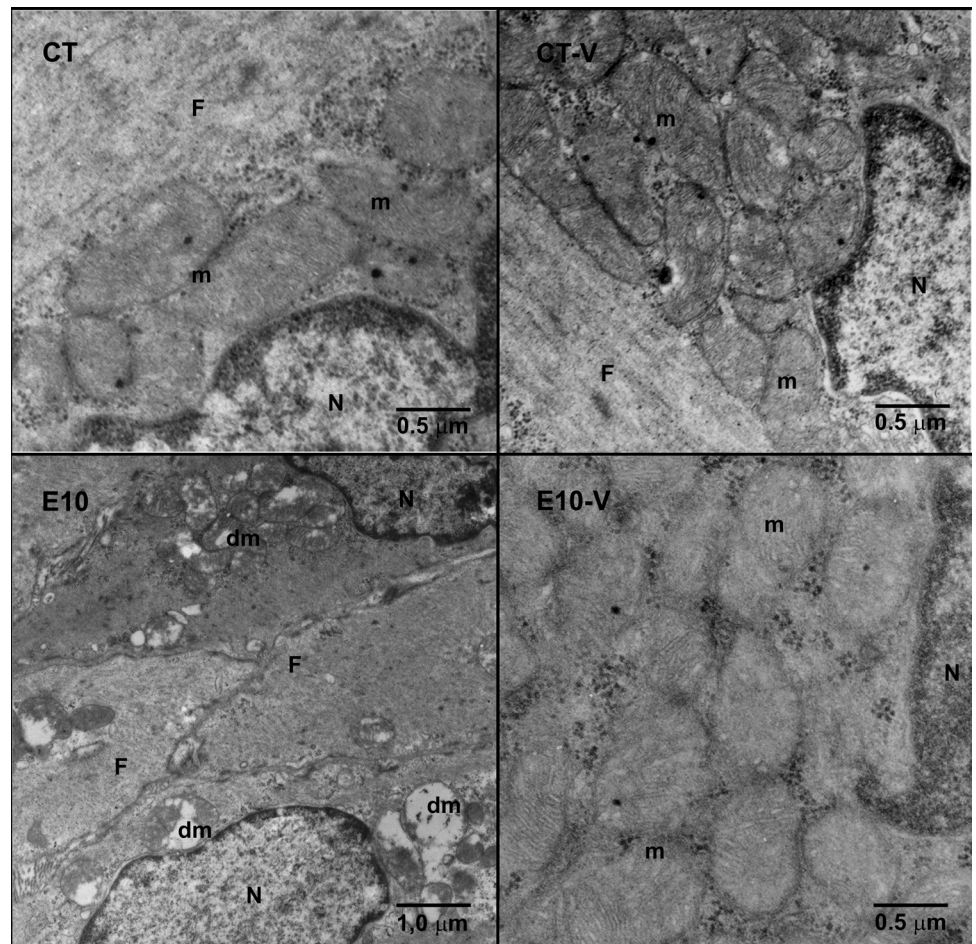

Fig. 1. Representative ultramicrographs from ileum isolated from sedentary (CT; *n* = 3), 10-day exercised (E10; *n* = 3; *right*), supplemented sedentary (CT-V; *n* = 3) and supplemented exercised (E10-V; *n* = 3; *left*) animals. F, muscular fiber; N, nucleus; m, mitochondria; dm, disorganized mitochondria.
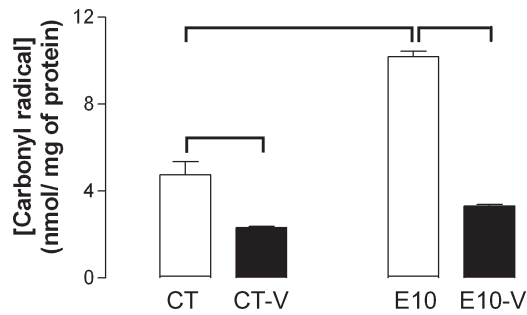
Fig. 2. Histogram of the protein carbonyl contents of the ileum from CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). Brackets indicate significant differences between indicated groups ($P < 0.05$).

content between CT-V and E10-V animals (Fig. 4A). The ileum isolated mitochondria superoxide dismutase activity was higher in E10 animals (0.183 ± 0.03 U·ml^{-1}·mg protein^{-1}) than CT animals (0.064 ± 0.006 U·ml^{-1}·mg protein^{-1}), corresponding to an increase of 186% (Fig. 4B). Interestingly, the exercised groups presented the same SOD activity (~0.2 U·ml^{-1}·mg protein^{-1}) regardless of vitamin supplementation, in contrast to the control groups in which a 113% increase was observed in CT-V animals vs. CT animals (Fig. 4B). Finally, no significant differences were observed between CT-V (0.136 ± 0.01 U·ml^{-1}·mg protein^{-1}) and E10-V animals (0.195 ± 0.02 U·ml^{-1}·mg protein^{-1}; Fig. 4B).

Citrate synthase activity in ileum mitochondria from E10 animals was 0.28 ± 0.01 μmol·ml^{-1}·min^{-1}/mg of protein, which corresponds to a reduction of 40% compared with the CT group (0.47 ± 0.02 μmol·ml^{-1}·min^{-1}/mg of protein; Fig. 5A). Vitamin supplementation led to a substantial increase of citrate synthase activity (0.62 ± 0.01 μmol·ml^{-1}·min^{-1}/mg of protein) in E10-V animals compared with E10 animals, which represents a 121% increase in mitochondrial activity among these animals (Fig. 5A). Last, a significant increase was observed in the CT-V animals (0.61 ± 0.02 μmol·ml^{-1}·min^{-1}/mg of protein, 30%) compared with the CT animals, while no differences were observed between E10-V and CT-V animals (Fig. 5A).

Ruptured mitochondria was 37 ± 2% and 39 ± 4% in CT and CT-V animals, respectively, relative to the total mitochondria content. The proportion of ruptured mitochondria increased to 62.3 ± 0.4% in E10 animals, but was reduced to 43 ± 3% in E10-V animals (Fig. 5B). Taken together, these results demonstrate that concurrent vitamin E and C supplementation of the animals protects ileum mitochondrial activity
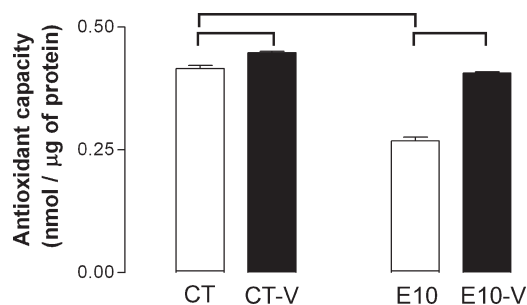

Fig. 3. Ileum total antioxidant capacity from CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). Brackets indicate significant differences between indicated groups ($P < 0.05$).

Fig. 4. A: histogram of the protein carbonyl contents of the isolated ileum mitochondria CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). B: ileum isolated mitochondrial superoxide dismutase activity from CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). Brackets indicate significant differences between indicated groups ($P < 0.05$).
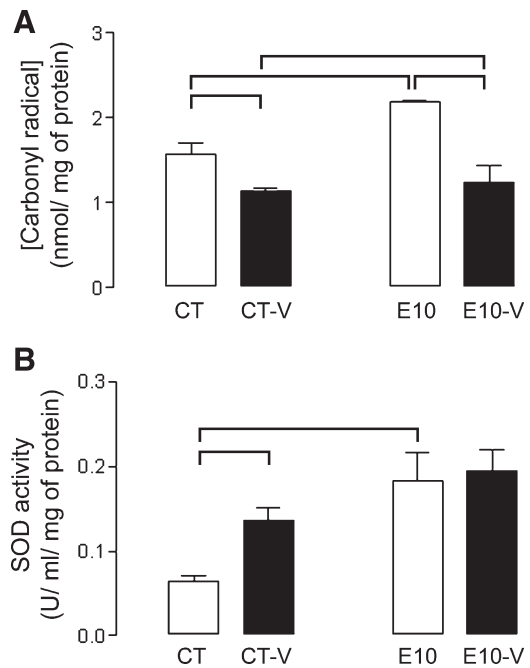
from exercise-induced impairments, akin to the effect on the whole ileum.

We also investigated the effects of exercise and/or vitamin supplementation on total blood redox status. Carbonyl content
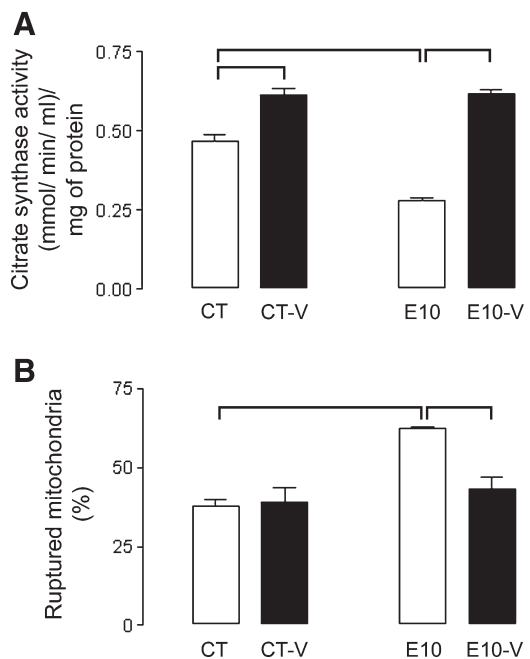

Fig. 5. A: ileum isolated mitochondrial citrate synthase activity from CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). B: histogram of the ruptured mitochondria of the isolated ileum from CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). Brackets indicate significant differences between indicated groups ($P < 0.05$).

increased by 30% in E10 animals (1.84 ± 0.08 nmol/mg of protein) compared with CT animals (1.42 ± 0.1 nmol/mg of protein). In contrast, carbonyl content decreased by 46% in E10-V animals (0.99 ± 0.02 nmol/mg of protein) compared with E10 animals (Fig. 6A), and decreased by 17% in CT-V animals (1.18 ± 0.04 nmol/mg of protein) compared with CT animals (Fig. 6A), and by 16% in E10-V animals vs. CT-V animals (Fig. 6A).

Concerning blood superoxide dismutase activity, there was a reduction of 23% in E10 animals (0.0410 ± 0.0007 $U \cdot ml^{-1} \cdot mg$ protein^{-1}) and of 19% in CT-V animals (0.043 ± 0.001 $U \cdot ml^{-1} \cdot mg$ protein^{-1}) compared with CT animals (0.053 ± 0.001 $U \cdot ml^{-1} \cdot mg$ protein^{-1}). No significant difference was observed between the enzyme activity of blood from E10-V animals (0.043 ± 0.001 $U \cdot ml^{-1} \cdot mg$ protein^{-1}) and blood from both E10 and CT-V animals (0.043 ± 0.001 $U \cdot ml^{-1} \cdot mg$ protein^{-1}; Fig. 6B). These results demonstrate that previous vitamin C and E supplementation also protects the animals' blood from the higher oxidant production caused by IEE.

DISCUSSION

The present results strongly corroborate our previous hypothesis that IEE leads to disturbances in intestinal mitochondria, mainly affecting redox status, and is also associated with impairment of whole intestinal redox status. This conclusion was reached by demonstrating that concurrent vitamin C and E supplementation of the animals 2 h before a single daily bout of IEE for 10 successive days leads to *1*) significant prevention of exercise-induced ileum mitochondria structural damage, associated with *2*) significant protection of ileum mitochondria redox status and protection of impaired redox status of the whole ileum. Finally, concurrent vitamin C and E supplementation also protected animals, albeit to a lesser degree, against

Fig. 6. A: histogram of the protein carbonyl contents of the blood from CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). B: blood SOD activity in CT (n = 5), CT-V (n = 5), E10 (n = 5), and E10-V (n = 5). Brackets indicate significant differences between indicated groups ($P < 0.05$).

the blood redox status alterations caused by this type of exercise program.

We recently reported that the IEE involved in the current program, in addition to the well-known deleterious effect on animals' physical performance, is associated with increased blood lactate concentration to levels above 4 mM and induction of time-dependent damage to the intestinal tissue by affecting its structure, contractility to various stimulants, and redox status (28). In the present study we further explored this point by focusing on the mitochondrial damage caused by this type of exercise. We hypothesized that IEE might be associated with compromised intestinal redox status, which may in turn be due to repetitive and short-term ischemic-reperfusion events caused by IEE in the splanchnic region (or a positive modulation of antioxidant enzymes), since effects were clearly detectable only after 10 days of this type of exercise (28).

Our novel findings support the above hypothesis, at least in murine models, both at the whole ileum and mitochondrial levels. In fact, IEE caused a marked increase (117%) in protein oxidation not only in the whole ileum (Fig. 2), but also in the mitochondria, albeit to a lesser degree (38%; Fig. 4A). Corroborating these data, citrate synthase activity in ileum mitochondria homogenates were significantly reduced (40%; Fig. 5A) while disruption of mitochondria rose by 25% after exercise (Fig. 5B). Finally, exercise-induced mitochondria injuries were also associated with significant impairment in ileum antioxidant defense (Fig. 3) thus confirming that IEE causes mitochondria degradation, as observed previously in morphologic studies (28; Fig. 1). These findings strongly point to an association between intestinal redox status disturbance and mitochondrial dysfunction as a result of IEE-induced oxidative stress.

If these mitochondria impairments were in fact due to increased oxidant reduction in response to IEE, we would expect to observe a protective effect in boosted animal antioxidant defense. Indeed, the reinforcement of the antioxidant defense of the animals by daily intraperitoneal supplementation of both vitamin C and E before each exercise session throughout the IEE exercise program proved effective in avoiding increased oxidant production in both ileum and mitochondria (Figs. 2 and 4A). This was unequivocally demonstrated by the presence of apparent healthy mitochondria on electron micrographies of the ileum of E10-V animals (Fig. 1, *right*) and by the behavior of all redox status and functional markers studied, except SOD activity. This behavior encompasses an initial dramatic reduction in protein oxidation by 67% in the whole ileum (Fig. 2) and by 43% in the mitochondria (Fig. 4A) followed by increased antioxidant capacity in the whole tissue (53%; Fig. 3) then significant reduction in mitochondria degeneration to control levels (Fig. 5B) and, finally, increased mitochondrial activity demonstrated by a significant increase in citrate synthase activity (121%) compared with exercised animals (Fig. 5A).

The possibility of an oxidant effect of vitamin C and E as opposed to an antioxidant effect can be ruled out since citrate synthase activity was increased (Fig. 5A; 30%) while both ileum (Fig. 2; 50%) and mitochondria (Fig. 4A; 28%) protein oxidation were reduced in CT-V animals. On the other hand, a protective effect induced by vitamin supplementation might also occur, such as inhibition of pro-inflammatory cytokine release (11), a phenomenon currently being investigated by our
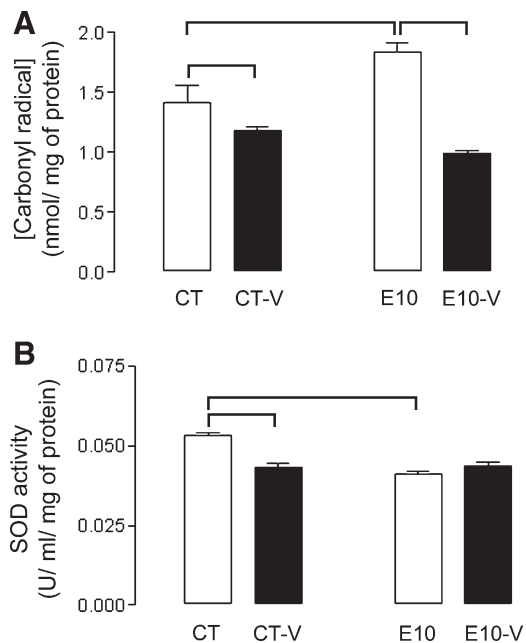
group. However, the direct observation that vitamin C and E supplementation prevented both exercise-induced intestine oxidative stress and mitochondrial damage greatly strengthens and supports the importance of the vitamin antioxidant features in this case.

The analysis of redox status disturbances in total blood caused by IEE revealed some similar modifications, although these were less marked than changes observed in the intestine. In fact, the protein oxidation level (Fig. 6*A*, increase of 30%) was much lower than in the ileum (117%; Fig. 2) while the vitamin supplementation protective effect was also lower at 46% (Fig. 6*A*) vs. 67% in the ileum (Fig. 2). Similarly, the effect of the vitamin was also lower at 17% in total blood (Fig. 6*A*) compared with 50% in the ileum (Fig. 2). On the basis of these findings, it seems reasonable to conclude that exercise-induced intestinal redox status modifications are not a direct consequence of systemic redox status disturbances, but stem from local redox status changes in the intestine. The observation of an imbalance between tissue redox status and systemic status is congruent with the proposal by Go and Jones (13) of a new definition of oxidative stress and their suggestions that cellular redox biology is compartmentalized by the structures of lipid-containing membranes (13, 19).

Concerning mitochondrial SOD activity, this enzyme is known to be the principal component of mitochondrial antioxidant defense and that its activity and expression is modulated by several substances, including superoxide anion (36). The results regarding its action in whole ileum mitochondria (Fig. 4*B*) and blood (Fig. 6*B*) remain controversial. One possibility is that IEE exerts such a high level of oxidative stress in the intestine, that it overwhelms the tissue antioxidant defense, leading to functional and morphological impairments in its mitochondria. In this case, a decrease in SOD activity would be expected likely accompanied by decreased production of ATP and/or increased hydrogen peroxide production in the tissue, and ileum contractility impairment. Vitamin supplementation would be expected to prevent these effects. However, this seems not to have been the case, as significantly high activity of mitochondrial SOD was observed in exercised animals, supplemented or otherwise (Fig. 4*B*), as well as an absence of impairments to whole ileum contractility in response to both membrane depolarization and muscarinic signaling (28). The second possibility is that IEE increases oxidant production at a higher degree than tissue antioxidant defense, such that an increase in mitochondria SOD activity would be expected in spite of tissue oxidative stress. In this case, previous supplementation of the animals with vitamins would lead to a reduction in mitochondrial SOD activity in exercised animals by boosting nonenzymatic tissue antioxidant defenses. Although IEE led to a significant increase (186%) in mitochondrial SOD activity (Fig. 4*B*), no expected reduction in enzyme activity following vitamin supplementation (Fig. 4*B*) was observed. Unexpectedly, enzyme activity was also greatly increased by vitamin supplementation compared with control animals (Fig. 4*B*). Therefore, the regulation of mitochondrial SOD activity appears to be complex and sensitive to several modulators. SOD activity is thus not a straightforward marker of mitochondrial oxidative stress. For instance, the observed exercise-induced increase of SOD activity might instead have been due to an upregulation of SOD2 expression (mitochondrial isoform) by superoxide anion (36), which may have been particularly high in the exercised animals (E10). Alternatively, the increase may have been due to the activity of other cell SOD modulators such as cytokines, lipopolysaccharide, and IFN-γ (9, 14, 21, 34, 35). The observation that SOD activity was positively modulated by vitamins is to be expected, since these vitamins have been identified as modulators of antioxidant defense in other tissues, including the intestine (31). Finally, although our data suggest that the absence of vitamin supplementation effects on mitochondrial SOD activity in exercised animals is the result of a common intermediary step in the underlying mechanisms triggered either by exercise or vitamin modulation of mitochondrial SOD activity, this notion requires confirmation by further experimental evidence.

Intense exercise has been associated with increased gastrointestinal distress in humans, mainly when either prolonged and intense exercise or an acute bout of treadmill running to exhaustion, were performed (12). These clinical symptoms are commonly ascribed to the fact that intestine may be subject to significant ischemic/reperfusion injury due to exercise-induced cardiac debt shift to active skeletal muscle. This would lead to as much as an 80% reduction in splanchnic blood flow (7, 25), which in turn can lead to ischemic/reperfusion injury to the delicate epithelium and mucosa (6) or result in a pro-oxidant state (17). There is now clear evidence for the dramatic change in intestinal redox status in response to a highly intense and exhaustive exercise program.

A novel time-efficient training paradigm involving short duration high-intensity interval training has been shown to improve aerobic function (3). This strategy is also expected to lead to greater adherence of sedentary populations compared with the strategies of time consuming traditional aerobic regimes (3). Nevertheless, the mitochondrial redox disturbances presented in our report require further discussion and suggest caution in prescribing such programs without antioxidant supplementation.

In sum, we provided compelling evidence of intestinal mitochondria and whole ileum damage caused by 10 days of intense and exhausting treadmill running exercise and subsequent exercise-induced mitochondrial redox disturbances. These findings are corroborated by the fact that deleterious effects are avoided by boosting animal antioxidant defenses with concurrent vitamin C and E supplementation 2 h before each exercise session.

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