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TRANSLATIONAL PHYSIOLOGY

Habitual exercise program protects murine intestinal, skeletal, and cardiac muscles against aging

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Rosa, Eloi F., Antonio C. Silva, Silvia S. M. Ihara, Oswaldo A. Mora, Jeannine Aboulafia, and Viviane L. A. Nouailhetas. Habitual exercise program protects murine intestinal, skeletal, and cardiac muscles against aging. *J Appl Physiol* 99: 1569–1575, 2005. First published June 16, 2005; doi:10.1152/japplphysiol.00417.2005.—Aging and aerobic exercise are two conditions known to interfere with health and quality of life, most likely by inducing oxidative stress to the organism. We studied the effects of aging on the morphological and functional properties of skeletal, cardiac, and intestinal muscles and their corresponding oxidative status in C57BL/6 mice and investigated whether a lifelong moderate exercise program would exert a protective effect against some deleterious effects of aging. As expected, aged animals presented a significant reduction of physical performance, accompanied by a decrease of gastrocnemius cross-sectional area and cardiac hypertrophy. However, most interesting was that aging dramatically interfered with the intestinal structure, causing a significant thickening of the ileum muscular layer. Senescent intestinal myocytes displayed many mitochondria with disorganized cristae and the presence of cytosolic lamellar corpuscles. Lipid peroxidation of ileum and gastrocnemius muscle, but not of the heart, increased in aged mice, thus suggesting enhanced oxidative stress. With exception of the intestinal muscle responsiveness, animals submitted to a daily session of 60 min, 5 days/wk, at 13 up to 21 m/min of moderate running in treadmill during animal life span exhibited a reversion of all the observed aging effects on intestinal, skeletal, and heart muscles. The introduction of this lifelong exercise protocol prevented the enhancement of lipid peroxidation and sarcopenia and also preserved cellular and ultracellular structures of the ileum. This is the first time that the protective effect of a lifelong regular aerobic physical activity against the deleterious effects of aging on intestinal muscle was demonstrated.

moderate exercise; C57B/6 mice; ileum; oxidative stress; isometric contractile response

AGING IS A COMPLEX BIOLOGICAL process defined as a general decline in organ function associated with a state of decreased adaptiveness to changes and capacity to restore disrupted homeostasis. The mechanisms underlying aging are far from being utterly understood and are under continual scrutiny to improve the quality of life of the growing number of elderly people (2). No single theory is generally accepted to account for the aging process. On the contrary, nowadays, aging is recognized as a process involving the interplay between a network of damaging agents and a network of cellular defenses (20).

The effects of aging on distinct organs and tissues have been widely demonstrated, as well as their relationship with the alteration of the organism oxidative stress (1, 19). Several are the cellular sources of reactive oxygen species (ROS), and, to limit the damage they inflict, organisms have evolved a complex antioxidant defense mechanism, composed of enzymatic and nonenzymatic substances, which scavenge free radicals, thus contributing to maintain an adequate redox status (for review, see Ref. 7). In physiological conditions the balance between prooxidant and antioxidant substances is kept slightly in favor of prooxidant products, thus favoring a mild oxidative stress state (9). According to the "aging free radical theory," introduced by Harman (13), aging is caused by an accumulative enhancement of the oxidative stress on various organs (29), tissues, and cell components. In addition, Meydani et al. (25) proposed that the redox balance is gradually more delicately set as the aging process develops, causing deregulation of cellular functions.

The most evident and well-understood age-related effects on skeletal muscle and heart are loss of muscle mass (or sarcopenia) and a significant hypertrophy, respectively (4, 28). On the other hand, there are few reports concerning the effects of aging on intestinal smooth muscle, despite recurring complaints of gastrointestinal motility disorders and chronic functional constipation by elderly people (14). Most studies focus mainly on the dysfunctions of intestinal mucosa absorption (10, 40). Deleterious effects of aging on human, rat, guinea pig, and mouse intestine have been associated with specific loss of cholinergic neurons from the myentheric plexus, leading to impaired motility (12) and muscular layer thickening (24). However, if aging is caused by increased tissue oxidative stress, it is quite possible that lipid peroxidation of plasma membrane, one of the most dramatic phenomena triggered by increased free radical production (36), might be contributing to changing membrane transport mechanisms, including ionic channels, carriers, and active pumps, thus disturbing signaling transduction mechanisms.

Aerobic exercise programs were introduced as a tool to improve cardiovascular conditioning and physical perfor-

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mance, whereas resistance exercise training programs are assigned for the development of muscle hypertrophy and strength. Hence, a prospective strategy to minimize the deleterious effect of aging is to take advantage of the beneficial effects of an appropriate exercise training either on antioxidant mechanisms (26, 29, 31, 35) or on its capacity to induce hypertrophy (28). However, because exercise benefits are intimately dependent on intensity and volume, it was first crucial to establish the appropriate type of exercise the aged animals should be submitted to. Considering that elderly people usually have a dramatic reduction in daily physical activity, we investigated whether a lifelong physical activity pattern is a good strategy to counteract the age-related deleterious effects.

In this study, we investigated the effects of aging on morphology, oxidative status, and reactivity of C57BL/6 murine ileum, heart, and gastrocnemius muscle and hypothesized that a lifelong moderate exercise program would have a protective role against aging deleterious effects.

MATERIAL AND METHODS

Animals. Inbred male C57BL/6 mice were obtained from Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia—Universidade Federal de São Paulo animal facilities, housed five animals/cage, with water and food ad libitum. The animals were kept on a 12:12-h light-dark cycle (0600 to 1800) and maintained at 23°C in our animal facility for at least 5 days before any experimental procedure. They were divided in three groups: sedentary young 3-mo-old (S 3), aged 18-mo-old (S 18), and continuously exercised animals from 3 to 18 mo old (E 3–18). Mice were killed by cervical dislocation, and the tissues of interest were isolated, frozen, and stored at −20°C for lipid peroxidation analysis. The animals' handling was approved by our University Ethics Committee, in adherence to the International Guiding Principles for Biomedical Research involving Animals (Geneva, 1985).

Exercise protocol. Mice were submitted to treadmill running, a kind of exercise in which intensity and duration can be easily manipulated and quantified, in opposition to voluntary wheel or swimming exercises (6). Animals from the E 3–18 group were initially acclimated to the treadmill environment by a 30-min running session at 13 m/min, 0% grade, for 5 successive days. Then they were submitted to an aerobic exercise-training program, which consisted of a daily session as follows: *1*) 3-min warm-up at 5 m/min; *2*) 60-min endurance run at speeds between 13 and 21 m/min, according to the tolerance of each animal; and *3*) 3-min warm-down at 5 m/min. The intensity of the applied exercise program was considered moderate, because it corresponded to 55–65% of C57BL/6 maximal oxygen uptake, and the speed range was adjusted between 13 and 21 m/min at 0% grade (34). Mice were motivated to run with gentle hand prodding. Electrical shock was avoided as a negative reinforcement because this would add undue stress not typically associated with volitional exercise. Sedentary animals (S 18 group) were exposed to the same environment conditions (handling, treadmill motor noise, vibration, and deprivation of food and water) while the other animals performed their daily exercise session.

Physical performance. Physical performance was assessed by determining the maximum treadmill speed reached by the animals during the incremental test. The schedule of this test consisted of 3 min of warm-up at 5 m/min, initial speed set at 10 m/min, followed by progressive increases of 1 m/min every min until animal exhaustion, and 3 min of cooling down at 5 m/min. Animals were killed 24 h after the last exercise session.

Histological studies. Fresh tissue sections were appropriately isolated and stained with hematoxylin and eosin. In brief, tissue samples were fixed in 10% buffered formalin dehydrated by graded concentrations of alcohol (from 50 to 85% ethylic alcohol), cleared in four rinses of xylene, embedded in paraffin wax at 58 ± 2°C, and sectioned at 4 μm. Morphological features of the ileum (100-fold magnification) and gastrocnemius muscles (200-fold magnification) were evaluated by sorting out 10 measurements from three fields per tissue sample. Muscular layer thickness of the ileum and cross-sectional areas from gastrocnemius muscle fibers were quantified by computer software (Image Tool 3.00 for Windows, University of Texas Health Science Center in San Antonio, San Antonio, TX).

Ultrastructure studies. Fresh ileum samples were fixed in phosphate-buffered 2% glutaraldehyde buffered at pH 7.2 with 0.2 M sodium phosphate for 4 h at 4°C. Then they were fixed in phosphate-buffered 1% osmium tetroxide for 1 h at 4°C, dehydrated in graded ethanol, treated with propylene oxide, and embedded in araldite epoxy resin. Ultrathin sections were cut with an ultramicrotome (Sorvall, Poter-Blum MT-1) and stained with uranyl acetate and lead citrate for transmission electron microscopy studies. Electron micrographs were taken with a digital camera, coupled to a 80-kV electron microscope (Carl Zeiss, Heidelberg, Germany).

Isometric contraction assay. The ends of an ileum strip, ~1.0 cm long, were tied up to a steel hook support, which was suspended in a 5-ml perfusion chamber containing Tyrode solution at 37°C, pH 7.4, and bubbled with air. Tissue strips were allowed to equilibrate under a 0.5-g basal tension for at least 30 min before any experimental procedure. During this rest period, the chamber solution was renewed every 10 min. Isometric tension was recorded by means of a force transducer (TRI 210, Letica, Barcelona, Spain) connected to an amplifier (model AECAD-0804, Solução Integrada, São Paulo, Brazil). Acquisition and analysis of the isometric contractions were done by means of the KitCad8 software (Software & Solutions, São Paulo, Brazil). Intestinal tissue responsiveness was evaluated by determining the potency and efficacy, obtained from noncumulative concentration-response curves to either carbachol (CCh) or KCl. The corresponding pharmacological parameters are EC_{50} (concentration of the stimulant that causes 50% of the maximum response, expressed in M) and maximum effect (expressed in g). Tissue strips were stimulated for 1.5 min at 5-min interval between two successive challenges. Just one concentration-contractile response curve was done per ileum segment.

Lipid peroxidation assay. Peroxidative damage to membrane lipid constituents from heart, gastrocnemius muscle, and ileum were determined by measuring the chromogen reaction product of 2-thiobarbituric acid (TBA) with one of the products of membrane lipid peroxidation, malondialdehyde (MDA), according to the technique described by Winterbourn et al. (38) and modified by Fraga et al. (11). In brief, homogenate tissue pools were incubated for 30 min with the reaction mixture at 95°C. The chromogen reaction product was extracted in *n*-butanol, and its concentration was determined spectrophotometrically (N-200, Hitashi, Tokyo, Japan) at 532 nm. Results are expressed as nanomoles per milliliter per gram dry tissue.

Solutions. The following solutions were used for lipid peroxidation assays: phosphate buffer solution (in mM) consisted of 20 KH_2PO_4, 150 KCl, and 40 HEPES; the reaction mixture contained phosphate buffer, 11% acetic acid, 0.1% tungstophosphoric acid, 0.5% SDS, and 0.2% TBA. For contractile assays, the composition of the Tyrode solution was (in mM) 135 NaCl, 2.68 KCl, 1.36 $CaCl_2 \cdot 2H_2O$, 0.49 $MgCl_2 \cdot 6H_2O$, 12 $NaHCO_3$, 0.36 NaH_2PO_4, and 5.5 D-glucose, pH 7.4.

Chemicals. All chemicals were analytical grade. Salts, D-glucose, *n*-butanol, TBA, tungstophosphoric acid, SDS, ethylic alcohol, acetic acid, and xylene were purchased from Merck (Darmstadt, Germany); carbachol, osmium tetroxide, glutaraldehyde, araldite epoxy resin, and HEPES were from Sigma (St. Louis, MO); and hematoxylin and eosin were from Nuclear (Diadema, Brazil).

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

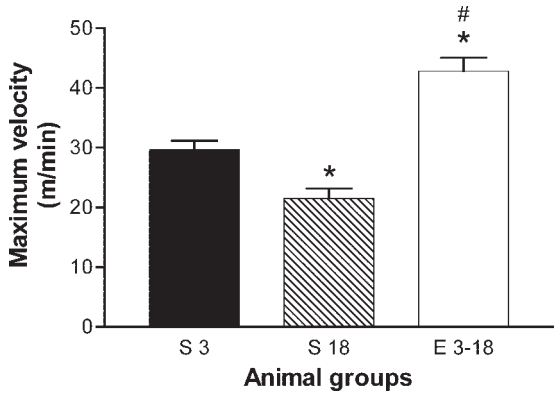

Fig. 1. Physical performance of young (S 3, $n = 8$), aged (S 18, $n = 5$), and continuously exercised (E 3–18, $n = 5$) C57BL/6 mice. The animal performance was evaluated by the maximum speed reached during a treadmill running incremental test. *Significant difference relative to S 3 animals, $P < 0.05$. #Significant difference relative to S 18 mice, $P < 0.05$.

Table 1. *Body weight, wet heart weight, and wet heart-to-body weight ratio from S 3 (n = 5), S 18 (n = 5), and E 3–18 (n = 5) animal groups*

	S 3	S 18	S 3–18
Body weight, g	27.6±1.1	32.8±0.9	31.7±1.0
Heart weight, g	0.116±0.009	0.222±0.020*	0.189±0.014*
Wet heart/body weight ratio, %	0.42±0.04	0.67±0.05*	0.60±0.05*

Values are means ± SE. S 3, sedentary young 3-mo-old mice, S 18, sedentary aged 18-mo-old mice, S 3–18, animals continually exercised from 3 to 18 mo. *Significant difference compared with S 3 animals, $P < 0.05$.

RESULTS

Physical performance

Figure 1 shows that the aged group (S 18) presented a 27% significant decrease of the maximum velocity compared with younger animals (S 3). On the other hand, animals submitted to a continuous moderated aerobic exercise during 15 mo (E 3–18) displayed not only a 100% enhancement in the maximum velocity compared with the 18-mo aged animals (S 18) but also a surprising 44% improvement of physical performance compared with younger ones.

Morphological studies. A common phenomenon associated with aging is the reduction of the skeletal muscle mass known as sarcopenia. Figure 2 illustrates that an 18-mo aging period caused a 47% reduction of the cross-sectional area of gastrocnemius fibers compared with the corresponding muscle from young animals. In contrast, the gastrocnemius muscle fibers from 18-mo-old animals submitted to a prolonged aerobic exercise program over 15 mo, with no interruptions (E 3–18), were well preserved with cross-sectional area ~2,000 μm^2, similar to those observed in the young animals (S 3) (Fig. 2).

Cardiac hypertrophy, commonly evaluated through the heart wet weight-to-body weight ratio (HW/BW ratio), is considered a good marker of endurance conditioning. However, because aging also promotes cardiac hypertrophy, it was interesting to verify whether this marker also stands for aged exercised animals. Table 1 illustrates the heart wet weight, body weight, and HW/BW ratio for the three animal groups. There was a significant enhancement of the HW/BW ratio in both S 18 and E 3–18 animal groups compared with that ratio exhibited by S 3 group. This increased ratio was rather due to a higher increase in the heart weight, which was of 97% for the S 18 animals and 62% for E 3–18 animals, than in body weight, which remained unchanged in aged mice, exercised or not (Table 1).

Lipid peroxidation. We investigated one of the well-known damaging processes associated to oxidative stress, the membrane lipid peroxidation (36), of distinct tissues of the three animal groups. As shown in Fig. 3, the S 3 level of lipid peroxidation was quite variable according to the organ studied. The heart presented the highest level, ~620 $nmol \cdot ml^{-1} \cdot g^{-1}$ dry tissue, and the gastrocnemius muscle and ileum intermediary levels, ~350 $nmol \cdot min^{-1} \cdot g^{-1}$ dry tissue. Aging caused 81 and 48% significant increase in gastrocnemius muscle and ileum membrane lipid peroxidation, respectively. However, when a continuous moderate exercise program was introduced as a daily routine (E 3–18 group), a drastic reduction to levels down to or lower than 200 $nmol \cdot min^{-1} \cdot g^{-1}$ dry tissue was observed in the MDA concentrations in heart, gastrocnemius muscle, and ileum.

Ileum cellular and ultracellular studies. The enhanced aged oxidative stress on the ileum and the protective effect exerted by 15 mo of aerobic exercise led us to explore the ileum

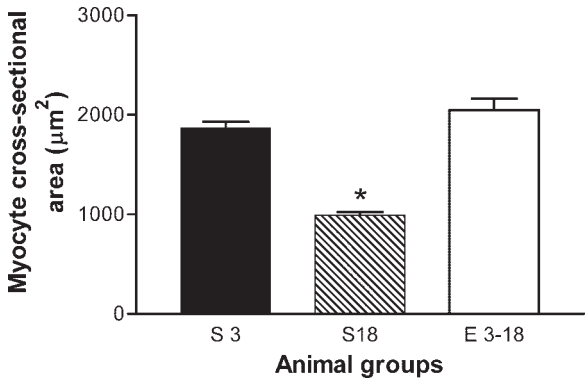

Fig. 2. Histograms of cross-sectional area of the gastrocnemius fiber muscles isolated from the S 3 ($n = 5$), S 18 ($n = 6$), and E 3–18 ($n = 5$) animal groups. *Significant difference relative to S 3 animals, $P < 0.05$.

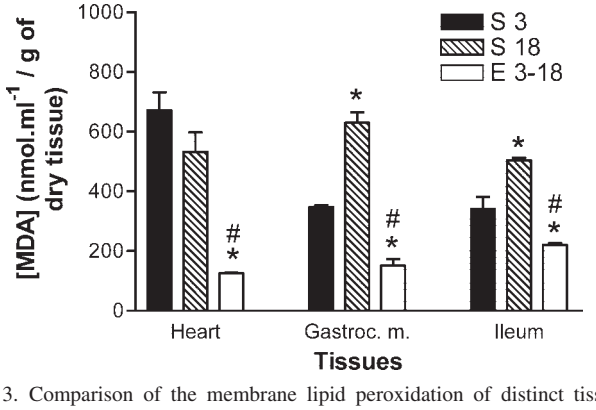

Fig. 3. Comparison of the membrane lipid peroxidation of distinct tissues isolated from S 3 ($n = 4$), S 18 ($n = 4$), and E 3–18 ($n = 4$) animal groups. [MDA], malondialdehyde concentration. *Significant difference in relation to S 3, $P < 0.05$. #Significant difference relative to S 18 animals, $P < 0.05$.

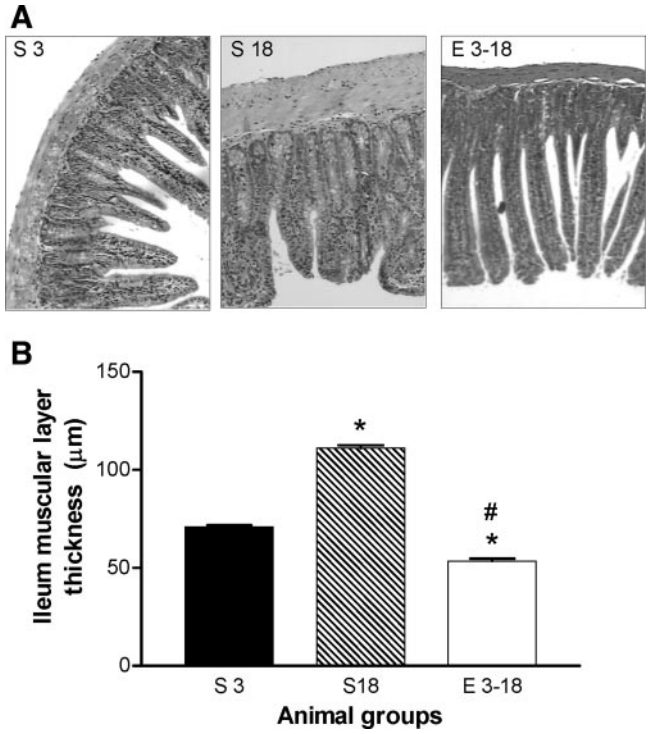

Fig. 4. *A*: representative ileum thin section light micrographs from S 3, S 18, and E 3–18 animal groups. Magnification, 100-fold. *B*: histogram of the muscular layer thickness of the ileum from S 3 ($n = 5$), S 18 ($n = 7$), and E 3–18 ($n = 5$) animal groups. *Significant difference in relation to S 3 animals, $P < 0.05$. #Significant difference in relation to S 18 mice, $P < 0.05$.

cellular and ultracellular structure alterations of the three animal groups.

Figure 4 illustrates representative light micrographs of hematoxylin-eosin-stained ileum sections isolated from the three animal groups. Aging and aging associated with prolonged aerobic exercise caused opposite cellular effects on the intestine structure. Compared with the ileum histological structure pattern from young animals (Fig. 4, S 3), the ilei isolated from the S 18 animal group (Fig. 4, S 18) exhibited a moderate to high damage of the mucosa, with some epithelial lifting of the epithelial layer, the presence of few denuded villi, and a mild level of architectural distortion. These structural alterations of the mucosa layer can be classified as grade 2 or 3, according to Chiu et al. (5). Besides damage of the mucosa, the most impressive aging effect was the significant thickening of the muscular layer (Fig. 4). These structural damages of both intestinal mucosa and muscular layer were not observed in the exercised aged animal group (Fig. 4, E 3–18), which exhibited an organized ileum architecture, presence of a normal mucosa with very regular villi and submucosal layers, and a muscular layer slightly thinner than in young animals (Fig. 4, S 3). Quantitatively, this impressive distinction corresponded to 57% significant increase of muscular layer thickness in aged animals and a small, but significant, 24% reduction in exercised aged animals compared with young ones. In contrast, there was a drastic and significant reduction, ~50%, of the muscular layer thickness in exercise aged animals compared with the aged ones (Fig. 4).

Considering these morphological results, it was interesting to perform ultrastructure analysis, mainly to evaluate the influence of aging and/or continuous aerobic exercise on cellular organelles. The representative electron micrograph of S 18 group ileum (Fig. 5) showed relaxed hypertrophied muscle fibers with a central elongated voluminous euchromatic nucleus, presence of interdigitated myofilaments, and elevated number of caveolae. Typically, some signals of organelle deterioration were observed in the aged animal micrographs, mainly the presence of mitochondria with distinct level of disorganization of the cristae, lamellar corpuscles, and lysosomes. In contrast, the electron micrographs of the muscular fibers of the ileum from E 3–18 group showed a more dense

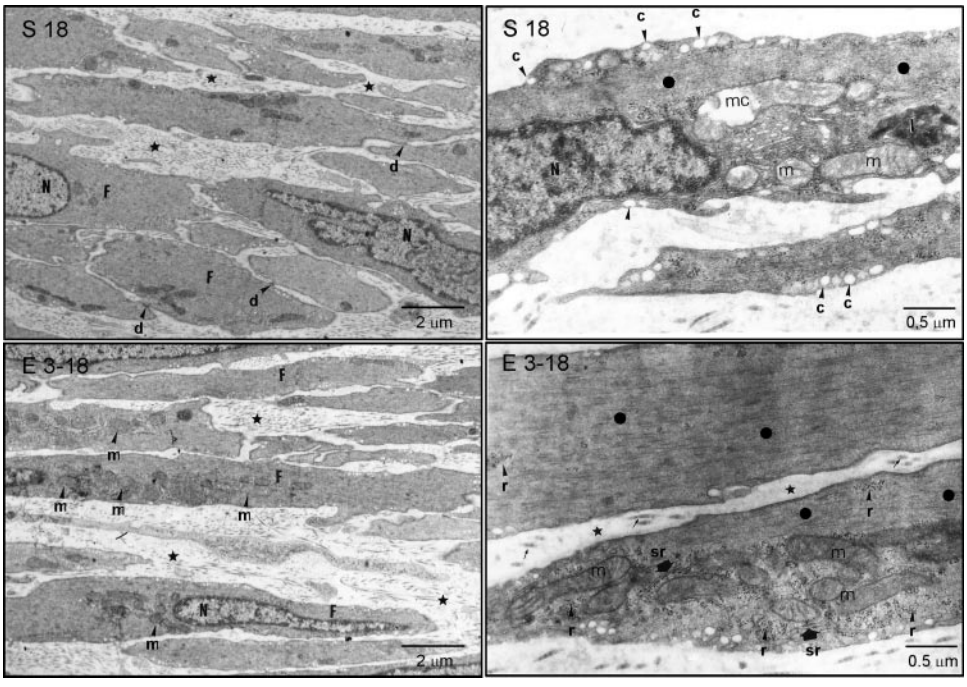

Fig. 5. Representative ileum electron micrographs isolated from S 18 ($n = 3$) and E 3–18 ($n = 3$) animal groups. F, muscular fiber; N, nucleus; ★, extracellular matrix; ●, myofilaments; m, mitochondria; mc, degenerated mitochondria; l, lysosomes; c, caveolae; d, desmosomes; sr, granular sarcoplasmic reticulum.

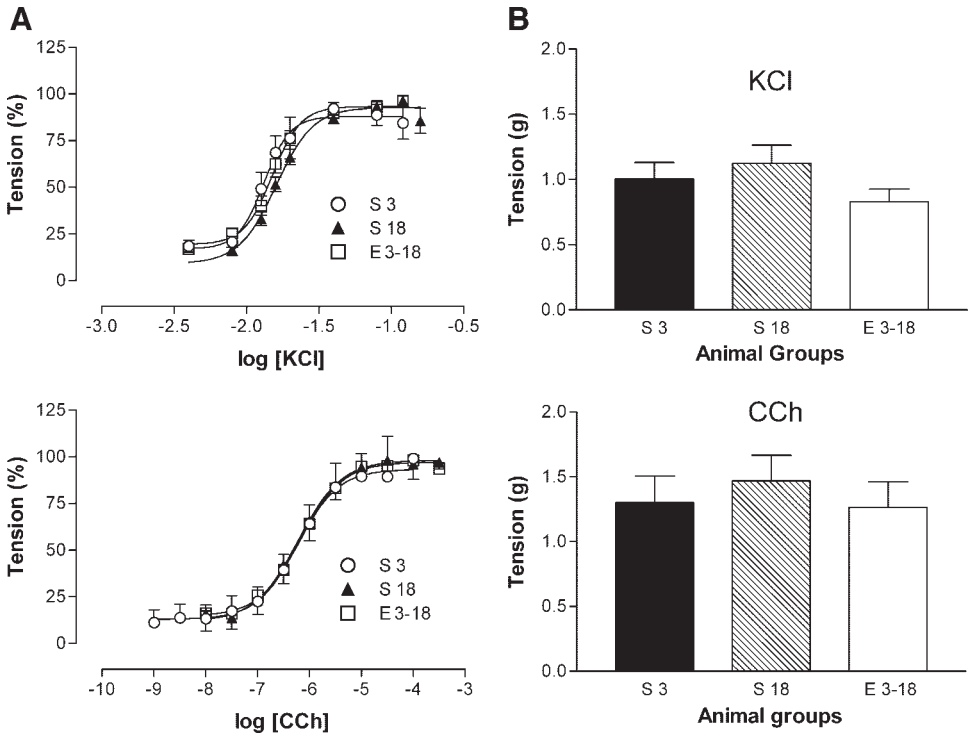

Fig. 6. *A*: comparison of isolated murine ileum responsiveness to KCl and carbachol (CCh), assessed by concentration-response curves from S 3 ($n = 6$), S 18 ($n = 12$), and E 3–18 ($n = 12$) animal groups. Tension is expressed relative to the stimulant maximum response. Brackets denote concentration. *B*: comparison of the maximum contractile responses elicited by KCl and CCh on isolated ileum from S 3 ($n = 6$), S 18 ($n = 12$), and E 3–18 ($n = 12$) animals.

interdigitated myofilament content, accumulation of numerous mitochondria, with different forms and sizes, located nearby the nuclear poles, and a central euchromatic nucleus (Fig. 5). In both animal groups, the extracellular matrix elements contained mainly type I collagen fibers and amorphous elements.

Ileum reactivity studies. To verify the effects of these structural features on the intestinal reactivity, the ileum was stimulated by increasing concentrations of a muscarinic agonist, CCh, or to KCl, in an attempt to infer on the pharmacomechanical and electromechanical couplings, respectively. As illustrated in Fig. 6*A*, there were no differences in the potency of the muscarinic receptor activation with CCh, as the EC_{50} values were ~0.6 μM for the three animal groups. Similar results were obtained for the contractile responses triggered by KCl depolarization. The EC_{50} values were similar and ~16 mM in young (S 3), aging (S 18), and exercised aged animals (E 3–18) (Fig. 6*A*). Regarding efficacy, there were no differences in the ileum maximum contractile responses triggered by addition of either CCh (~1.3 g) or KCl (~1 g) in the three animal groups (Fig. 6*B*). These results suggest the presence of compensatory mechanisms in each group to obtain the same response despite the morphological damage induced by aging.

DISCUSSION

We provide evidence, for the first time, that lifelong physical activity pattern has beneficial effects on C57BL/6 mice intestinal tissue, mainly by reversing the aging deleterious effects on the structure and oxidative status of the ileum. Despite these alterations, its contractile response was unaffected by aging or exercise. In addition, we confirmed that this exercise program exerts a clear positive effect on some well-known deleterious responses triggered by aging, such as reduced physical activity, sarcopenia of the gastrocnemius, cardiac hypertrophy, and oxidative status of these tissues.

An increasingly number of authors claim that lifestyle factors, including diet, regular aerobic exercise, vitamin supplementation, and nonsmoking habits, may favorably modulate the related adverse physiological and pathophysiological consequences of aging (3). Herein, we evaluated the effect of a continuous 15-mo period of moderate treadmill running program, starting at 3 mo old, on the murine physical performance, gastrocnemius muscle sarcopenia, HW/BW ratio, and lipid peroxidation level of distinct tissues or organs. We chose this period instead of the usual 8- to 16-wk period reported in most studies involving the beneficial effects of a prolonged exercise program (22, 39) because 8 wk cover only 15–30% of the animal life span and thus cannot be considered as a habitual practice.

One consistent deleterious effect of aging described in animal or human models is the impairment of physical performance (28). The maximum velocity reached by aged mice in the incremental running test was significantly reduced to 50% of the value attained by young animals (Fig. 1). However, by exercising during 15 mo, the animals preserved their capacity of adaptive response to aerobic exercise. Indeed, there was a drastic physical performance improvement in aged exercised animals, which was not only higher than their aged-matched pairs but also 44% higher than the young animal values (Fig. 1). These results may be attributed to intrinsic aspects of the designed exercise program. Although not intended to improve performance, because the principles of overloading and overcompensation were not applied (15) and the exercise stimulus was maintained ~60% of maximal oxygen uptake all over the animal life span, one might suppose that the mice were still submitted to a certain level of overloading considering they were growing older at the same time. Corroborating the beneficial effect of exercise, the expected age-induced sarcopenia of the gastrocnemius muscle was totally prevented in aged

animals continuously exercised (Fig. 2). This shows once again the preservation of the adaptive response of the elder animals to exercise stimulus, as they are able to develop the specific musculature engaged in the movement. In contrast, the increased HW/BW ratio, widely accepted as a good marker of aerobic conditioning (8), must be interpreted with caution, because it is known that both aging and endurance exercise induce cardiac hypertrophy (4, 8). The same heart weight values observed in aged animals regardless of exercise routine suggest that the daily physical activity somehow protected the senescent heart against the pathological age-related hypertrophy (Table 1). Obviously, this is a mere speculation, albeit an attractive one, because we did not carry out further morphological studies of the heart to better clarify this point. Consequently, cardiac hypertrophy cannot be used as an aerobic conditioning marker in aging research, as usually done in exercise physiology studies (8). As far as we know, this is the first time that such a prolonged moderate exercise program was investigated and its remarkable beneficial effect against aging deleterious effect was so clearly demonstrated.

Aging has been hypothesized to be caused by the cumulative and deleterious effects of ROS over the life span (13). On the other hand, exercise intensity is also known to interfere with the cell redox status, by altering the balance between oxidant production and antioxidant defense mechanisms (19). Whereas a moderate exercise program can improve the organism redox status by augmenting the defense mechanisms to a higher level, thus compensating any simultaneous increase of oxidant products, strenuous physical exercise, in turn, increases ROS production and oxidative stress (18). The better physical performance of aged exercised animals compared with the physical performance of aged animals led us to suppose that this could be due to a better overall oxidative status of the former animal group. So, to clarify this point, we indirectly evaluated the redox status by measuring membrane lipid peroxidation of distinct tissues and organs isolated from the young, aged, and exercised aged animals (Fig. 3). As expected, and already described by other authors (23, 37), the level of oxidative stress was quite variable according to the tissues or organs studied (Fig. 3). Nevertheless, the main point here is to remark that the gastrocnemius muscle, which is one of the muscles directly engaged in the treadmill running, underwent a drastic and significant prooxidative shift of its redox status with aging, because there was 81% increase in the MDA concentration, one of the end products of the membrane lipid peroxidation (Fig. 3). More interesting was the clear and strong improvement of the redox status of the gastrocnemius muscle when the regular moderate exercise program was associated with the aging process (Fig. 3). This improvement was also observed for the heart, which was the organ with the highest lipid peroxidation level among the organs and tissues studied, in both aged and young animals (Fig. 3). These data strongly suggest that the heart is under a high oxidative stress condition in this rodent species, which is likely due to their accelerated aerobic metabolism (6). In addition, these results also evidenced that aerobic heart conditioning is a powerful way to counteract this unfavorable oxidative condition. Navarro et al. (29) have recently described that moderate exercise, namely 52 wk of 5-min daily session throughout the week of treadmill running, decreased the aging-associated development of oxidative stress in other tissues by preventing the increase of lipid and protein oxidation, decrease in antioxidant enzyme activities, and decrease in mitochondrial oxidase and reductase enzyme activities. Finally, it might be argued that the lipid peroxidation data presently described were not fair enough because of the nonspecificity and low sensitivity of the methodology employed for measuring lipid peroxidation, i.e., the MDA reaction with thiobarbituric acid-reactive substances (16). However, the differences observed were so expressive that if any correction had to be made, this would be for an underestimation of the differences described, therefore not interfering with our interpretation of the data.

The main and most interesting point we addressed in this study was the aging effects on the structure and responsiveness of the ileum, considering that intestinal problems such as constipation, inflammation, and cramps are very frequent complaints among elderly people (14). We thus raised the hypothesis that aging-associated intestine dysfunctions could be related to the increase of its oxidative stress, which could be counteracted by prolonged moderate exercise. Thus appropriate exercise could promote a favorable shift of the oxidant-antioxidant balance as a consequence of the activation of tissue antioxidant mechanisms triggered in response to ischemia-reperfusion tissue hypoxia (17, 32). In fact, ileum hypoxia induced by submaximal exercise in old rats has recently been demonstrated (27). So, we have studied the aging effects on ileum structure by means of light and electron microscopy studies, lipid peroxidation, and contractile responsiveness as a functional test. In addition, we investigated whether a regular long-term moderate exercise program might contribute to preserve intestinal tissue against aging effects.

Murine ileum was sensitive to aging regarding structure and redox status, because it showed clear signs of degeneration at both cellular and ultracellular levels, and higher oxidative stress (Figs. 3–5). On the other hand, the introduction of daily moderate treadmill running sessions during a 15-mo period, corresponding to 80% of the animal life span, exerted a striking protective influence against aging deleterious effects in C57BL/6 mice (Figs. 4 and 5). Moreover, this exercise program not only diminished aging oxidative stress by reducing ileum lipid peroxidation but also remarkably improved the redox status of the senescent intestine even compared with young animals (Fig. 3).

Surprisingly, all these morphological and redox status changes did not have any functional consequences on tissue responsiveness, because neither the electromechanical coupling nor the muscarinic pharmacomechanical coupling was influenced by aging (Fig. 6). This might indicate that either the signaling transduction pathways are well preserved or there are some compensatory mechanisms leading to the same contractile response. The scarce literature focusing on ileum responsiveness in aged animals or in experimentally induced ileum hypertrophy conveys the notion that the contractile response is diminished. Lofgren et al. (24), when inducing guinea pig ileum hypertrophy by partial occlusion, observed modification of the ileum contractile function, mainly by altering the expression of myosin isoforms. The only study dealing with aging on ileum was reported by Ochillo and Tsai (30), who observed a diminished responsiveness of the muscarinic receptor activation in the longitudinal muscle of guinea pig ileum, which could not be explained by a reduction in the number of receptors in aged animals. Besides the distinct animal species, the discrepancy of the present results relative to those described by Ochillo and Tsai may be attributed to the

different muscarinic agonists used. The influence of aging on acetylcholine-evoked contraction has been explained by an effect on the activity of the enzyme acetylcholinesterase (21), but in the present study the influence of this enzyme was ruled out because the ileum was stimulated with choline instead of acetylcholine (Fig. 6). So, the absence of aging and/or exercise effect on ileum responsiveness, despite the loss of cholinergic nervous terminations and swallowing of nitrergic ones (12), is likely due to the large reserve capacity of this tissue as a constituent organ of the gastrointestinal system (33).

In summary, we demonstrated for the first time that a lifelong physical activity pattern is a suitable strategy to counteract the observed cellular and ultracellular morphological alterations and the oxidative stress caused by aging on the isolated murine ileum. In addition, the beneficial effects of this kind of exercise are also the reversion of the well-known effects of aging, such as impairment of the physical performance, sarcopenia, heart hypertrophy, and enhanced oxidative stress of skeletal and cardiac muscles.

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