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Original Contribution

Systemic and mitochondrial adaptive responses to moderate exercise in rodents

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Abstract

The systemic and nonmuscular adaptive response to moderate exercise is reviewed and compared with muscle responses to moderate and exhaustive exercise. Rats participating in voluntary wheel running and mice subjected to treadmill exercise on a lifelong basis showed 10–19% increased median life span. Mice also showed improved neurological functions, such as better (35–216%) neuromuscular coordination (tightrope test) and better (11–27%) exploratory activity (T maze). These effects are consistent with the systemic effects of moderate exercise lowering hyperglycemia, hypercholesterolemia, and hypertension. Mitochondria isolated from brain, liver, heart, and kidney of exercised mice show a 12–32% selectively increased complex IV activity, with a significant correlation between complex IV activity and performance in the tightrope test. Chronic exercise decreases (10–20%) the mitochondrial content of TBARS and protein carbonyls in the four organs after 24 weeks of training. Protein carbonyls were linearly and negatively related to complex IV activity. Exercise increased the levels of nNOSμ in human muscle and of nNOS in mouse brain. It is concluded that chronic moderate exercise exerts a whole-body beneficial effect that exceeds muscle adaptation, likely through mechanosensitive afferent nerves and β-endorphin release to brain and plasma that promote mitochondrial biogenesis in distant organs.

Keywords: Moderate exercise; Systemic adaptation; Life span; Neurological function; Electron transfer; Oxidation products; mtNOS; Free radicals

There is an active interest in the research involving exercise and its physiological effects that extend from the adaptive responses associated with regular moderate exercise to the tissue damage that follows high-intensity exercise. An operative distinction is made between strenuous or high-intensity exercise, performed by athletes or by experimental animals that are subjected to exhaustive exercise, and the moderate regular exercise of most humans and of experimental animals. In turn, high-intensity exercise is divided into two types: sprint exercise, in which anaerobic muscle work is mainly involved, as in short fast runs and in weight lifting, and endurance exercise, with its high-intensity aerobic muscle work and full involvement of the lung–heart–muscle unit. Exhaustive exercise has been long associated with oxidative stress and oxidative damage based on experimental data showing an increase in oxidative stress markers after high-intensity exercise. Davies et al. [1] early reported increased free radicals, detected by EPR, in the muscle of rats subjected to exhaustive exercise. More recently, Gomez-Cabrera et al. [2] pointed out the role of xanthine oxidase in the oxidative damage that occurs after strenuous exercise in rats and humans. Moderate exercise is similar to endurance exercise, with different quantitative intensity but with the characteristic difference that moderate exercise is not accompanied by muscle tissue damage as is the case with exhaustive exercise. The beneficial effects of regular moderate exercise have been consistently reported in a series of human situations and diseases and in studies with experimental animals. There is ample evidence of the reduction in skeletal

Abbreviations: EPR, electron paramagnetic resonance; GSSG, glutathione disulfide (oxidized form); HSP70, heat-shock protein 70; LPS, Escherichia coli lipopolysaccharide; NADH and NAD, reduced and oxidized forms of nicotinamide adenine dinucleotide; NOS, nitric oxide synthase; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; mtNOS, mitochondrial NOS; TBARS, thiobarbituric acid-reactive substances.

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muscle mass upon aging and of the beneficial effects of regular exercise in increasing muscle mass and strength in elderly humans. The evidence extends from experimental animals to humans and from biochemical markers to physiological parameters and behavioral performance [3]. In this article we review the systemic and nonmuscular beneficial effects of moderate exercise according to our experience utilizing the mouse–treadmill model [4,5].

Moderate exercise and rodent life span

It has been reported that chronic exercise produces a slight to moderate increase in life span in rodents. Holloszy et al. [6] observed that male rats with access to voluntary wheel running exhibited an about 10% increase in median life span, considering as controls ad libitum or per-faid rats which do not show change in maximal life span (Fig. 1A). Voluntary wheel running started at full rat adulthood (350–420 g) and declined with rat age, with 43 and 4.8 km/week run at 8 and 34 months of age.

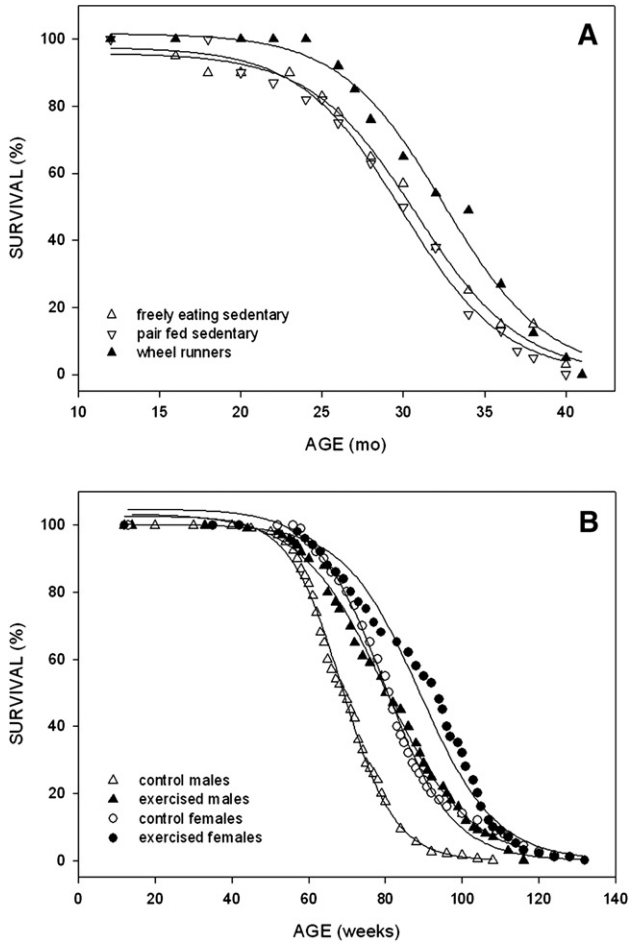

Fig. 1. Effects of chronic moderate exercise on rodent life span. (A) Voluntary wheel exercise in rats. Median life span: sedentary control pair fed (30 rats), 30 months; sedentary control fed ad libitum (54 rats), 31 months; voluntary runners (32 rats), 34 months. Redrawn from Holloszy et al. [6]. (B) Treadmill weekly exercise in mice (40 mice/group). Median life span: control males, 69±2 weeks; exercised males, 81±2 weeks; control females, 81±2 weeks; exercised females, 89±3 weeks. Maximal life span (last survivor): control males, 108 weeks; exercised males, 124 weeks; control females, 116 weeks; exercised females, 136 weeks. The mice used, Swiss CD1-UCadiz, belong to a senescence-accelerated strain [21]. Reproduced from [5] by permission of the publisher.

Navarro et al. [5] subjected male and female mice to moderate treadmill exercise, 5 min at each velocity (10, 15, and 20 cm/s) in one session and with one session per week [5], equivalent to running about 60 min/week or 10 km/week for humans. Chronic moderate exercise showed the important result of an increased median life span in males (17%) and females (10%), with similar effects in increasing maximal life span, 15% in males and 17% in females [5] (Fig. 1B).

This highly significant effect of moderate exercise in increasing rat and mice median life span, the most sensitive parameter to describe effects on aging and survival by chronic conditions or treatments [7], is properly described as a shift-to-the right of the survival curve.

The systemic beneficial effects of moderate physical exercise

The assessment of whole-body beneficial effects of chronic moderate exercise was approached by the determination in mice of the quality of the neurological response as a function of age. The neurological performances of exercised and control mice were followed from 28 weeks of age (young mice) to 78 weeks of age (senescent mice) with the tightrope and the T-maze tests. In the tightrope test for the evaluation of neuromuscular coordination, mice are placed in the middle of a 60-cm tightrope by hanging from their anterior legs and the assay is considered successful if the mice reach the column at the end of the tightrope in less than 60 s [5]. In the T-maze test for the evaluation of exploratory and cognitive activities, mice are challenged in a T maze with 50-cm arms and the assay is considered successful if the mice move toward the intersection in less than 60 s [5]. The level of success of the experimental mice in the tests, initially high at 28 weeks of age, about 58% in the tightrope and 80% in the T maze, decreased continuously as a function of age up to 78 weeks of age to values of 8 and 15% in males and females, respectively, in the tightrope and to 35 and 45% in males and females, respectively, in the T maze (Figs. 2 and 3). Most interestingly, chronic moderate exercise showed the important effect of improving mouse performance in both assays, revealing a higher quality of neuromuscular and neurological activities. Concerning the tightrope test, moderate exercise significantly and markedly improved success by 35 and 39% in males and females, respectively, at 52 weeks and by 163 and 216% in males and females at 78 weeks of age (Fig. 2). Similarly, moderate exercise improved, significantly but more moderately, mouse performance in the exploratory assay of the T maze, by 13 and 11% in males and females at 52 weeks of age and by 23 and 27% in males and females at 78 weeks of age (Fig. 3).

The current clinical knowledge of the past 2 decades has recognized the beneficial effects of exercise in lowering hyperglycemia [8,9], hypercholesterolemia [10], and hypertension [11]. Consequently, exercise has been regularly prescribed as part of the treatment for diabetes [12], hypertension, and cardiovascular disease [13–15] and to lower the risks of metabolic syndrome [16,17] and neurodegenerative diseases

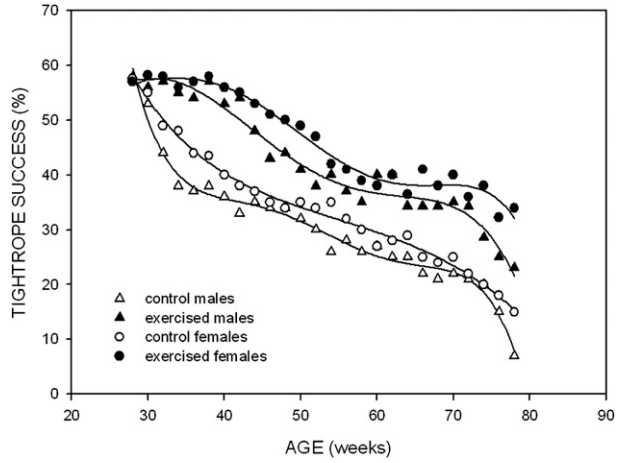

Fig. 2. Effects of chronic moderate exercise on mouse performance in the tightrope test as a function of age (40 mice/group). Points correspond to mean values of pooled mice in tests performed every 2 weeks. Reproduced by permission of the publisher from [5].

[18,19]. However, the mechanisms by which muscle exercise exerts these multiple effects are yet poorly understood, with the exception of the exercise-induced increase in the insulin-sensitive glucose transporter 4 [12] and in the insulin receptor [20] of skeletal muscle plasma membrane, which play logical roles in ameliorating type 2 diabetes.

It is concluded that chronic moderate exercise exerts a whole-body beneficial effect that exceeds muscle adaptation.

Effects of moderate exercise on the mitochondrial function of organs other than exercised muscle

It is well known that muscle adaptation to regular exercise involves mitochondrial biogenesis and synthesis of new components of the respiratory chain to match increased energy demands [21]. As an approach to the above-mentioned systemic and nonmuscular effects of exercise, the rate of electron transfer by mitochondrial complexes I, II, III, and IV was studied in brain, liver, heart, and kidney from exercised mice. Mice of 28 weeks of age were subjected to the same exercise protocol that improved survival and neurological function, and mitochondria were isolated after 24 and 50 weeks of regular moderate exercise. At the first point, 24 weeks of chronic exercise, complex IV (cytochrome oxidase), the enzyme that catalyzes the ATP-yielding mitochondrial O_2 uptake, showed a consistently increased activity, in the range of 12–32%, in brain, liver, heart, and kidney of male and female mice [5]. The effect was almost selective on complex IV; the activity of complex I was increased in mitochondria only from male and female brain and from female heart and kidney. The activities of complexes II and III were not affected by exercise in any of the four organs of male or female mice and served as negative controls to give specificity to the finding [5]. Complex I and IV activities decrease upon aging [22] and regular moderate exercise for 24 to 50 weeks exhibited a beneficial effect that was able to partially prevent the age-associated decline in the activities of mitochondrial complexes IV and I in the four mentioned organs in male and female mice [5]. As an example, Table 1 shows the data corresponding to the effects of chronic regular exercise on brain and liver mitochondria of male mice.

The increased activity of complex IV in mitochondria from brain, liver, heart, and kidney was linearly related ($r^2 = 0.75$–0.88; $p < 0.01$) to the successes in the tightrope test, considering male and female mice [5]. This fact links the quality of mitochondrial function, in terms of enzymatic activity, to the quality of the neuromuscular response. Thus, regular physical exercise is recognized as a factor in retarding the cell damage and physiological dysfunction that are characteristic of the aging process in organs other than the directly involved skeletal muscle. The adaptive beneficial effects of exercise have been observed in heart [5,23], kidney [5], liver [5], and brain [5,24,25]. These systemic and nonmuscular beneficial effects of moderate exercise support the concept of a complex regulatory system involving nervous regulation, systemic cytokine release, and genomic modulation. Radak et al. [26,27] have reported that moderate exercise activates DNA repair systems, involving

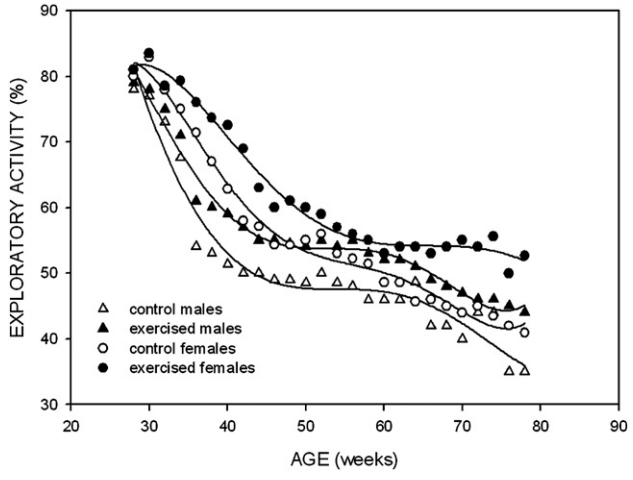

Fig. 3. Effects of chronic moderate exercise on mouse exploratory activity in the T-maze test as a function of age (40 mice/group). Points correspond to mean values of pooled mice in tests performed every 2 weeks. Reproduced by permission of the publisher from [5].

Table 1
Effects of chronic moderate exercise on the enzymatic activities of respiratory complexes in brain and liver mitochondria of male mice

Exercise duration	Brain		Liver	
	24 weeks	50 weeks	24 weeks	50 weeks
Complex IV				
Exercised mice	108±5 *	90±6	127±5 *	81±5
Control mice	93±5	77±9	101±5	74±6
Complexes I and III				
Exercised mice	306±12 *	225±10	341±15	232±16
Control mice	269±10	214±10	349±14	214±14
Complexes II and III				
Exercised mice	111±6	114±6	162±9	150±6
Control mice	120±6	117±9	158±8	142±8

Values are means±standard error and the enzymatic activities are expressed in nmol cytochrome *c* reduced or oxidized/min × mg of mitochondrial protein. Adapted from [5].

* $p < 0.05$.

oxoguanine and uracyl DNA-glycosylases, and improves the resistance against oxidative stress and aging in rat skeletal muscle.

Both moderate and high-activity exercise are understood to produce an increase in mitochondrial mass in muscle [21]. This response may be well extended to other organs, a point that has not yet been explored. For years it was assumed that during exercise muscle would have an increased mitochondrial production of oxygen free radicals, about linearly related to the rate of respiration [28,29]. This was a misconception because moderate exercise is accompanied by a decreased mitochondrial production of superoxide radicals and hydrogen peroxide in muscle and in heart, in agreement with the lower rate of hydrogen peroxide production in resting (State 4) mitochondria compared with active (State 3) mitochondria [30]. Exercise by lifelong voluntary wheel running reduced subsarcolemmal and interfibrillar mitochondrial H_2O_2 production in the heart [31].

The current knowledge points to an overproduction of cellular oxidizing free radicals during exhausting exercise due to xanthine oxidase activation [2].

Oxidation products, antioxidant enzymes, and moderate exercise

It has long been recognized that excess oxidizing free radicals and oxidation products of free radical-mediated reactions, such as GSSG and lipid peroxidation products, are increased in muscle during exhaustive exercise [1,32–35], along with myocyte damage, which is expressed by the release of enzymes and proteins to plasma. The released muscle enzymes include lactate dehydrogenase, creatine kinase, and aspartate–alanine transaminase and the released muscle proteins include myoglobin, troponin, and HSP70 [33,36–38]. Acute exercise also affects the antioxidant profile of other organs, as reported for liver GSSG and HSP70 [34,38]. Systemic oxidative stress occurs after acute intense exercise and is sensed as increased plasma levels of TBARS, GSSG, 8-isoprostane, conjugated dienes, and 8-HO-deoxyguanosine [33–35,37,39].

Chronic moderate exercise shows opposite effects compared to acute high-intensity exercise. The word hormesis, taken from toxicology to refer to the dual effects of a substance, beneficial at low doses or levels and toxic at high doses or levels, has been used to describe the effects of exercise [40]. Chronic moderate exercise decreases the mitochondrial contents of oxidation products, TBARS, and protein carbonyls in brain, liver, heart, and kidney [5] (Table 2). The effect was evident in adult mice after 24 weeks of training (about 20% for males and 10% for females) and not noticeable in senescent mice, after 50 weeks of exercising. Interestingly, mitochondrial protein carbonyls were linearly and negatively related to the activity of complex IV; the more oxidative damage, the lower the complex IV activity [5].

For years there was an active interest in the determination of the activities of the antioxidant enzymes in exercised muscle due to the knowledge of the oxidative damage that follows intense exercise [1,2,28,29]. The mitochondrial and cytosolic isoforms of superoxide dismutases, Mn-SOD and Cu,Zn-SOD, were generally reported to be up regulated in muscle after regular bouts of high-intensity exercise or after chronic moderate exercise. Other antioxidant enzymes, such as catalase and glutathione peroxidases, did not show the same consistent up regulation [4,41,42]. Chronic moderate exercise shows a moderate effect in increasing the activities of Mn-SOD, Cu,Zn-SOD, and catalase in brain, heart, liver, and kidney of mice exercised for 24 to 50 weeks. Antioxidant enzyme activities were increased by about 15–20% after 24 weeks of regular exercise, but the effect almost disappeared in senescent mice after 50 weeks of exercise [5].

Table 2
Effect of chronic moderate exercise on the content of oxidation products, TBARS and protein carbonyl groups, in brain and liver mitochondria of male mice

Exercise duration	Brain		Liver	
	24 weeks	50 weeks	24 weeks	50 weeks
TBARS				
Exercised mice	5.7±0.5 *	9.1±0.4	4.0±0.2	4.6±0.3
Control mice	8.5±0.4	8.9±0.4	4.5±0.2	4.8±0.2
Protein carbonyl groups				
Exercised mice	61±3 *	81±3 *	140±7 *	184±8
Control mice	79±5	93±5	169±7	187±7

Values are means±standard error and the content of oxidation products is expressed in nmol/mg of mitochondrial protein. Reproduced by permission of the publisher from [5].

* $p<0.05$.

Nitric oxide and mitochondrial nitric oxide synthases in exercise

Nitric oxide has a physiological role in the regulation of blood flow during exercise; endothelial NO controls exercise-induced hyperemia in the heart coronary arteries and in contracting skeletal muscle. It has been accepted that the three isoforms of NOS (nNOS, iNOS, and eNOS) are expressed in the skeletal muscles of all mammals and that nNOS is the predominant isoform [43,44]. Muscle NOSs are highly regulated, their expression and localization are regulated by complex factors, including muscle activity, nervous innervation, exposure to cytokines and growth factors, and nonphysiological effectors, such as LPS [43,45]. Conflicting results have been reported concerning the immunoreactivity and localization of muscle NOS isoforms [43,45]. The 51–57% homology reported for nNOS, iNOS, and eNOS with the cross-reactivity to the anti-NOS antibodies can explain some of the conflicting observations [46]. It can be considered, extending to skeletal muscle what has been found in heart muscle in relation to NOS [47], that the mitochondrial isoform (mtNOS) serves the physiological functions of regulation of myocyte respiration and of intracellular signaling, whereas the isoform of the sarcoplasmic reticulum (eNOS) is involved in the activation of the GMP cyclase and in cGMP production to activate the sarcoplasmic reticulum Ca^{2+}pump and Ca^{2+}sequestration after muscle contraction.

Human muscle mainly expresses nNOS μ (the splice variant μ of nNOS), with barely detectable levels of iNOS and eNOS.

Table 3
MtNOS activity in mitochondria isolated from tissues of mice subjected to moderate exercise on the treadmill with 12 daily sessions at 10, 15, and 20 cm/s per session

Tissue	Control mice	Exercised mice
Whole brain	0.62±0.05	1.58±0.10 * (154%)
Heart	0.76±0.05	1.10±0.08 * (45%)
Kidney cortex	1.30±0.09	1.70±0.10 * (31%)
Liver	1.36±0.10	1.54±0.11 (13%)
Skeletal muscle (leg)	0.84±0.06	1.18±0.10 * (40%)

MtNOS activity in nmol NO/min × mg mitochondrial protein. In parentheses, percentage of increase. Authors' original observation.
* $p<0.05$.

Moreover, the levels of nNOS μ in human muscle were found to be up regulated by exercise: nNOS μ was 60% higher in trained athletes than in sedentary individuals and 10 days of intense exercise increased nNOS μ levels in type I, IIa, and IIx muscle fibers [48,49].

The effects of exercise in up regulating mtNOS activity were also observed at a distant organ, in the brain. The mtNOS activity of brain mitochondria was 47% (from 0.36 to 0.53 nmol NO/mg protein; $p<0.05$) and 55% (from 0.20 to 0.31 nmol NO/mg protein; $p<0.05$) increased after 24 and 50 weeks of chronic moderate exercise in mice [50]. The same exercise protocol during only 12 days confirmed the up regulation of whole-brain mtNOS by moderate exercise, with a 154% increase in enzyme activity. Other organs, such as heart, kidney, and liver, and the target leg muscle showed a more moderate up regulation, in the range of 13–45% (Table 3).

Hypothesis for the systemic effects of moderate exercise

The systemic effects of moderate exercise constitute a challenge in terms of the formulation of a hypothesis. The physiological condition of repeated muscle contraction generates intramyocyte and extramyocyte changes that constitute physiological signals.

In the myocyte, the repeated ATP-consuming contractions change the redox state of the mitochondrial respiratory chain to a more oxidized state. The ratio of the mitochondrial NADH/NAD redox pair is about 80–100 under muscle resting conditions (mitochondrial State 4) but in intense exercise (mitochondrial State 3) the ratio is about 2–3 [51]. Sirtuin-2, the histone deacetylase that controls gene silencing and expression, is activated by NAD and it has been reported to be activated in myocytes after exercise [52]. A speculation of this hypothesis is that activated sirtuin-2 promotes the expression of muscle NOS, specially the mtNOS isoform of the inner mitochondrial membrane. Increased mtNOS activity leads to higher levels of mitochondrial NO that inhibit electron transfer at complexes IV and III and lead to enhanced H_2O_2 production [53]. Increased myocyte levels of NO, cGMP, and H_2O_2 are known signals that promote mitochondrial biogenesis [21,54,55].

Concerning the systemic effects of moderate exercise, it is considered that the repeated muscle contraction triggers, by acetylcholine from the neuroplaque or by shearing forces from the vascular system or the myocytes, mechanosensitive afferent nerve fibers (group 3). The afferent fibers activate central opioid systems that respond with β-endorphin delivery to other brain areas and with β-endorphin release to the plasma [56,57]. Systemic endorphins and released muscle proteins (such as HSP70) activate specific receptors in peripheral organs that activate mtNOS expression, which in turn promotes mitochondrial biogenesis in those distant organs [54]. The pleiotropic association between mtNOS activity and cellular homeostasis is interpreted as a NO and H_2O_2 signal from mitochondria that reflects highly energized mitochondria and promotes mitochondrial biogenesis [50,54]. The decreased level of oxidation products (TBARS and protein carbonyls) is consistent with a faster mitochondrial turnover and new mitochondrial biosynthesis from nonoxidized lipids and proteins.

Acknowledgments

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