Original Article

Oxidative Stress is a Central Target for Physical Exercise Neuroprotection Against Pathological Brain Aging

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Abstract

Physical exercise is suggested for preventing or delaying senescence and Alzheimer’s disease (AD). We have examined its therapeutic value in the advanced stage of AD-like pathology in 3xTg-AD female mice through voluntary wheel running from 12 to 15 months of age. Mice submitted to exercise showed improved body fitness, immunorejuvenation, improvement of behavior and cognition, and reduced amyloid and tau pathology. Brain tissue analysis of aged 3xTg-AD mice showed high levels of oxidative damage. However, this damage was decreased by physical exercise through regulation of redox homeostasis. Network analyses showed that oxidative stress was a central event, which correlated with AD-like pathology and the AD-related behaviors of anxiety, apathy, and cognitive loss. This study corroborates the importance of redox mechanisms in the neuroprotective effect of physical exercise, and supports the theory of the crucial role of oxidative stress in the switch from normal brain aging to pathological aging and AD.

Key Words: Alzheimer’s disease—3xTg-AD mice—Physical exercise—Oxidative stress—Behavior tests—Cognition—Amyloid β—Phospho-tau.

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Alzheimer’s disease (AD) is considered the major cause of dementia in the elderly. Age is the principal risk factor for sporadic AD, the most common form of the disease compared to less than 5% of cases of inherited familial AD. The mechanisms triggering brain pathological aging are unknown, although oxidative stress is one deleterious process common to both brain aging and AD (1). As stated in the free radical theory of aging, there is a general progressive imbalance (in favor of the former) between the generation of intracellular reactive oxygen species and the levels of antioxidant defenses, that is the major determinant of lack of functionality, pathological changes, and
ultimately the death of the individual (2) Mitochondria are particularly vulnerable to oxidative damage and are central to the theory of aging. Increased mitochondrial oxidative damage has been found to be related to sarcopenia in skeletal muscle (3). Moreover, a shortening of telomeres was reported in isolated mouse muscle fibers submitted to an oxidative injury (4). There is an association between oxidative stress markers and all-cause mortality (5). Furthermore, resistance against protein damage is associated with longevity in a range of mammal and avian species (6). Protein oxidative damage in peripheral blood is a proposed biomarker of age-related decline and severe dependence (7). Similarly, increased lipid and protein oxidative damage was shown in lymphocyte mitochondria of AD patients (8). The brain is very sensitive to oxidative stress because of its high metabolic activity, high density of oxidizable substrates, and relatively low antioxidant defense. Many studies showed increased oxidative stress in AD brains (9, 10) and AD experimental models (11, 12), although the link between amyloid and tau pathology is not well understood.

Several data suggest that unhealthy diet and lifestyle can play an important role in developing sporadic AD (13), irrespective of the significant role played by genetic factors, and physical exercise may have a major preventive value (14, 15). Cognitive benefits of aerobic exercise are well documented in older adults, including short-term interventions of 3 months (16). However, there is no consensus on the possible therapeutic value of physical exercise in advanced stages of dementia. In recent years, different running strategies involving voluntary exercise (running wheel) or forced exercise (treadmill) have been assayed in aging and AD animal models to study the ability of physical exercise to offset progressive neuropathological processes and age-related dementia (12, 17–22). The effects of physical exercise were found to be multiple and acting at different levels of brain complexity. Such effects included improvement in mitochondrial condition and reduction in oxidative damage, neurogenesis and angiogenesis through secretion of neurotrophic factors, strengthening of synaptic function and neurotransmission function, increase of brain volume, epigenetic changes, partial reduction of amyloid pathology, and systemic effects of cardiovascular and neuroimmunoendocrine rejuvenation (12, 17, 19–21, 23–27). These beneficial mechanisms are also believed to be present in humans (15, 28, 29).

Corresponding to these investigations, in previous studies using the triple-transgenic mouse model of AD (3xTg-AD) (30), we found that the physical exercise has protective effects at early and moderate stages of the disease, in both male and female mice. The 3xTg-AD mice mimic many of the critical hallmarks of AD neuropathology, including the Aβ and tau pathologies, impaired learning and memory (30), and others such as presence of behavioral and psychological symptoms of dementia (BPSDs) (12), oxidative stress (12) and immunosenescence (31). They also show age-related progression of their AD pathology. Sex differences in the pathology progression included transient cognitive loss worsening in young females (32) and earlier neuroimmunoendocrine senescence associated to lower longevity in males (31). Interestingly, mitochondria dysfunction in these mice (23, 33) mimics that of AD and other age-related diseases (34). Bearing all of these points in mind, therefore, we aimed to analyze the therapeutic value of voluntary physical exercise on the late stages of AD-like pathology and cognitive loss in the aged 15-month-old 3xTg-AD female mice, with emphasis on the centrality of the oxidative stress changes in the AD brain.

Methods

Animals

Female 3xTg-AD mice and nontransgenic (NonTg) mice with the same genetic background hybrid (129 × C57BL/6) (30) were used in the present study. Animal breeding, physical exercise treatment, behavioral studies, and necropsies were performed in the facilities of the animal house of the University of Barcelona, under approval from the local animal ethics committee (Ref: DMAH-3933, CEEA, UB), in accordance with Spanish legislation and the EU Directive 2010/63/EU for animal experiments.

Administration of Physical Exercise

The mice had free access to a running wheel at 12 months of age for 3 months. To avoid the distress of individual housing, four to five animals from different litters, grouped by genotype (NonTg or 3xTg-AD), were housed in a cage with a computerized running wheel (Activity Wheel Cage System for mice, Tecniplast, Buguggiate, Italy), as previously described (12). The sedentary control mice were housed in similar cages without a running wheel.

Immunoeendocrine Status

The body weight and running activity of the mice were controlled during the study. At necropsy, the weight of the intra-abdominal white adipose tissue (WAT), the spleen, and the thymus were recorded as an indirect measure of the immunoenodocrine status of the mice.

Behavioral and Cognitive Tests

Behavioral and cognitive tests were performed as previously described (12). BPSD-related behavior was evaluated by three tests performed on successive days, with single trial of 5 minutes each. The open-field test was used to evaluate vertical (rearings) and horizontal (ambulatory movement) activity and general behavior. The light/dark box test was used to evaluate anxiety-like behavior. The Boissier’s four-hole board test was used to assess exploratory behavior and emotionality (defecation). Finally, spatial learning and memory were measured using the Morris water maze (MWM) test, with 1 day of cue learning, 6 days of acquisition of learning of the location of the escape platform, and one last day of memory retrieval tested through the removal of the hidden platform.

Brain Samples

The animals were decapitated after evaluation of behavior and cognitive patterns. Hippocampus and cerebral cortex were dissected and stored at –80°C for further analysis.

Western Blotting

The cerebral cortical and hippocampal tissues were processed for protein determination by Western blot as previously detailed (25). Primary antibodies used were: anti-Aβ clone 6E10 (1:1,000, 4 kDa; Signet, Emeryville, CA), antiphospho-tau clone AT8 (1:1,000, phospho-Ser202, 55 kDa; Pierce, Rockford, IL), and antitau-actin (1:10,000, 30 kDa, Sigma, Madrid, Spain). Secondary antibodies used were: antimouse (1:2,000) and antirabbit (1:5,000) conjugated to peroxidase (Amersham, Arlington Heights, IL). The immunoreactive bands were detected by a chemiluminiscence method using the ECL Prime Western Blotting Detection Reagent kit (Amersham). Densitometric analysis was performed using Image Lab v3.0.1 (Bio-Rad), and protein values were referred to the corresponding pan-actin content.

Amyloid β ELISA

Soluble Aβ40 and Aβ42 were analyzed in cerebral cortical tissue using commercial ELISA kits (KHB348 and KHB344,
respective, Invitrogen, Camarillo, CA), following the manufacturer’s instructions.

Lipid Peroxidation, Superoxide Dismutase, Glutathione Peroxidase, and Glutathione Reductase Assays

The cerebral cortical tissues were homogenized in ice-cold potassium buffer (0.1 M, pH 7.0). For that purpose, 100 mg/ml were sonicated in a Labasonic M (Sartorius, Goettingen, Germany) with 3 cycles of 10-s sonication/10-s rest at a frequency of 20 kHz and a power of 100 W. Samples were maintained on ice during the procedure and then centrifuged at 12,000g for 30 minutes at 4°C. The supernatants were collected and stored at −80°C until assay. Lipid peroxidation and enzymatic activities of glutathione peroxidase (GPx) and both CuZn and Mn isoforms of superoxide dismutase (SOD) were determined as previously described (35). The glutathione reductase (GR) activity was measured by oxidation of NADPH coupled to GR-mediated reduction of GSSH.

Reduced Glutathione and Oxidized Glutathione Assays

For glutathione extraction, the cerebral cortical tissues were sonicated as described above to homogenize 200 mg/ml in ice-cold 3.3% sulfosalicylic acid in 6.6-mM HCl. The acid homogenates were centrifuged at 12,000g for 30 minutes at 4°C, and the resultant deproteinized supernatant fractions were stored at −80°C until assay. The levels of reduced glutathione (GSH) and oxidized glutathione disulfide (GSGG) were determined as previously described (12).

Statistics

The results were expressed as the mean ± SEM. Data were analyzed with two-way analysis of variance followed by Bonferroni post hoc test, by using GraphPad Prism 4 (GraphPad Software, La Jolla, CA). Column statistics were used in the removal test of the MWM. Association between all pairs of variables was evaluated by 2-tailed Pearson correlation analysis using IBM SPSS Statistics 19.0 software (IBM Corporation, Armonk, NY).

Network Analysis

Graph theory tools were used to jointly analyze the results of all the variables measured in the study. Statistically significant correlations between pairs of variables were selected to make a network. Thecorrelation network was analyzed with the PageRank centrality algorithm (36) using Mathematica v9 software (Wolfram Research, Champaign, IL). This analysis determines the importance of each variable from the global network structure and not just from the number of links (correlations between pairs of variables) to their direct neighbors. The PageRank centrality algorithm assigns a weight to each node of the network and this weight ranks its importance within the set of nodes. This numerical weight is referred to as the PageRank of the node. The algorithm is an improvement with respect to the degree centrality method, which only accounts for direct links to a node. The PageRank of a node, instead, is computed recursively and depends on the number and PageRank of all nodes in the network; nodes that are several links apart contribute less to the calculation. Thus, a node that is linked (directly and through other nodes) to many nodes which have a high PageRank value receives a high rank itself and it is said to be more central in the network.

Results

Physical Exercise Ameliorated Immunoendocrine Function in 3xTg-AD Mice

All experimental groups had a similar body weight at the beginning of the study (not shown). Physical exercise treatment induced a reduction of body weight (Figure 1A) [exercise factor, F(1,31) = 17.64, p < .001]. The WAT, spleen, and thymus weights were expressed as a percentage of body weight (Figure 1B–D). In Tg SED mice, the WAT and spleen percentages were higher, whereas the thymus percentage showed a slight tendency to be smaller, compared to NonTg SED mice [genotype factor, F(1,31) = 4.68, p = .039 and F(1,31) = 7.22, p = .011, on the WAT and spleen weights, respectively]. All tissue changes observed in the Tg SED mice were reversed in the Tg EXE animals by physical training in the running wheel [exercise, F(1,31) = 5.37, p = .025 and interaction of exercise × genotype, F(1,31) = 6.66, p = .015, on the WAT weight]. Tg EXE mice run a distance of 16.7 ± 3.82 km/wk, calculated as the mouse average from the wheel turns in each cage. NonTg EXE mice run 11.16 ± 3.29 km/wk. Running performance of both strains was similar to that in previous studies with younger females (12,24).

Physical Exercise Normalized BPSD-Like Behaviors in 3xTg-AD Mice in a Battery of Tests

In the open field test (Figure 2A–F), the horizontal, vertical, and self-grooming activities (Figures 2A–C) were significantly reduced in Tg SED mice compared to NonTg SED mice [genotype factor, F(1,32) = 5.78, p = .022 and F(1,32) = 7.68, p = .009, on the distance ambulated and grooming activity, respectively]. Physical exercise partially recovered these exploratory and adaptive responses in Tg EXE mice [interaction exercise × genotype, F(1,32) = 7.09, p = .012 and F(1,32) = 6.00, p = .002, on the distance ambulated and the number of rearings, respectively]. The latency of initial horizontal movement (Figure 2D) was increased and that of vertical activity decreased (Figure 2E) in Tg SED mice compared to NonTg SED mice, whereas the latency of self-grooming activity (Figure 2F) also increased [genotype factor, F(1,32) = 10.48, p = .003 and F(1,32) = 9.93, p = .003, on latency of vertical activity and latency of grooming, respectively]. Physical exercise ameliorated these behaviors in Tg EXE mice [interaction exercise × genotype, F(1,32) = 9.01, p = .005 and F(1,32) = 9.22, p = .007, on latency of horizontal activity and latency of grooming, respectively].

In the light/dark box test (Figure 2G–I), the Tg SED mice showed an increased number of entries into the lit area (Figure 2G) but a lower time in lit area (Figure 2H), and a higher latency of entry into the lit area (Figure 2I) than NonTg SED mice. Therefore, anxious mice that entered into the lit compartment moved quickly back to the dark compartment, this rising the number of entries even though the total time in lit area was maintained low. Physical exercise treatment recovered this anxious behavior in Tg EXE mice [interaction exercise × genotype, F(1,32) = 12.97, p = .001 and F(1,32) = 4.46, p = .042, on the latency of entry into the lit area and on the time spent exploring this area, respectively].

The results of the four-hole board test (Figure 2J–L) showed that the Tg SED mice realized a lesser number of head-dipping behaviors and showed a higher latency of dipping to the four holes than NonTg SED mice (Figure 2J) and K, respectively [genotype factor, F(1,32) = 11.47, p = .002, on the number of head-dipping behaviors]. These behavioral changes were reversed by physical exercise in Tg EXE mice [exercise factor, F(1,32) = 5.73, p = .023, on the number of head-dippings, and interaction exercise × genotype, F(1,32) = 13.97, p = .001, on the latency to explore the four holes]. The number of
defecation boli was higher in Tg SED mice than in NonTg SED mice (Figure 2L) [genotype factor, \(F(1,32) = 4.49, p = .042\)]. This emotionality indicator was reduced by physical exercise [interaction exercise \(\times\) genotype, \(F(1,32) = 6.36, p = .017\)].

Unexpectedly, NonTg EXE mice showed a worsening of latency parameters in the dark/light box test (Figure 2B) and hole-board test (Figure 2K).

**Physical Exercise Ameliorated Memory Response in 3xTg-AD Mice in the Morris Water Maze Test**

The distances covered in place task acquisition and the time spent in the platform quadrant in the removal test of the MWM test are shown in Figure 3A and B. The acquisition curves did not differ among the groups (Figure 3A) [day factor, \(F(4,155) = 3.11, p = .017\)]. In the retrieval of learning (Figure 3B), both NonTg groups and Tg EXE group spent significantly greater-than expected time in the platform quadrant than the time spent at random (column statistics, at least \(p < .05\), see Figure 3B), indicating good memory response. This effect was not observed in Tg SED mice, which swam at random in the pool, unaware of the former position of the escape platform. Time spent in the platform quadrant was also greater than that in the opposite quadrant [quadrant factor, \(F(1,54) = 29.39, p < .001\); interaction exercise \(\times\) quadrant, \(F(1,54) = 8.99, p < .001\)]. Therefore, physical exercise significantly increased memory retrieval in Tg EXE mice (Figure 3B).

**Physical Exercise Reduced Amyloid \(\beta\) Pathology**

The analysis of immunoblotting from cerebral cortical protein extracts incubated with 6E10 antibody showed a decrease in A\(\beta\) oligomers within the exercised group Tg EXE as compared to Tg SED mice (Figure 4A) [genotype factor, \(F(1,12) = 51.34, p < .001\); exercise factor, \(F(1,12) = 7.684, p = .0169\); interaction exercise \(\times\) genotype, \(F(1,12) = 5.182, p < .0419\)].

Levels of both A\(\beta\)40 and A\(\beta\)42, determined by ELISA in the cerebral cortical tissue were significantly increased in the Tg SED group as compared to NonTg mice, whose levels were negligible (Figure 4B). Exercise treatment induced a significant reduction in A\(\beta\)42 levels (59%) and a partial reduction in A\(\beta\)40 levels (30%) in Tg EXE mice [amyloid species factor, \(F(1,20) = 12.23, p = .023\); mouse group factor, \(F(2,20) = 32.54, p < .001\); and interaction between both factors, \(F(2,20) = 5.86, p = .010\)].

**Physical Exercise Ameliorated Phospho-Tau Pathology**

Physical exercise decreased the content of phospho-tau in hippocampus of Tg EXE mice as analyzed by western blot with AT8 antibody (Figure 5A) [genotype factor, \(F(1,12) = 20.16, p < .0007\); exercise factor, \(F(1,12) = 7.082, p = .0207\)]. Exercise also reduced phospho-tau content in cerebral cortex of Tg EXE mice (Figure 5B) [genotype factor, \(F(1,12) = 6.409, p = .0263\); exercise factor, \(F(1,12) = 10.73, p = .0066\)]. Absence of statistical difference in the cortex phospho-tau level of Tg SED versus NonTg SED was probably due to the variability of the data and the lower phospho-tau levels in cerebral cortex as compared to those in hippocampus of 3xTg-AD mice (37).

**Physical Exercise Normalized Cerebral Cortical Redox Homeostasis in 3xTg-AD Mice**

Lipoperoxidation levels were significantly increased in Tg SED mice compared to NonTg groups, but this effect was reversed by physical exercise (Figure 1).
exercise (Figure 6A) [genotype factor, F(1,23) = 10.37, p = .004; exercise factor did not reach significance].

The levels of GSH were similar among all mice groups (Figure 6B), whereas GSSG levels were significantly higher in Tg SED mice compared to NonTg mice (Figure 6C) [genotype factor, F(1,22) = 4.30, p = .050]. Physical exercise reversed the GSSG values in 3xTg-AD mice [exercise factor, F(1,22) = 5.71, p = .026].

Tg SED mice showed a significantly greater enzymatic activity of GPX and GR compared to NonTg SED group (Figure 6D and E), but both values were reduced to control level by physical exercise. [genotype factor, F(1,22) = 7.33, p = .012, on GPX activity; interaction exercise × genotype, F(1,22) = 15.27, p < .001, on GR activity].

CuZn-SOD activity was significantly lower in Tg SED mice than in NonTg SED group (Figure 6F). Physical exercise treatment increased CuZn-SOD activity in Tg mice until reaching activity levels similar to the NonTg SED group [interaction effect of exercise × genotype, F(1,23) = 3.23, p = .081]. The Mn-SOD activity did not differ among the groups (data not shown).

Correlations Between Variables Showed Centrality of Oxidative Stress

A network of connections between variables was created by using all correlations between pairs of variables in the study (Figure 7) (significance was set at p < .05). Forty-four correlations (links) were detected between 22 variables (nodes). The PageRank centrality algorithm indicated that lipoperoxide (LPO) and GSSG were the most highly connected to the global network. The enzymes GPX and GR, included in the GSH cycle, correlated positively with the former, therefore indicating oxidative stress. The analysis showed that in general, oxidative stress levels (LPO and GSH cycle variables) positively correlated with variables of amyloid and tau pathology (amyloid and phospho-tau brain levels), BPSDs (anxiety, emotionality, apathy, and nonadaptive behaviors), and frailty (obesity and immunosenescence). However, levels of CuZn-SOD did not, suggesting this enzyme is rather indicative of redox homeostasis in these mice. Furthermore, variables of oxidative stress, BPSDs and frailty negatively correlated with those of cognitive level (memory), and well-being-like behavior (active exploration and adaptive behaviors).

Discussion

The present study is a characterization of the physical, behavioral, and cognitive conditions as well as the oxidation state of the brain of 15-month-old female 3xTg-AD mice. We also determined the therapeutic efficacy of 3 months of physical exercise at an advanced age, as treatment was initiated at 12 months of age, when AD-like pathology is overtly present (23,30,38).

Fifteen-month-old mice showed a frail phenotype with disruption in the immunoendocrine axis as shown by increased abdominal fat, splenomegaly and a tendency to body weight gain and thymus involution. This is in agreement with a premature immunosenescence hypothesized for 3xTg-AD mice (31) and the suggestion of analyzing function and redox state of peripheral immune cells as an indicator of the progression of AD (39). The exercise regimen induced a
systemic physiological amelioration. Interestingly, body weight and abdominal fat positively correlated with brain oxidative damage and negatively with cognition, in agreement with previous studies with aged obese mice (40). Physical exercise also reduced a trend to WAT gain at earlier pathology stages in 9-month-old female (24) and 12-month-old male 3xTg-AD (23). Exercise treatment improved body fitness and decreased physical frailty markers, in agreement with previous reports in old mice (41). Interestingly, these benefits correlated with the improvement of cognition. These results in mice support the inverse correlation between physical frailty and cognitive function described in older adults (42–45). Age-related brain and systemic deterioration might share some common pathologic mechanisms as suggested by these studies and supported by the robust links observed in our graph theory analysis between immunoeendocrine markers and brain pathology markers.

In the assessment of behavioral patterns, we found strong evidence of anxiety and higher emotionality and indicators of apathetic and nonadaptive behaviors. These results showed a progressive deterioration in behavior patterns at older ages in comparison with those shown at 4–12 months of age (12,38). BPSDs behaviors positively correlated with oxidative stress and negatively with cognition and adaptive behaviors.

Progressive loss of memory is one of the most relevant affectations in AD patients and an essential trait for the experimental models used for the disease. In the measure of cognitive affectations through the MWM test, 3xTg-AD mice showed a lack of spatial memory retrieval. This in agreement with cognitive deficits shown at younger ages (12,38). However, acquisition of learning curve was worse than those of younger animals in both strains 3xTg-AD and NonTg. Memory measures negatively correlated with oxidative stress, amyloid and tau pathology, frailty and BPSDs. Voluntary exercise has protected several AD mouse models from cognitive loss at early and moderate stages of pathology progression (13,18,20,24). However, protection against cognitive loss in aged AD mice was controversial and only reported for Tg2576 mice (18). The exercise treatment of old 3xTg-AD females from 12 to 15 months of age also protected against memory loss.

It has been suggested that behavioral disorders and memory loss in AD patients may be due to neuronal dysfunction and/or neuronal death occurring in the hippocampus, the amygdala, and other cortical regions of their brains. These areas are susceptible to a degeneration of synapses and neurons, and such degeneration has been associated with the accumulation of Aβ and tau hyperphosphorylation (46). In line with what happens in humans, 3xTg-AD mice presented Aβ deposits and hyperphosphorylated tau in these AD-sensitive areas (12,30). Accordingly, amyloid and phospho-tau levels positively correlated with BPSDs and negatively with adaptive behaviors and cognition. Furthermore, they correlated with oxidative stress.

Running wheel exercise induced a general reduction in amyloid β pathology in aged 3xTg-AD, in agreement with previous results obtained in 12-month-old male 3xTg-AD mice previously submitted to 6 months of voluntary physical exercise (23). A reduction of amyloid β oligomers levels in these studies is not reinforced with a reduction of APP-C99 levels by physical exercise, which was not obtained in previous studies of 6-month exercise in 7-month olds (25) or 3-month exercise in 9-month-old mice (24). We may hypothesize that the exercise treatment increases the disposal of amyloid β but barely modifies APP processing. Tau pathology was reduced in the hippocampus and cerebral cortex of exercised 3xTg-AD mice. Reduction in the levels of phospho-tau was not found in previous studies with younger 3xTg-AD mice (12,23–25) or other TgAD mice. It may indicate that running exercise is a relatively higher endurance intervention in old mice than in younger ones. Treadmill endurance exercise and long-term voluntary running have been shown to ameliorate the accumulation of phospho-tau in mouse models with tauopathy (47,48). Although forced exercise has been found to be less neuroprotective than voluntary exercise in Tg2576 mice (19) lacking tau pathology, endurance exercise could induce higher decrease of tau pathology than moderate exercise. Accordingly, in a study of AD biomarkers in cognitively normal older adults, the group that met the exercise guidelines set by the American Heart Association showed significant improvement in amyloid-related biomarkers but not in tau markers in the CSF (49). Therefore, a
physically active life decreases AD risk (50) but is less effective in reversing memory loss in established dementia (51). The decrease in phospho-tau found in the hippocampus of 3xTg-AD added more evidence to the effects of physical exercise against the decline in hippocampal function in aging and AD (27). These results may help to set a more neuroprotective pattern of exercise, supporting that a requirement of moderate-intensity physical activity to protect against dementia (52).

The 15-month-old 3xTg-AD mice suffer brain oxidative stress with increased LPO, disruption of glutathione metabolism, and changes in the activity of antioxidant enzymes compared to their NTg counterparts. Levels of the markers of oxidative stress LPO and GSSG were more elevated in 15-months than in 7-month-old female tissue (12). Oxidative changes in these mice might be related to defects in their mitochondrial electron chain (12) in addition to the presence of amyloid pathology, and they mimic oxidative AD changes (8). In addition, at 15 months we found an increased response in the GSH recycling system GPX/GR and a higher exhaustion of the antioxidant enzyme CuZn-SOD than at 7 months (12).

Male mice of the 3xTg-AD strain have shown higher brain oxidative damage and higher antioxidant response than females (12,23). Twelve-month-old sedentary males had shown nearly twice the values of LPO than 15-month-old females without any increase in GSSG (23). In addition to a possible more efficient GSG detoxification, CuZn-SOD activity increases in 7- and 12-month-old male mice without exhaustion signs (12,23). Similarly to 15-month females, an increase in LPO levels coupled to a reduction in CuZn-SOD activity has also been reported in the brain of Thy1-APP751(SL) transgenic mice (53). Increasing the enzymatic activity of GPX/GR complex can produce a better detoxification of free radicals, as this complex regulates the balance between GSH and GSSG, and GSH is one of the most important nonenzymatic antioxidant. Furthermore, it has been reported that GPX is an important enzyme for protection from lipid peroxidation. Both GSH and GPX are essential in the defense against oxidative stress in the AD brain (9,54).

In all measured oxidative stress patterns, the physical exercise treatment made possible the restoration of values in 3xTg-AD to normal values shown by NTg SED mice. These results are in line...
with previous studies in which a diminution in oxidative stress markers has been observed in different animal models of aging and AD by using different treatments of physical exercise (20,23,55). However a longer period of exercise of 6 months had induced instead a further increase of GSSG in 7-months 3xTg-AD mice, but jointly with also a decrease of the oxidative damage marker LPO (12). Exercise treatment normalized levels of all antioxidant enzymes tested. Interestingly, CuZn-SOD activity has been found instead exacerbated in the former 7-month-old mice possibly as defense against the discussed increase of GSSG (12). Moreover, the regulation of enzymatic activity due to chronic physical exercise has been well documented in rodents, showing variable effects according to the brain region (12,20,23,55–57). Exercise training in humans has also been reported to increase antioxidant defense (58). Also, a hormetic stress effect of low oxidative damage induced by physical activity might promote changes that induce protective gene expression (59–61). In this regard, physical exercise intensity should be adapted to prefrail elders to prevent such deleterious effects (62), as the slightly worsened behaviors detected in NonTg EXE as opposed to the general improvement of Tg EXE mice. Indeed, young healthy animals were reported to be less responsive to the activation of a protective pathway by exercise than older ones (63). Interventions that decrease oxidative damage may contribute to a reversal of cognitive deficits, as has been reported in old mice treated with CuZn-SOD mimetics (64). Furthermore, redox-based therapeutic interventions have been proved to counteract aging-related diseases and promote longevity (65–68).

In this study, physical exercise in the running wheel was initiated at an advanced stage of the AD pathology and it was able to ameliorate the 3xTg-AD behavioral alterations and oxidative

Figure 6. Oxidative condition measured in the cerebral cortex of 3xTg-AD (Tg) and nontransgenic (NonTg) mice after 3 months of physical exercise (EXE) or in sedentary conditions (SED). (A) The indicator of oxidative stress lipoperoxide (LPO) showed higher levels in Tg SED. (B) Reduced glutathione (GSH) levels did not differ between treatment groups. (C) Oxidized glutathione (GSSG), another indicator of oxidative stress, was higher in Tg SED. (D, E) The antioxidant enzymes of the GSH system, glutathione peroxidase (GPX) (D) and GR (E), increased their level of activity. (F) Levels of the antioxidant enzyme CuZn-SOD were decreased. All changed parameters were reversed by EXE to values similar to those of NonTg SED. Values are the mean ± SEM, n = 6. Statistics: *p < .05, **p < .01 compared to NonTg SED; #p < .05 compared to Tg SED.
stress changes in addition to decreasing the Aβ and phospho-tau levels and restoring memory deficits. Also, normalization of fitness and immunoendocrine parameters induced by running exercise suggests a physiological rejuvenation in these mice. The high connectivity shown by the oxidative stress parameters confirms a central role of redox homeostasis both in the AD-like pathology of aged 3xTg-AD mice and in the neuroprotective effects of physical exercise.

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Conflict of Interest
The authors declare that there are no financial or commercial conflicts of interest.

References

Figure 7. Network of the connectivity between body status, behavior, cognition, amyloid and tau pathology, and oxidative stress variables, analyzed by PageRank centrality algorithm. Nodes show the variables measured in the study and links indicate positive (continuous line) or negative (dotted line) correlations between pairs of them (Pearson’s, at least $p < .05$). Links with greater thickness indicate higher correlation value. Nodes with greater size indicate higher centrality of the variables in the network. Centrality rank of the variables is as follows: 1, lipoperoxide level in the cerebral cortex; 2, oxidized glutathione; 3, Aβ40 level; 4, phospho-tau in hippocampus; 5, spleen weight; 6, distance covered in the open field; 7, body weight; 8, time in platform quadrant in the removal test; 9, Aβ6-mer; 10, white abdominal fat weight; 11, defecation boili in the hole board; 12, latency to ambulation; 13, latency of entry into lit area in the dark and light box; 14, Aβ42 level; 15, grooming activity; 16, glutathione peroxidase; 17, ratio between time in platform quadrant and in opposed quadrant; 18, latency of grooming in the open field; 19, glutathione reductase; 20, phospho-tau in cerebral cortex; 21, CuZn superoxide dismutase; and 22, head-dipping activity. Node colors sorted variables as follows: red, oxidative stress; dark red, antioxidant defense; brown, amyloid and phospho-tau; orange, body frailty and unfitness; yellow, behavioral and psychological symptoms of dementia (BPSD)-like behaviors such as anxiety, apathy and emotionality; blue, exploratory and adaptive behavior; and green, cognition.