Treadmill Running Reverses Cognitive Declines due to Alzheimer Disease

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ABSTRACT

CHO, J., M.-K. SHIN, D. KIM, I. LEE, S. KIM, AND H. KANG. Treadmill Running Reverses Cognitive Declines due to Alzheimer Disease. Med. Sci. Sports Exerc., Vol. 47, No. 9, pp. 1814–1824, 2015. Purpose: This study investigated the effect of treadmill running on cognitive declines in the early and advanced stages of Alzheimer disease (AD) in 3xTg-AD mice. Methods: At 4 months of age, 3xTg-AD mice (N = 24) were assigned to control (AD + CON, n = 12) or exercise (AD + EX, n = 12) group. At 24 months of age, 3xTg-AD mice (N = 16) were assigned to AD + CON (n = 8) or AD + EX (n = 8) group. The AD + EX mice were subjected to treadmill running for 12 wk. At each pathological stage, the background strain mice were included as wild-type control (WT + CON, n = 8–12). Results: At the early stage of AD, 3xTg-AD mice had impaired short- and long-term memory based on Morris water maze along with higher cortical Aβ deposition, higher hippocampal and cortical tau pathology, and lower hippocampal and cortical PSD-95 and synaptophysin. A 12-wk treadmill running reversed the impaired cognitive declines and significantly improved the tau pathology along with suppression of the decreased PSD-95 and synaptophysin in the hippocampus and cortex. At the advanced stage of AD, 3xTg-AD mice had impaired short- and long-term memory along with higher levels of Aβ deposition, soluble Aβ1–40 and Aβ1–42, tau pathology, and lower levels of brain-derived neurotrophic factor, PSD-95, and synaptophysin in the hippocampus and cortex. A 12-wk treadmill running reversed the impaired cognitive declines and significantly improved the Aβ and tau pathology along with suppression of the decreased synaptic proteins and brain-derived neurotrophic factor in the hippocampus and cortex. Conclusions: The current findings suggest that treadmill running provides a nonpharmacological means to combat cognitive declines due to AD pathology. Key Words: PHYSICAL EXERCISE, COGNITIVE FUNCTION, ALZHEIMER DISEASE, NEUROPATHOLOGY

Alzheimer disease (AD) is an age-dependent and neurodegenerative disorder characterized by learning, memory, and communication deficits and accounts for 60%–80% of dementia cases worldwide (6). Pathologically, AD is characterized by the accumulation of extracellular aggregates of β-amyloid peptide (Aβ) and intracellular aggregates of hyperphosphorylated tau proteins (14).

The triple transgenic mouse model of AD (3xTg-AD) overexpresses human amyloid precursor protein (APPsw), presenilin 1 (PS-1M146V), and tauP301L and develops a progressive AD-like brain pathology and deterioration in multiple aspects of brain physiology and behavior (25). The cognitive and behavioral deterioration with corresponding brain pathology mimics AD progression in patients with AD, making the 3xTg-AD mouse a suitable model for clinical studies in AD pathology (34).

Although anticholinesterase therapies have greatly improved symptomatic treatment of AD, they have not been demonstrated to significantly slow disease progression (37). Consequently, the lack of effective therapies to treat AD coupled with the projected dramatic increase in the number of persons with AD in the coming decades has put medical research in a crisis to urgently find effective and inexpensive interventions. A promising evidence-based and relatively adverse effect-free lifestyle approach such as physical activity is emerging as an alternative or adjunct to anticholinesterase therapies (4).

Physical activity improves cognitive functions by promoting brain-derived neurotrophic factor (BDNF)-dependent synaptic plasticity, neurotransmission, and neurogenesis in animal studies (34), and it reduces the risk of AD and delays the onset or progression of AD with minimal cost and adverse effects (11). However, biological mechanisms underlying the therapeutic effect of physical exercise in AD-related cognitive declines over time are not fully understood. Thus, obtaining mechanistic insights into the effect of physical exercise on AD pathology at different pathological stages would certainly contribute to the development of new and improved options to treat and/or prevent clinical conditions associated with this disease.

To the best of our knowledge, only a few studies have explored the benefits of physical exercise as a nonpharmacological means of minimizing cognitive declines and reversing AD...
pathology in 3xTg-AD mice. These investigators reported on the beneficial effects of voluntary wheel running against cognitive declines and AD pathology (9,10). Unlike voluntary wheel running, however, Giménez-Lloret et al. (12) found no beneficial effect of treadmill running against Morris water maze (MWM)-based cognitive declines in 3xTg-AD mice. Treadmill running is a type of forced exercise that activates the stress response including elevated corticosterone levels. However, circulating stress hormones increase acutely in response to exercise and are generally restored to their baselines as exercise progresses or adaptation occurs (29). In addition, some studies showed that voluntary wheel running and treadmill running might differentially affect brain and behavior (20). In this study, we investigated the role of treadmill running as a nonpharmacological means of preventing cognitive declines and reducing AD neuropathology at early and advanced stages of the disease in 3xTg-AD mice.

MATERIALS AND METHODS

Animals. The construction of 3xTg-AD mice harboring APPSwe PS1ΔE9, and tauP301L human transgenes is well documented along with the pathological characteristics of the AD mice (25). The genotypes were confirmed by polymerase chain reaction analysis of DNA obtained from tail biopsies. Mice were bred and kept at a pathogen-free facility located at the Sungkyunkwan University School of Medicine (12:12 h light–dark cycle) and were given 1 wk to acclimate to the housing condition before study commencement. Mice were allowed ad libitum access to food and water. Animal handling and procedures were reviewed and approved by the Sungkyunkwan University School of Medicine Institutional Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International Guidelines for animal experiments.

Experiment design and exercise training protocol. At 4 months of age, 3xTg-AD mice (N = 24) were randomly assigned to either control (AD + CON, n = 12) or exercise (AD + EX, n = 12) group. A third group of background strain mice (C57BL/6) of the same age was included as a wild-type control (WT + CON, n = 12). At 24 months of age, 3xTg-AD mice (N = 16) were randomly assigned to either AD + CON (n = 8) or AD + EX (n = 8) group. A third group of the background strain mice at the same age was included as a WT + CON group (n = 8).

At both 4 and 24 months of age, mice in the exercise groups were made to run on a motor-driven and low-noise animal treadmill (Columbus Instruments, Inc., Columbus, OH) with duration of 30 min per session and a frequency of 5 d·wk⁻¹ for 12 wk. Each treadmill session started with 5-min warm-up at a speed of 5 m·min⁻¹, followed by 20-min main exercise at a speed of 10 m·min⁻¹, and finished with 5-min cooldown at a speed of 5 m·min⁻¹. Mice in the AD + EX group tolerated the treadmill running well with mild prodding with the hand, if necessary; electric shock was not used in this test. On the other hand, mice in the WT + CON and AD + CON groups had their food removed and exposed to the noise of the treadmill for the same duration as the mice in the AD + EX group.

MWM. MWM was conducted within 1 wk after the 12-wk treadmill running. The modified MWM used in this study is described elsewhere (27). The water maze consisted of a circular pool (120 cm in diameter and 40 cm in height) made of silver metal. A translucent acrylic escape platform was placed in a fixed location away from the wall in the center of a quadrant arbitrarily labeled as the southeast (SE) quadrant. The pool was filled with water 1.5 cm above the escape platform. To prevent the use of reflected light from objects in the room as visual landmarks, all mice were tested under dim red light illumination provided by three red bulbs in upward-facing clamp lamps that were located at opposite ends of the room. The visual cues covered all four quadrants.

Although no single cue served as a beacon for the platform location, the mice had the opportunity to learn that the escape platform was located in the area with no tactile cues. A camera mounted above the pool was used to capture images of the mice swimming during behavioral testing. These images were analyzed with a computerized video tracking system (ANY-maze TM; Stoelting) using several of the available measures, as follows: (a) escape latency (i.e., time in seconds to reach the escape platform), (b) number of platform crossings, (c) total time spent in the target quadrant, (d) average swim speed (m·s⁻¹), and (e) path lengths. The present study was carried out in three replications with similar numbers of 3x-Tg-AD mice and wild-type mice per replication. All the MWM procedures began at 11:00 p.m. (±30 min). The acquisition task consisted of four trial sessions per day for three consecutive days, with each trial 15 min apart. In each trial, the mouse was gently released from one randomly selected starting point (i.e., east, west, north, or south) and allowed to swim until it escaped onto the platform (always in the SE quadrant). Mice that failed to find the platform within 60 s were placed on it for 20 s, the same period as that allowed for the successful animals. One and a half hour after the four trials of place learning, the platform was removed from the maze, and the mice performed a probe trial test of 60 s for 1.5-h short-term retention of memory. On the following day, the mice were tested for the cue learning of a visual platform for 24 h long-term retention of memory. After the probe test, mice were anesthetized with a mixture of zoletil and rompun and transcardially perfused with 1× phosphate buffered solution (PBS). For immunohistochemistry, brains were fixed in 4% paraformaldehyde and then incubated in 30% sucrose at 4°C. The other brains were flash-frozen in liquid nitrogen and stored at −80°C until immunoblotting and enzyme-linked immunosorbent assay (ELISA) analysis.

Immunohistochemistry. Fixed brains were cut on a microtome (CM3050S; Leica Microsystems, Nussloch, Germany) in 45 μm-thick slices, and tissue sections were collected in a cold cryoprotectant solution (80-mM K2HPO4, 20-mM KH2PO4, 1 for 12 wk. Each treadmill session started with 5-min warm-up at a speed of 5 m·min⁻¹, followed by 20-min main exercise at a speed of 10 m·min⁻¹, and finished with 5-min cooldown at a speed of 5 m·min⁻¹. Mice in the AD + EX group tolerated the treadmill running well with mild prodding with the hand, if necessary; electric shock was not used in this test. On the other hand, mice in the WT + CON and AD + CON groups had their food removed and exposed to the noise of the treadmill for the same duration as the mice in the AD + EX group.
154-mM NaCl, 0.3 g·mL\(^{-1}\) of sucrose, 0.01 g·mL\(^{-1}\) of polyvinylpyrrolidone, 30% v/v ethylene glycol). Brain sections were pretreated with 70% formic acid for 10 min at room temperature and washed three times for 5 min in 1× PBS. Sections then were blocked in 5% goat serum for 30 min, and the sections were immunostained with anti-\(\alpha\)B antibody. Incubations were performed overnight at 4°C at dilutions of 1:1000 for \(\alpha\)B antibody (6E10; Covance Research Products, Dedham, MA). After subsequent washes to remove primary antibody excess, the sections were incubated with Rhodamine RedTM-X goat antimouse IgG (H + L) (1:500; Invitrogen, Eugene, OR) for 2 h and washed three times with 1× PBS for 5 min. Immunostaining was performed in six brain sections of each animal. A blinded expert was provided with the stained brain sections and took pictures of them using a Zeiss LSM 510 META Duoscan confocal microscope with 20× objective and manually counted plaque numbers.

**Western blotting.** Western blotting was performed as described previously (32). In brief, protein extracts from hippocampal and cortical tissues were prepared by homogenization. Homogenates were centrifuged, and protein concentrations of supernatants were determined by the Bradford assay (Bio-Rad, Hercules, CA). Ten to fifteen micrograms of total proteins were boiled in laemmli sample buffer and loaded on 7.5%–15% sodium dodecyl sulfate/polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. For study of CTF-\(\beta\), proteins were electrophoresed in Tris-Tricine SDS PAGE gels. Membranes were blocked in 5% nonfat dry milk/0.05% Tris-buffered saline with primary antibody excess, the sections were incubated with primary antibody (for \(\alpha\)B antibodies. The primary antibodies included rabbit anti-p-Tau T205, T231, S404 (ThermoScientific, Rockford, IL), mouse anti-Tau, rabbit anti-presenilin 1, rabbit anti-PSD-95, rabbit anti-BACE1 (Cell Signaling, Beverly, MA), rabbit anti-ADAM17, rabbit anti-Nephrilysin (Santa Cruz Biotechnology, San Diego, CA), rabbit anti-IDE (Abcam, Cambridge, United Kingdom), rabbit anti-BDNF (Alomone Labs, Jerusalem, Israel), rabbit anti-APP c-terminal fragment (Sigma-Aldrich, St. Louis, MO), mouse antisynaptophysin (Millipore, Temecula, CA), and rabbit anti-\(\beta\)-actin (Bethyl Laboratories, Montgomery, TX). The membrane was subsequently incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Finally, blots were developed with a chemiluminescent HRP substrate kit (Millipore, Billerica, MA). The intensity of the bands was determined by ImageJ version 1.42 software (National Institutes of Health, Bethesda, MD) and normalized with \(\beta\)-actin densitometric values.

**Amyloid-\(\beta\) ELISA.** Soluble \(\alpha\)B\(_{1-40}\) and \(\alpha\)B\(_{1-42}\) levels in hippocampal and cortical tissues were measured with commercial ELISA kits (IBL, Minneapolis, MN) as described (32). In brief, diluted standard and soluble brain samples were incubated in the antiimmunob \(\alpha\)B mouse IgG monoclonal antibody (for \(\alpha\)B\(_{1-40}\) measurement) or affinity purified anti-immunob \(\alpha\)B rabbit IgG (for \(\alpha\)B\(_{1-42}\) measurement) precoated plate at 4°C overnight. Next, each well was washed more than seven times and then incubated in affinity-purified HRP conjugated antiimmunob \(\alpha\)B mouse IgG for 1 h at 4°C. After incubation with the antibody and subsequent washing steps, plates were incubated in 3,3’,5,5’-Tetramethylbenzidine solution for 30 min at room temperature in the dark. Finally, the reaction was stopped by addition of 1 N H\(_2\)SO\(_4\) and colorimetric values were measured at 450 nm using a plate reader (Tecan Systems, Inc., San Jose, CA).

**Statistics.** Data are expressed as means ± SD. Three (within-subject factor, 3 times) × three (between-subject factor, WT + CON vs AD + CON vs AD + EX) mixed-model ANOVA were used to test any time–group interaction effect in average escape latency. One-way ANOVA followed by the least significant difference post hoc multiple comparison tests, if necessary, were used to compare any significant differences in the other outcomes among WT + CON, AD + CON, and AD + EX mice. Statistical significances were tested at \(P = 0.05\). All statistics were conducted using the SPSS-PC software (version 20.0).

**RESULTS**

**Treadmill running prevents cognitive declines due to AD pathology.** At 7 months of age, there was a significant time–group interaction effect (\(P = 0.049\)) for average latency escape. In addition to mice in the WT + CON group, mice in the AD + CON and AD + EX groups significantly reduced latency escape over the 3-d trial course (\(P = 0.006\) for WT + CON mice, \(P = 0.003\) for AD + CON mice, and \(P = 0.004\) for AD + EX mice), suggesting that in the early pathological stage, AD mice had intact acquisition for spatial learning, yet mice in the AD + EX group had significantly greater reductions in the latency escape (\(P = 0.002\)) than mice in the AD + CON group, with no significant differences between AD + EX and WT + CON mice (\(P = 0.365\)). In the probe test, mice in the WT + CON group had significantly greater 1.5-h short-term (\(P = 0.027\) for time spent in target quadrant and \(P = 0.001\) for number of platform crossings) and 24-h long-term memory (\(P = 0.017\) and \(P = 0.041\) for time spent in target quadrant and number of platform crossings, respectively) than mice in the AD + CON group. Mice in the AD + EX group had significantly greater 1.5-h short-term (\(P = 0.006\) and \(P = 0.001\) for time spent in target quadrant and number of platform crossings, respectively) and 24-h long-term memory (\(P = 0.016\) and \(P = 0.014\) for time spent in target quadrant and number of platform crossings, respectively) than mice in the AD + CON group. On the other hand, there were no significant differences in both 1.5-h short-term (\(P = 0.514\) and \(P = 0.972\) for time spent in target quadrant and number of platform crossings, respectively) and 24-h long-term memory (\(P = 0.631\) and \(P = 0.588\) for time spent in target quadrant and number of platform crossings, respectively) between WT + CON and AD + EX mice (Fig. 1A–C).

At 27 months of age, there was a significant time–group interaction effect (\(P = 0.018\)) for average latency escape. Mice in the WT + CON and AD + EX groups had significant
reductions in latency escape over the 3-d trial course \((P = 0.006\) and \(P = 0.050\) for WT + CON and AD + EX mice, respectively), whereas mice in the AD + CON group had no significant change in latency escape over the same trial course \((P = 0.288)\). This finding suggests that 12 wk of treadmill running fully reversed the impaired acquisition for spatial learning due to AD pathology. In the probe test, mice in the AD + EX group had significantly greater 1.5-h short-term \((P = 0.039\) and \(P = 0.032\) for time spent in target quadrant and the number of platform crossings, respectively) and 24-h long-term memory \((P = 0.025\) and \(P = 0.025\) for time spent in target quadrant and the number of platform crossings, respectively) than mice in the AD + CON group. However, there were no significant differences in both soluble \(\text{A} \beta_{1-40}\) and \(\text{A} \beta_{1-42}\) in the hippocampus or in the cerebral cortex between WT + CON and AD + CON mice (Fig. 2C–D). Likewise, there were no significant differences in both soluble \(\text{A} \beta_{1-40}\) and \(\text{A} \beta_{1-42}\) levels in the hippocampus or in the cerebral cortex between AD + CON and AD + EX mice (Fig. 2C–D).

At 27 months of age, mice in the AD + CON group had significantly higher \(\text{A} \beta\) deposition in the cerebral cortex than mice in the WT + CON group, with no such difference in the hippocampus between the two groups of mice (Fig. 2A–B). There were no significant differences in both soluble \(\text{A} \beta_{1-40}\) and \(\text{A} \beta_{1-42}\) in the hippocampus or in the cerebral cortex between WT + CON and AD + CON mice (Fig. 2C–D). Likewise, there were no significant differences in both soluble \(\text{A} \beta_{1-40}\) and \(\text{A} \beta_{1-42}\) levels in the hippocampus or in the cerebral cortex between AD + CON and AD + EX mice (Fig. 2C–D).

At 27 months of age, mice in the AD + CON group had significantly higher \(\text{A} \beta\) deposition (Fig. 2E–F) as well as higher levels of soluble \(\text{A} \beta_{1-40}\) and \(\text{A} \beta_{1-42}\) (Fig. 2G–H) in the hippocampus and cerebral cortex than mice in the WT + CON group. The elevated \(\text{A} \beta\) accumulation and soluble \(\text{A} \beta_{1-42}\) were significantly decreased after 12 wk of treadmill
running, but this positive effect was not noted for Aβ₁₋₄₀ in those areas of the brain (Fig. 2G–H).

At 7 months of age, mice in the AD + CON group had significantly higher levels of tau and p-tau proteins at T205, T231, and S404 phosphorylation sites in the hippocampus and the cerebral cortex than mice in the WT + CON group (Fig. 3A–B). The elevated p-tau proteins at the T205 site in the hippocampus as well as at the T205 and T231 sites in the cortex were significantly reduced after 12 wk of treadmill running (Fig. 3A–B).

At 27 months of age, mice in the AD + CON group had significantly higher levels of tau and p-tau proteins at the T205, T231, and S404 sites in the hippocampus and cortex than mice in the WT + CON group, more elevated APP proteins in the hippocampus, and more elevated presenilin 1 proteins in the cortex (Fig. 4A–C). However, the elevated APP, presenilin 1, and CTFβ proteins in the hippocampus and cortex than mice in the WT + CON group, more elevated APP proteins in the hippocampus, and more elevated presenilin 1 proteins in the cortex (Fig. 4A–C). However, the elevated APP, presenilin 1, and CTFβ proteins in the hippocampus and cortex were not reduced after 12 wk of treadmill running (Fig. 4A–C). In addition, no significant genotype- or exercise training-dependent differences were found in ADAM10 and 17, BACE1, IDE, neprilysin, and CTFβ in the hippocampus and cerebral cortex.

At 7 months of age, mice in the AD + CON group had significantly higher levels of APP, presenilin 1, and CTFβ proteins in the hippocampus and cortex than mice in the WT + CON group, more elevated APP proteins in the hippocampus, and more elevated presenilin 1 proteins in the cortex (Fig. 4A–C). However, the elevated APP, presenilin 1, and CTFβ proteins in the hippocampus and cortex were not reduced after 12 wk of treadmill running (Fig. 4A–C). In addition, no significant genotype- or exercise training-dependent differences were found in ADAM10 and 17, BACE1, IDE, neprilysin proteins in the hippocampus or in the cerebral cortex (Fig. 4A–B).

At 27 months of age, mice in the AD + CON group had significantly higher APP and presenilin 1 proteins in the hippocampus and cortex than mice in the WT + CON group, which were not alleviated after 12 wk of treadmill running.
On the other hand, mice in the AD + EX group had significantly lower BACE1 proteins in the hippocampus and cortex than mice in the AD + CON group (Fig. 5A–B). Consistent with the suppressed BACE1 proteins, we also found that mice in the AD + EX group had significantly lower CTF proteins in the hippocampus and cortex than mice in the AD + CON group (Fig. 5C). Collectively, the findings of this study suggest that treadmill running suppresses the amyloidogenic pathway at least in the advanced stage of AD pathology by decreasing \( A \)-secretase protein expression and/or perhaps the enzyme activity in the hippocampus and cerebral cortex of 3xTg-AD mice.

**Treadmill running ameliorates downregulated synaptic proteins and BDNF.** Synaptic loss in the cerebral cortex is considered one of the earliest cellular events in AD. The number of synapses in the brain is severely reduced and may contribute to 20%–30% of the brain weight loss in AD (39). In this study, we assessed whether treadmill running contributes to pre- and postsynaptic connection and/or synaptic stability by measuring synaptic proteins in the hippocampus and cerebral cortex such as PSD-95, synaptophysin, BDNF, and nerve growth factor (NGF).

At 7 months of age, mice in the AD + CON group had significantly lower PSD-95 and synaptophysin proteins in the hippocampus and cortex than mice in the WT + CON group, which were recovered after 12 wk of treadmill running. However, there were no significant differences in BDNF and NGF between AD + CON and AD + WT + CON mice or between AD + CON and AD + EX mice (Fig. 6A–C).

At 27 months of age, mice in the AD + CON group had significantly lower levels of BDNF, PSD-95, and synaptophysin in the hippocampus and cortex than mice in the WT + CON group, which were fully or partially recovered after 12 wk of treadmill running. However, there were no significant differences in NGF between AD + CON and AD + WT + CON mice or between AD + CON and AD + EX mice (Fig. 6D–F). Collectively, the findings of this study suggest that regardless of severity of AD pathology, treadmill running promotes synaptic connection and/or plasticity by overcoming...
AD-related downregulation of synaptic proteins and BDNF in 3xTg-AD mice.

**DISCUSSION**

The 3xTg-AD transgenic mice developed age-dependent and progressive AD-like brain neuropathology that causes cognitive and neuropsychiatric-like symptoms of dementia (25). At 7 months of age, we found that 3xTg-AD mice had both short- and long-term memory impairments on the basis of the MWM. As expected, the cognitive declines observed in the early pathological stage became exacerbated in the advanced pathological stage. In the advanced pathological stage, 3xTg-AD mice had also impaired acquisition for spatial learning. Our findings are consistent with previous studies. García-Mesa et al. (9) and García-Mesa et al. (10) demonstrated that 3xTg-AD mice had cognitive loss and behavioral and psychological symptoms of dementia-like behaviors along with long-term potentiation impairment in vivo, with more severe disturbances at the older age tested. Little is known about the effectiveness of treadmill running as a nonpharmacological means of preventing and/or alleviating cognitive declines at different stages of AD pathology. In this study, two groups of 4- and 24-month-old 3xTg-AD mice were subjected to treadmill running for 12 wk and they were killed at 7 months (i.e., in the early stage of AD pathology) and 27 months (i.e., in the advanced stage of AD pathology) of age, respectively. We found that 12 wk of treadmill running fully reversed the short- and long-term memory impairments in the early and advanced stages of AD pathology in 3xTg-AD mice. Consistent with the current findings, previous studies demonstrated that voluntary wheel running alleviated cognitive declines in animals with AD (2,40), patients with AD (7,39), and elderly persons with mild cognitive impairment (31,33), yet we are the first to report that regardless of severity of AD pathology, chronic treadmill running also provides a

![Figure 4](http://www.acsm-msse.org)

**FIGURE 4**—In the early stage of AD pathology, treadmill running did not alter the APP and APP processing enzymes and the Aβ degrading enzymes in the hippocampus and cerebral cortex. A. Representative Western blots of APP, ADAM10, ADAM17, BACE1, presenilin 1, IDE, neprilysin and β-actin. B. Densitometric analyses of the Western blot bands normalized to β-actin. C. Western blot and densitometric analyses of CTFβ. Values are means ± SD. ***P < 0.001 in AD + CON mice vs WT + CON mice. Twelve mice per group were used.
nonpharmacological means to combat cognitive decline in 3xTg-AD mice.

Some studies reported suppressive effects of exercise on markers of Aβ pathology (2), and other studies did not (24). The contradictory findings of previous studies may stem from the use of different genetic animal models (9), the age and stage of disease when tested (19), and the type, intensity, or duration of the exercise intervention (9). In the early stage of AD pathology, we found that treadmill running did not induce any suppressive or alleviating effect against Aβ deposition in 3xTg-AD mice. In agreement with our findings, García-Mesa et al. (10) showed that voluntary wheel running did not change any of the Aβ pathology in 6- to 7-month-old 3xTg-AD mice. In the advanced stage of AD pathology, however, we found that treadmill running alleviated Aβ deposition in the hippocampus and cerebral cortex in 3xTg-AD mice. In addition, we also found that treadmill running reduced expression of BACE1 and its end-product CTFβ proteins, indicating that the suppressive effect of treadmill running on Aβ deposition is related to its suppressive effect on the amyloidogenic pathway by decreasing β-secretase protein expression and/or perhaps the enzyme activity in the hippocampus and cerebral cortex of 3xTg-AD mice (22).

The biological activity of tau is regulated by phosphorylation. In tauopathies, however, tau protein is abnormally hyperphosphorylated and aggregates within neurons in the form of neurofibrillary tangles (14). In this study, we found that 3xTg-AD mice had significantly higher p-tau at the T205 site in the hippocampus and at the T205 and T231 sites in the cortex in the early and advanced stages of AD pathology.

FIGURE 5—In the advanced stage of AD pathology, treadmill running decreased BACE1 and CTFβ expression in the hippocampus and cerebral cortex. A. Representative Western blots of APP, ADAM10, ADAM17, BACE1, presenilin 1, IDE, neprilysin, and β-actin. B. Densitometric analysis of the Western blot bands normalized to β-actin. C. Western blot and densitometric analysis of CTFβ. Values are means ± SD. †P < 0.05 and †††P < 0.001 in AD + CON mice vs WT + CON mice; **P < 0.01 and ***P < 0.001 in AD + EX mice vs AD + CON mice. Eight mice per group were used.
which were significantly alleviated by 12 wk of treadmill running. Consistent with our findings, Leem et al. (21) showed that 12 wk of treadmill running resulted in significantly decreased p-tau levels in the CA3 subregion of the hippocampus in Tg-NSE/htau23 mice. Similarly, Um et al. (38) also showed that 12 wk of treadmill running suppressed tau phosphorylation levels at the Ser404, Ser202, and Thr231 residues in the hippocampus in Tg-NSE/hPS2m. On the other hand, Belarbi et al. (5) found that voluntary wheel running did not change total tau or phosphorylated tau levels in THY-Tau22 mice. Together, the findings to date suggest that treadmill running rather than voluntary wheel running may provide greater suppressive/alleviating effect on tau pathology.

Synaptic loss is an early event of AD neuropathology (26) and is the best pathological correlate of cognitive declines due to AD (36). Pathologically, decreased synaptic proteins such as synaptophysin and PSD-95 are attributed to synaptic loss and thereby cognitive declines in AD (36). In addition, extracellular Aβ deposition in the hippocampus and cerebral cortex is related to synaptic loss, which inhibits the induction of long-term potentiation, an electrophysiological correlation of memory formation (17). Consequently, there is mounting evidence to suggest that AD is primarily a disease of synaptic dysfunction.

In this study, we found that synaptic proteins such as PSD-95 and synaptophysin were significantly decreased in the early and advanced stages of AD pathology in 3xTg-AD mice, which were reversed after 12 wk of treadmill running. Consistent with our findings, recent studies showed that synaptophysin and PSD-95 proteins were significantly decreased in 7-month-old 3xTg-AD mice (30) and 15- to 16-month-old 3xTg-AD mice (18). In addition, Revilla et al. (30) demonstrated that the decreased synaptophysin and PSD-95 proteins observed in 7-month-old 3xTg-AD mice were fully recovered after 6 months of voluntary wheel running (8,35).
BDNF is considered one of the most important neurotrophic factors involved in synaptic connection and/or plasticity (3) and long-term potentiation (2) in the brain. Decreased BDNF levels are also attributed to Aβ toxicity (27). Furthermore, hippocampal BDNF levels were decreased in AD (28) and normal aging (23). On the other hand, exercise has been shown in a large number of studies to be an effective means of increasing serum (13) and brain BDNF levels (1). Likewise, we also found that in the advanced stage of AD pathology, BDNF proteins were significantly decreased in the hippocampus and cerebral cortex of 3xTg-AD mice, which were reversed after 12 wk of treadmill running. Together, the findings of the current study suggested that treadmill running-induced restoration of decreased BDNF and synaptic proteins contributed to preventing the cognitive declines due to AD pathology by promoting synaptic connection and/or stability in 3xTg-AD mice (3).

In summary, the hippocampus plays a key role in the consolidation of information from short-term memory to long-term memory and the cerebral cortex promotes long-term memory formation. In the early stage of AD pathology, we found that, in the absence of amyloidosis modulation, treadmill running suppressed the downregulation of synaptic proteins, such as PSD-95 and synaptophysin and alleviated tau hyperphosphorylation in the hippocampus and cerebral cortex. In the early stage of AD pathology, therefore, we speculate that enhancement of synaptic transmission (15) and/or alleviation of synaptic loss and altered synaptic function (16,21) played critical roles in restoring short- and long-term memory impairments after 12 wk of treadmill running. In the advanced stage of AD pathology, on the other hand, treadmill running suppressed the downregulation of the synaptic proteins and BDNF in the hippocampus and cerebral cortex. Furthermore, treadmill running reduced Aβ accumulation, β secretase expression, and hyperphosphorylation of tau proteins in the hippocampus and cerebral cortex in the advanced stage of the disease. Therefore, we speculate that in the late stage of AD pathology, enhancement of synaptic transmission and synaptic plasticity and/or neurogenesis (13,23,29) in conjunction with alleviation of amyloidosis and tauopathy (2,5) played concurrent roles in restoring short- and long-term memory impairments after 12 wk of treadmill running.

In conclusion, the findings of the current study suggest that regardless of the severity of the disease, treadmill running provides a protective effect against cognitive declines by reducing the severity of AD neuropathology in 3xTg-AD mice. Collectively, the benefits of treadmill running on spatial learning and retention of short- and long-term memory, synaptic transmission, synaptic stability and/or neurogenesis, and Aβ and tau pathology demonstrated in this study using the 3xTg-AD mouse model further support the value of physical exercise as a nonpharmacological means to combat neurodegenerative clinical consequences.

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REFERENCES


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