FUNCTION american physiological society

*FUNCTION*, 2023, 5(1): zqad066

https://doi.org/10.1093/function/zqad066
Advance Access Publication Date: 4 December 2023
Original Research

RESEARCH ARTICLE

# Neuromuscular Dysfunction Precedes Cognitive Impairment in a Mouse Model of Alzheimer's Disease

Matthew H. Brisendine[1], Anna S. Nichenko[1], Aloka B. Bandara[1], Orion S. Willoughby[1], Niloufar Amiri[1], Zach Weingrad[2], Kalyn S. Specht[1], Jacob M. Bond[3], Adele Addington[1], Ronald G. Jones, III[4], Kevin A. Murach[4], Steven Poelzing[3], Siobhan M. Craige[1,3], Robert W. Grange[1], Joshua C. Drake[1,3,*]

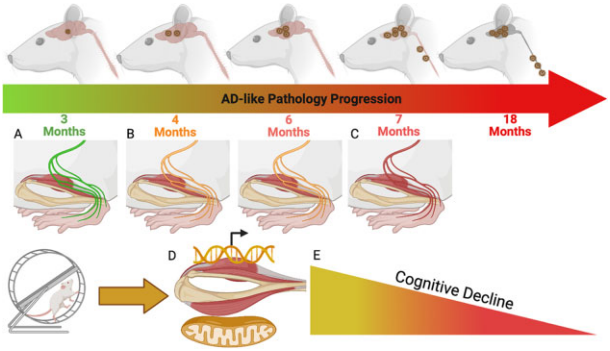
[1]Department of Human Nutrition, Foods, and Exercise, Virginia Tech, Blacksburg, VA 24061, USA, [2]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA, [3]Translational Biology, Medicine, and Health Program, Virginia Tech, Roanoke, VA 24016, USA and [4]Department of Health, Human Performance, and Recreation, University of Arkansas, Fayetteville, AR 72701, USA

*Address correspondence to J.C.D. (e-mail: joshuacd@vt.edu)

## Abstract

Alzheimer's disease (AD) develops along a continuum that spans years prior to diagnosis. Decreased muscle function and mitochondrial respiration occur years earlier in those that develop AD; however, it is unknown what causes these peripheral phenotypes in a disease of the brain. Exercise promotes muscle, mitochondria, and cognitive health and is proposed to be a potential therapeutic for AD, but no study has investigated how skeletal muscle adapts to exercise training in an AD-like context. Utilizing 5xFAD mice, an AD model that develops AD-like pathology and cognitive impairments around 6 mo of age, we examined in vivo neuromuscular function and exercise adapations (mitochondrial respiration and RNA sequencing) before the manifestation of overt cognitive impairment. We found 5xFAD mice develop neuromuscular dysfunction beginning as early as 4 mo of age, characterized by impaired nerve-stimulated muscle torque production and compound nerve action potential of the sciatic nerve. Furthermore, skeletal muscle in 5xFAD mice had altered, sex-dependent, adaptive responses (mitochondrial respiration and gene expression) to exercise training in the absence of overt cognitive impairment. Changes in peripheral systems, specifically neural communication to skeletal muscle, may be harbingers for AD and have implications for lifestyle interventions, like exercise, in AD.

**Submitted:** 28 September 2023; **Revised:** 17 November 2023; **Accepted:** 26 November 2023




**Key words:** exercise; neuromuscular; mitochondria; sciatic nerve; alzheimer's disease; 5xFAD

## Introduction

Alzheimer's disease (AD) develops along a continuum, beginning with a preclinical phase that spans several years to possibly decades before clinical manifestation as mild cognitive impairment (MCI) and eventual diagnosis as AD.[1,2] This prolonged pre-clinical phase has led to speculation that therapeutic strategies might be developed that delay or prevent AD onset. As a portion of AD cases are linked to modifiable risk factors,[3,4] understanding phenotypes and related mechanisms of preclinical or early stage AD could lead to earlier diagnostic markers and novel therapeutic interventions. In particular, loss in muscle mass and associated motor functions, such as reduced muscle strength (dynapenia) and gait speed, predict the rate of cognitive decline and development of MCI, and those with more muscle mass and strength are less likely to show deficits.[5–10] Therefore, while AD is an age-associated disease of the brain, accumulating evidence suggests peripheral physiologic phenotypes, such as declining skeletal muscle health, may be harbingers for AD before clinically evident as cognitive impairment. But what initiates skeletal muscle decline in the context of early stage AD is unknown.

Skeletal muscle contraction is induced by the depolarization of $\alpha$ motor neurons that send action potentials along their axons to the neuromuscular junctions of innervated myofibers. Mitochondria are critical for meeting the energetic demands of contracting skeletal muscle and neurons by utilizing nutrient substrates to generate ATP. Mitochondrial dysfunction in neurons is well established as a characteristic of both AD-diagnosed humans and mouse models of AD. Recent evidence in humans has demonstrated skeletal muscle mitochondrial function to be a predictor of AD pathology,[8] and impaired skeletal muscle mitochondrial respiration has been reported in individuals with MCI.[9] Mitochondrial function, particularly within skeletal muscle, can be maintained into advancing age through regular exercise. Low physical activity has been identified as a modifiable risk factor for AD and other age-related pathologies that are associated with increased risk for developing AD (eg obesity, hypertension, and diabetes) are modifiable through regular exercise.[3] However, support in the literature for a benefit of exercise on AD is less clear.[10] Data from two multi-decade-long studies suggest there is no preventative effect on AD, though exercise did mitigate other forms of dementia.[11,12] Studies that explored exercise as an interventional strategy in mouse models of AD have produced conflicting results.[11,13,14] Given the loss in muscle mass and associated strength and motor impairments during the early stages of AD,[5–7,15–17] it is possible that underlying neuromuscular dysfunction in early AD affects adaptation to exercise. However, to our knowledge, no studies have examined how skeletal muscle adapts to exercise training in AD, as measures of adaptive responses to exercise focus on brain pathology and cognitive health.[11,14,13]

The purpose of this study was to characterize neuromuscular function in an AD-like context while minimizing age-related neuromuscular dysfunction as a possible variable in wild type (WT) animals. Furthermore, we aimed to assess whether the adaptation of skeletal muscle to aerobic exercise is altered in early AD-like pathology prior to the manifestation of overt cognitive impairment. 5xFAD mice are an established mouse model of AD that develops significant extracellular A$\beta$ plaques in the brain by 4 mo of age with declines in cognition starting around 6 mo of age.[18,19] Additionally, 5xFAD mice have previously been reported to develop motor dysfunction around 9 mo of age.[18–20] While no single animal model of AD recapitulates all aspects of the pathology, we posited that such early development of AD-like pathology makes the 5xFAD model appropriate for investigating early stage AD-like phenotypes in peripheral systems, such as skeletal muscle, without advancing age in WT counterparts being a confounding variable.[20] We hypothesized neuromuscular dysfunction would be an early characteristic in 5xFAD mice and that exercise-mediated adaptation of skeletal muscle would be altered in the absence of overt cognitive impairment.

## Materials and Methods

### Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Virginia Tech. Mice were housed in temperature-controlled (21°C) quarters with 12:12 h light-dark cycle and ad libitum access to water and chow (Purina). Heterozygous 5xFAD mice were obtained commercially (Jackson Laboratories, strain #034840). The 5xFAD mouse model develops aggressive AD-like pathology due to the overexpression of APP and PSEN1 containing five familial AD mutations in forebrain neurons under the control of a *Thy1* mini-gene.[19] Male and Female heterozygous mice and WT littermates were from colonies bred in the house. Genotypes were confirmed via PCR of DNA isolated from tail snip.

### In-vivo Muscle Function

Force-frequency relationship of the plantar flexor muscles was assessed monthly, as previously described,[21–23] at 3, 4, 5, and 6 mo of age to precede the development of significant A$\beta$ plaques

(4 mo) and end around the earliest reported development of cognitive impairment (6 mo).[19] Briefly, Plantar flexor torque was determined by the force (mN) applied to and multiplied by the length of the foot pedal (m). The units were set to mN × m. Isometric torque was determined with the foot at 90° to the tibia (neutral position) over a series of stimulations at frequencies: 1, 10, 30, 50, 65, 80, 100, 120, 150, 180, and 200 Hz via F-E2 platinum-tipped needle electrodes (Natus Manufacturing, Gort, Ireland) to apply stimulations from the 701C stimulator (ASI, Aurora, ON, Canada). For tibial nerve depolarization, two electrodes were taped together ~3 mm apart and inserted percutaneously, distal from the knee, parallel along the tibia. For direct muscle stimulation, electrodes were placed within the proximal and distal heads of the gastrocnemius to recruit approximately the same number of motor units as with tibial nerve depolarization,[24] thus bypassing motor nerve-directed input. Data were plotted as torque normalized to mouse body mass (mN × m/g). Relative torque data were calculated as each respective mouse value for each time point/baseline torque × 100%. Nerve-stimulated torque production at 100 Hz was normalized to direct muscle-stimulated torque production at 100 Hz as a proxy of nerve transmission to skeletal muscle via neuromuscular junctions.[25]

## Compound Nerve Action Potential (CNAP) of Sciatic Nerve

Compound nerve action potential (CNAP) duration, an index of conduction in nerve bundles[26] was measured by a stimulus electrode placed at distance greater than 3 mm from a bipolar sensing electrode in differential amplifier mode with electrode spacing at 2 mm ($2x10^{-3}$ m). The common ground for the bipolar sensing electrode and stimulation electrode were placed in the foot of the mouse. Signal from the bipolar electrode was amplified by a Hugo Sachs Electonik D-79232 amplifier and digitized by a Powerlab 4/35 at a sampling rate of 4 kHz. The left sciatic nerve was stimulated at a period of 1 s, 10 mV, and 1 m/s monophasic pulse using a model 4100 isolated high-powered stimulator (A-M Systems, Sequim, WA, USA).

## Exercise Training

Heterozygous 5xFAD and WT littermates were randomly assigned to exercise training or sedentary groups at 10 wk of age. Mice selected for exercise training were individually housed in cages with running wheels with the wheels locked for two days to acclimate before releasing the wheel on day three. Mice in running wheels were monitored daily for the first week and any mouse that declined to utilize the running wheel during this time was removed from the study. Sedentary mice were group housed in cages between 2 and 5 mice per cage without running wheels. Exercising mice were given free access to the running wheel and both exercised and sedentary mice were provided food and water ad libitum. Daily running distance was recorded via Lab View software.

## Exercise Capacity

After 6-8 wk of running (16-18 wk of age), all mice were acclimated for 10 min at 13 m/min at 0% incline for 3 consecutive days prior to testing endurance capacity, as previously described.[27,28] The night before testing, the running wheels were locked. On the fourth day, mice performed an exhaustive exercise test on a treadmill set to a 5% incline. Mice ran at 13 m/min for 30 min, after which treadmill speed was increased by 3 m/min every 30 min, up to 28 m/min, until perceived exhaustion. Exhaustion was defined as refusal to run, despite prodding from a brush at the back of the treadmill, for 20 s. Blood lactate was measured prior to and at the end of exercise when exhaustion was perceived.

## Cognitive Function via T-Maze

After 7-9 wk of running (17-19 wk of age) and at least 1 wk after exercise capacity testing, we assessed mice for cognitive function via T-maze.[29] Mice were placed in the testing room for 20 min, undisturbed, to acclimatize. Mice were individually introduced to the bottom of the T-maze with a divider isolating the mice from the rest of the maze. When the mouse was facing away from the divider, the divider was removed, allowing the mouse access to the maze. After choosing an arm of the T-maze, defined as the entire body of the mouse having passed the entrance wall, a guillotine door was closed, and the mouse was isolated in the chosen arm of the maze for 30 s (trial test). The mouse was then removed and placed in a dark container for 1 min before being reintroduced to the bottom of the maze and the test repeated (a sample test). Selection of the opposite arm from the trial test was counted as an alternation, thus assessing working memory due to their nature to explore unfamiliar areas.[29] Time taken to make an arm choice was recorded and a given test was stopped if a mouse exceeded 90 s without a decision made. The T-maze and surface were cleaned with 10% ethanol between each mouse tested and flipped 180° to minimize olfactory cues between mice.

## Tissue Collection

Following cognitive testing, mice were allowed to run for an additional 3 wk. After 12 wk (22 wk of age) and following an overnight fast, mice were anesthetized with 3% isoflurane. Tibialis anterior (TA) was collected antemortem for mitochondrial respiration. After which, mice were euthanized via cervical dislocation and tissues were collected and snap frozen in $LN_2$.

## Skeletal Muscle Mitochondria Respiration and Citrate Synthase Activity

Mitochondrial respiration was assessed via oxygen consumption rates of permeabilized muscle fibers measured through a high-resolution respirometry device (O2k, Oroboros Instruments, Innsbruck, Austria). Methods for dissection, permeabilization, and specific substrate-uncoupler-inhibitor titration (SUIT) protocols were performed as previously.[30–32] Briefly, one TA muscle from each mouse was dissected in half. One half was weighed and set aside for citrate synthase enzyme kinetics the other half of the TA was dissected into fiber bundles ranging in size from 5 to 15 myofibers per bundle and permeabilized with 100 μg/μL of saponin in buffer x. Fiber bundles were then rinsed for 15 min in buffer Z before being separated, and ~2.5 mg portions were loaded into respirometry chambers. Measurements were performed on 3-6 technical replicates at 25°C with constant stirring and oxygen concentrations maintained between 300 and 500 μM/L. Baseline respiration rate was recorded after fiber bundles were given time to equilibrate to the O2K chamber and before the addition of any respiration substrates. Substrates (2.5 mM malate, 5 mM succinate, 5 mM glutamate, 2.5 mM ADP, and 0.5 μM rotenone) were sequentially injected into each chamber once respiration was stable for 2 min. Integrity of the outer mitochondrial membrane was assessed by injection of 10 μM

Cytochrome C. Oxygen consumption rates were normalized to the wet weight of tissue loaded into the chamber. Initial baseline respiration rates were subtracted from all rates before analysis. For the citrate synthase enzyme kinetics the other half of the TA muscle was homogenized in 33 milimolar $PO_4$ (1 mg/40 μL), freeze thawed 3 times at −80°C, then combined with acetyl-CoA and oxaloacetate and read in a spectrophotometer (Nexgen 5) at 412 nm for 3 min every 15 s.

### RNA Extraction, Sequencing, and Analyses

Snap frozen Gastrocnemius muscle was homogenized with a mortar and pestle in LN2, then added to Trizol. Total RNA was isolated using the Qiagen RNeasy Mini Kit (RNeasy Mini Kit, QIAGEN, 74106). For the RNA sequencing (Novogene, Durham, NC, USA), mRNA was purified from total RNA using poly T oligo-attached magnetic beads. After fragmentation, first-strand cDNA was synthesized using random hexamer primers, followed by second-strand cDNA synthesis using either dUTP for directional library or dTTP for non-directional library. Libararies were checked with Qubit and real-time PCR for quantification and a bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms according to effective library concentration and data amount. Index of the reference genome was built using Hisat2 v2.0.05 and paired-end clean reads were aligned to the reference genome. Reads were maped to each gene using featureCounts v1.5.0-p3 and then fragments per kilobase of transcript sequence per millions base pairs sequenced (FPKM) of each gene were calculated based on the length of the gene and read count mapped. Differential expression analysis was performed using the DESeq2Rpackage(1.20.0). Resulting P-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value $< 0.05$ found by DESeq2 and were set as the threshold for significantly differentially expressed. Gene ontology (GO) enrichment analysis of differentially expressed genes was implemented by the cluster Profiler R package, in which gene length bias was corrected. Gene ontology terms with a corrected P-value $< 0.05$ were considered significantly enriched by differential expressed genes. Ingenuity Pathway Analysis (Qiagen) was used to analyze differentially regulated pathways between sedentary WT and 5xFAD at ∼6 mo (22 wk) of age. Chord diagrams were generated using the GOplot R package.

### Statistics

Data are presented as mean ± SEM and were analyzed and plotted via GraphPad Prism 9.5.1. Data were analyzed via Student's t-test when one variable was present, two-way ANOVA when two variables were present, or repeated measures two-way ANOVA when two variables were measured in the same animals over time. Post-hoc analyses were only performed when a significant interaction between a categorical and a quantitative variable was found, which is indicated in the figures where applicable. Statistical significance was established a priori as $P < 0.05$.

## Results

### Longitudinal Assessment of Neuromuscular Function in Vivo

To assess neuromuscular function in 5xFAD mice, we longitudinally measured muscle torque production monthly in male and female mice beginning at 3 mo of age (prior to the development of significant A$\beta$ plaques[18]) until 6 mo of age, when AD-like cognitive impairment has been reported to manifest.[18,19] Muscle torque production of the plantar flexors (ie gastrocnemius, plantaris, and soleus) was determined first via tibial nerve-stimulated contraction, then via direct muscle (gastrocnemius) stimulation 48 h later (Figure 1A). This two-pronged approach allows for discernment between the major physiological processes responsible for skeletal muscle contraction; signal transmission along motor nerves and muscle contractile proteins.[33,21] At 3 mo of age, there was no difference in nerve- or direct muscle-stimulated torque production between WT and 5xFAD mice in either males or females (Supplementary Figure S1A, E, I, M). In male mice beginning at 4 mo of age, nerve-stimulated torque production significantly declined in 5xFAD mice and remained so at 5 and 6 mo of age, whereas nerve-stimulated torque production in WT mice remained stable over time (Figure 1B, C, J; Supplementary Figure S1B-D). While torque production in female mice also significantly declined in 5xFAD by 6 mo of age (Figure 1E; Supplementary Figure S1L), the decline was not as consistent as in males (Figure 1D, E, K; Supplementary Figure S1J-L). Interestingly, in both males and females, no differences in torque production were found when inducing muscle contraction through direct stimulation (Figure 1F-I, L, M; Supplementary Figure S1E-H, M-P). Expressing nerve-stimulated torque production relative to direct muscle-stimulated torque production (Supplementary Figure S1Q, R),[25] reinforced the notion that nerve-stimulated torque production was impaired in 5xFAD mice. No differences in body weight between groups were noted throughout the study, and therefore differences in torque production are not due to differing mouse size (Figure 1N, O). Collectively, these findings suggest the capacity for skeletal muscle to produce torque remains intact in 5xFAD mice up to at least 6 mo of age. Further, these findings implicate impaired peripheral motor nerve conduction to skeletal muscle is an early event in the AD-like pathology of 5xFAD mice.

### Impaired Peripheral Nerve Function in 5xFAD Mice

To confirm whether nerve-stimulated plantar flexor torque production was due to impaired nerve conduction, we assessed the compound nerve action potential (CNAP) of the sciatic nerve at 7 mo of age. Sciatic nerve CNAP was measured in vivo by exposing the nerve mid thigh and placing a stimulus electrode and a bipolar sensing electrode along the axonal body of the nerve, the common ground for the bipolar sensing electrode and stimulation electrode was placed in the foot of the mouse (Figure 2A). CNAP duration was significantly slower in both male and female 5xFAD mice compared to WT mice (Figure 2B, C). Importantly, sciatic CNAP was normal between groups when measured in male mice at 3 mo of age (Supplementary Figure S2), corresponding to the age when nerve-stimulated muscle torque production was also normal (Figure 1B, C; Supplementary Figure S1A). To gain further insights into possible changes in related nerve function, we assessed gene pathway changes via RNA sequencing of RNA isolated from gastrocnemius muscle of 6 mo WT and 5xFAD mice. We found altered expression of pathways involved in neurotransmitter and nervous system signaling in both male and female 5xFAD mice (Supplementary Figure S3A-G), which may allude to further impairment in neuromuscular junctions. Taken together with the reduced nerve-stimulated muscle torque production in Figure 1, the development of peripheral nerve dysfunction and altered conduction to skeletal muscle in 5xFAD

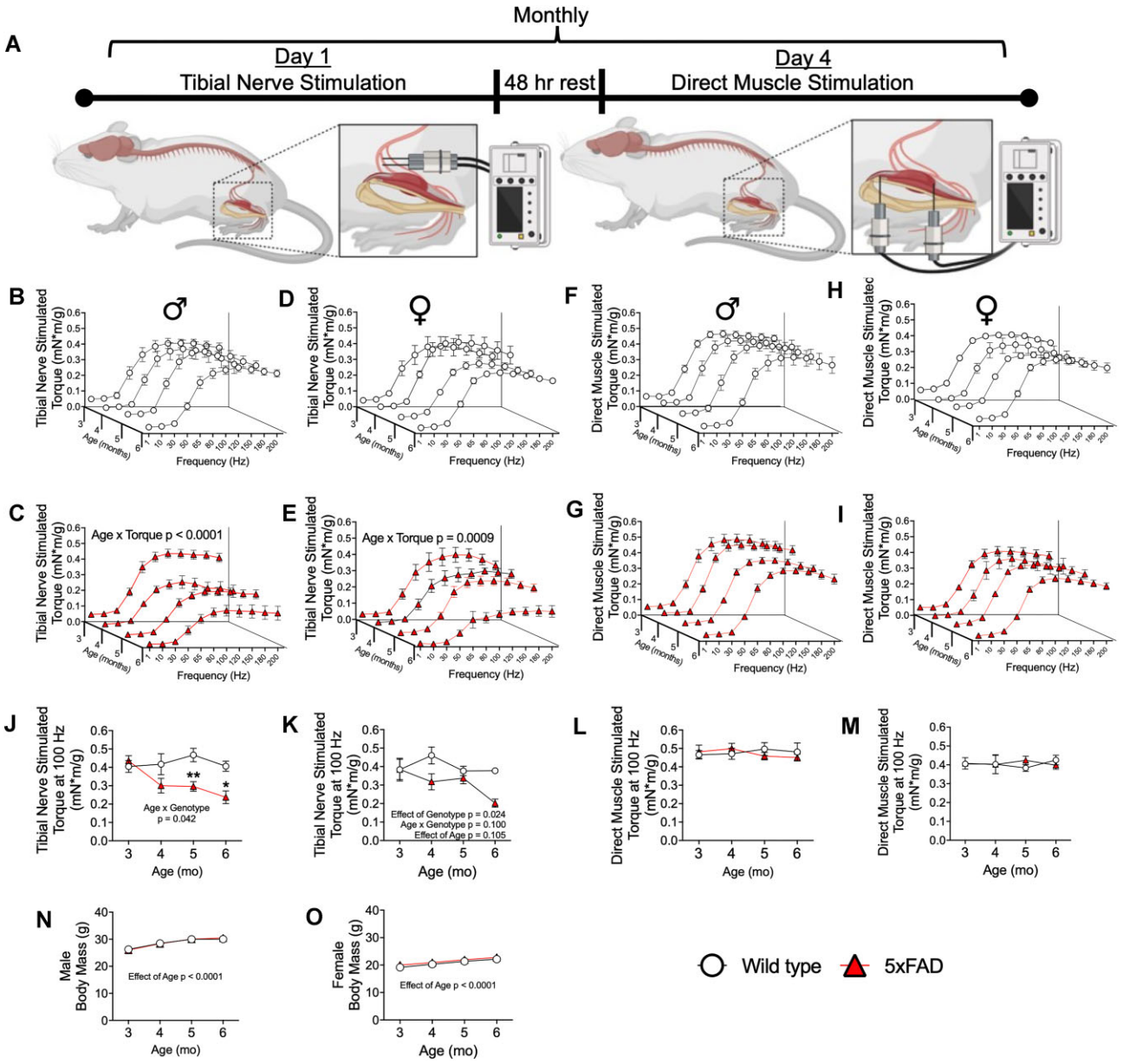



**Figure 1.** Development of neuromuscular dysfunction in male and female 5xFAD mice. (A) Study the design of tibial nerve-stimulated plantar flexor torque production followed by direct muscle stimulation 48 h later, performed monthly from 3 to 6 mo of age. B-I Torque-Frequency Curves; (B) Male WT tibial nerve stimulation. (C) Male 5xFAD tibial nerve stimulation. (D) Female WT tibial nerve stimulation. (E) Female 5xFAD tibial nerve stimulation. (F) Male WT direct muscle stimulation. (G) Male 5xFAD direct muscle stimulation. (H) Female WT direct muscle stimulation. (I) Female 5xFAD direct muscle stimulation. J-M Torque at 100 Hz; (J) Male torque production at 100 Hz via tibial nerve stimulation. (K) Female torque production at 100 Hz via tibial nerve stimulation. (L) Male torque production at 100 Hz via direct muscle stimulation. (M) Female torque production at 100 Hz via direct muscle stimulation. (N) Male body mass. (O) Female body mass. $n = 5$ per group and sex. Data presented as mean ± SEM and repeated measures two-way ANOVA was performed and Sidak's post-hoc test performed when significant interaction between variables was observed ($^{*}P < 0.05$ and $^{**}P < 0.01$). Panel A created in Biorender.

mice is analogous to early stage AD pathology found in AD and MCI subjects.[5,15–17,34]

## Improved Exercise Capacity with Exercise Training

Exercise has been proposed as a potential interventional strategy to delay AD onset and/or cognitive decline, although support for this notion in the literature is less clear.[10] Beginning at 10 wk of age (prior to the development of neuromuscular or cognitive dysfunction), male and female 5xFAD and WT mice were randomly assigned to a sedentary group or given ad libitum access to running wheels for 12 wk (Figure 3A, D). Exercise capacity significantly increased with exercise training in both males and females, irrespective of genotype (Figure 3B, C, E, F). However, there was a significant effect of genotype for exercise capacity to be lower in female 5xFAD mice (Figure 3E). In both male and female, heart wet weight increased with exercise training regardless of genotype (Supplementary Figure S4A, B), consistent with improved exercise capacity. However, female 5xFAD mice had significantly reduced heart wet weight compared to WT females, and a similar trend was observed in male

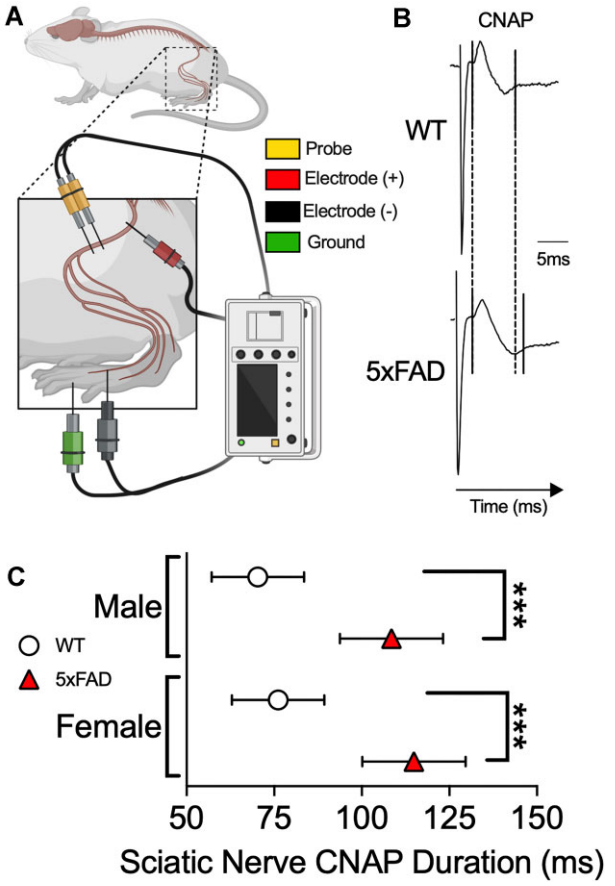



**Figure 2.** Impaired compound nerve action potential (CNAP) in the sciatic nerve of male and female 5xFAD mice: (A) Graphic of sciatic CNAP stimulation. (B) Rerepresentative WT versus 5xFAD CNAP waveform. (C) Male and female WT and 5xFAD sciatic CNAP duration. $n = 5$ per group and sex. Data presented as mean ± SEM and students *T*-test performed (**$P < 0.01$). Panel A created in Biorender.

5xFAD (Supplementary Figure S4A, B). In sum, these findings are consistent with improved cardiopulmonary adaptation to exercise training in 5xFAD mice.

## Altered Adaptation of Skeletal Muscle to Exercise Training in 5xFAD Mice

Increased mitochondrial respiration in skeletal muscle is one of the primary adaptive responses to aerobic exercise training, aiding increased exercise capacity.[35] However, to our knowledge, no study has investigated how skeletal muscle adapts to exercise training in an AD-like context. In light of the development of neuromuscular dysfunction in 5xFAD mice (Figure 1), we hypothesized that adaptation of muscle to exercise training would be altered. Following the completion of 12 wk of exercise training, we assessed TA muscle mitochondrial respiration capacity via high-resolution respirometry. No differences were observed between sedentary and exercised groups with the addition of glutamate/malate or succinate (Supplementary Figure S4C, D, E); however, female 5xFAD mice did have reduced respiration compared to WT with the addition of succinate (Supplementary Figure S4F). Exercise-trained male WT mice had significantly increased ADP-stimulated State III respiration compared to sedentary counterparts; however, there was no improvement in State III respiration in 5xFAD male mice with exercise training, nor did exercise training increase skeletal muscle mitochondrial respiration when the entire respiration curve was analyzed (Figure 3 G, H). In female mice, no difference was found in ADP-stimulated State III respiration in 5xFAD compared to WT with or without exercise (Figure 3I). However, there was a significant genotype effect for 5xFAD females to have reduced skeletal muscle mitochondrial respiration when the entire respiration curve was analyzed (Figure 3J). Addition of rotenone to inhibit complex I of the electron transport chain resulted in similar rate patterns to ADP-stimulated state 3 respiration with no differences in female mice (Supplementary Figure S4I). However, in male mice, there was a significant effect of exercise training to increase mitochondrial respiration in both WT and 5xFAD mice following the addition of rotenone (Supplementary Figure S4G), suggesting an impairment in complex I-mediated respiration in the presence of ADP in exercise-trained 5xFAD male mice (Supplementary Figure S4G). No differences were seen in citrate synthase activity in male or female mice between sedentary and exercise groups (Supplementary Figure S4H, J), suggesting respiration differences were not due to altered mitochondrial content.

To gain additional insight into skeletal muscle adaptation to exercise training in 5xFAD mice, we isolated RNA from the gastrocnemius and performed RNA sequencing. Sedentary WT and 5xFAD male and female mice had different transcriptional signatures in gastrocnemius muscle (Figure 4A, D), with 5xFAD male and female sedentary mice expressing 117 and 119 unique genes, respectively, from WT sedentary counterparts (Figure 4B, E). With exercise training, 5xFAD male and female mice expressed 82 and 118 unique genes, respectively, from exercise-trained WT counterparts (Figure 4B, E). In particular, in male WT mice, exercise training resulted in significant upregulation of genes related to mitochondrial respiratory chain, oxidative phosphorylation, and proton transporters, as well as cytosolic translation and ribosome biogenesis (Figure 4C), consistent with improved mitochondrial respiration post-exercise training (Figure 3G, H). However, in male 5xFAD exercise-trained mice, these same pathways were significantly downregulated compared to exercise-trained WT counterparts (Figure 4C), again consistent with a lack of exercise-induced adaptation in the skeletal muscle mitochondrial respiration (Figure 3G, H). Interestingly, in female WT mice, exercise training resulted in significant upregulation of different genes from those of exercise training in WT male mice. Specifically, genes significantly upregulated with exercise training in skeletal muscle of WT female mice were related to immune response, extracellular matrix organization, neurotransmitter biosynthesis, and dendritic spine maintenance (Figure 4F). However, these same pathways were significantly downregulated in exercise-trained 5xFAD female mice relative to their WT counterparts (Figure 4F). Taken together, these data suggest an altered adaptive response of skeletal muscle to exercise training in 5xFAD mice despite increased exercise capacity.

## Cognitive Health With Exercise Training

To understand the response of skeletal muscle to exercise training in relation to cognitive health, we assessed spatial learning and memory via T-maze alternation prior to the collection of tissues used in Figures 3 and 4. The T-maze leverages the mouse's nature to explore a new environment, and thus, with successive trials, mice prefer to visit a new arm of the maze rather than the previously visited arm, providing an indication of working memory.[29] There were no differences among male mice in successful alternations across genotype or with exercise (Figure 5A).

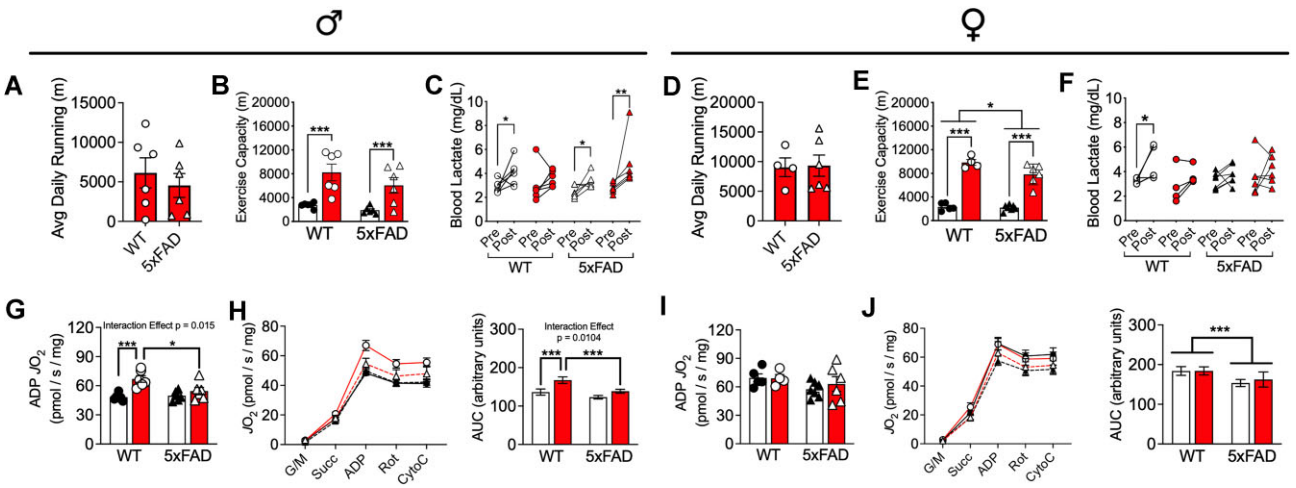



Figure 3. Running wheel exercise and skeletal muscle adaptations. (A) Male WT and 5xFAD Ex average daily running. (B) Male exercise capacity. (C) Male blood lactate pre- and post-exercise capacity test. (D) Female WT and 5xFAD Ex average daily. (E) Female exercise capacity. (F) Female blood lactate pre- and post-exercise capacity test. (G) Male ADP-stimulated State 3 respiration. (H) Male SUIT protocol and respiration rates area under the curve. (I) Female ADP-stimulated State 3 respiration. (J) Female SUIT protocol and respiration rates area under the curve. Males $n = 6$ per group; females $n = 4$-7 per group. Data presented as mean ± SEM and analyzed via Student's *t*-test (one variable) or two-way ANOVA (two variables) with post-hoc analysis performed when significant interaction effect was found ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

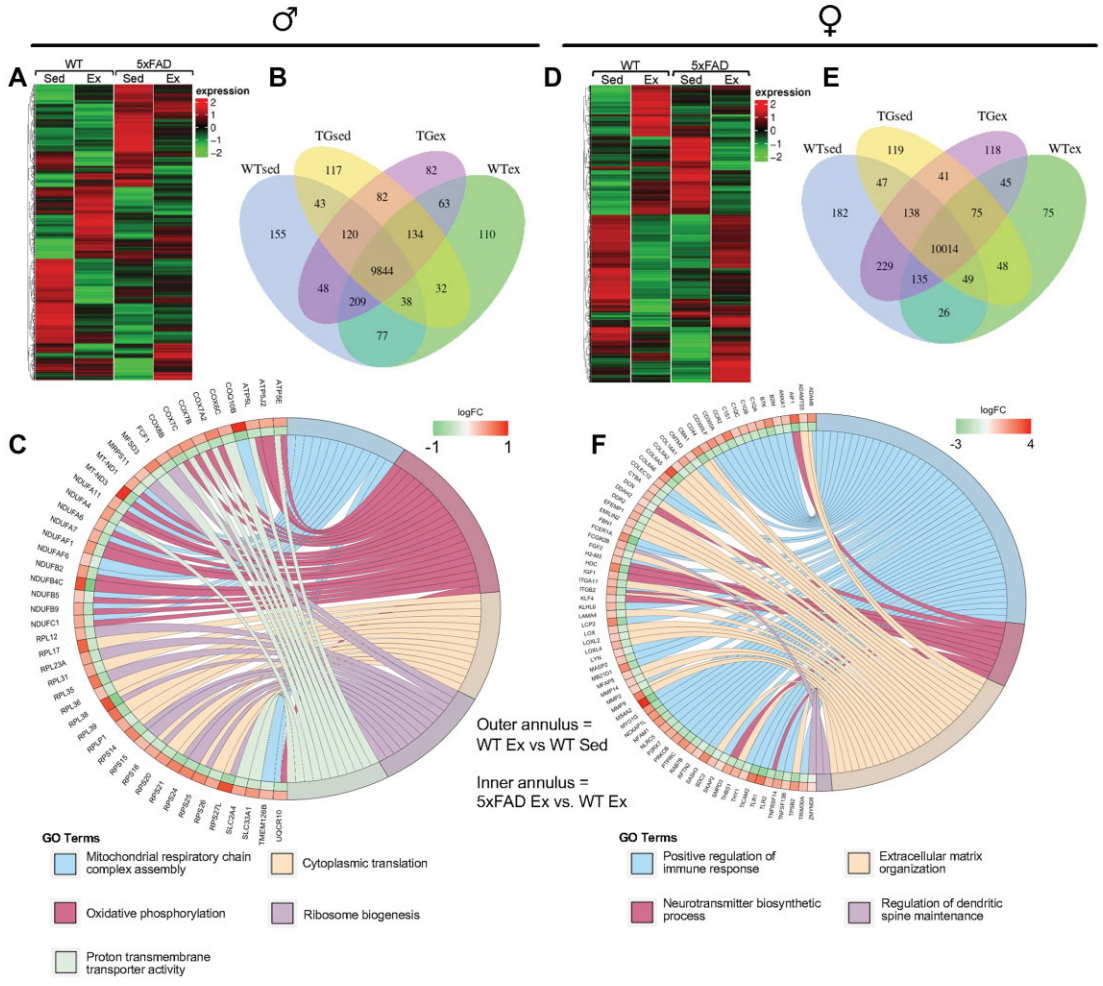



Figure 4. Altered transcriptional changes in skeletal muscle with exercise training between male and female 5xFAD mice. (A) Heat map of global gene changes in male mice. (B) Differently expressed gene (DEG) between male groups. (C) Chord diagram of representative gene pathway differences between male WT Ex versus WT sed and 5xFAD Ex versus WT Ex. (D) Heat map of global gene changes in female mice. (E) Differently expressed gene (DEG) between female groups. (F) Chord diagram of representative gene pathway differences between female WT Ex versus WT sed and 5xFAD Ex versus WT Ex. Males $n = 6$ per group; females $n = 4$-7 per group.

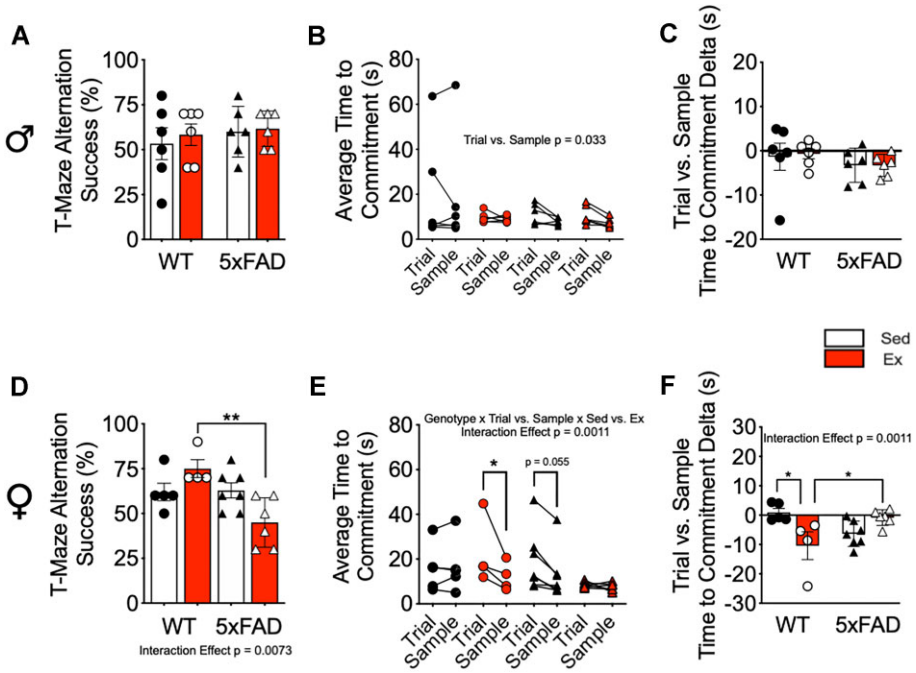



**Figure 5.** T-maze assessment of cognitive function in male and female 5xFAD mice following exercise training: Mice were introduced to the T-maze and tested 10 times over 3 d. (A) Male percent success rate in alternation from initial T-arm choice. (B) Male average time to T-arm commitment. (C) Male time to commitment difference between trial and sample test. (D) Female percent success rate in alternation from initial T-arm choice. (E) Female average time to T-arm commitment. (F) Female time to commitment difference between trial and sample test. Males $n = 6$ per group; females $n = 4$-7 per group. Data presented as mean ± SEM and analyzed via two-way ANOVA with post-hoc analysis performed with significant interaction effect was found ($^{*}P < 0.05$ and $^{**}P < 0.01$).

Alternatively, there was a significant decline in alternation success in exercise-trained female 5xFAD compared to exercise-trained WT mice (Figure 5D). Time to commitment, defined as the time taken for a mouse to commit to a single arm of the T-maze, was measured as an indicator of the sensorimotor effect of exercise.[29] In male mice, average time to commitment improved in all groups (Figure 5B, C), which, in combination with the lack of difference in alternation suggests no overt cognitive impairment in 5xFAD male mice at the time of testing. In female exercise-trained WT and sedentary 5xFAD mice, there was a significant decrease from trial time to commitment and sample time to commitment (Figure 5E). Furthermore, there was a significant difference in exercise-trained WT females sample time to commitment compared to exercise-trained 5xFAD mice (Figure 5F), which, in combination with decreased alternation suggests ad libitum exercise training in 5xFAD female mice accelerated cognitive decline. No differences were found in brain wet weight across groups or sexes (Supplementary Figure S5A, B). In sum, these data suggest that the development of neuromuscular dysfunction and the maladaptive response of skeletal muscle to exercise training (Figures 1-4) may be independent of overt indices of impaired cognitive health in 5xFAD mice.

## Discussion

In this study, we investigated neuromuscular function over time (ages 3-7 mo) in a mouse model of AD and the adaptive response of skeletal muscle to exercise training prior to the development of overt cognitive impairment. In 5xFAD mice, we discovered a decline in neuromuscular function manifests as early as 4 mo of age. Notably, in both males and females, impaired neuromuscular function was due to impaired neural conduction to skeletal muscle, as evidenced by impaired nerve-stimulated muscle torque production and sciatic CNAP, in contrast to maintained torque production when peripheral nerve conduction was bypassed[24] through direct muscle stimulation. In response to aerobic exercise training via voluntary wheel running, we found both male and female 5xFAD mice increased exercise capacity compared to sedentary counterparts, but the adaptive response of skeletal muscle differed from WT and between sexes. Male 5xFAD mice did not increase mitochondrial State 3 respiration or have gene changes associated with improved mitochondrial function with exercise training in skeletal muscle. Alternatively, female 5xFAD mice had lower overall mitochondrial respiration in muscle with no effect of exercise training in either genotype but with differing transcriptional responses. Finally, we showed evidence that neuromuscular dysfunction and altered adaptive responses of skeletal muscle to exercise training occurred prior to the development of overt cognitive dysfunction in 5xFAD mice, particularly males.

Loss of muscle strength is a known phenotype of the preclinical phase of AD and is shown to predict cognitive decline.[5–7,15–17] Our findings provide the first direct evidence of impaired neural conduction to skeletal muscle in vivo in a mouse model of AD that precedes overt cognitive impairment, providing support to the notion that neuromuscular dysfunction is an early stage characteristic of AD-like pathology that may explain motor deficits associated with pre-clinical AD. Skeletal muscle contraction is induced through the relaying of chemical signals originating in the brain along motor neuron axons and across neuromuscular junctions to innervated myofibers. Impaired nerve-stimulated muscle torque production and slower sciatic CNAP in the present study could be reflective of demyelination or alteration of sodium channels, among other possibilities.[36] Of note, the development of impaired nerve-stimulated muscle torque production at 4 mo of age corresponds with the timing

of impaired long-term potentiation and synaptic transmission in the hippocampus of 5xFAD mice,[18] suggesting early brain changes in AD have systemic consequences.

Muscle atrophy and contractile dysfunction occur following nerve injury and as a result of aging.[21,23,37–41] Previous findings in the 3xTG AD mouse model also showed impaired nerve-stimulated muscle contraction and loss of NMJ innervation but only at 17-20 mo of age, well after the onset of cognitive impairment.[25] Our functional and RNAseq data in young 5xFAD mice suggests impaired nerve conduction to skeletal muscle prior to overt cognitive impairment is part of the AD-like pathology and not an accelerated aging phenotype associated with AD. A recent study in humans revealed a positive correlation between peripheral motor nerve conduction velocity and cognitive function in subjects diagnosed with MCI or AD compared to cognitively intact controls.[34] Impaired neural communication to skeletal muscle in 5xFAD mice may be a clinically relevant phenotype of early stage AD that suggests measures of peripheral nerve conduction may serve to identify individuals at increased risk for developing AD.

Next, we tested the adaptive response of skeletal muscle to exercise training that was begun (2.5 mo) prior to the onset of neuromuscular (4 mo) and cognitive impairment (≥6 mo) in 5xFAD mice. Although both male and female 5xFAD mice develop neuromuscular dysfunction and improved exercise capacity with exercise training, the adaptive response in muscle from WT counterparts diverged between sexes. Divergent adaptive transcriptional changes in skeletal muscle between sexes, despite a similar phenotypic adaptive response of improved exercise capacity have been observed in humans.[42] In particular, we show male WT mice improved ADP-stimulated, State 3 mitochondrial respiration in skeletal muscle following exercise training, an adaptive response that was absent in male 5xFAD mice following exercise training and supported by RNA sequencing. In contrast, female mice did not have an exercise training-mediated increase in ADP-stimulated mitochondrial respiration, which may be due to State 3 respiration in WT female mice already being at the level attained by exercise-trained male WT mice, suggesting female mice are at physiological capacity of mitochondrial respiration in skeletal muscle. However, female 5xFAD mice had significantly lower total mitochondrial respiration rates in the skeletal muscle compared to WT mice. Collectively, our findings support clinical data reporting deficiencies in mitochondrial State 3 respiration in the skeletal muscle of subjects with MCI[9] and recent findings that skeletal muscle mitochondrial energetic deficiencies predict the development of MCI and AD.[43]

Additionally, we showed skeletal muscle gene expression differs between WT and 5xFAD mice, suggesting that AD-like pathology fundamentally alters the transcriptional landscape of skeletal muscle. A recent report identified sex-specific pathways may underlie resilience to AD. In particular, resilience to AD in females was linked, in part, to upregulation of immune-related pathways.[44] We found increased expression of genes related to positive regulation of immune responses upregulated in the skeletal muscle of exercise-trained female WT mice but not in exercise-trained female 5xFAD mice. Further sexual dimorphic transcriptional responses to exercise in the skeletal muscle of 5xFAD mice suggest exercise as an interventional strategy for AD-like pathology may require personalization.

Loss of neural connectivity to skeletal muscle (ie motor units) occurs in aged rodents and humans[45–47] and is associated with loss of mitochondrial function.[45] Therefore, it is possible that impaired neuromuscular function during the early stage of AD-like pathology in 5xFAD mice underlies altered sex-dependent functional and gene transcriptional changes in the skeletal muscle at baseline and in response to an exercise stimulus, as well as impaired skeletal muscle mitochondrial respiration during early stage AD. As treatment with the acetylcholinesterase inhibitor donepezil normalizes mitochondrial respiration in skeletal muscle of MCI subjects,[9] future studies examining the adaptive response of skeletal muscle as well as peripheral and central nervous systems in combination with established pharmacological treatments used in AD may provide a means to optimize lifestyle intervention to delay or improve AD.

To assess the adaptive response of skeletal muscle to exercise training in context with the cognitive health of 5xFAD mice, we performed T-maze alternation testing.[29] Collectively, our findings suggest that neuromuscular dysfunction and an altered adaptive response to exercise occurred without overt cognitive impairment, particularly in males. Although physical activity is considered a modifiable risk factor for AD, epidemiological studies have reached mixed conclusions as to the benefits of exercise training on reducing AD occurrence. Yu *et al.* found 6 mo of cycling minimized memory and executive function losses; however, language and attention processing speed continued to decline despite exercise treatment.[48] Also, exercise benefits were not maintained after 12 mo, as declines were seen in all measures.[48] Others have found aerobic exercise offers functional benefits to those with AD with minor effects seen on memory but only in individuals who showed improvement in cardiorespiratory fitness; that may imply a link between disease stage, training volume, and adaptive benefits.[49] Exercise training of 5xFAD mice has also shown mixed results as to positive benefits on indices of cognitive health.[14,16,17] Exercise volume may explain this discrepancy, as limiting female mice to 3 h running per day and returning them to communal cages improved cognition, hippocampal neurogenesis, and reduced A$\beta$ load.[16] Alternatively, individualized ad libitum access to running wheels (as we performed here) did not slow the development of AD-like pathology, neuroinflammation, or preserve cognitive health.[11,14] In humans, excessive exercise volume and intensity have been shown to cause impairments in skeletal muscle mitochondrial function, despite improved performance.[50] Thus, exercise volume may need to be closely monitored and system-dependent adaptive responses assessed for endurance exercise to be an effective lifestyle intervention during the early stages of likely AD. In sum, our findings highlight a need for a more integrative understanding of AD and AD-like pathology across a range of cognitive states, particularly within the context of lifestyle interventions (eg exercise), which influence multiple systems.

Accumulating evidence has shown a link between skeletal muscle and mitochondrial function and the development of AD.[5–12] Our findings in 5xFAD mice suggest neuromuscular dysfunction and altered adaptive responses of skeletal muscle to running wheel exercise occur prior to the development of overt AD-like cognitive impairments (Figure 5). While aggressive development of AD-like pathology in the 5xFAD model can be advantageous for investigating age-independent aspects of AD-like etiology, this also creates a limitation in potential translatability. Gene changes in the brain of 5xFAD mice with age show general agreement with that of humans with AD,[18] but additional studies of potential neuromuscular dysfunction in human subjects with MCI or AD are needed. Future studies modifying neuromuscular function and exercise through drug treatments, modified exercise prescription/regimen and modalities

are all worthy avenues of investigation. A better mechanistic understanding for the neuromuscular dysfunction and disconnect between exercise and AD could lead to new therapeutic approaches for AD.

## Acknowledgments

We thank the Virginia Tech Metabolism Core for technical assistance with skeletal muscle contraction and mitochondrial respiration experiments. We also thank Drs Jill Morris and John Thyfault (KUMC), as well as other members of JCD's laboratory for critical feedback and discussion.

## Author Contributions

Study design: M.H.B. and J.C.D. Conducting experiments: M.H.B., A.S.N., A.B.B., O.S.W., N.A., Z.W., K.S.S., J.M.B., A.A., S.M., and J.C.D. Analyzing data: M.H.B., A.S.N., R.G.J., K.A.M., S.P., R.W.G., and J.C.D. Manuscript drafting: M.H.B., A.S.N., A.B.B., O.S.W., N.A., Z.W., K.S.S., J.M.B., A.A., R.G.J., K.A.M., S.P., S.M.C., R.W.G., and J.C.D.

## Supplementary Material

Supplementary material is available at the *APS Function* online.

## Funding

This work was supported by a National Institutes of Health grant [R01-AG080731-01] from the National Institute on Aging to J.C.D. Other support for the authors include R00AG057825 (to J.C.D.), K01-AR073332 ( to S.M.C.), and R01-AG080047 (to K.A.M.).

## Conflict of Interest

The authors have no conflicts to disclose.

## Data Availability

The data within this article are available in the article and in its online supplementary material.

## References

1. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;**7**(3):280–292.
2. Linn RT, Wolf PA, Bachman DL, et al. The "preclinical phase" of probable Alzheimer's Disease: a 13-year prospective study of the Framingham cohort. *Arch Neurol* 1995;**52**(5):485–490.
3. Norton S, Matthews FE, Barnes DE, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol* 2014;**13**(8):788–794.
4. Hwang PH, Ang TFA, De Anda-Duran I, et al. Examination of potentially modifiable dementia risk factors across the adult life course: the Framingham Heart Study. *Alzheimers Dement* 2023;**19**(7):2975–2983.
5. Burns JM, Johnson DK, Watts A, Swerdlow RH, Brooks WM. Reduced lean mass in early Alzheimer disease and its association with brain atrophy. *Arch Neurol* 2010;**67**(4):428–433.
6. Soto ME, Secher M, Gillette-Guyonnet S, et al. Weight loss and rapid cognitive decline in community-dwelling patients with Alzheimer's disease. *J Alzheimers Dis* 2012;**28**(3):647–654.
7. Ogawa Y, Kaneko Y, Sato T, Shimizu S, Kanetaka H, Hanyu H. Sarcopenia and muscle functions at various stages of Alzheimer Disease. *Front Neurol* 2018;**9**(710):1–7.
8. Tian Q, Mitchell BA, Zampino M, Fishbein KW, Spencer RG, Ferrucci L. Muscle mitochondrial energetics predicts mobility decline in well-functioning older adults: the baltimore longitudinal study of aging. *Aging Cell* 2022;**21**(2):e13552.
9. Morris JK, McCoin CS, Fuller KN, et al. Mild cognitive impairment and donepezil impact mitochondrial respiratory capacity in skeletal muscle. *Function* 2021;**2**(6):zqab045.
10. Brisendine MH, Drake JC. Early stage Alzheimer's disease: are skeletal muscle and exercise the key? *J Appl Physiol (1985)* 2023;**134**(3):515–520.
11. Hansson O, Svensson M, Gustavsson AM, et al. Midlife physical activity is associated with lower incidence of vascular dementia but not Alzheimer's disease. *Alzheimers Res Ther* 2019;**11**(1):87.
12. Najar J, Östling S, Gudmundsson P, et al. Cognitive and physical activity and dementia: a 44-year longitudinal population study of women. *Neurology* 2019;**92**(12):e1322–e1330.
13. Choi SH, Bylykbashi E, Chatila ZK, et al. Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer's mouse model. *Science* 2018;**361**(6406):eaan8821.
14. Svensson M, Andersson E, Manouchehrian O, Yang Y, Deierborg T. Voluntary running does not reduce neuroinflammation or improve non-cognitive behavior in the 5xFAD mouse model of Alzheimer's disease. *Sci Rep* 2020;**10**(1):1346.
15. Boyle PA, Buchman AS, Wilson RS, Leurgans SE, Bennett DA. Association of muscle strength with the risk of Alzheimer disease and the rate of cognitive decline in community-dwelling older persons. *Arch Neurol* 2009;**66**(11):1339–1344.
16. Buchman AS, Wilson RS, Boyle PA, Bienias JL, Bennett DA. Grip strength and the risk of incident Alzheimer's disease. *Neuroepidemiology* 2007;**29**(1-2):66–73.
17. Aggarwal NT, Wilson RS, Beck TL, Bienias JL, Bennett DA. Motor dysfunction in mild cognitive impairment and the risk of incident Alzheimer disease. *Arch Neurol* 2006;**63**(12):1763–1769.
18. Forner S, Kawauchi S, Balderrama-Gutierrez G, et al. Systematic phenotyping and characterization of the 5xFAD mouse model of Alzheimer's disease. *Sci Data* 2021;**8**(1):270.
19. Oakley H, Cole SL, Logan S, et al. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* 2006;**26**(40):10129–10140.
20. O'Leary TP, Mantolino HM, Stover KR, Brown RE. Age-related deterioration of motor function in male and female 5xFAD mice from 3 to 16 months of age. *Genes Brain Behav* 2020;**19**(3):e12538.
21. Wilson RJ, Drake JC, Cui D, et al. Mitochondrial protein S-nitrosation protects against ischemia reperfusion-induced denervation at neuromuscular junction in skeletal muscle. *Free Radic Biol Med* 2018;**117**:180–190.
22. Cui D, Drake JC, Wilson RJ, et al. A novel voluntary weightlifting model in mice promotes muscle adaptation and insulin sensitivity with simultaneous enhancement of autophagy and mTOR pathway. *FASEB J* 2020;**34**(6):7330–7344.

23. Wilson RJ, Drake JC, Cui D, et al. Voluntary running protects against neuromuscular dysfunction following hindlimb ischemia-reperfusion in mice. *J Appl Physiol* 2019;**126**(1):193–201.
24. Okuma Y, Bergquist AJ, Hong M, Chan KM, Collins DF. Electrical stimulation site influences the spatial distribution of motor units recruited in tibialis anterior. *Clin Neurophysiol* 2013;**124**(11):2257–2263.
25. Xu H, Bhaskaran S, Piekarz KM, et al. Age related changes in muscle mass and force generation in the triple transgenic (3xTgAD) mouse model of Alzheimer's disease. *Front Aging Neurosci* 2022;**14**(876816):1–14.
26. Bhatt NK, Park AM, Al-Lozi MT, Gale DC, Paniello RC. Compound motor action potential duration and latency are markers of recurrent laryngeal nerve injury. *Laryngoscope* 2017;**127**(8):1855–1860.
27. Laker RC, Drake JC, Wilson RJ, et al. Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nat Commun* 2017;**8**(1):548.
28. Drake JC, Wilson RJ, Cui D, et al. Ulk1, Not Ulk2, is required for exercise training-induced improvement of insulin response in skeletal muscle. *Front Physiol* 2021;**12**(876816):1–7.
29. Deacon RMJ, Rawlins JNP. T-maze alternation in the rodent. *Nat Protoc* 2006;**1**(1):7–12.
30. Drake JC, Wilson RJ, Laker RC, et al. Mitochondria-localized AMPK responds to local energetics and contributes to exercise and energetic stress-induced mitophagy. *Proc Natl Acad Sci U S A* 2021;**118**(37):e2025932118.
31. Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R, Kunz WS. Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. *Nat Protoc* 2008;**3**(6):965–976.
32. Perry CGR, Kane DA, Lanza IR, Neufer PD. Methods for assessing mitochondrial function in diabetes. *Diabetes* 2013;**62**(4):1041–1053.
33. Wilson RJ, Drake JC, Cui D, et al. Voluntary running protects against neuromuscular dysfunction following hindlimb ischemia-reperfusion in mice. *J Appl Physiol (1985)* 2019;**126**(1):193–201.
34. Qian X, Yue L, Mellor D, Robbins NM, Li W, Xiao S. Reduced peripheral nerve conduction velocity is associated with Alzheimer's Disease: a cross-sectional study from China. *Neuropsychiatr Dis Treat* 2022;**18**:231–242.
35. Granata C, Oliveira RSF, Little JP, Renner K, Bishop DJ. Mitochondrial adaptations to high-volume exercise training are rapidly reversed after a reduction in training volume in human skeletal muscle. *FASEB J* 2016;**30**(10):3413–3423.
36. Seidl AH. Regulation of conduction time along axons. *Neuroscience* 2014;**276**:126–134.
37. Zhao L, Lv G, Jiang S, et al. Morphological differences in skeletal muscle atrophy of rats with motor nerve and/or sensory nerve injury. *Neural Regen Res* 2012;**7**(32):2507–2515.
38. Yang X, Li M, Ji Y, et al. Changes of gene expression patterns of muscle pathophysiology-related transcription factors during denervated muscle atrophy. *Front Physiol* 2022;**13**(923190):1–16.
39. Tomlinson BE, Irving D. The numbers of limb motor neurons in the human lumbosacral cord throughout life. *J Neurol Sci* 1977;**34**(2):213–219.
40. McKinnon NB, Montero-Odasso M, Doherty TJ. Motor unit loss is accompanied by decreased peak muscle power in the lower limb of older adults. *Exp Gerontol* 2015;**70**:111–118.
41. Sheth KA, Iyer CC, Wier CG, et al. Muscle strength and size are associated with motor unit connectivity in aged mice. *Neurobiol Aging* 2018;**67**:128–136.
42. Chapman MA, Arif M, Emanuelsson EB, et al. Skeletal muscle transcriptomic comparison between long-term trained and untrained men and women. *Cell Rep* 2020;**31**(12):107808.
43. Tian Q, Bilgel M, Walker KA, et al. Skeletal muscle mitochondrial function predicts cognitive impairment and is associated with biomarkers of Alzheimer's disease and neurodegeneration. *Alzheimers Dement* 2023;**19**(10):4436–4445.
44. Eissman JM, Dumitrescu L, Mahoney ER, et al. Sex differences in the genetic architecture of cognitive resilience to Alzheimer's disease. *Brain J Neurol* 2022;**145**(7):2541–2554.
45. Spendiff S, Vuda M, Gouspillou G, et al. Denervation drives mitochondrial dysfunction in skeletal muscle of octogenarians. *J Physiol* 2016;**594**(24):7361–7379.
46. Burke SK, Fenton AI, Konokhova Y, Hepple RT. Variation in muscle and neuromuscular junction morphology between atrophy-resistant and atrophy-prone muscles supports failed re-innervation in aging muscle atrophy. *Exp Gerontol* 2021;**156**:111613.
47. Power GA, Allen MD, Gilmore KJ, et al. Motor unit number and transmission stability in octogenarian world class athletes: can age-related deficits be outrun? *J Appl Physiol (1985)* 2016;**121**(4):1013–1020.
48. Yu F, Vock DM, Zhang L, et al. Cognitive effects of aerobic exercise in Alzheimer's Disease: a pilot randomized controlled trial. *J Alzheimers Dis* 2021;**80**(1):233–244.
49. Morris JK, Vidoni ED, Johnson DK, et al. Aerobic exercise for Alzheimer's disease: a randomized controlled pilot trial. *PLoS One* 2017;**12**(2):e0170547.
50. Flockhart M, Nilsson LC, Tais S, Ekblom B, Apró W, Larsen FJ. Excessive exercise training causes mitochondrial functional impairment and decreases glucose tolerance in healthy volunteers. *Cell Metab* 2021;**33**(5):957–970.e6.


**Submitted:** 28 September 2023; **Revised:** 17 November 2023; **Accepted:** 26 November 2023