Title

Age-Dependent Effects of Voluntary Wheel Running Exercise on Voiding Behavior and Potential Age-Related Molecular Mechanisms in Mice.

Journal

The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences, 79(6)

Authors

Liu, Teresa
Pascal, Laura
Bauer, Scott
et al.

Publication Date

2024-06-01

DOI

10.1093/gerona/glae007

Peer reviewed

The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences, 2024, **79**(6), 1–10
https://doi.org/10.1093/gerona/glae007
Advance access publication 10 January 2024
Special Issue: Urinary Incontinence and Voiding Dysfunction: Special Article

Age-Dependent Effects of Voluntary Wheel Running Exercise on Voiding Behavior and Potential Age-Related Molecular Mechanisms in Mice

Teresa T. Liu, PhD,[1] Laura E. Pascal, PhD,[2,3] Scott R. Bauer, MD, ScM,[4,5] Hannah N. Miles, BA,[1,6] Jules B. Panksepp, PhD,[7] Granville L. Lloyd, MD,[8] Lingjun Li, PhD,[6,9] Donald B. DeFranco, PhD,[2] and William A. Ricke, PhD[1,*]

[1]Department of Urology, George M. O'Brien Center of Research Excellence, University of Wisconsin – Madison, Madison, Wisconsin, USA.
[2]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
[3]UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.
[4]Department of Medicine, Urology, Epidemiology and Biostatistics, University of California San Francisco, San Francisco, California, USA.
[5]San Francisco VA Medical Center, San Francisco, California, USA.
[6]School of Pharmacy, University of Wisconsin – Madison, Madison, Wisconsin, USA.
[7]Waisman Center, University of Wisconsin – Madison, Madison, Wisconsin, USA.
[8]Division of Urology, Department of Surgery, Rocky Mountain Regional VA Medical Center, University of Colorado Anschutz School of Medicine, Aurora, Colorado, USA.
[9]Department of Chemistry, University of Wisconsin – Madison, Madison, Wisconsin, USA.

*Address correspondence to: William A. Ricke, PhD. E-mail: rickew@urology.wisc.edu

Decision Editor: Lewis A. Lipsitz, MD, FGSA (Medical Sciences Section)

Abstract

Background: Older men frequently develop lower urinary tract symptoms attributed to benign prostatic hyperplasia (LUTS/BPH). Risk factors for LUTS/BPH include sedentary lifestyle, anxiety/depression, obesity, and frailty, which all increase with age. Although physical exercise may reduce the progression and/or severity of LUTS/BPH, the age-related mechanisms responsible remain unknown.

Methods: Voiding symptoms, body mass, and frailty were assessed after 4-weeks of voluntary wheel running in 2-month (n = 10) and 24-month (n = 8) old C57Bl/6J male mice. In addition, various social and individual behaviors were examined in these cohorts. Finally, cellular and molecular markers of inflammation and mitochondrial protein expression were assessed in prostate tissue and systemically.

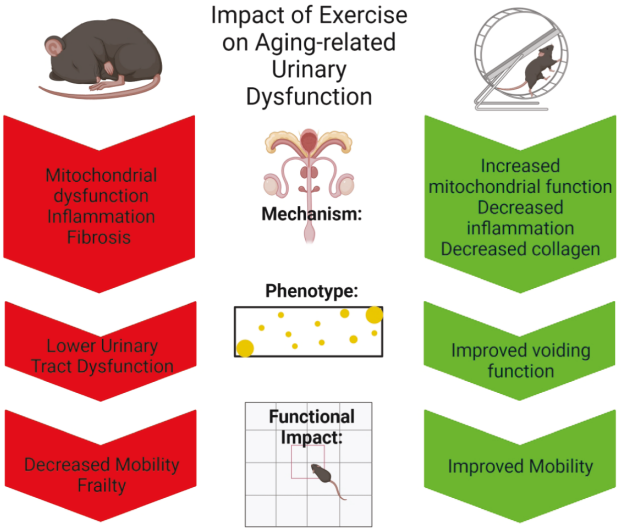
Results: Despite running less (aged vs young $\overline{X}$ = 12.3 vs 30.6 km/week; p = .04), aged mice had reduced voiding symptoms ($\overline{X}$ = 67.3 vs 23.7; p < .0001) after 1 week of exercise, which was sustained through week 4 ($\overline{X}$ = 67.3 vs 21.5; p < .0001). Exercise did not affect voiding symptoms in young mice. Exercise also increased mobility and decreased anxiety in both young and aged mice (p < .05). Exercise decreased expression of a key mitochondrial protein (PINK1; p < .05) and inflammation within the prostate (CD68; p < .05 and plasminogen activator inhibitor-1; p < .05) and in the serum (p < .05). However, a frailty index ($\overline{X}$ = 0.17 vs 0.15; p = .46) and grip strength ($\overline{X}$ = 1.10 vs 1.19; p = .24) were unchanged after 4 weeks of exercise in aged mice.

Conclusions: Voluntary aerobic exercise improves voiding behavior and mobility, and decreases prostatic mitochondrial protein expression and inflammation in aged mice. This promising model could be used to evaluate molecular mechanisms of aerobic exercise as a novel lifestyle intervention for older men with LUTS/BPH.

Keywords: Benign prostatic hyperplasia, Lower urinary tract symptoms, Physical activity, Preclinical mouse model, Translational

Received: April 13 2023; Editorial Decision Date: December 23 2023.

Graphical Abstract



Benign prostatic hyperplasia (BPH) is a pathological condition affecting the male accessory sex gland, the prostate. The single most significant risk factor for BPH is age with steady increases in incidence as men age. Thus, although 50% of men between 50 and 60 years of age exhibit histological BPH (eg, prostate proliferation, smooth muscle dysfunction, increased prostatic, and periurethral fibrosis), the incidence increases to 60%–80% for men aged 61–69 (1,2). The clinical manifestation of BPH in many men is lower urinary tract symptoms (LUTS) including frequent urination, urinary urgency, and nocturia, which can be debilitating and disruptive to quality of life (3). In addition to older age, metabolic and inflammatory conditions (eg, diabetes, obesity), depression/anxiety disorders, and frailty are risk factors for LUTS/BPH (4–8). Treatment for LUTS/BPH includes behavioral and lifestyle changes, medication, and/or surgery (9–11).

Exercise has been postulated as a potential first-line intervention for urinary symptoms caused by BPH, and several studies have shown that exercise is inversely associated with LUTS as well as several LUTS risk factors (eg, metabolic syndrome, depression, frailty, and so on) (11–15). Exercise interventions are varied and range from aerobic activity to strength training. Although results in human studies have been mixed, the efficacy of exercise to resolve preclinical models of frailty in rats has been well-characterized in the Molecular Transducers of Physical Activity Consortium (16). Other studies in mice have shown significant changes in relevant metabolic, inflammatory, and hormonal pathways upon both forced treadmill and voluntary wheel running (17–19). Additionally, aerobic exercise decreases anxiety-like behaviors in young mice provided with voluntary running wheels (20). However, the effect and potential mechanisms of an exercise intervention on lower urinary tract function and related urinary symptoms and voiding behavior in both humans and mouse models remain unknown.

Increased age in rodents is associated with lower urinary tract dysfunction and related voiding behaviors consistent with LUTS/BPH in humans, such as increased prostate volume/mass, prostate hyperplasia surrounding the prostatic urethra, and periurethral fibrosis (21). Although the prostates between human and murine are anatomically different, the human prostate is a solid organ surrounding the prostatic urethra, while the murine prostate is composed of lobes (eg, anterior, ventral, dorsolateral), histological alterations that lead to voiding dysfunction are well conserved (22). Collectively, the age-related lower urinary tract dysfunction and voiding behavior observed in mice makes them an ideal model to study the nexus of aging and voiding dysfunction with an exercise intervention in a controlled setting with the ability to eliminate the many confounding factors contributing to LUTS in older men (22,23).

Here, we report our findings regarding the impact of aging and voluntary exercise in male mice on voiding behavior as well as potential age-related mechanisms including body mass, grip strength, mobility, frailty, anxiety, and social behavior. Additionally, we examined the association of increased exercise with well-established molecular hallmarks of aging (eg, mitochondrial dysfunction) and BPH (eg, inflammation) in both prostate tissue and serum.

Method

Mice

Male C57Bl/6J mice were obtained from The Jackson Laboratory (JAX Stock# 000664, Bar Harbor, ME) at 6 weeks of age or directly from the National Institute of Aging's Division of Aging Biology colony housed at Charles River Laboratories (Wilmington, MA) at 22 months of age. Although young and aged mice were obtained from 2 separate facilities, the C57Bl6/J NIA colony are derived from the C57Bl6/J mice from the Jackson Laboratory and rederived every 6–7 years, minimizing genetic drift between the colonies. Animals were singly housed at the beginning of the behavioral experiments under standard laboratory conditions with 12:12 light/dark cycle and provided with food and water ad libitum. Mice were euthanized with CO_2, followed by cardiac puncture. Urogenital tracts were carefully dissected, and mass of tissue was determined as previously described (24). Young mice (n = 10) were 2 months of age, while aged mice (n = 8) were 24 months of age at the initiation of the exercise intervention. A corresponding group of young (n = 10) and aged (n = 9) mice were singly housed

but sedentary. All mice included survived the duration of the study. Upon euthanasia with CO_2, 3 caliper measurements (x, y, z) were taken of each bladder to calculate volume ($4/3 \cdot \pi \cdot ((x \cdot y \cdot z)/8)$) as our group has done previously (22). Residual urine was removed from the bladder, and the dried bladder was weighed. The prostate lobes (anterior, ventral, and dorsolateral prostate) were dissected, and the mass of prostate lobes and bladder was measured. No gross cancerous growths were observed within the urogenital tract or throughout the body in any mice.

Void Spot Assays

Void spot assays were performed as previously described to assess voiding behavior (22,25). Briefly, mice were individually placed in cages lined with chromatography filter paper (Ahlstrom, Kaukauna, WI) for 4 hours with free access to food, but water restricted. Void spot assays were performed once a week throughout the duration of the study with 2 baseline void spot assays performed prior to exposure to exercise. Filter paper was imaged under UV light and analyzed with VoidWhizzard (25). VoidWhizzard outputs spot counts binned by size as well as area of voided spots.

Frailty Index and Grip Strength

The noninvasive 31-item frailty index, based on established clinical signs of decline, was performed in young and old mice (26). Briefly, the assessment included an evaluation of the integument, musculoskeletal system, vestibulocochlear/auditory systems, ocular and nasal systems, digestive system, urogenital system, respiratory system, signs of discomfort, body weight (g), and body surface temperature (°C). Each variable was assessed as none, mild, or severe, and all values were averaged to give a frailty index score. Using a force meter, grip strength was measured 5 times per mouse by recording the maximum force measurement for each test. This testing was conducted during the second half of the circadian light phase. All 5 observed values were averaged to get the final force reading.

Voluntary Wheel Running

Young and aged mice were given free access to voluntary running wheels (Lafayette Instrument, Lafayette, IN). Each wheel was individually monitored throughout the duration of the study. Mice were run on a standard running wheel with evenly spaced rungs for week 1, 3, and 4. For week 2, the mice were given a complex wheel with random missing rungs to assess motor skill and cognitive function (27,28).

Open Field Testing

Mice were brought into the open field-testing room in their home cages and allowed to acclimate to the room for a minimum of 30 minutes prior to the start of testing. Mice were placed into a polycarbonate arena (42 × 42 × 42 cm) and their spatial position was monitored for 30 minutes using a 16 × 16 photobeam array, which included one set of vertical photobeams (Omnitech Electronics, Columbus, OH). This occurred during the second half of the circadian light phase. Mice were tested prior to the introduction of the exercise wheels and after 4 weeks of exercise intervention. Fusion software (v6.5) was used to quantify the position of the mouse (center vs periphery), distance traveled, and "sterotypy" (ie, increased grooming and sniffing). These assessments measure both locomotor function and anxiety-like behavior (29).

Social Investigation and Ultrasonic Vocalizations

Social investigation (SI) and ultrasonic vocalizations were assayed as previously (30). Mice were assessed prior to and immediately after exercise intervention. Twenty-four hours prior to testing, male "test" mice were placed individually into a clean home cage. Ten minutes prior to testing, the cage top was replaced with a thin sheet of transparent Plexiglas containing a central 2.5-cm hole. Five minutes before testing, the cage was placed under a Sony 4K Handycam camcorder (FDR-AX43) mounted on a tripod, and an ultrasound recording microphone was positioned over the hole. Testing began when a female C57Bl/6J "stimulus" mouse (2–3 months old) was placed inside the cage of the test mouse. Females from the same home cage were always equally represented in young and aged groups of male mice. The total duration of SI that a test mouse directed toward a stimulus mouse was assessed for a 5-minute period using Stopwatch + (v1.6) software. The SI phenotype entails all aspects of social approach and interaction, including pursuit of the stimulus mouse within one body length, sniffing at any part of its body, and allo-grooming. SI was calculated by summing the duration that the test mouse engaged in each of these behavioral elements.

Ultrasonic vocalizations emission was monitored with an UltraSoundGate CM16 microphone (2–250 kHz flat frequency range) that was connected to an UltraSoundGate 416H A/D converter (Avisoft Bioacoustics, Glienicke, Germany). Signals were recorded at a 300 kHz (16-bit) sampling rate and saved as.wav files with Avisoft-Recorder software (v4.2.31). Spectrograms were generated in SASLab Pro (v4.52) using a fast Fourier transform (Hamming window, length = 256, frame size = 100%). Specific settings for the ultrasonic vocalization segmentation using the "whistle tracking" algorithm in SASLab Pro were max change = 6 pixels, hold time = 8 ms, minimum duration = 3 ms, and high-band pass filter = 40 kH.

Histopathology and Immunohistochemistry

Tissues for immunohistochemistry (IHC) were fixed in 10% normal buffered formalin and paraffin embedded. All immunostaining procedures were performed as previously described (31). Sections were stained with anti-PINK1 (1:100; NBP1-49678, Novus Biologicals, Centennial, CO), anti-CD68 (KP1, CM033, BioCare Medical), and anti-PAI-1 (1:1 000; ab66705, Abcam, Cambridge, UK). PINK1, a protein involved in mitophagy whose altered expression is an established marker for mitochondrial dysfunction, was detected by indirect immunofluorescence using the OPAL620 fluorophore (Akoya Biosciences, Marlborough, MA) (32). CD68, a marker for macrophages, and plasminogen activator inhibitor-1 (PAI-1), a component of the fibrinolysis pathway whose dysregulation is associated with tissue fibrosis, detected by immunohistochemistry using a 3,3′-diaminobenzidine (DAB; Cell Signaling, Danvers, MA) detection system (33). Images were captured using Mantra 2 (Akoya Biosciences) and quantified using InForm (Akoya Biosciences).

Serum Protein Preparation

Serum was diluted in 6M urea in 50 mM Tris-HCl buffer pH8 with 1X protease inhibitor (Roche). Proteins were reduced using 5 mM dithiothreitol for 1 hour. Alkylation was performed using 15-mM iodoacetamide incubation for 30 minutes. Samples were diluted to 1.5 M urea using 50 mM

Tris-HCl. Proteins were trypsin (Promega) digested at a 50:1 ratio for 16 hours at 37°C and quenched using 20% trifluoroacetic acid to 1% concentration. Peptides were desalted using Sep-Pak C18 cartridges (Waters Corporation), concentrated via SpeedVac concentrator (Thermo Scientific), and concentrations determined by peptide assay prior to instrumental analysis.

Liquid Chromatography With Tandem Mass Spectrometry Acquisition

Tryptic peptides were suspended in 0.1% formic acid (Sigma) at 0.5 mg/mL. Samples were analyzed using a Waters nanoACQUITY UPLC coupled with Thermo Orbitrap Elite mass spectrometer. Capillary column (15 cm length, 75 µm inner diameter) was self-fabricated and packed using Bridged Ethylene Hybrid C18 materials (1.7 µm, 130 Å, Waters Corporation). Solvents consisted of 0.1% formic acid in water (buffer A) and 0.1% formic acid in 80% acetonitrile (buffer B). Liquid chromatography (LC) gradients include: trapping phase from 0 to 18 minutes at 5% B, moving to 30% at 100 minutes, 95% B from minutes 100.5 to 110, and 0% B from minutes 110.5 to 125. Survey scans from 360 to 1 600 m/z were performed using a resolving power of 60 000 and automatic gain control (AGC) target of 1 × 106 and maximum injection time of 150 ms. The top 20 precursors were selected for higher energy collisional dissociation fragmentation with normalized collision energy of 35, an isolation width of 1.0 Da, 15 000 resolving power, 5×10^4 AGC target, maximum 150 ms injection time, and lower mass limit of 120.0 m/z. Precursors were subject to dynamic exclusion for 15 seconds with a 10-ppm mass tolerance. Each sample was acquired in technical duplicate.

Spectral Processing and Analysis

Serum peptides were identified using Proteome Discoverer (version 2.5, Thermo Scientific) and searched against UniProt-reviewed mouse proteome with SequestHT with trypsin as the enzyme allowing for 2 missed cleavages. Precursor mass tolerance of 20 ppm and a fragment mass tolerance of 0.02 Da were set for the searching. Carbamidomethylation of cysteine residues (+57.021 Da) was chosen as a static modification, while dynamic modifications selected consisted of oxidation of methionine residues (+15.995 Da). Search results were filtered to 1% FDR at both peptide and protein levels. The INFERYS 1.0 rescoring algorithm (MSAID) was used after initial search to increase peptide identifications and scoring confidence (34).

Statistics and Pathway Analysis

Comparisons between young and aged mice were analyzed using a Student's *t* test in instances of one comparison and 2-way ANOVA with the Sidak's multiple comparison post hoc test. All pre- and postexercise measurements taken in young and aged mice were analyzed using a repeated measures 2-way ANOVA with the Sidak's multiple comparison post hoc test. Pathway analysis on significant serum proteins was performed using DAVID (Database for Annotation, Visualization, and Integrated Discovery) (35,36). Proteins were clustered using average linkage and Euclidean distance measure.

Study Approval

All animal experiments were conducted under the protocols approved by the University of Wisconsin and University of Pittsburgh Animal Care and Use Committee.

Results

Aging Increases Urinary Frequency and Frailty in Mice

We observed a significantly increased bladder mass (p value < .001) and bladder volume (p value < .05) in aged (24-month-old) compared to young adult (2-month-old) male mice (Figure 1A and B; Supplementary Figure 1A and B). Additionally, aged mice had a significantly increased anterior, ventral, and dorsolateral prostate mass compared to young mice (Figure 1C–E; Supplementary Figure 1C–E). Aged mice also exhibited a significant increase in the number of voiding spots (Figure 1F) (22). Using established paradigms to measure skeletal muscle strength and relevant frailty outcomes in mice, aged mice had a significantly higher frailty index score and a lower grip strength compared to young mice (Figure 1G and H) (26).

Aging Alters Social Behavior and Anxiety in Mice

Aged mice spent a greater amount of time within the center of an open field when compared to young mice, which increases over the duration of testing, consistent with decreased anxiety (Figure 2A and B) (29). Additionally, aged mice showed lower stereotypic behaviors such as grooming and sniffing, another measure of decreased anxiety (Figure 2C). Aged mice also showed overall less exploratory movement in the first 10 minutes, suggesting a decrease in mobility (Figure 2D). We also examined the social behavior of male mice when placed in proximity to female mice by measuring both proximity-based SI and ultrasonic vocalizations. Aged mice showed significantly lower SI and ultrasonic vocalizations compared to young mice (Figure 2E and F).

Aging Alters Ability to Exercise and Cognition in Mice

We used voluntary wheel running to elicit aerobic exercise in young and aged mice. Running was monitored in individual animals, and all mice ran daily irrespective of age. However, aged mice ran significantly less per week than young mice (Figure 3A). To provide an additional enhancement of this aerobic exercise paradigm, after 1 week of running on a normal wheel, mice were switched to a complex wheel for a week and then switched back to a normal wheel for the duration of the study. Although we observed a slight decrease in running with the complex wheel in young mice, there was no significant difference in running distance between the different wheels (Figure 3B). In contrast, in aged mice, there was a significant decrease in running distance with the altered wheel, suggesting that aged mice have altered motor skills and/or cognition relative to their younger cohorts; aging mice resumed running activity upon return to a normal wheel (Figure 3C) (27,28).

Short-Term Exercise Did Not Improve Body Mass, Frailty, or Grip Strength in Mice

While all mice exercised daily, there was no significant change in body mass after exercise (Figure 3D). In our 4-week aerobic exercise paradigm, there was no statistical decrease in FI

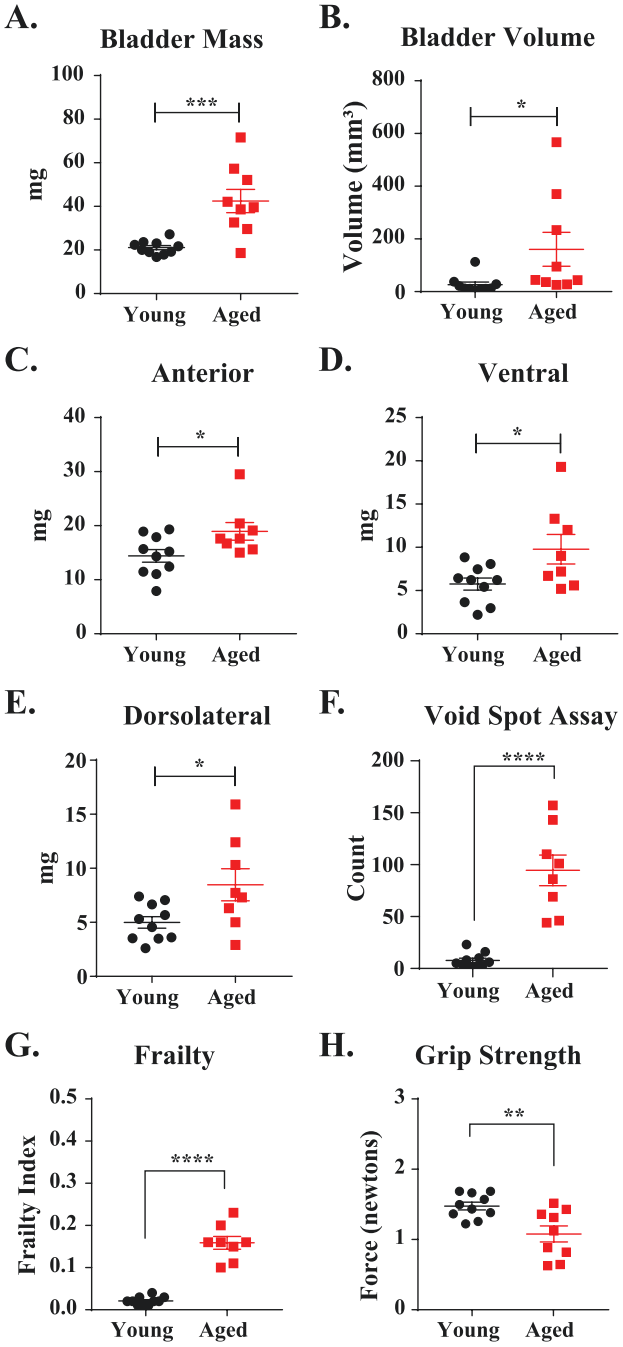


Figure 1. Impact of aging on bladder voiding and frailty. (A) Bladder mass was significantly increased in aged mice compared to young. (B) Bladder volume was increased in aged mice compared to young. (C) Anterior prostate mass was increased in aged mice compared to young. (D) Ventral prostate mass was increased in aged mice compared to young. (E) Dorsolateral prostate mass was increased in aged mice compared to young. (F) Aged, male mice had significantly more urination spots as measured by void spot assays compared to young. (G) Aged, male mice had an increased frailty index compared to young mice as measured by the clinical frailty assessment survey (27). (H) Aged, male mice had a decrease in grip strength as measured by a hand-held force meter. *, *p* value < .05; ***, *p* value < .001; ****, *p* value < .0001.

score in aged mice after exercise (Figure 3E). In addition, we did not observe a change in grip strength in aged mice after exercise, although there was a significant decrease in grip strength in young mice after exercise (Figure 3F).

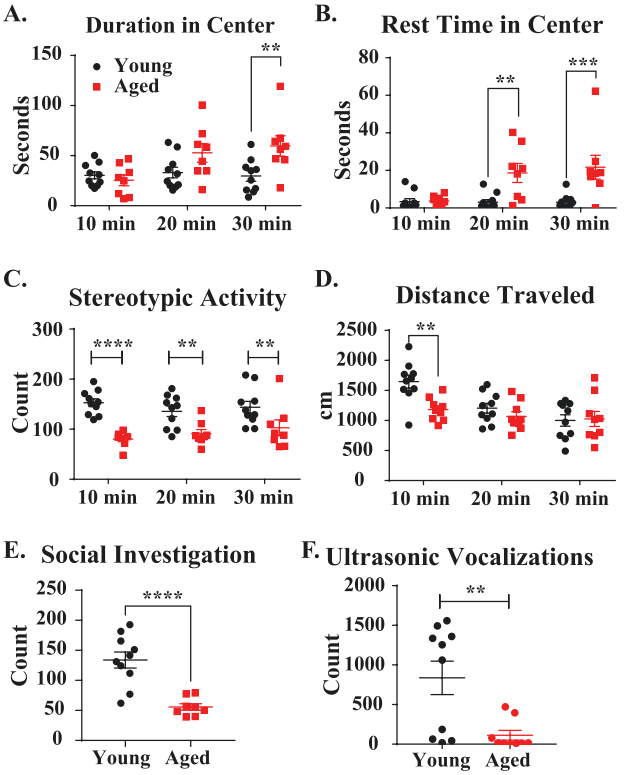


Figure 2. Aging adversely affects movement, anxiety, and social behavior. (A) Aged mice spend a significant amount of time in the center of the open field test compared to young mice in the last 10-minute testing interval. (B) Aged mice also spend a longer duration of sedentary time in the center of the open field test compared to young mice in the second- and third 10-minute testing interval. (C) Aged mice have significantly less stereotypic movements compared to young mice in all testing intervals. (D) Overall movement as measured by distance traveled through the open field apparatus was significantly decreased in aged mice compared to young within the first 10-minute testing interval. (E) Aged, male mice investigated unfamiliar female mice significantly less than young, male mice. (F) Aged, male mice produced significantly less ultrasonic vocalizations than young, male mice when placed in vicinity of female mice. **, *p* value < .01; ***, *p* value < .001; ****, *p* value < .0001.

Exercise Decreases Urinary Frequency in Aged Mice

Examination of urinary frequency using a void spot assay revealed a significant decrease in the number of void spot counts (indicating improved function) in aging mice after exposure to voluntary wheel running (Figure 3G). Despite the decrease in distance run on the altered wheel, there remained a significant decrease in urinary frequency in week 2 (Figure 3C and G). The bladder volume remained unchanged in aged mice (Figure 3H; Supplementary Figure 2A). Urinary frequency for young mice was low (void spot count $\overline{X}$ = 7.8) at baseline and there was no change following voluntary wheel running. Although voiding behavior as measured by void spot assay was not altered by exercise in young mice, bladder mass increased slightly following exercise (Figure 3I; Supplementary Figure 2B). Examination of the prostate lobes showed a slight increase in anterior prostate mass (*p* value = .06) but no change in ventral prostate mass following exercise in both young and aged mice (Figure 3J and K; Supplementary Figure 2C and D). However, there was a significant increase in dorsolateral prostate mass following exercise in the aged animals compared to both the sedentary

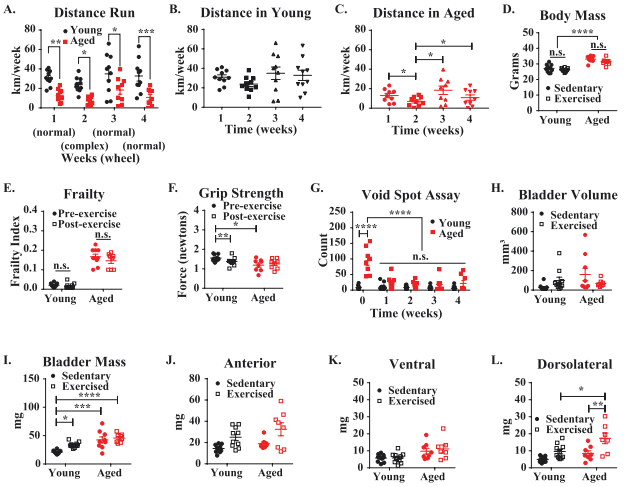


Figure 3. Aerobic exercise alters voiding function in aging male mice. (A) Given free access to a voluntary running, both young and aged mice ran daily. Aged mice on average ran significantly less than young mice. (B) Young mice given access to normal and complex running wheels showed no significant decrease in running with the complex wheel. Regardless of running wheel modality, young mice ran on average the same amount weekly showing no alterations in cognitive function. (C) Aged mice showed a significant decrease in running when given a complex wheel compared to a normal running wheel, suggesting cognitive alterations in the aged mice. Upon reintroduction of a normal wheel, aged mice resume their running with no significant decrease compared to initial running behavior. (D) Body mass was monitored throughout the exercise period, and there was no change in mass with exercise. (E) Frailty index score was unchanged in both young and aged mice upon exercise. (F) Grip strength was significantly decreased in young mice following exercise intervention. There was no change in grip strength in aged mice following exercise. (G) Urinary frequency as measured by void spot assay was significantly decreased in aged mice upon 1 week of exercise and remained low throughout the study. There was no dysfunction in the young mice, and the voiding function remained the same throughout the study. (H) Bladder volume was not significantly different between sedentary and exercised mice regardless of age. (I) Bladder mass was significantly increased in aged mice with and without exercise intervention compared to young, sedentary mice. Although the bladder mass remained unchanged in aged mice with exercise, young mice with exercise showed a significant increase in bladder mass compared to young, sedentary mice. (J) Anterior prostate mass was slightly increased after exercise in both young and aged mice. (K) Ventral prostate mass was unchanged after exercise in both young and aged mice. (L) Dorsolateral prostate mass was increased in aged mice after exercise compared to sedentary aged mice and exercised young mice. Solid data points—sedentary/preexercise; open data points—exercise/postexercise; *, p value < .05; **, p value < .01; ***, p value < .001; ****, p value < .0001.

aged and the exercised young mice (Figure 3L; Supplementary Figure 2E).

Exercise Alters Social Behavior, Mobility, and Anxiety in Mice

Unlike the differences observed between young and aged mice, we observed the most significant changes in the open field apparatus within the first 10 minutes of testing, mice were also assessed for 30 minutes (Supplementary Table 1). Thus, when we restricted our assessment to the first 10 minutes of testing, we observed a trend toward increased entries into the center with young mice (p value = .08). In contrast, aged mice made significantly more entries into the center following exercise (Figure 4A). Additionally, the duration and rest time in the center were also significantly increased after exercise in aged mice but remained unchanged in young mice (Figure 4B and C).

Total distance traveled in the open field was significantly increased in both young and aged mice after exercise (Figure 4D). Overall, aged mice explored less than young mice, prior to and after 4 weeks of voluntary wheel running (Figure 4D). Examination of stereotypic activity revealed a significant increase in both young and aged mice after exercise with aged mice exhibiting overall less stereotypic behavior than young mice (Figure 4E). Total vertical activity, measuring mobility, trended toward an increase in both young mice and aged mice after exercise (p value = .052; Figure 4F). Assessment of SI and ultrasonic vocalization showed no significant change with exercise in either young or aged mice (Figure 4G and H).

Exercise Increases Expression of a Key Mitochondrial Protein and Inflammation Markers in Serum and Prostatic Tissue

Aged mice showed a significant increase in PINK1 expression, which is a measure of mitochondrial dysfunction, after exposure to voluntary wheel running in all prostate lobes (Figure 5A; Supplementary Figure 1). Additionally, the greater accumulation of macrophages (ie, CD68 + staining by IHC) within the prostate stroma of all 3 lobes in aged mice was decreased following exposure to voluntary wheel running (Figure 5B). Furthermore, PAI-1, an important contributor to collagen accumulation in fibrotic tissue, was also decreased following exercise in aged mice (Figure 5C) (37). Serum proteomics was used to provide a more comprehensive and unbiased assessment of exercise effects on systemic changes in circulating proteins in young versus aged mice. This analysis identified 273 proteins significantly altered in aged mice compared to young (p value < .05; Figure 5D; Supplementary Table 2). Of these, 127 proteins were differentially regulated in aged mice with exercise compared to sedentary. Pathway analysis of these showed a significant enrichment of KEGG pathways including extracellular matrix-receptor interaction (p value = 7.8e-5), complement and coagulation cascades (p value = 1.0e-4), and PI3K-Akt signaling (p value = 1.4e-2).

Discussion

The key findings of this study are that exposure to voluntary wheel running in male mice alleviates aging-related voiding behavior and improves mobility and behavioral deficits. These improvements occur alongside a restoration of age-related declines in a key mitochondrial protein involved in mitophagy (ie, PINK1) and markers of inflammation (ie, CD68, PAI-1) in the prostate.

Many of the cellular, organ-based, and systemic hallmarks of aging can be improved by a variety of exercise interventions in humans and other mammals (20,38–40). However, the heterogeneous responses of individual organ systems in aged organisms to exercise are likely due to a combination of both shared and distinct mechanisms. The decrease in voiding frequency in response to exercise could prove to be insightful for both identifying novel LUTS/BPH mechanisms and targets as well as providing translational evidence to support the development of a structured exercise intervention for older men suffering from LUTS/BPH.

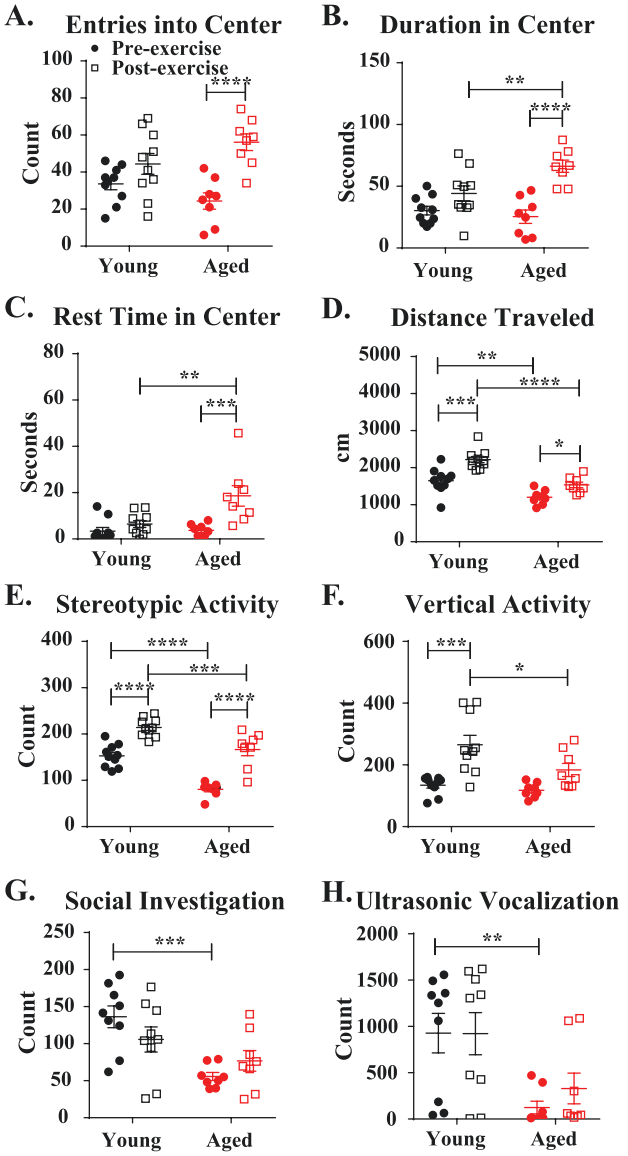


Figure 4. Aerobic exercise improves anxiety, movement, and social behavior. (A) Aged mice following exercise significantly increased the number of entries into the center of the open field test. (B) Aerobic exercise increased the amount of time male mice (young and aged) spent in the center portion of the open field test. (C) Aged mice following exercise spent more sedentary time in the center of the open field test. (D) Overall distance traversed by mice as measured by the open field test increased upon exercise regardless of age. (E) Stereotypic activity was significantly increased with exercise in young and aged mice following voluntary running wheel. (F) Vertical activity was significantly increased in male mice after exercise in both young and aged groups. (G) Social investigation was unchanged in male mice following exercise but slightly improved in aged mice following exercise. (H) Ultrasonic vocalizations were unchanged in young mice following exercise. Aged mice following exercise showed a slight increase in vocalizations. One young mouse was excluded from social investigation and ultrasonic vocalization analysis due to significant aggression toward female mouse. Solid data points—sedentary/preexercise; open data points—exercise/postexercise; *, *p* value < .05; **, *p* value < .01; ***, *p* value < .001; ****, *p* value < .0001.

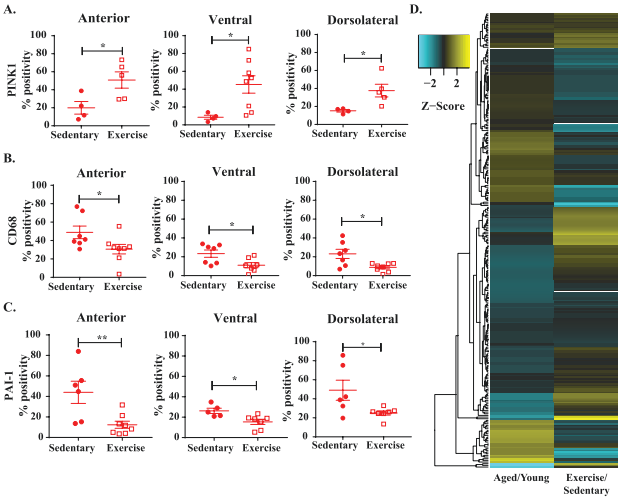


Figure 5. Local and systemic alterations in mitochondrial function and inflammation are modulated with exercise. (A) PINK1 was significantly increased upon exercise intervention in aged mice in anterior, ventral, and dorsolateral prostate. (B) Stromal CD68 showed a significant decrease in anterior, ventral, and dorsolateral prostate in aged mice following exercise intervention. (C) PAI-1 expression in aged anterior, ventral, and dorsolateral prostate was decreased upon exercise compared to sedentary control. (D) Heat map of 287 statistically significant proteins (*p* value < .05) identified by serum proteomics in aged mice compared to young and aged exercise compared to aged sedentary. *, *p* value < .05; **, *p* value < .01.

In older men, who are likely to have some degree of histologic BPH, physical inactivity, anxiety/depression, and frailty are risk factors for LUTS development and progression, with increasing frailty associated with concurrent worsening LUTS (5,41,42). We have previously shown that aged mice have increased urinary frequency and decreased urinary volume per void that correlate with an increase in periurethral prostatic tissue (eg, duct number), prostate enlargement, increased bladder mass, and increased bladder, urethral, and prostate fibrosis (22). These age-related changes may give insight into the underlying etiology of voiding behavior and voiding dysfunction in mouse models of LUTS/BPH (43). The current study is consistent with prior studies demonstrating that aged mice also have greater frailty, lower mobility and anxiety, and lower interest in female mice (22,26,29). Importantly, our study demonstrated that aerobic exercise reversed urinary frequency in aged male mice within a week of treatment. Interestingly, bladder mass and volume exhibited no significant difference when compared to age-matched sedentary animals with no access to exercise. Although bladder volume measured at time of euthanasia cannot be considered maximal bladder volume, the changes in the bladder correlate between mass and volume, even when normalized to body mass (Supplementary Figures 1 and 2). Thus, exercise-induced urinary improvements may precede measurable changes in bladder pathology or may be driven by central or systemic adaptations (ie, central nervous system, kidney function) in the regulation of voiding behavior that overcome age-related changes in organ pathology. Although longer exercise interventions may lead to improvements in age-related bladder pathology, clinical improvements in bothersome urinary symptoms are most relevant for patient well-being and are likely to lead to greater patient compliance with prescribed exercise regimens. Future studies with longitudinal assessments of voiding behavior, voiding function, and bladder pathology following prolonged or variable exercise interventions in aged mice are needed. Examination of the prostate lobes showed no change in ventral prostate

mass but a slight increase in anterior prostate mass; exercise significantly increased the mass of the dorsolateral prostate. Patients with urinary symptoms attributed to BPH often experience greater anxiety, depression, and sexual dysfunction, which are major determinants of their quality of life (4,44). Exercise in the aged mice led to increased mobility, decreased anxiety-like behaviors assessed by open field testing, and increased SI and ultrasonic vocalization suggesting that the psychological symptoms that frequently co-occur with urinary symptoms attributed to LUTS/BPH may also improve with exercise intervention (29,30).

Although other exercise paradigms achieve a consistent intensity (ie, VO_2Max) between animals, studies have shown that voluntary wheel running decreases overall stress with little loss of efficacy (17,45–47). In addition to lower stress levels, which could adversely affect urinary symptom assessments and mask the positive effects of exercise, an additional benefit of using the voluntary running wheel over a treadmill is the ability to use a complex wheel to assess for motor skill and cognition alterations (27,28). In young mice, there was no significant decrease in distance run regardless of wheel modality. However, in the aged mice, there was a significant decrease in distance run upon switching to the complex wheel, which was recovered upon switching back to the normal wheels. This suggests that there are potential motor and cognition deficits with age that could be linked to symptoms and voiding dysfunction. Interestingly, the decrease in distance run on the complex wheel was not associated with a recurrence of urinary frequency in aged mice. These findings suggest that high-intensity, long-term, or constant exercise may not be required for the alleviation of urinary symptoms.

Although our exercise intervention was effective in modifying urination, social behavior, and mobility, grip strength and frailty were unchanged. Unlike measurement of frailty in humans, which can be defined using a clinical phenotype (eg, physical frailty) or an index (eg, cumulative deficit frailty) (48), frailty assessment in rodents is currently limited to indices such as the clinical frailty index. This frailty index evaluates a wide range of parameters including physical ability, integument alterations, auditory and ocular assessments, and overall discomfort (26). Although it has the sensitivity to assess alterations in a variety of disease processes and with aging, assessment of frailty-related voiding behavior and voiding dysfunction may require additional urogenital-focused measurements. In a similar previous study of the impact of exercise on frailty, a 2-month aerobic exercise intervention significantly increased grip strength (17,46,47). Four weeks of voluntary exercise might not significantly increase grip strength in aged mice even though it was sufficient to decrease urinary frequency. In human aging, biological aging or molecular mechanisms that underlie normal organ homeostasis begin to decline well before phenotypic and functional aging manifests (49). This suggests that the biological changes within the tissues (ie, mitochondrial dysfunction, inflammation) that drive urination may change with exercise before phenotypic and functional changes are measurable. The heterogeneity in these aging measures in an inbred strain of mice subjected to tightly controlled living conditions could provide useful insight into how lifelong exposure to even minor fluctuations in environmental and social stresses could affect organ-specific individual responses to aging in humans.

The ability of exercise to mitigate or prevent urinary symptoms suggests an underlying mechanism that contributes to the broad development of anxiety, cellular stress, and voiding dysfunction. BPH is often associated with a variety of metabolic and inflammatory conditions (eg, diabetes, obesity), that along with age, can be triggered at the cellular level by mitochondrial dysfunction in multiple tissue types. Exercise has been shown to produce organ-specific and systemic "exerkines" that reverse the inflammatory effects of disease and aging (50). With exercise in aged mice, there is both a systemic decrease in serum and prostate proinflammatory and profibrotic factors. Within the serum, 2 pathways significantly altered by exercise and aging were the complement/coagulation and the PI3K-Akt pathways. Both significantly affect inflammation, and the proteomics data suggest that components of these pathways are altered with age but restored with exercise. Additionally, components of fibrosis were found to be increased with age but alleviated with exercise. Within the prostate tissue, there is an increase in PINK1, a protein essential for the mitophagy pathway, CD68, a marker of macrophages, and PAI-1, a proinflammatory mediator of fibrosis. This suggests that inflammation and accumulation of dysfunctional mitochondria, due in part to reduced mitophagy, accumulated through normal aging could promote the development of LUTS/BPH, and the reduction of these factors through exercise could effectively improve urinary symptoms.

Although this study examines both the physiological and molecular outcomes upon exercise intervention, there are some limitations to the study. For example, we only assessed mice at 2 and 24 months of age, and our results may reflect changes in maturation as well as aging alone. Furthermore, young and aged mice were generated in 2 different facilities, which may introduce genetic and behavioral differences. Additionally, while gross tumors throughout the body were not observed during prostate necropsy, we did not perform detailed histological analyses on nonprostate tissues. Thus, the complex behaviors we assessed could be affected by benign or metaplastic dysfunction in other organ systems. With voluntary wheel running, we are unable to control and normalize exercise intensity throughout the cohort. This study was also limited to 4 weeks in duration. Future studies would benefit from examination of more regimented exercise that could be translated to patient care. Finally, we only assessed a handful of markers important to aging-mediated mitochondrial dysfunction and inflammaging and utilized void spot assays as our sole measure of voiding behavior rather than other direct measures of voiding function such as cystometry. Nonetheless, the results obtained support a conclusion that voluntary exercise intervention can concurrently improve voiding behavior as well as mobility and behavioral deficits in mice. Furthermore, our results support the use of mice as a model to explore detailed metabolic and systemic physiological responses resulting from structured exercise that can delay, prevent, or reverse age-related voiding behavior and voiding dysfunction.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

This work was supported by the National Institutes of Health (K01 AG059899 to T.T.L., NIDDK CAIRIBU Convergence Award to L.E.P., R03AG067937 and K76 AG074903 to S.R.B., and U54 DK104310 and R01 DK131175 to W.A.R.). This study was supported in part by a core grant to the Waisman Center from the National Institute of Child Health and Human Development (P50 HD105353).

Conflict of Interest

None.

Data Availability

Graphical abstract created with BioRender.com. The mass spectrometry proteomics data have been deposited to the ProteomXchange Consortium via the PRIDE partner repository with the data set identifier PXD044453.

Acknowledgments

The authors would like to thank Emily Ricke, Alexis Adrian, Vilena Maklakova, and the Ricke Lab for all of their help and support.

Author Contributions

All authors participated equally in the development, data analysis, and writing of this study. T.T.L., L.E.P., and J.B.P. performed the mouse experiments. T.T.L. and L.E.P. performed the IHC experiments. H.N.M. performed the serum proteomics.

References

1. Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. *J Urol*. 1984;132:474–479. https://doi.org/10.1016/s0022-5347(17)49698-4
2. Roehrborn CG, McConnell JD. Etiology, pathophysiology, epidemiology and natural history of benign prostatic hyperplasia. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ, eds. *Campbell's Urology*. Philadelphia, PA: W.B. Saunders Company; 2002:1297–1330.
3. Chute CG, Panser LA, Girman CJ, et al. The prevalence of prostatism: a population-based survey of urinary symptoms. *J Urol*. 1993;150:85–89. https://doi.org/10.1016/s0022-5347(17)35405-8
4. Rosen R, Altwein J, Boyle P, et al. Lower urinary tract symptoms and male sexual dysfunction: the multinational survey of the aging male (MSAM-7). *Eur Urol*. 2003;44:637–649. https://doi.org/10.1016/j.eururo.2003.08.015
5. Coyne KS, Wein AJ, Tubaro A, et al. The burden of lower urinary tract symptoms: evaluating the effect of LUTS on health-related quality of life, anxiety and depression: EpiLUTS. *BJU Int*. 2009;103(Suppl 3):4–11. https://doi.org/10.1111/j.1464-410X.2009.08371.x
6. Rodriguez-Nieves JA, Macoska JA. Prostatic fibrosis, lower urinary tract symptoms, and BPH. *Nat Rev Urol*. 2013;10:546–550. https://doi.org/10.1038/nrurol.2013.149
7. Lloyd GL, Wiesen B, Atwell M, Malykhina A. Marijuana, alcohol, and ED: correlations with LUTS/BPH. *Curr Urol Rep*. 2021;22:21. https://doi.org/10.1007/s11934-020-01031-9
8. Bauer SR, McCulloch CE, Cawthon PM, et al. Longitudinal associations between concurrent changes in phenotypic frailty and lower urinary tract symptoms among older men. *J Frailty Aging*. 2023;12:117–125. https://doi.org/10.14283/jfa.2022.33
9. Roehrborn CG, Oyarzabal Perez I, Roos EP, et al. Efficacy and safety of a fixed-dose combination of dutasteride and tamsulosin treatment (Duodart(R)) compared with watchful waiting with initiation of tamsulosin therapy if symptoms do not improve, both provided with lifestyle advice, in the management of treatment-naive men with moderately symptomatic benign prostatic hyperplasia: 2-year CONDUCT study results. *BJU Int*. 2015;116:450–459. https://doi.org/10.1111/bju.13033
10. Kim EH, Larson JA, Andriole GL. Management of benign prostatic hyperplasia. *Annu Rev Med*. 2016;67:137–151. https://doi.org/10.1146/annurev-med-063014-123902
11. Moul S, McVary KT. Lower urinary tract symptoms, obesity and the metabolic syndrome. *Curr Opin Urol*. 2010;20:7–12. https://doi.org/10.1097/MOU.0b013e3283336f3f
12. Silva V, Grande AJ, Peccin MS. Physical activity for lower urinary tract symptoms secondary to benign prostatic obstruction. *Cochrane Database Syst Rev*. 2019;4:CD012044. https://doi.org/10.1002/14651858.CD012044.pub2
13. Rohrmann S, Katzke VA, Kaaks R. Lifestyle and progression of lower urinary tract symptoms in German men-results from the EPIC-Heidelberg cohort. *Urology*. 2018;120:192–196. https://doi.org/10.1016/j.urology.2018.06.013
14. Singh B, Olds T, Curtis R, et al. Effectiveness of physical activity interventions for improving depression, anxiety and distress: an overview of systematic reviews. *Br J Sports Med*. 2023;57:1203–1209. https://doi.org/10.1136/bjsports-2022-106195
15. Arrieta H, Rezola-Pardo C, Gil SM, et al. Effects of multicomponent exercise on frailty in long-term nursing homes: a randomized controlled trial. *J Am Geriatr Soc*. 2019;67:1145–1151. https://doi.org/10.1111/jgs.15824
16. Sanford JA, Nogiec CD, Lindholm ME, et al.; Molecular Transducers of Physical Activity Consortium. Molecular Transducers of Physical Activity Consortium (MoTrPAC): mapping the dynamic responses to exercise. *Cell*. 2020;181:1464–1474. https://doi.org/10.1016/j.cell.2020.06.004
17. Kim YJ, Kim HJ, Lee WJ, Seong JK. A comparison of the metabolic effects of treadmill and wheel running exercise in mouse model. *Lab Anim Res*. 2020;36:3. https://doi.org/10.1186/s42826-019-0035-8
18. Wang SS, Li K, Liu Z, Gui S, Liu N, Liu X. Aerobic exercise ameliorates benign prostatic hyperplasia in obese mice through downregulating the AR/androgen/PI3K/AKT signaling pathway. *Exp Gerontol*. 2021;143:111152. https://doi.org/10.1016/j.exger.2020.111152
19. Bisset ES, Heinze-Milne S, Grandy SA, Howlett SE. Aerobic exercise attenuates frailty in aging male and female C57Bl/6 mice and effects systemic cytokines differentially by sex. *J Gerontol A Biol Sci Med Sci*. 2022;77:41–46. https://doi.org/10.1093/gerona/glab297
20. Binder E, Droste SK, Ohl F, Reul JM. Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. *Behav Brain Res*. 2004;155:197–206. https://doi.org/10.1016/j.bbr.2004.04.017
21. Sandhu JS, Bixler BR, Dahm P, et al. Management of lower urinary tract symptoms attributed to benign prostatic hyperplasia (BPH): AUA guideline amendment 2023. *J Urol*. 2023;211:101097JU0000000000003698. https://doi.org/10.1097/JU.0000000000003698
22. Liu TT, Thomas S, McLean DT, et al. Prostate enlargement and altered urinary function are part of the aging process. *Aging (Albany NY)*. 2019;11:2653–2669. https://doi.org/10.18632/aging.101938
23. Pascal LE, Igarashi T, Mizoguchi S, et al. E-cadherin deficiency promotes prostate macrophage inflammation and bladder overactivity in aged male mice. *Aging (Albany NY)*. 2022;14:2945–2965. https://doi.org/10.18632/aging.203994
24. Nicholson TM, Ricke EA, Marker PC, et al. Testosterone and 17beta-estradiol induce glandular prostatic growth, bladder outlet obstruction, and voiding dysfunction in male mice. *Endocrinology*. 2012;153:5556–5565. https://doi.org/10.1210/en.2012-1522

25. Wegner KA, Abler LL, Oakes SR, et al. Void spot assay procedural optimization and software for rapid and objective quantification of rodent voiding function, including overlapping urine spots. *Am J Physiol Renal Physiol*. 2018;315:F1067–F1080. https://doi.org/10.1152/ajprenal.00245.2018
26. Whitehead JC, Hildebrand BA, Sun M, et al. A clinical frailty index in aging mice: comparisons with frailty index data in humans. *J Gerontol A Biol Sci Med Sci*. 2014;69:621–632. https://doi.org/10.1093/gerona/glt136
27. Hibbits N, Pannu R, Wu TJ, Armstrong RC. Cuprizone demyelination of the corpus callosum in mice correlates with altered social interaction and impaired bilateral sensorimotor coordination. *ASN Neuro*. 2009;1:e00013. https://doi.org/10.1042/AN20090032
28. Liebetanz D, Baier PC, Paulus W, Meuer K, Bahr M, Weishaupt JH. A highly sensitive automated complex running wheel test to detect latent motor deficits in the mouse MPTP model of Parkinson's disease. *Exp Neurol*. 2007;205:207–213. https://doi.org/10.1016/j.expneurol.2007.01.030
29. Seibenhener ML, Wooten MC. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *J Vis Exp*. 2015;96:e52434. https://doi.org/10.3791/52434
30. Panksepp JB, Jochman KA, Kim JU, et al. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. *PLoS One*. 2007;2:e351. https://doi.org/10.1371/journal.pone.0000351
31. Nicholson TM, Sehgal PD, Drew SA, Huang W, Ricke WA. Sex steroid receptor expression and localization in benign prostatic hyperplasia varies with tissue compartment. *Differentiation*. 2013;85:140–149. https://doi.org/10.1016/j.diff.2013.02.006
32. Gautier CA, Kitada T, Shen J. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. *Proc Natl Acad Sci U S A*. 2008;105:11364–11369. https://doi.org/10.1073/pnas.0802076105
33. Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. *Cardiovasc Ther*. 2010;28:e72–e91. https://doi.org/10.1111/j.1755-5922.2010.00171.x
34. Zolg DP, Gessulat S, Paschke C, et al. INFERYS rescoring: boosting peptide identifications and scoring confidence of database search results. *Rapid Commun Mass Spectrom*. 2021:e9128. https://doi.org/10.1002/rcm.9128
35. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4:44–57. https://doi.org/10.1038/nprot.2008.211
36. Sherman BT, Hao M, Qiu J, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res*. 2022;50:W216–W221. https://doi.org/10.1093/nar/gkac194
37. Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol*. 2012;227:493–507. https://doi.org/10.1002/jcp.22783
38. Angulo J, El Assar M, Alvarez-Bustos A, Rodriguez-Manas L. Physical activity and exercise: strategies to manage frailty. *Redox Biol*. 2020;35:101513. https://doi.org/10.1016/j.redox.2020.101513
39. Cadore EL, Rodriguez-Manas L, Sinclair A, Izquierdo M. Effects of different exercise interventions on risk of falls, gait ability, and balance in physically frail older adults: a systematic review. *Rejuvenation Res*. 2013;16:105–114. https://doi.org/10.1089/rej.2012.1397
40. Nian L, Shukang G, Shasha W, Xiangyun L. Aerobic exercises ameliorate benign prostatic hyperplasia via IGF-1/IGF-1R/ERK/AKT signalling pathway in prostate tissue of high-fat-diet-fed mice with insulin resistance. *Steroids*. 2021;175:108910. https://doi.org/10.1016/j.steroids.2021.108910
41. Bauer SR, Jin C, Kamal P, Suskind AM. Association between lower urinary tract symptoms and frailty in older men presenting for urologic care. *Urology*. 2021;148:230–234. https://doi.org/10.1016/j.urology.2020.09.041
42. Bauer SR, Walter LC, Ensrud KE, et al. Assessment of frailty and association with progression of benign prostatic hyperplasia symptoms and serious adverse events among men using drug therapy. *JAMA Netw Open*. 2021;4:e2134427. https://doi.org/10.1001/jamanetworkopen.2021.34427
43. Ruetten HM, Henry GH, Liu TT, et al. A NEW approach for characterizing mouse urinary pathophysiologies. *Physiol Rep*. 2021;9:e14964. https://doi.org/10.14814/phy2.14964
44. Kirby RS. The natural history of benign prostatic hyperplasia: what have we learned in the last decade? *Urology*. 2000;56:3–6. https://doi.org/10.1016/s0090-4295(00)00747-0
45. Parrott CR, Ghosh P, Tedeschi J, Gunasekara G, Broderick TL. Urinary corticosterone and normetanephrine levels after voluntary wheel and forced treadmill running in the db/db mouse. *J Diabetes Mellit*. 2011; 01(04):8. https://doi.org/10.4236/jdm.2011.14011
46. Graber TG, Ferguson-Stegall L, Liu H, Thompson LV. Voluntary aerobic exercise reverses frailty in old mice. *J Gerontol A Biol Sci Med Sci*. 2015;70:1045–1058. https://doi.org/10.1093/gerona/glu163
47. Liu H, Graber TG, Ferguson-Stegall L, Thompson LV. Clinically relevant frailty index for mice. *J Gerontol A Biol Sci Med Sci*. 2014;69:1485–1491. https://doi.org/10.1093/gerona/glt188
48. Walston J, Bandeen-Roche K, Buta B, et al. Moving frailty toward clinical practice: NIA intramural frailty science symposium summary. *J Am Geriatr Soc*. 2019;67:1559–1564. https://doi.org/10.1111/jgs.15928
49. Ferrucci L, Levine ME, Kuo PL, Simonsick EM. Time and the metrics of aging. *Circ Res*. 2018;123:740–744. https://doi.org/10.1161/CIRCRESAHA.118.312816
50. Chow LS, Gerszten RE, Taylor JM, et al. Exerkines in health, resilience and disease. *Nat Rev Endocrinol*. 2022;18:273–289. https://doi.org/10.1038/s41574-022-00641-2