

# Voluntary wheel running is a non-invasive test to measure physical performance in aging mice

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## Abstract

Physical and cognitive abilities are important elements of healthy aging with many complexities for which tests have been established to quantitatively measure age-related declines. Mouse studies can effectively model human phenotypic traits and parameters that accompany aging by addressing questions that are not realistic in human clinical trials. The shortened timeline of aging mouse models provides researchers to investigate intervention approaches in a shorter time period. Over the years, a number of tests have been developed to measure physical and cognitive abilities in mice that have a wide range of stress and invasiveness. Voluntary wheel running (VWR) is a non-invasive representation of natural behavior in a mouse, as it measures the voluntary limit of physical capacity in a non-stressful scenario. Archived data from a mouse healthspan study were used to determine if there was an independent relationship between VWR and grip strength force, maze learning and memory retention, and physical coordination on a rotating rod, in male mice from age groups of 4, 12, 20, and 28 months. Wheel running distance over three days was not associated with performance data from any of the three physical tests, suggesting that VWR can be used independently as a stand alone or in concert with other performance tests to help determine the anti-aging effects of a drug or other intervention approaches.

**Keywords:** Voluntary wheel running, aging, non-invasive performance test, resilience to aging, aging mouse model, self-motivated exercise

## Introduction

Aging affects cognitive and physical function in animals and humans. Memory retention, learning ability, coordination, and physical strength are impaired with aging. Such deterioration causes stress and limits independence which is additionally harmful to quality of life [1]. Mouse models are practical for studying aging because mice undergo similar functional declines as humans. Mouse studies can effectively model human phenotypic traits and parameters that accompany aging by answering questions that cannot feasibly be investigated in a clinical trial with humans. The short lifespan of a mouse proves advantageous for aging studies because the full impact of an anti-aging treatment can be observed in a matter of months, whereas in humans the outcomes remain unknown for years. The

shortened timeline in aging mouse models allows for an accelerated treatment protocol and provides researchers the ability to investigate more intervention approaches in a much shorter time period.

In this regard, it is imperative to have meaningful and non-stressful tests to measure any changes in aging parameters as a result of a specific treatment regimen. Many tests have been developed to measure physical and cognitive abilities in mice with a wide range of invasiveness. Some physical and cognitive tests include shocking mice or putting mice in gallons of water and requiring them to perform. The Morris water maze [2, 3] is invasive and stressful because it forces mice to swim, while the radial water tread maze measures similar cognitive parameters but is less stressful and noninvasive [4]. Other tests are relatively non-invasive such as grip strength and a rotating rod (rotarod), but these are generally performed during the sleep cycle so there is still some degree of stress. On the other hand, voluntary wheel running (VWR) is noninvasive since performance measurements are fully self-motivated, and the mice are tested during their active cycle and as such are not disturbed [5]. Additionally, VWR has been well characterized in literature [6-9].

This brief report provides evidence that distance ran over three days in a running wheel is not dependent on any of

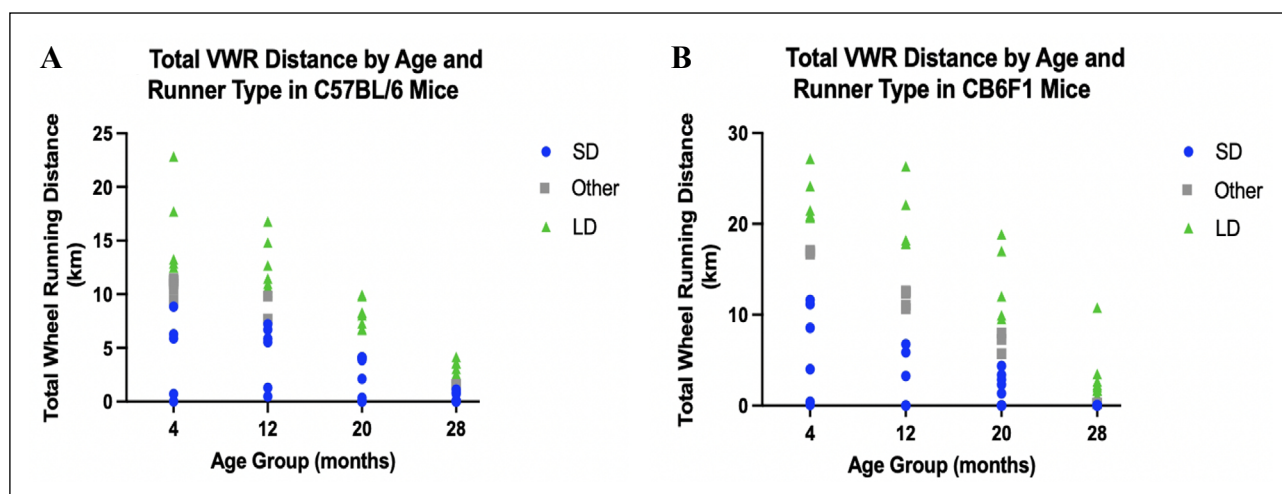
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**Figure 1.** VWR distance declined with age in (A) C57BL/6 mice ( $N = 15-16$  per age group) and (B) CB6F1 mice ( $N = 15-24$  per age group). Differences in distance between ages were analyzed using two-tailed t-tests and were found to be significant in both strains between the 4-month age group and both the 20-month and 28-month age groups.  $P < 0.05$ .

several standard performance tests used to measure the degree of physical aging in mice. Therefore, it can be used as an independent performance test as a stand, alone or in concert with other tests to help determine the anti-aging effects of a drug or other intervention approaches. Researchers must continue to develop less invasive assays to accomplish the same testing requirements to be more relevant for comparative medicine. Less invasive tests are better translated for use in human aging studies in addition to the importance of mouse health and well-being.

## Materials and Methods

Data were collected from archived records of a study performed at the University of Washington as part of a project to define mouse health span [10]. For this study, 22 C57BL/6 and 24 CB6F1 (C57BL/6×BALB/c) male mice from each of four age groups at 4 months, 12 months, 20 months, and 28 months were obtained from the NIA aged mouse colony (Charles River, Inc.). The archived data used for this study were from cross-sectional cohorts from four different age groups, so the mice were not followed throughout their lifetimes.

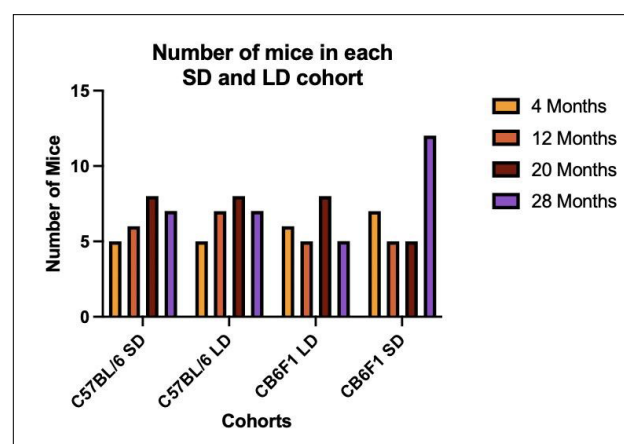
VWR was performed as described [5]. Briefly, mice were individually housed in cages with a slanted running wheel (Med Associates, Inc.) with additional nestlets as enrichment. There was a 1-day acclimation period with the wheels locked before the start of data collection. The wheels transmitted data in the form of distance run per minute wirelessly to a nearby hub connected to a computer. The distance was exported to a spreadsheet in kilometers run every minute for the next 72 hours.

The radial water tread (RWT) maze tested the cognitive ability of the mice by looking at memory retention and learning abilities as described [4]. Briefly, the maze was composed of a circular enclosure filled with one inch of water and had nine escape holes, all closed except one, which led out of the enclosure to a darkened standard

cage. During the four training days, each mouse had three trials. On days 5 and 12, the location of the escape hole was changed. This allowed for testing of learning as well as short- and long-term memory retention.

The ability of a mouse to walk on a rotating rod (rotarod) at increasing speeds was used to test physical coordination and agility [11]. Mice were placed on a rod with four lanes, and rotation speed of the rod was increased until all mice fell onto a soft cushion below. A sensor determined when each mouse fell and the time was noted in seconds. The mean time of three trials per mouse was recorded.

Physical strength was tested by measuring maximum grip strength force [12]. Each mouse was placed on a horizontal metal bar with their front paws pulled backwards by an operator until they released their grip. The sensor connected to the bar recorded the peak force. There were five trials per mouse and the highest force reading was kept. Mice were also weighed on the trial date and the force was normalized by dividing the reading by body weight. Numerical data were analyzed using Student's t-tests and the calculation of  $P$ -values. Differences between runner types were analyzed using two-factor analysis of variance.



**Figure 2.** Number of mice in each cohort of long-distance (LD) runners and short-distance (SD) runners, by strain.

Linear regression and best-fit curves were determined using GraphPad Prism analysis and Microsoft Excel data analysis.

## Results and Discussion

The range and variation of total running distance decreased with advancing age in the age groups of 4, 12, 20, and 28 months (Figure 1). There was a decrease in maximum distance from young to old age with regular fluctuation within each age group. The variation within each group also decreased with increasing age. This observation suggests that VWR performance can be used to test physical aging decline.

The average running distance and standard deviation were calculated for each age group, and mice that ran 0.25 standard deviation further than the average were classified as long distance (LD) runners, and mice that ran 0.25 standard deviation less than the average were short distance (SD) runners. Mouse numbers for each SD and LD cohort are shown in Figure 2.

LD or SD distance runners did not have either strong or weak performances in the grip strength, RWT maze, or rotarod assays (Figure 3). T-tests showed that there were no significant differences in test data between LD and SD runners. The lack of a direct association between the VWR and these tests suggests that the VWR assesses independent aspects of physical and possibly cognitive age-related impairment.

It is worth noting that only males were used in this study. CB6F1 and C57BL/6 male mice run further than female mice (Keely and Ladiges, unpublished observations), so female mice of these strains would be expected to show

lower numbers and less variation in the results. Therefore, the VWR should be informative in both sexes, though repeating this project with female mice for more cross examination of differences between the sexes would be informative.

Observations from this study suggest that VWR performance declines with age and can provide additional insight into aging that can be used as an independent assay, either in a stand-alone manner or included in a test panel to assess aging decline. Running wheels can be expensive and this addition has a cost consideration. Another consideration is that mice must be individually housed for four days and additional enrichment must be added to the cages.

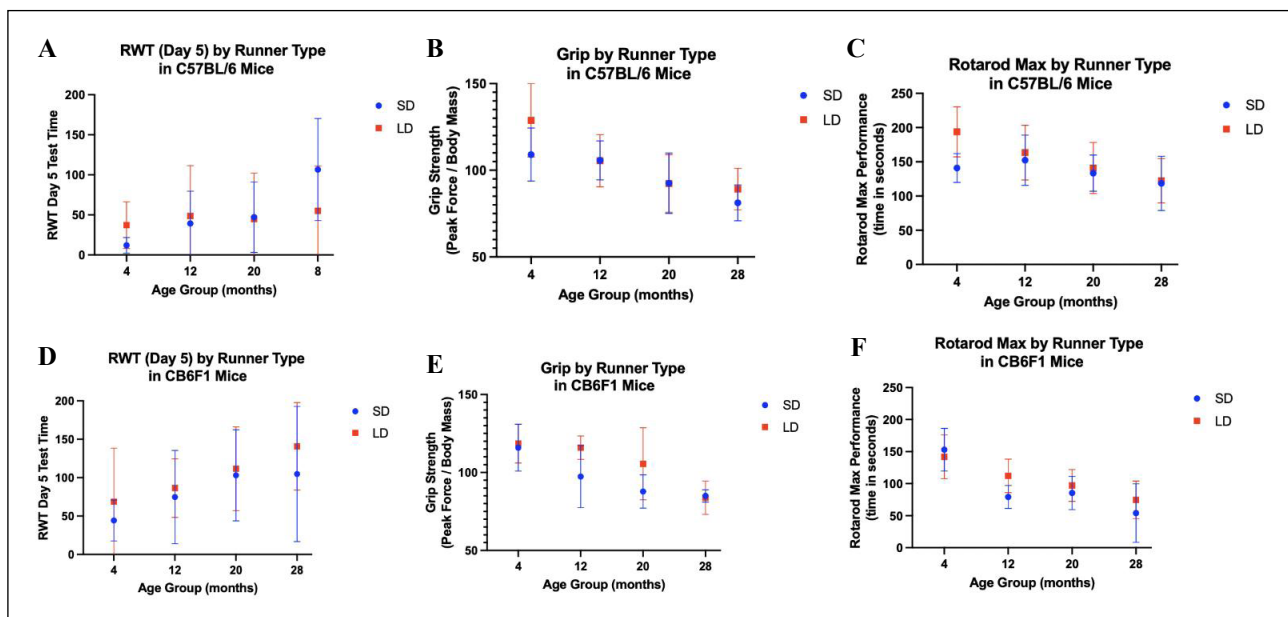
A long-term study following the same mice from young to older ages would be informative as this was a cross-sectional study that used different mice at these age points and did not follow mice as they aged. Because the current study investigated the association of voluntary wheel running with other assays over the span of a few weeks, the question of whether VWR can be predictive of aging resilience could be addressed by a longer-term study.

## Declarations

**Financial support and sponsorship:** None.

**Ethical approval:** The study was approved by the University of Washington IACUC.

**Availability of data and materials:** The data supporting the study results are available from the corresponding author upon reasonable request.



**Figure 3.** There was no association between the distance run by either short-distance runners or long-distance runners and the results of other assays. (A) RWT performance by runner type in B6 mice. (B) Grip strength by runner type in B6 mice. (C) Rotarod performance by runner type in B6 mice. (D) RWT performance by runner type in F1 mice. (E) Grip strength by runner type in F1 mice. (F) Rotarod performance by runner type in F1 mice. Differences between SD and LD runners were analyzed by t-test and found not to be significant ( $P > 0.05$ ).  $N = 5-12$  mice per cohort for each age group.

**Conflicts of interest:** Warren Ladiges is a member of the editorial board of *Aging Pathobiology and Therapeutics*. The authors declare that they have no conflicts and were not involved in the journal's review or decision regarding this manuscript.

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