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Original Article

A New Frailty Score for Experimental Animals Based on the Clinical Phenotype: Inactivity as a Model of Frailty

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Abstract

The development of animal models to study human frailty is important to test interventions to be translated to the clinical practice. The aim of this work was to develop a score for frailty in experimental animals based in the human frailty phenotype. We also tested the effect of physical inactivity in the development of frailty as determined by our score. Male C57Bl/6J mice, individually caged, were randomly assigned to one of two groups: sedentary (inactive) or spontaneous wheel-runners. We compared the sedentary versus the active lifestyle in terms of frailty by evaluating the clinical criteria used in humans: unintentional weight loss; poor endurance (running time); slowness (running speed); weakness (grip strength), and low activity level (motor coordination) at five different ages: 17, 20, 23, 26 and 28 months of age. Each criterion had a designated cut-off point to identify the mice with the lowest performance. Lifelong spontaneous exercise significantly retards frailty. On the contrary sedentary animals become frail as they age. Thus, physical inactivity is a model of frailty in experimental animals. Our frailty score provides a tool to evaluate interventions in mice prior to translating them to clinical practice.

Keywords: Sarcopenia—Exercise—Health—Mice

Research in aging has changed substantially. For years the focus was on interventions that successfully enhance survival but the time for living better has come (1). Longevity has traditionally been the method through which an intervention is considered successful in ageing studies (2). However, the gains in life-years have been accompanied by an increase in the rates of disability and as a consequent absence of autonomy, independence, and well-being (1). Frailty is an age-associated, biological syndrome, characterized by decreased biological reserves due to dysregulation of several physiological systems, which puts an individual at risk when facing minor stressors, and is associated with "bad" outcomes like disability, hospitalization and finally, death (3,4). Frailty is a good predictor of disability. The prevalence of frailty in the old population can be established at around 15% (1).

Two characteristics are important in the context of frailty. The first one is that if left untreated, it will eventually evolve into disability and later on, death. This is a major personal and social concern. European Union analysis have shown that by the year 2020, approximately half of the population over 70 years will be at high risk of disability (1). The second characteristic is that it is reversible, that is, that it can be prevented and even treated. Exercise is one of the most important interventions to prevent frailty. A few studies have been published (5–10) showing that exercise can improve some characteristics of frailty. However, the effects of the exercise interventions

were not conclusive and did not show convincing evidence of effectiveness (1). This is due to the differences in the outcomes assessed (mainly physical determinants and functional abilities) between the different studies and the different types of training interventions. We have recently proposed a multicomponent exercise intervention that reverses frailty in community dwelling frail persons (11).

Frailty is defined in clinical practice by the presence of three or more of the following criteria: unintentional weight loss, self-reported exhaustion, weakness, slow walking speed, and low physical activity (12). Thus, inactivity is critical for the diagnosis of frailty (13). Inactivity causes 9% of premature mortality, or more than 5.3 of the 57 million deaths that occurred worldwide in 2008 (14). Physical inactivity has a deleterious effect that is comparable to smoking and obesity. Furthermore, it is estimated that physical inactivity is a significant predictor of poor skeletal muscle health and poor quality of life (15).

Given the influence of frailty on the health of old persons the development of animal models for frailty represents a very important challenge in basic gerontological research (16,17). Even though clinical interest in frailty has grown in recent years (1,18), to our knowledge, research in experimental animal models of frailty is very scarce. Four models have been reported in the literature (17,19–21). Moreover, there is a lack of a longitudinal study in which frailty is evaluated at different ages.

Physical activity is a very promising intervention for the modulation of both health and lifespan. The benefits of regular exercise go beyond longevity (22). Being physically active is a major contributor to one's overall physical and mental wellbeing. Lifelong physical exercise has become one of the key strategies in the prevention and treatment of chronic, degenerative diseases, among the elderly (23). We believe exercise is so important that should be considered *as a* drug (24) and even as a lifelong supplement for healthy aging (25).

Thus, the major objective of our study was (a) to develop a score for frailty in rodents that is based on the human clinical parameters and thus, easy to extrapolate to humans. We have based our score (that we have named the "Valencia Score") on the construct described by Linda Fried and co-workers (12). A second aim was to determine if a lifelong spontaneous exercise *intervention* reverses frailty as measured by our new frailty score.

Material and Methods

Experimental Animals

Adult male C57BL/6J mice, 3-month-old, were randomly assigned to one of two groups: sedentary control (n = 60) or spontaneous exercise (n = 60). The animals were housed in individual cages. Mice of the active group had 24 hours access to a 11.5 cm diameter running wheel connected to an electronic wheel-revolution count built at the top of the cage. The sedentary mice were free to move around their cage but did not have access to a running wheel. We chose to wait until 3 months of age to allow our animal's access to exercise wheels, as this is the age at which mice reach musculoskeletal maturity, and we postulated that our animals would achieve maximal performance if they were exposed to running wheels at this age (26).

The average temperature in the animal house was 23 ± 1°C, relative humidity was 60%, and 12 hours day/night cycles were maintained. Mice were checked daily. Water and food were available ad libitum. The number of running revolutions was recorded weekly. Food consumption was determined weekly by subtracting the amount of food remaining from the amount offered (Table 1).

Animals were studied at 17, 20, 23, 26, and 28 months of age. Maximal lifespan in our mice was 31.7 months in both groups (sedentary and wheel-runners) while the average lifespan was 25.0 months for sedentary mice and 25.7 for wheel-runners (22). The 17- and 20-month-old animals come from the flat part of the longevity curve so we can consider these animals middle-aged adult mice. By contrast, the aged animals come from the survival curve where mortality is most accelerated (23-month-old) and where more than 50% of the animals have died (26- and 28-month-old). We started the measurements of the components of the "Valencia Score" for frailty when the animals were 17-month-old. When the measurements of the "Valencia Score" were performed in young animals (3- and 6-month-old) none of them fulfilled any of the frailty criteria established for the 17-, 20-, 23-, 26-, and 28-month-old animals (data not shown).

The experimental protocol was approved by the Committee of Ethics in Research of the Faculty of Medicine, University of Valencia.

Body Weight

Animal's body weight was recorded weekly by using a PB3002 Delta Range balance (Mettler Scales, Toledo, OH) (Supplementary Table 1).

Motor Coordination Test (Tight-Rope Test)

The tight-rope test was based on the method previously described by Miquel (27) and extensively used by our team (28). Mice were placed in the middle of a 60 cm long and 1.5 cm wide rope. The test results were considered successful if the mouse reached any end of the rope or was maintained on it for 60 seconds. All the animals had five chances to complete the test. We determined the percent of mice that succeeded in passing the test.

Incremental Treadmill Test

The animals were submitted to a graded intensity treadmill test (Model 1050 LS Exer3/6; Columbus Instruments, Columbus, OH) to determine their endurance (running time) and running speed along the study. We followed a modification of the protocol of Davidson and co-workers (29). After a warm up period the treadmill band velocity was increased until the animals were unable to run further. The initial bout of 6 minutes at 6 m/min was followed by consecutive 2 m/min increments every 2 minutes. Exhaustion was defined as the third time a mouse could no longer keep pace with the speed of the treadmill and remained on the shock grid for 2 seconds rather than running. Exercise motivation was provided for all rodents by means of an electronic shock grid at the treadmill rear. However, the electric shock was used sparingly during the test. We recorded the running time (endurance) and the maximal running speed achieved by the mice.

Table 1. Mean Food Intake and Running Distance in 17-, 20-, 23-, 26-, and 28-Month-old Male Mice

	Food Intake (grams/wk)		Running Distance (Km/d)	
Age (mo)	Sedentary	Wheel-runners	Sedentary	Wheel-runners
17	32.2 ± 1.2	31.5 ± 1.9	—	0.7 ± 0.3
20	37.4 ± 4.6	35.5 ± 4.1	—	0.5 ± 0.3
23	37.8 ± 3.4	38.7 ± 3.6	—	0.5 ± 0.3
26	31.2 ± 5.9	32.2 ± 6.7	—	0.3 ± 0.1
28	28.2 ± 5.9	32.2 ± 7.2	—	0.2 ± 0.1

Note: Values are shown as mean ± *SD*.

Grip Strength Test

The Grip Strength Meter (Panlab. Harvard Apparatus) was employed in assessing neuromuscular function by sensing the peak amount of force that the mice applied in grasping specially designed pull bar assemblies. Metering was performed with precision force gauges in such a manner as to retain the peak force applied on a digital display. Mice were randomly chosen to grasp the pull-bar with their forelimb for a few seconds. The animals were then drawn along a straight line leading away from the sensor. The animals released at some point and the maximum force attained was stored on the display. Peak force was automatically registered in grams-force by the apparatus. Data were recorded, and four additional trials were immediately given (30).

Data Analysis

Differences in body weight, maximal running time and speed, grip strength test, motor coordination, and frailty score were tested using Pearson's chi-squared test for each age group and parameter. Differences were considered significant at $p < .05$. Statistical calculations were performed using SPSS (version Pasw Statistics 17.0).

Results

Description of the "Valencia Score" for Frailty

This score is based on the previous one for frailty developed for humans by Linda Fried and co-workers (12). We have adapted it to experimental animals. The test consists of the measurement of five components: weight loss (change in body weight), weakness (grip strength), poor endurance and slowness (incremental treadmill test), and low activity level (motor coordination) (Figure 1).

Body weight

Animals' body weights were recorded throughout their lifespan. At 3 months of age, when we started the exercise intervention in our mice, the average weights were similar in both groups, sedentary (26.1 ± 1.5 g) and wheel-runners (25.3 ± 1.0 g) (Supplementary Table 1). The reference value for all the animals studied (at 17, 20, 23, 26, and 28 months of age) was obtained as follows: we selected ten 17-month-old mice (five sedentary and five exercised) and weighted them out. The weights were averaged and this was our 100% of the weight of a mouse. When an animal lost more than 5% of the weight at 17 months of age was considered positive for this frailty criterion (body weight). As the animals grew older, wheel-runners lost less weight than the sedentary ones. The differences were significant in 20- and 23-month-old animals (Figure 2). We only weighted five animals per group because the weights were very similar in all the mice from the same experimental group (Supplementary Table 1). We randomly selected the mice to be weighted at the different time points.

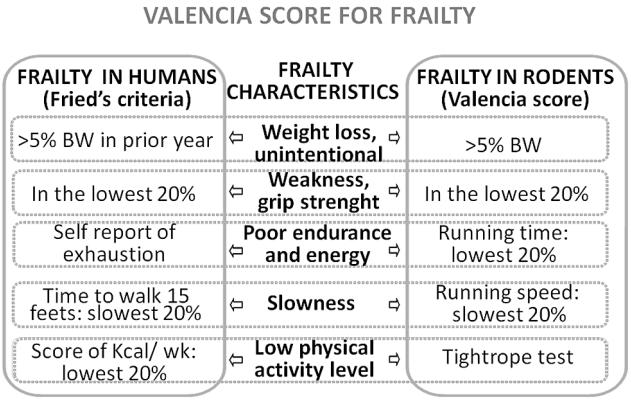

Figure 1. Frailty components in mice vs humans.

Grip strength

We first measured the grip strength of our mice at different ages (17-, 20-, 23-, 26-, and 28-month-old). We established the 20th percentile as a cut-off point. Animals that ranked below the 20th percentile fulfilled the frailty criteria of weakness. For instance, at 17 months of age, we measured the grip strength of 120 mice (60 sedentary and 60 wheel-runners). The 20th percentile was 77.8 grams. Those animals that ranked below 77.8 grams indicated weaker strength and were considered positive for this frailty criterion at that particular age. We made these calculations for 20-, 23-, 26-, and 28-month-old mice.

Figure 3 shows the percentage of mice that did not fulfill the grip strength frailty criterion in each of the two experimental groups. Animals belonging to the wheel-running group were significantly less positive for this frailty parameter than controls.

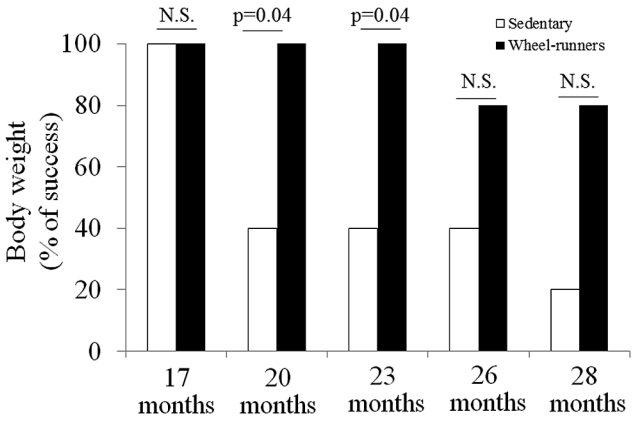

Figure 2. Unintentional weight loss. Animals' body weights were recorded throughout their lifespan. When an animal lost more than 5% of the weight at age 17, we considered that it was frail for this parameter. Data are expressed as percentage of mice that did not lose more than the 5% of body weight in the sedentary (S) and wheel-runners' (WR) groups. The number of animals tested was 5 in all the experimental groups and ages (17, 20, 23, 26 and 28 months). Statistical differences were tested using Pearson's chi-squared test.

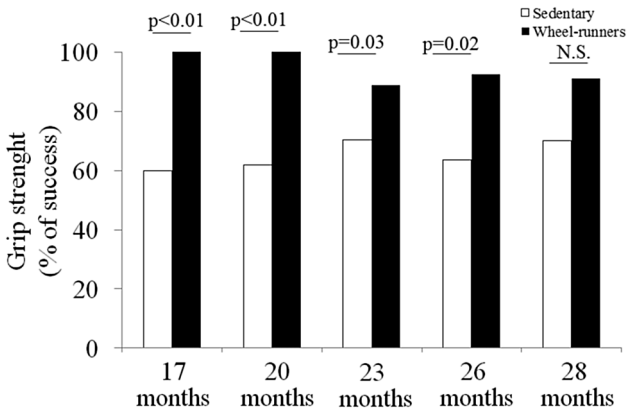

Figure 3. Weakness. Reference mice grip strength values at the different ages were obtained. Data are expressed as percentage of mice that did not fulfill this frailty criterion. The number of animals tested were: 17 m (60S and 60WR), 20 m (50S, 46 WR), 23 m (44S, 45WR), 26 m (22S, 27WR), 28 m (11S, 11WR). Statistical differences were tested using Pearson's chi-squared test.

Running time and running speed

Endurance is a key component of the diagnosis of frailty in the clinical setting (12). Thus, we determined the endurance of mice by measuring the running time values when performing an incremental intensity test in a treadmill at the different ages studied (17-, 20-, 23-, and 26-month-old). For instance, we measured the running time of nineteen, 17-month-old mice (9 sedentary and 10 wheel-runners). We established the 20th percentile as a cut-off point. We got a threshold of 14 minutes for this parameter. Those animals that reported less than 14 minutes of running time during the endurance test, fulfilled this frailty criterion at 17 months of age. We made these calculations for mice of 20, 23, and 26 months of age. We could not perform an endurance test in the oldest animals (28 months of age) because they were unable to keep even the lowest running intensities. In the clinical practice, subjects that are unable to perform one test are categorized as positive for that criterion. Thus, all the animals at 28 months of age were considered positive for the endurance parameter. Figure 4A shows that wheel-runners performed significantly better in the incremental running test than sedentary mice at 17, 23, and 26 months of age.

Apart from the running time, we measured the running speed achieved while performing an incremental intensity test, as an index of "slowness" (Figure 4B). For instance, we measured the running speed of twenty-six, 20-month-old mice (13 sedentary and 13 wheel-runners). Then, we determined the 20th percentile for the running speed parameter in our mice. We got a threshold of 16 m/min for this parameter. Those animals that reported less than 16 m/min of running speed fulfilled this frailty criterion at 20 months of age. We made these calculations for mice at 17, 23, and 26 months of age.

As mentioned previously, the oldest animals (28 months of age) were all considered positive for the running speed parameter because they were unable to perform the incremental treadmill test. Figure 4B shows that wheel-runners performed better in the incremental running test than sedentary mice at 17, 23, and 26 months of age. We randomly selected the mice to perform the endurance and running speed test at the different time points.

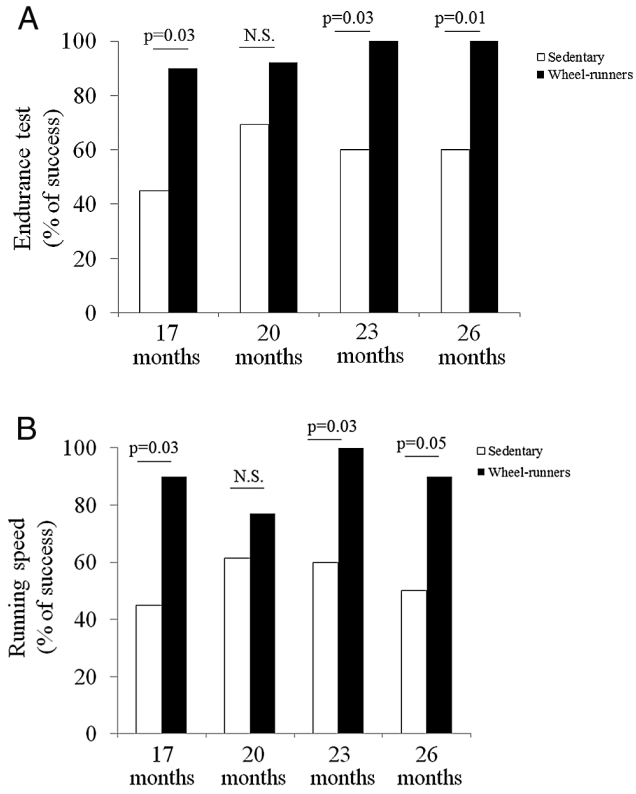

Figure 4. Poor endurance and slowness. We determined the reference running time (**A**) and running speed (**B**) values at the different ages in our mice. Data are expressed as the percentage of mice that did not fulfill these frailty criteria. The number of animals tested were: 17m (9S, 10WR), 20m (13S, 13WR), 23m (10S, 8WR), 26m (10S, 10WR). Statistical differences were tested using Pearson's chi-squared test.

Motor coordination

The tightrope test is a widely used and extensively validated behavioral marker of ageing (28,31). Neuromuscular coordination was quantified as the percentage of mice that successfully passed the tightrope test and was considered as a good marker of motor coordination and physical strength. We determined the reference coordination values at the different ages (17-, 20-, 23-, 26-, and 28-month-old) (Figure 5). As mentioned in the Material and Methods Section, the results were considered successful if the mouse reached any end of the rope or was maintained on it for 60 seconds. All the animals had five chances to complete the test. Figure 5 shows that as age advanced the exercise group, with more passing members, had better results than the control group in the tightrope test. We randomly selected the mice to perform the tightrope test at the different time points.

Frailty score

The frailty score for each age group of animals was calculated as follows: total number of test failed by the animals at each age group, divided by the total number of tests performed by these animals, expressed in percentage (Figure 6). For every age studied, the percentage of frail mice was significantly higher in the sedentary group than in the wheel-running group.

Discussion

Frailty is a geriatric syndrome with a tremendous impact on the older individual, their family, and society as a whole. The development of animal models, as well as clinically relevant scores for frailty represents an important step forward in the development of intervention studies to prevent this clinical syndrome. As recently highlighted very few studies track declines with ageing in living animals (32). To

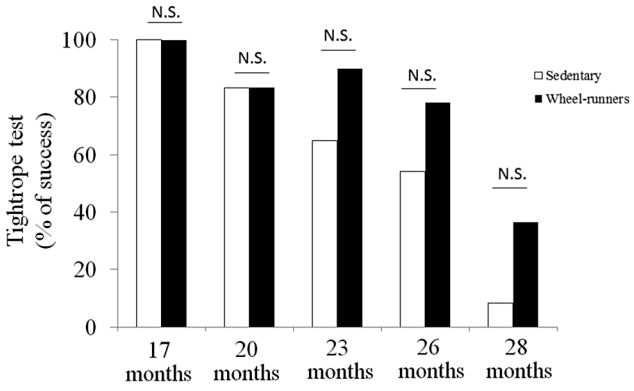

Figure 5. Motor coordination. It was determined as the percentage of animals that successfully passed the tightrope test at the different ages. The number of animals tested were 17m (12S, 12WR), 20m (12S, 12WR), 23m (20S, 20WR), 26m (24S, 23WR), 28 m (12S, 11WR). Statistical differences were tested using Pearson's chi-squared test.

	17 months		20 months		23 months		26 months		28 months	
	W-R	S	W-R	S	W-R	S	W-R	S	W-R	S
Running time (endurance)	1	4	2	4	0	4	1	4	11	12
Running speed (slowness)	1	4	1	4	0	4	0	4	11	12
Motor coordination	0	0	2	2	2	7	5	11	7	11
Body weight	0	0	0	3	0	3	1	3	1	4
Grip strenght	0	24	0	19	5	13	2	8	1	4
Total number test failed (A)	2	32	5	32	7	31	9	30	31	43
Total number test performed(B)	97	95	89	93	90	89	75	71	49	52

(A/B)	0.02	0.34	0.06	0.34	0.08	0.35	0.12	0.42	0.63	0.83
Frailty Score: (A/B)x100	2	34	6	34	8	35	12	42	63	83

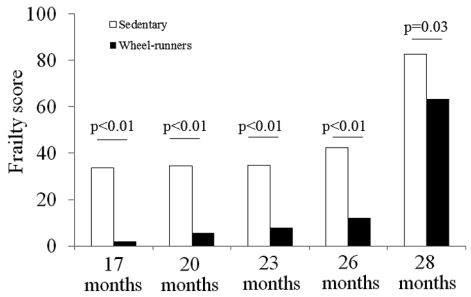

Figure 6. Frailty score in mice. The frailty score for each age group of animals was calculated as follows: total number of test failed by the animals at each age group divided by the total number of tests performed by these animals, expressed in percentage. Statistical differences were tested using Pearson's chi-squared test.

determine how best to preserve function with ageing in humans, we must create common grounds between animal studies and clinical assessments. Several functions known to decline with age in humans can also be assessed in rodents. But many physiological tests that have been established in humans either do not exist or are not routinely performed on ageing animals (32).

Even if clinical interest in frailty has grown in recent years (18) research in experimental animal models of frailty is scarce. Only four mice models have been reported in the literature (17,19–21). In the first one, the authors found that the genetically altered IL-10 deficient mice develops inflammation and strength decline consistent with human frailty (17). However, the authors did not report a frailty index for the animals.

In the second one, Parks and co-workers determined 31 invasive and noninvasive variables, including activity levels, body composition, hemodynamic, and metabolic measurements to generate a unique score for frailty for each mouse (19). Although very complete, the translation of this frailty index to the clinical practice seems difficult especially because the parameters determined do not coincide with the accepted measures used clinically to define frailty in humans. For instance, the authors do not consider relevant deficits that are predictors of frailty such as muscle grip strength. A more simplified index based on readily apparent signs of clinical deterioration has been used by the same research group to characterize frailty in aging mice (21). This tool has been successfully applied to quantify the effect of dietary and pharmaceutical interventions on frailty (33). However, in our opinion in this frailty index authors do not consider relevant deficits that are predictors of frailty such as walking-running speed or physical activity.

In the fourth study, a Frailty Index that matched the clinical criteria used in humans (slow walking speed, weakness, low activity level, and poor endurance) was developed in old mice (20). However, this score has been tested in a low number of animals (n = 11) of the same age (27- to 28-month-old mice) and a longitudinal study is required, with different ages, to develop animal models for frailty.

Here we report a new, clinically relevant, score to determine frailty in experimental animals that we have named "Valencia Score". In our score we include two clinically relevant parameters that have not been taken into account in the previous ones, changes in body weight and an incremental treadmill test (to approach the human frailty criteria: poor endurance and slowness).

At this point of the discussion it is important to state that there is not yet a standardized and valid method of clinically screening for frailty in humans. More than 20 different instruments have been used to measure frailty clinically (34). The most commonly used model of frailty is that of Linda Fried (12). Others include the frailty index (35), the classification of frailty and vigorousness (36), and the Edmonton frailty scale (37). In the study by Linda Fried and co-workers it was concluded that frailty is a combination of five components: unintentional weight loss, poor endurance, weakness, slow walking speed, and low physical activity. Based on this work we developed the "Valencia Score," adapting Fried's test to experimental animals (Figure 1).

We found that as the animals grew old, they showed poorer results in the tests that determined the five components of frailty: weight loss (Figure 2), grip strength (Figure 3), endurance and running speed (Figure 4A and B), and motor coordination (Figure 5). Our results suggest that the selected criteria determined were a good choice to establish a score of frailty (Figure 6).

One important feature of frailty is that it is reversible, that is, that it can be prevented and treated. Exercise is one of the most important interventions to prevent frailty because it can potentiate resilience (38). Physiological resiliency can be defined as the ability of an organism to cope with a challenge, and return to normal baseline function following a perturbation (38). Importantly, the gradual loss of resiliency with age contributes to, and may underlie the onset of aging-related conditions, including frailty (38). There is little consensus in the literature to pinpoint which kind of exercise is more effective for frail individuals (39). We have recently found in humans, that a combined program of endurance, strength, coordination, balance, and flexibility exercises can reverse frailty (11). In the present study, those mice that had free access to the running wheels performed significantly better than the sedentary animals in all the frailty criteria measured. Moreover, we also found a significant improvement in the frailty index for each age group. In all cases, the percentage of frail mice was significantly higher in the sedentary animals than in the wheel-runners. It has been recently shown that a late onset voluntary exercise intervention, for 4 weeks, can reverse frailty in old mice (28-month-old) (40). Our results confirm these data but with a longer exercise intervention and in a wider range of ages (17-, 20-, 23-, 26-, and 28-month-old mice).

Elderly individuals often say they would rather keep on feeling healthy than merely live longer (41). In this study we have shown that lifelong spontaneous exercise prevents age-associated frailty in mice, providing an animal model of frailty and a way to measure it in non-human experimental models.

In is important to comment on the limitations of this study. Due to technical problems, we could not perform all the measurements in every mouse of the cohort. This is a limitation of our study especially because it made it impossible for us to establish an individual score of frailty in every mouse. Another limitation is that we have

not performed the test in both males and females so we cannot take into consideration potential sex differences in the rate of functional decline.

Supplementary Material

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

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Conflict of Interest

The authors declare that no conflict of interest exists.

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