

Phenotypic Frailty Assessment in Mice: Development, Discoveries, and Experimental Considerations

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The underlying mechanisms contributing to the onset of frailty, its progression, and its mortality risk remain unknown. Recently, the two most common human frailty assessments were reverse-translated to mice. Here, we highlight the development of the mouse frailty phenotype, unique discoveries, experimental considerations, and future perspectives.

aging; index; function; physiology; resilience

Introduction

Frailty is generally defined as an age-associated biological syndrome characterized by marked vulnerability to adverse health outcomes (16, 17), a reduced capacity to react to stressors, and an overall loss in physiological function (7, 48). Over the past two decades, the topic of human frailty has gained a tremendous amount of popularity (FIGURE 1A). A primary reason for the increased popularity was the development of human frailty assessments in the early 2000s. The two most widely used assessments are the frailty phenotype and the frailty index pioneered by Fried et al. (17) and Rockwood et al. (47), respectively. The frailty index conceptualizes frailty by using a multidimensional approach and is based on a comprehensive geriatric assessment that measures deficits in physiological, psychological, cognitive, and social function (47). In contrast to the frailty index, the frailty phenotype primarily focuses on physical components of unsuccessful aging (i.e., physical frailty), including weakness, poor endurance/exhaustion, slowness, and low activity (17). Although these two clinical assessments provide important information on frailty identification, we and others developed preclinical mouse frailty assessments (20, 40, 49). Preclinical mouse assessments for frailty have the potential to address the underlying physiological and cellular mechanisms necessary for the development of interventions to prevent or treat frailty. Because of the shortened lifespan, preclinical mouse assessments are poised to fully characterize the states within the frailty spectrum (e.g., pre-frail) across the lifespan for trajectory, prediction, and prognosis, which are also needed to devise effective interventions. Additionally, the development of frailty assessment tools in mice provides an opportunity to investigate characteristics of the assessment tool itself for discriminative power across the lifespan.

The preclinical mouse assessments of frailty were developed to recapitulate the human frailty index and phenotype, and were published in 2012 (49) and 2014 (40), respectively. Since their development, a growing number of mouse frailty studies have been published (FIGURE 1B), mimicking the growth observed in the human literature (FIGURE 1A). As a consequence, these preclinical models have made it possible to bypass many of the ethical, logistical, and biological complications of working with older adults.

Recognizing how preclinical frailty assessments were developed and optimized, the discoveries achieved, the experimental considerations (possible limitations), and the future perspectives provides guidance to delve into questions underlying mechanisms of frailty or treatment interventions. In this review, we focus specifically on the mouse frailty phenotype, since several recent reviews have been published on the mouse frailty index (26, 28, 32). The overall goal of this review is to highlight the key concepts we learned from its initial development to our most recent publications. We hope our unique outlook for this review sets a forward-thinking attitude for preclinical reverse-translational research on frailty.

Development

The clinical human phenotype developed by Fried et al. (17) includes five measures: four components of physical function (weakness, poor endurance/exhaustion, slowness, and low activity) and unintentional weight loss. Defining frailty begins with identifying physical performance tests in mice that measure strength, endurance, walking speed, and physical activity levels. The next step in defining frailty requires determining the cutoff percentile, which Fried and colleagues (17) identified as the lowest 20% of the cohort's baseline (or the lowest quintile). Being in the lowest 20% within a measure

gave a positive frailty marker, with more positive markers indicative of greater frailty. Frailty states or subgroups included non-frail, intermediate (i.e., pre-frail), and frail. Our preclinical mouse phenotype was developed to mimic Fried's frailty phenotype, be simple to use, be noninvasive, and have the potential to translate to clinical settings (4, 36, 40). Here, we briefly describe the development of the original mouse frailty phenotype and how it has been modified in recent years.

Our original frailty phenotype was completed on 11 male C57BL/6 mice at 27–28 mo of age (50% survival rate) (61) and published in 2014 (40). It included measures of strength (inverted grip hang), walking speed (rotarod), physical activity (voluntary wheel running), and an endurance score (inverted grip hang plus rotarod). Mice with three or more positive frailty markers were identified as frail, mice with two positive markers were identified as pre-frail, and mice with one or no positive frailty marker were considered non-frail. In this initial frailty phenotype, we selected a cutoff percentile corresponding to 1.5 standard deviation (SD) below the cohort mean (i.e., the lowest seventh percentile of the group.) Following this seminal publication, several other groups, including our own, modified our original frailty phenotype for the C57BL/6 mouse strain (4, 5, 20, 24, 36, 42).

We began conducting two studies in 2015; the first in males ($n = 29$) and the second in females ($n = 27$) (4, 36). In these studies, we made four significant modifications to our original preclinical frailty phenotype: we 1) included body weight, 2) added a measure of endurance that is reliable and valid (treadmill fatigue test), 3) replaced the inverted grip hang test for strength

with the electronic grip meter test, and 4) adjusted our cutoff to the lowest 20th percentile (versus 7th percentile). Most important, because we were interested in investigating the relationship between frailty status and mortality risk, we also used a longitudinal research design, testing mice every 3 months starting at a younger age. The longitudinal research design also provided us the opportunity to detect changes in physical function and body weight of our mice at both the frailty subgroup and the individual mouse levels, and allowed us to establish prevalence of frailty across the lifespan and the age at which frailty was first observed (4, 36). Although we acknowledge cross-sectional studies are important, they typically provide a “snapshot” of frailty at a single moment in time (i.e., specific age) (1, 33, 44, 56). Furthermore, without a longitudinal approach, it is difficult to determine what has happened before or after the snapshots. We discovered, by using the mouse frailty phenotype assessment, frailty characteristics in mice parallel many frailty characteristics observed in humans.

Discoveries

By using additional frailty measures (i.e., body weight and endurance), a more liberal cutoff percentile, and a longitudinal research design, we were able to describe many additional characteristics of frailty, including the onset of frailty, prevalence of frailty, and mortality risk of frail mice (4, 36). The onset of frailty represents the age at which frailty is first observed and is useful for determining the biological mechanisms leading up to frailty, which could ultimately be used to design interventions to prevent or delay frailty. In male and female C57BL/6 mice, the onset of frailty occurred at ~17 mo, an age when the survival rate is ~100% (4, 36). Another important characteristic of frailty is prevalence, which reflects the proportion of a particular population that is affected by frailty. We observed that the prevalence of frailty increased from 3% to over 60% across 17–32 mo of age in male and female C57BL/6 mice (FIGURE 2A). These findings are consistent with those seen in humans, where the prevalence of frailty in males and females increases from 3 to 11% at 65–75 yr old to 60% at 90 yr old (11, 12, 18). Last, we demonstrated that non-frail mice live longer than frail mice in both sexes. In fact, non-frail male and female mice lived 20% (6.7 mo) and 7% (2.1 mo) longer than frail mice, respectively (4, 36). Similar to the findings reported by Fried and colleagues (17), we confirm that measures of physical function in the C57BL/6 mouse are valuable indicators and predictors of frailty.

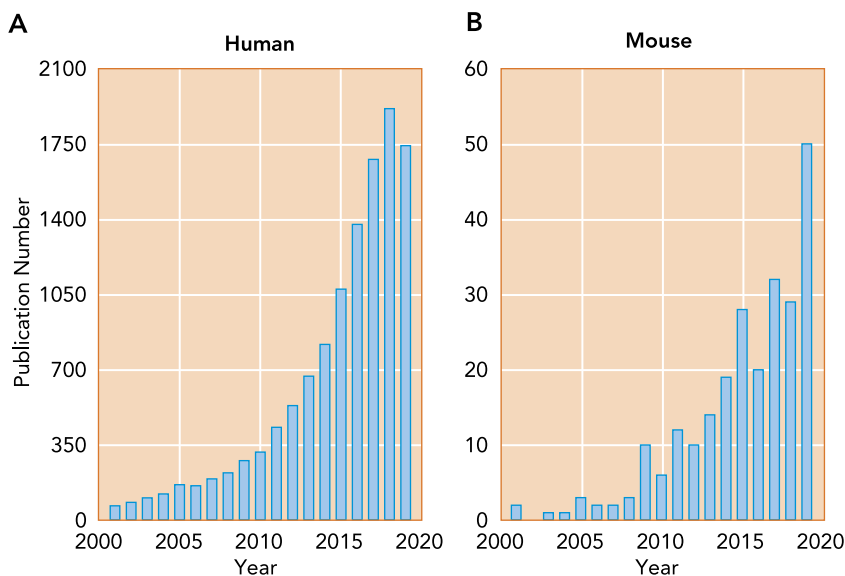


FIGURE 1. Frailty publications have drastically increased from 2000 to 2019
Publication searches were done in PubMed using “((human[tw] OR humans[tw]) AND frailty[tw])” (A), and “((mouse[tw] OR mice[tw]) AND frailty[tw])” (B).

Experimental Considerations

Measures of Physical Function

Physical function is an important component in laboratory and clinical settings. Measures of physical function are generally conducted for the purpose of training or performance in healthy, young individuals, whereas in the old, it is more common for physical function to be assessed for general health (29) or determination of frailty (17). Regardless of the specific purpose, various components of physical function are assessed using a wide array of equipment and methods. Components of physical function often include tests that incorporate multiple whole-body systems (e.g., neuromuscular, cardiorespiratory) to measure aspects of strength, endurance, and coordination (51). These tests can be as intensive as a maximal treadmill test with simultaneous analysis of gas exchange to as basic as a timed walk/run over a set distance. When considering all ages and their respective health status (or limitations), the availability of equipment, cost, and general expertise of the individuals conducting the assessments, the more basic approaches are typically utilized (6). By using these basic approaches, it is well documented that physical function declines with age and that physical dysfunction leads to increased morbidity and mortality in humans (37, 43, 59). For example, age-related reductions in muscular strength (43) and walking speed (59) have both been linked to disability and mortality in older adults. Importantly, and as noted above, Fried's frailty phenotype includes several measures of physical function (e.g., weakness, poor endurance/exhaustion, slowness, and low activity) that not only identify frailty states but also predict mortality (17). Collectively, measures of physical function are valuable when determinants of longevity are studied.

The human aspects of physical function, aging, and mortality are also observed in mice. Indeed, our laboratory and many others have reported physical function declines in aging C57BL/6 mice (4, 20, 30, 36). Some mouse assessments may not completely recapitulate those used in humans, yet they remain fundamentally translatable and valuable (30). As previously mentioned, we used a voluntary running wheel, motorized treadmill, grip meter, and rotarod to test activity level, endurance, strength, and walking speed, respectively. In **FIGURE 3**, we have depicted a simplified visual of how these various measures of physical function decline in aging C57BL/6 mice. These measures of physical function should therefore be able to identify frailty states (i.e., non-frail, pre-frail, and frail) and predict mortality, similar to that of Fried's human frailty phenotype (17). We confirmed this concept by using our updated frailty phenotype

and indeed observed that physical dysfunction (and body weight) could be used to identify frailty and subsequently predict mortality in male and female C57BL/6 mice, as described above in *Discoveries* (4, 36).

We and others have published the value of using a phenotype that emphasizes physical function to assess markers of frailty, including identification, onset, prevalence, and mortality risk in C57BL/6 mouse strain (4, 5, 20, 36). In recent years, the phenotypic assessment tool has also been utilized to determine mechanisms of frailty and the efficacy of potential therapies (56, 60). With usage increasing, we would like to point out several significant factors to consider when developing or implementing a frailty phenotype. First, it is important to incorporate a battery of measures that will assess multiple components of physical function. Second, the metabolic demand (i.e., aerobic versus anaerobic) should also be considered when selecting these measures. Tests that challenge the mouse, such as a treadmill run to exhaustion, may

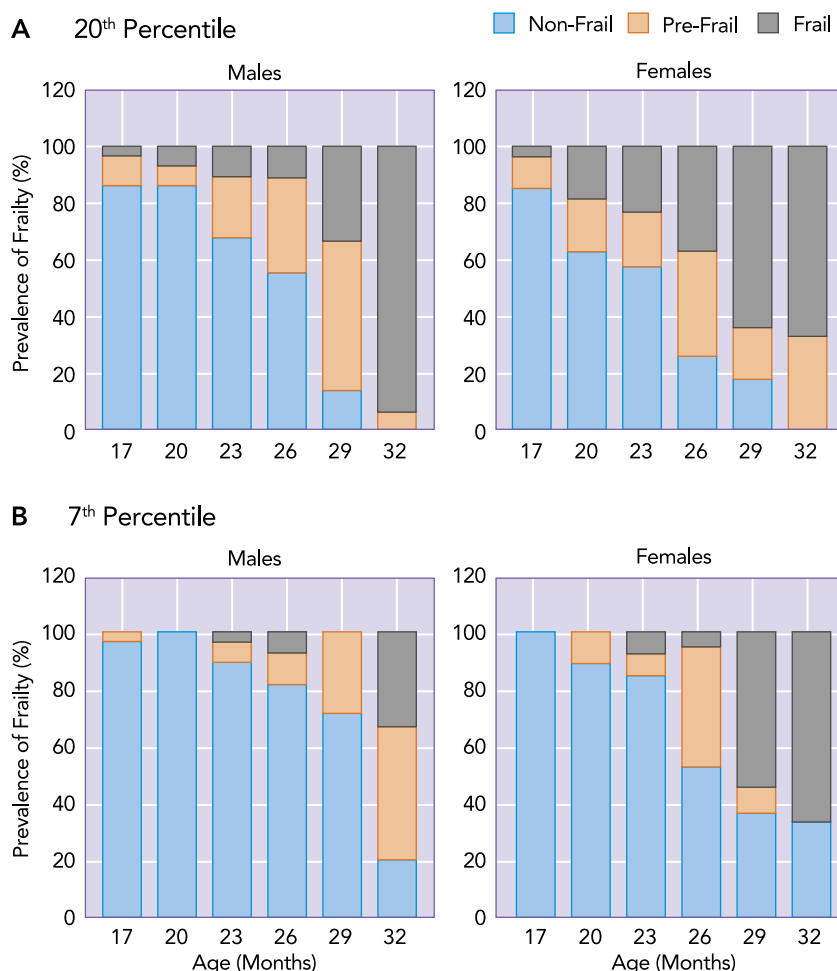


FIGURE 2. Prevalence of frailty in C57BL/6 mice
Prevalence of frailty using a 20th percentile (A) or 7th percentile (B) cutoff in C57BL/6 mice will yield different results. For male and female mice, prevalence of frailty was determined in the same cohort across a lifespan by using identical frailty criteria with the exception of the cutoff percentiles. Data is from Thompson and colleagues (4, 36).

yield more information about how the neuromuscular and/or cardiorespiratory systems respond to stress. Third, it should be accepted that not all measures of physical function decline at the same age or similar rate. This fact can clearly be seen in **FIGURE 3** when comparing physical activity assessed by voluntary wheel running distance to strength measured with a grip meter. Last, not every mouse will become frail or demonstrate frailty in the same manner. For example, one mouse may be observed as frail by weakness and walking speed, whereas in a different mouse it may be endurance and physical activity, but, nonetheless, both mice would be considered frail. Kane et al. (34) articulated this concept by writing, “there are different ways to become frail and each of them is valid for the person or mouse who becomes frail in that way.”

In summary, physical function declines with age and is associated with morbidity and mortality. In this way, measures of physical function can be used to predict or identify frailty. However, no single measure can accurately test for frailty; rather, the power of the frailty phenotype relies on using a battery of assessments that examine distinct components of physical function. Furthermore, measures of physical function decline at different ages and various rates. If these salient factors are considered, we believe the frailty phenotype can be a powerful tool for researchers to assess frailty in rodents (and in humans).

Body Weight

An important and novel component of our mouse frailty phenotype is the measure of a high or heavy body weight (4), which does not follow unintentional weight loss outlined by Fried et al. (17). As previously published (4), we would like to emphasize several key concepts. First, with our frailty phenotype and the conclusions published by other laboratories, mice that are heavy during mid- to late-adulthood are prone to dying earlier in life (4, 14, 45, 46). Our reports further indicate that high body weight in C57BL/6 mice is largely due to an excess of body fat not fat-free mass (4, 36). These findings support the use of heavy body weight (or a high body fat percentage) as one of the measures within the mouse frailty phenotype. Human data may also support this concept, since Blaum et al. (8) reported obesity was associated with the frailty syndrome in older women, suggesting that weight loss is a possible but not necessary component of the frailty phenotype. Second, body weight steadily decreases in aging mice (55), and, under the right circumstances, unintentional weight loss can be used as a frailty marker (20). In fact, mice may lose a dramatic amount of weight in a fairly short period before dying. But, to capture this rapid body weight loss, it is critical to measure body weight weekly or even daily. Therefore, it is possible heavy adult mice that were identified as frail in our recent studies did lose weight before dying, but it was not captured due to the time between assessments (3 mo) (4, 5, 36). The third concept emphasizes the important role measures of frailty play across the lifespan (i.e., heavy body weight in mid- to late-adulthood versus low body weight near death). In our work, when using heavy body weight as a marker of frailty, it became impossible for this measure to be a positive frailty marker in old mice because of steady age-associated weight loss. We did observe that, at the older ages, measures of low physical function became very important positive markers in frailty identification. The last concept focuses on designing interventions to prevent frailty where it is advantageous to initiate the intervention as soon as possible, before any weight loss. Hence, the measure of heavy body weight in mid- to late-adulthood can be effective within the mouse frailty phenotype.

In summary, investigating frailty across the lifespan with a longitudinal research design to identify characteristics of frailty (e.g., onset, mortality), the measure of body weight, and its cutoff value within the frailty phenotype requires careful consideration because of well-established, age-associated changes in body weight. When identifying frail mice in mid- to late-adulthood, our data strongly suggest that heavy body weight is a positive marker

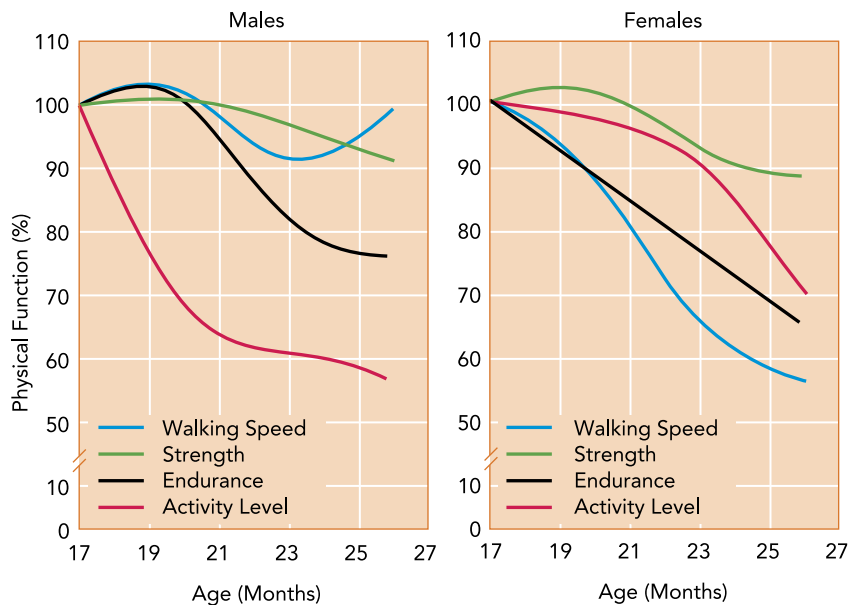


FIGURE 3. Aged-related changes are observed in measures of physical function in C57BL/6 mice

For male and female mice, measures of physical function were expressed relative to values obtained at 17 mo of age (i.e., 17 mo was set as 100%). Walking speed, strength, endurance, and activity level were assessed using a rotarod, grip meter, motorized treadmill, and voluntary running wheel, respectively. Data is from Thompson and colleagues (4, 36).

for frailty. In contrast, when identifying frailty in old mice, rapid unintentional weight loss becomes a positive marker for frailty.

Cutoff Values

Various methods have been used to determine suitable cutoff values in mice (4, 20, 33, 36, 40). In general, establishing the cutoff value for each frailty measure comprises 1) selecting a cutoff percentile or a standard deviation (SD) and 2) identifying a specific age at which the designated cutoff percentile is applied. For example, in our updated mouse frailty phenotype, cutoff criteria in males corresponded to values determined at 23 mo of age based on the lowest 20th percentile for activity level, endurance, strength, and walking speed, and the top 20th percentile for body weight (4). By using the values obtained at this percentile and age, mice at other ages were classified as non-frail, pre-frail, and frail, according to the number of positive frailty markers they possessed. See **FIGURE 4** for a general schematic of a mouse frailty assessment.

Selection of how stringent or liberal the cutoff percentile is will impact the prevalence of frailty. For example, our initial frailty phenotype used a strict cutoff of the lowest seventh percentile (40). Using this percentile, no mice were considered frail at 24 mo (33), and 9% were frail at 27–28 mo (40). In our more recent studies, we observed that the seventh percentile was too stringent and likely would underestimate the prevalence of frailty, as reported by Kane et al. (33). To address this potential underestimation, we used a more liberal cutoff of the 20th percentile, which corresponds to Fried's human model (17) and other mouse frailty phenotypes (20, 33). With the cutoff of the 20th percentile, we were able to accurately identify frail mice and predict mouse mortality (4, 36). To depict the impact of selecting different cutoff percentiles, we compared the 20th versus 7th percentiles in **FIGURE 2**.

The age at which to determine the cutoff values requires careful consideration. We found that it was important to consider the physical deficits observed among the mouse cohort, where the age falls on the mouse survival curve and how closely it equates to Fried's human model (17). As mentioned above, we initially selected mice at 27–28 mo of age (the only age group investigated); however, we recently published frailty cutoff values using 23 and 20 mo of age for male and female mice, respectively (4, 36). **FIGURE 3** highlights that measures of physical function begin to or have declined at ~20 mo of age. This is an important consideration because the age selected should represent a time when deficits in physical function can be seen in the mouse cohort but before these

deficits become too severe. Along with this reasoning, mouse survival rate requires consideration, since identification should occur before the frail mice begin to die. Furthermore, because frailty is thought to be reversible, the age of 23 and 20 mo for male and female mice also allows adequate time to implement possible life-changing interventions. Last, these ages within the C57BL/6 mouse strain are equal to ~60–75 human years (13, 31), which correspond to the initial age brackets assessed by Fried et al. (17).

To reiterate, selecting an appropriate cutoff percentile and identifying the age at which the cutoff is applied are essential to a successful frailty phenotype. We suggest that careful consideration go into both these variables to ensure the prevalence of frailty is not grossly underestimated (or overestimated). We and others have found that the 20th percentile cutoff in 17- to 24-mo-old C57BL/6 mice to be favorable (4, 20, 33, 36).

Sex Differences

Many age-related biochemical, physiological, and behavioral changes, including frailty, are already known to be sex-specific (39, 41, 50, 52). In fact, women have higher frailty index scores than men at all ages but paradoxically have a lower mortality risk, a concept termed the sex-frailty paradox (21, 22). These data suggest each sex may have different physiological reserves, and the link between health status and mortality is not necessarily fixed in humans. As with humans, many sex differences are also observed in mice (3, 5, 34, 63). Therefore, sex should be considered when developing and implementing a mouse frailty phenotype.

In our recent investigations, we compared male and female C57BL/6 mice by using our frailty phenotype (4, 36). These results revealed that the prevalence of frailty was greater in female mice but was age-dependent and only transient (5). However, regardless of sex, frail mice died at a similar rate after the frailty assessment and had comparable mean lifespans. In fact, nearly 50% of the frail male and female mice died within 6 mo after being identified as frail. It is essential to point out that, in our hands, male C57BL/6 mice lived ~3–4 mo longer than female C57BL/6 mice (mean lifespan; 31.8 versus 28.1 mo). This 13% difference therefore prompted us to adjust the age of cutoff value determination to 23 and 20 mo for male and female, respectively.

Another sex-related aspect of frailty we uncovered was that sex-specific anthropometric and physical functional measures had significant influences on the frailty cutoff values. For instance, although some frailty values were similar between sex, others appeared to be dependent on being male or female (5). Fried's human phenotype (17)

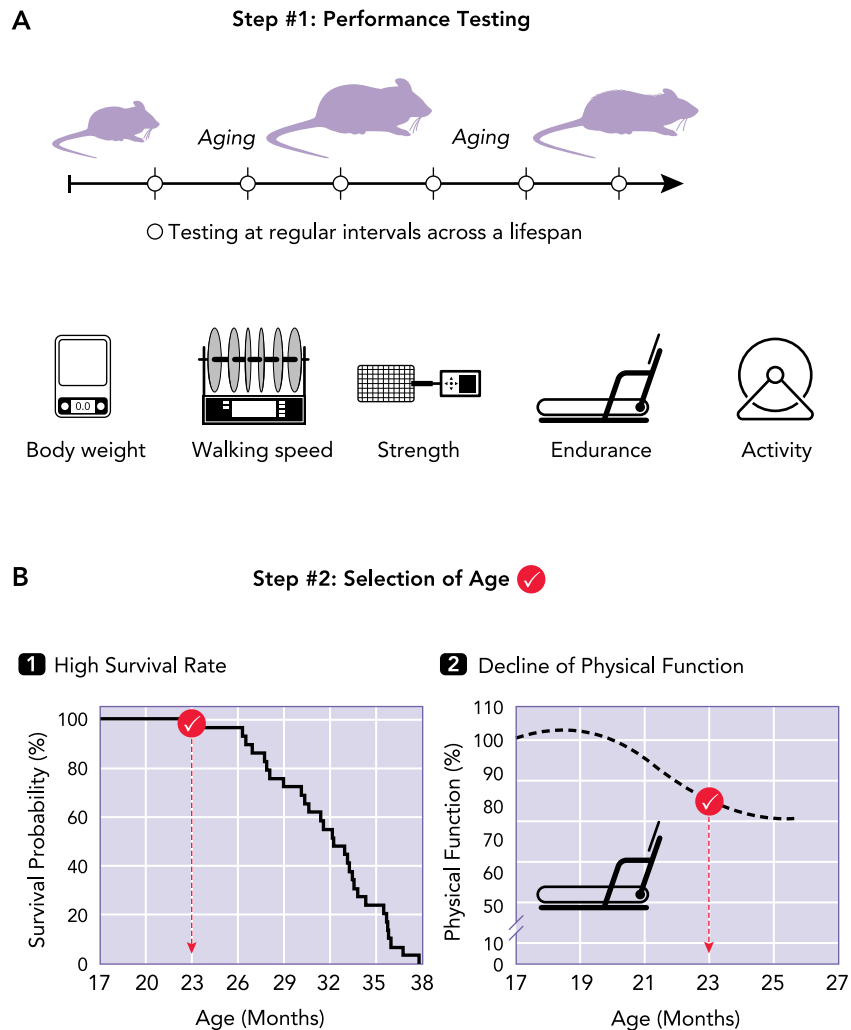


FIGURE 4. General schematic of a mouse frailty assessment

A: in Step #1, body weight and measures of physical function are assessed at regular testing intervals across a lifespan. Measures of physical function include walking speed, strength, endurance, and activity level as assessed using a rotarod, grip meter, motorized treadmill, and voluntary running wheel, respectively. B: during Step #2, selection of mouse age occurs based on (1) survival rate and (2) rate of age-related deficits in physical function (pictured is endurance). The age selected should correspond with an age with a high survival rate (i.e., prior to frail mice dying) and when deficits in physical function can be seen in the mouse cohort but prior to these deficits becoming too severe. We found that this age was ~23 mo for male C57BL/6 mice, as indicated by the check marks in the figure. (C and D continue on next page.) C: in Step #3, (1) cutoff values are determined for body weight and each measure of physical function using the 20th percentile from 23-mo-old mice, and then (2) mice are categorized as non-frail, pre-frail, and frail based on the number of positive frail markers. Each letter (e.g., B, T, Y) in (1) and (2) of Step #3 corresponds to a mouse ID for each individual mouse in the study cohort. D: last, in Step #4, discoveries regarding frailty characteristics can be made such as (1) the onset and prevalence of frailty and (2) mortality risk of frail mice. In the figure, we demonstrate in male C57BL/6 mice that the onset of prevalence of frailty occurred at ~17 mo of age, that frailty (and pre-frailty) increased with age, and that frail (and pre-frail) mice died early in life compared with non-frail mice.

acknowledged this by adjusting cutoff values according to sex, and Antoch et al. (3) alluded to this in mice with their physiological frailty assessment. Collectively, these studies suggest that males and females should be investigated separately (not grouped together).

Together, we recommend that sex be considered when frailty is assessed and emphasize that there are different ways to become frail—one of which may be sex. When designing a frailty study, being cognizant of the anthropometric, physical functional, and lifespan differences between males and females is critical. Because these variables have

significant sex differences, the identification of the frailty cutoff values should be based on sex. Last, because sex hormones fluctuate with age and are known to influence function (e.g., physical activity) (9, 23, 25), their impact on frailty identification requires consideration.

Forward Thinking

Frailty Domains

The frailty phenotype described in this review emphasizes the domain of physical frailty; however, other domains such as cognition and depression

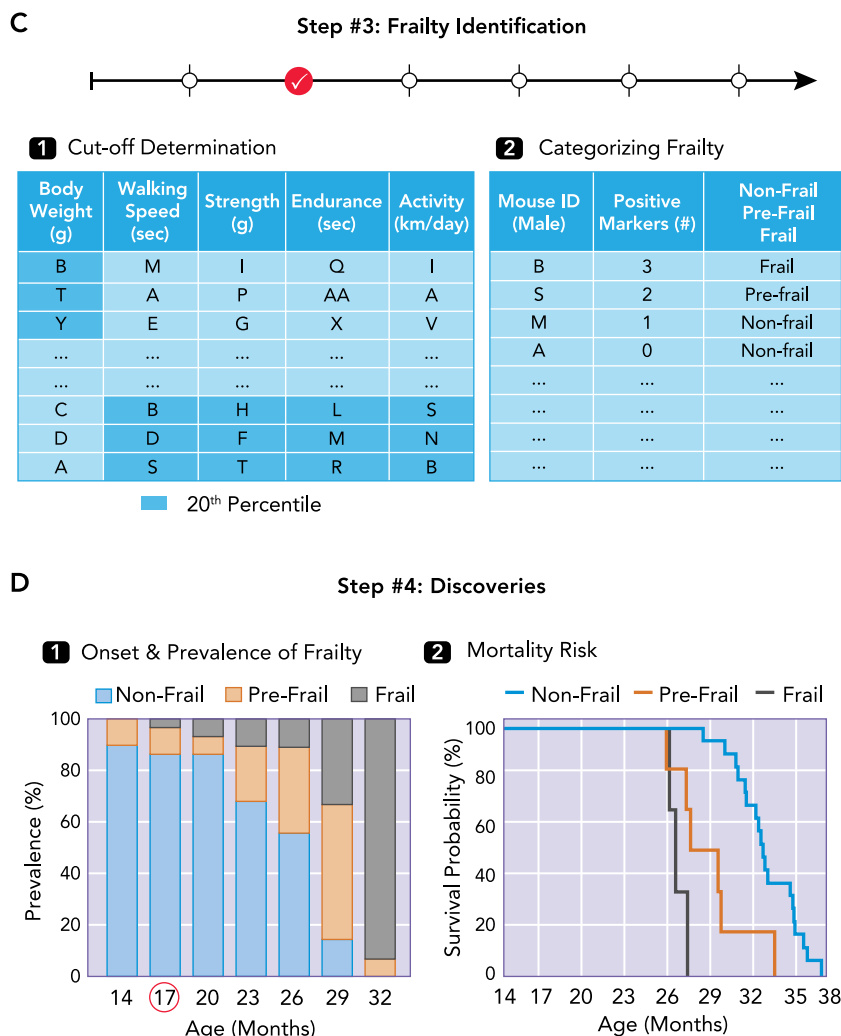


FIGURE 4. Continued

influence the status of frailty (53, 58, 62). Clinically, domains of cognitive frailty, social frailty, and psychological frailty (encompassing motivation and mood) also predict disability and mortality (2, 27). Among these different domains, cognitive frailty is increasingly recognized as a fundamental determinant of an individual's vulnerability and resilience to stressors (2). To date, preclinical assessments for frailty identification do not emphasize measures for cognition, depression, motivation, etc. Because these frailty domains share clinical characteristics with physical frailty (e.g., exhaustion), it is important to consider their impact on the assessment power or discriminative power of the mouse frailty phenotype in terms of identifying frailty onset, prevalence, and mortality risk. Another important consideration in preclinical assessments of frailty is to determine whether the presence of multiple frailty domains increases the risk for negative outcomes of frailty. Collectively, understanding the impact of these frailty domains may provide avenues for potentially reversing or preventing frailty.

Frailty States and Transitions

Frailty is recognized as a dynamic syndrome that exists on a continuum, in that movement can be bi-directional between the frailty states (i.e., non-frail, pre-frail, or frail) (19, 38). Characterizing the frailty states should ultimately provide an avenue by which to detect the underlying mechanisms, delineate their etiology, identify risks factors associated with frailty transitions (i.e., pre-frail to frail), and provide means for developing beneficial treatment strategies. Significant changes in physical function play a role in frailty states and transitions. Although we stated that frailty is recognized along a continuum, it is possible there is a threshold for physical function, i.e., a point at which frailty is no longer remediable or reversible. We believe further work with preclinical assessments has the potential to enhance our understanding of frailty states, their transitions, and the presence of a threshold for physical function.

Frailty and Multimorbidity

With age, there is an increase in multimorbidity (having two or more diseases), and its prevalence reaches 30% in people younger than 65 yr and ranges between 55 and 98% in people older than 65 yr (10). Describing the co-occurrence of frailty and multimorbidity, and assessing their association have the potential to further decipher the mechanisms governing both the aging and the frailty processes. Genetically modified mouse models have been developed to understand the etiology and treatment for numerous diseases (cardiovascular disease, Alzheimer's disease, various cancers, Type 2 diabetes mellitus) (15, 34, 35, 57, 60), and, now in parallel with the frailty phenotype assessment, it is possible to describe the physical health, the relationships between frailty progression and the specific disease, and the impact of interventions (35, 54). One caveat in genetically modified mouse models is that it is difficult to mimic the timeline of disease development and to assess the impact of the disease across the lifespan that are observed in humans. Although this caveat currently exists, the advancement of biomedical technology in preclinical mouse studies of multimorbidity and the assessments of frailty across the lifespan should help define not only associations but also causality among these chronic conditions and frailty.

Conclusions

To make significant advancements in the field of frailty, the well-established frailty phenotype developed by Fried et al. (17) was reverse-translated using male and female C57BL/6 mice. Using the mouse frailty phenotype that stresses measures of physical function, we found that frailty characteristics in mice parallel frailty characteristics observed in humans (4, 5, 36). By investigating frailty across the lifespan in mice, we report the onset of frailty occurs in mid- to late-adulthood, the prevalence of frailty increases with age, and mice identified as frail have a high mortality risk. These findings support the use of the mouse frailty phenotype to investigate the underlying mechanisms associated with frailty and the development of effective therapies. The journey in developing the mouse frailty phenotype has also yielded new concepts to consider, including heavy body weight as a frailty measure and implications when setting cut-off values for each frailty measure to determine positive frailty markers. ■

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C.W.B., D.K., and L.V.T. conceived and designed research; C.W.B. and D.K. performed experiments; C.W.B., D.K., and L.V.T. interpreted results of experiments; C.W.B., D.K., and L.V.T. prepared figures; C.W.B., D.K., and L.V.T. drafted manuscript; C.W.B. and L.V.T. edited and revised manuscript; C.W.B. and L.V.T. approved final version of manuscript; D.K. and L.V.T. analyzed data.

References

- Andreux PA, van Diemen MPJ, Heezen MR, Auwerx J, Rinsch C, Groeneveld GJ, Singh A. Mitochondrial function is impaired in the skeletal muscle of pre-frail elderly. *Sci Rep* 8: 8548, 2018. doi:10.1038/s41598-018-26944-x.
- Andrew MK, Mitnitski AB, Rockwood K. Social vulnerability, frailty and mortality in elderly people. *PLoS One* 3: e2232, 2008. doi:10.1371/journal.pone.0002232.
- Antoch MP, Wrobel M, Kuropatwinski KK, Gitlin I, Leonova KI, Toshkov I, Gleiberman AS, Hutson AD, Chernova OB, Gudkov AV. Physiological frailty index (PFI): quantitative in-life estimate of individual biological age in mice. *Aging (Albany NY)* 9: 615–626, 2017. doi:10.18632/aging.101206.
- Baumann CW, Kwak D, Thompson LV. Assessing onset, prevalence and survival in mice using a frailty phenotype. *Aging (Albany NY)* 10: 4042–4053, 2018. doi:10.18632/aging.101692.
- Baumann CW, Kwak D, Thompson LV. Sex-specific components of frailty in C57BL/6 mice. *Aging (Albany NY)* 11: 5206–5214, 2019. doi:10.18632/aging.102114.
- Beaudart C, Rolland Y, Cruz-Jentoft AJ, Bauer JM, Sieber C, Cooper C, Al-Daghri N, Araujo de Carvalho I, Bautmans I, Bernabei R, Bruyère O, Cesari M, Cherubini A, Dawson-Hughes B, Kanis JA, Kaufman JM, Landi F, Maggi S, McCloskey E, Petermans J, Rodríguez Mañas L, Reginster JY, Roller-Wirnsberger R, Schaap LA, Uebelhart D, Rizzoli R, Fielding RA. Assessment of muscle function and physical performance in daily clinical practice: a position paper endorsed by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). *Calcif Tissue Int* 105: 1–14, 2019. doi:10.1007/s00223-019-00545-w.
- Bisset ES, Howlett SE. The biology of frailty in humans and animals: understanding frailty and promoting translation. *Aging Med (Milton)* 2: 27–34, 2019. doi:10.1002/agm2.12058.
- Blaum CS, Xue QL, Michelon E, Semba RD, Fried LP. The association between obesity and the frailty syndrome in older women: the Women's Health and Aging Studies. *J Am Geriatr Soc* 53: 927–934, 2005. doi:10.1111/j.1532-5415.2005.53300.x.
- Cabelka CA, Baumann CW, Collins BC, Nash N, Le G, Lindsay A, Spangenburg EE, Lowe DA. Effects of ovarian hormones and estrogen receptor α on physical activity and skeletal muscle fatigue in female mice. *Exp Gerontol* 115: 155–164, 2019. doi:10.1016/j.exger.2018.11.003.
- Calderón-Larrañaga A, Vetrano DL, Onder G, Gimeno-Feliu LA, Coscollar-Santaliestra C, Carfi A, Pisciotto MS, Angleman S, Melis RJF, Santoni G, Mangialasche F, Rizzuto D, Welmer AK, Bernabei R, Prados-Torres A, Marengoni A, Fratiglioni L. Assessing and measuring chronic multimorbidity in the older population: a proposal for its operationalization. *J Gerontol A Biol Sci Med Sci* 72: 1417–1423, 2017.
- Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *Lancet* 381: 752–762, 2013. doi:10.1016/S0140-6736(12)62167-9.
- Collard RM, Boter H, Schoevers RA, Oude Voshaar RC. Prevalence of frailty in community-dwelling older persons: a systematic review. *J Am Geriatr Soc* 60: 1487–1492, 2012. doi:10.1111/j.1532-5415.2012.04054.x.
- Dutta S, Sengupta P. Men and mice: relating their ages. *Life Sci* 152: 244–248, 2016. doi:10.1016/j.ifs.2015.10.025.
- Economou AC. Taxonomic differences in the mammalian life span-body weight relationship and the problem of brain weight. *Gerontology* 26: 90–98, 1980. doi:10.1159/000212400.

15. Fielder E, Weigand M, Agneessens J, Griffin B, Parker C, Miwa S, von Zglinicki T. Sublethal whole-body irradiation causes progressive premature frailty in mice. *Mech Ageing Dev* 180: 63–69, 2019. doi:10.1016/j.mad.2019.03.006.
16. Fried LP, Ferrucci L, Darer J, Williamson JD, Anderson G. Untangling the concepts of disability, frailty, and comorbidity: implications for improved targeting and care. *J Gerontol A Biol Sci Med Sci* 59: 255–263, 2004. doi:10.1093/gerona/59.3.M255.
17. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA; Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 56: M146–M156, 2001. doi:10.1093/gerona/56.3.M146.
18. Gale CR, Cooper C, Sayer AA. Prevalence of frailty and disability: findings from the English Longitudinal Study of Ageing. *Age Ageing* 44: 162–165, 2015. doi:10.1093/ageing/afu148.
19. Gill TM, Gahbauer EA, Allore HG, Han L. Transitions between frailty states among community-living older persons. *Arch Intern Med* 166: 418–423, 2006. doi:10.1001/archinte.166.4.418.
20. Gomez-Cabrera MC, Garcia-Valles R, Rodriguez-Mañas L, Garcia-Garcia FJ, Olaso-Gonzalez G, Salvador-Pascual A, Tarazona-Santabalbina FJ, Viña J. A new frailty score for experimental animals based on the clinical phenotype: inactivity as a model of frailty. *J Gerontol A Biol Sci Med Sci* 72: 885–891, 2017. doi:10.1093/gerona/glw337.
21. Gordon EH, Hubbard RE. The pathophysiology of frailty: why sex is so important. *J Am Med Dir Assoc* 19: 4–5, 2018. doi:10.1016/j.jamda.2017.10.009.
22. Gordon EH, Peel NM, Samanta M, Theou O, Howlett SE, Hubbard RE. Sex differences in frailty: a systematic review and meta-analysis. *Exp Gerontol* 89: 30–40, 2017. doi:10.1016/j.exger.2016.12.021.
23. Gorzek JF, Hendrickson KC, Forstner JP, Rixen JL, Moran AL, Lowe DA. Estradiol and tamoxifen reverse ovariectomy-induced physical inactivity in mice. *Med Sci Sports Exerc* 39: 248–256, 2007. doi:10.1249/01.mss.0000241649.15006.b8.
24. Graber TG, Ferguson-Stegall L, Liu H, Thompson LV. Voluntary aerobic exercise reverses frailty in old mice. *J Gerontol A Biol Sci Med Sci* 70: 1045–1058, 2015. doi:10.1093/gerona/glu163.
25. Greising SM, Carey RS, Blackford JE, Dalton LE, Kosir AM, Lowe DA. Estradiol treatment, physical activity, and muscle function in ovarian-senescent mice. *Exp Gerontol* 46: 685–693, 2011. doi:10.1016/j.exger.2011.04.006.
26. Heinze-Milne SD, Banga S, Howlett SE. Frailty assessment in animal models. *Gerontology* 65: 610–619, 2019. doi:10.1159/000501333.
27. Holt-Lunstad J, Smith TB, Baker M, Harris T, Stephenson D. Loneliness and social isolation as risk factors for mortality: a meta-analytic review. *Perspect Psychol Sci* 10: 227–237, 2015. doi:10.1177/1745691614568352.
28. Howlett SE. Assessment of frailty in animal models. *Interdiscip Top Gerontol Geriatr* 41: 15–25, 2015. doi:10.1159/000381131.
29. Jantunen H, Wasenius N, Salonen MK, Perälä MM, Osmond C, Kautiainen H, Simonen M, Pohjolainen P, Kajantie E, Rantanen T, von Bonsdorff MB, Eriksson JG. Objectively measured physical activity and physical performance in old age. *Age Ageing* 46: 232–237, 2017.
30. Justice JN, Carter CS, Beck HJ, Gioscia-Ryan RA, McQueen M, Enoka RM, Seals DR. Battery of behavioral tests in mice that models age-associated changes in human motor function. *Age (Dordr)* 36: 583–592, 2014. doi:10.1007/s11357-013-9589-9.
31. Justice JN, Cesari M, Seals DR, Shively CA, Carter CS. Comparative approaches to understanding the relation between aging and physical function. *J Gerontol A Biol Sci Med Sci* 71: 1243–1253, 2016. doi:10.1093/gerona/glv035.
32. Kane AE, Hilmer SN, Mach J, Mitchell SJ, de Cabo R, Howlett SE. Animal models of frailty: current applications in clinical research. *Clin Interv Aging* 11: 1519–1529, 2016. doi:10.2147/CIA.S105714.
33. Kane AE, Huizer-Pajkos A, Mach J, Mitchell SJ, de Cabo R, Le Couteur DG, Howlett SE, Hilmer SN. A comparison of two mouse frailty assessment tools. *J Gerontol A Biol Sci Med Sci* 72: 904–909, 2017. doi:10.1093/gerona/glx009.
34. Kane AE, Shin S, Wong AA, Fertan E, Faustova NS, Howlett SE, Brown RE. Sex differences in healthspan predict lifespan in the 3xTg-AD mouse model of Alzheimer's disease. *Front Aging Neurosci* 10: 172, 2018. doi:10.3389/fnagi.2018.00172.
35. Keller K, Kane A, Heinze-Milne S, Grandy SA, Howlett SE. Chronic treatment with the ACE inhibitor enalapril attenuates the development of frailty and differentially modifies pro- and anti-inflammatory cytokines in aging male and female C57BL/6 mice. *J Gerontol A Biol Sci Med Sci* 74: 1149–1157, 2019. doi:10.1093/gerona/gly219.
36. Kwak D, Baumann CW, Thompson LV. Identifying characteristics of frailty in female mice using a phenotype assessment tool. *J Gerontol A Biol Sci Med Sci* 75: 640–646, 2020. doi:10.1093/gerona/glz092.
37. Landi F, Calvani R, Tosato M, Martone AM, Bernabei R, Onder G, Marzetti E. Impact of physical function impairment and multimorbidity on mortality among community-living older persons with sarcopenia: results from the iSIRENTE prospective cohort study. *BMJ Open* 6: e008281, 2016. doi:10.1136/bmjopen-2015-008281.
38. Lang PO, Michel JP, Zekry D. Frailty syndrome: a transitional state in a dynamic process. *Gerontology* 55: 539–549, 2009. doi:10.1159/000211949.
39. Le Couteur DG, Anderson RM, de Cabo R. Sex and aging. *J Gerontol A Biol Sci Med Sci* 73: 139–140, 2018. doi:10.1093/gerona/glx221.
40. Liu H, Graber TG, Ferguson-Stegall L, Thompson LV. Clinically relevant frailty index for mice. *J Gerontol A Biol Sci Med Sci* 69: 1485–1491, 2014. doi:10.1093/gerona/glt188.
41. López-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 153: 1194–1217, 2013. doi:10.1016/j.cell.2013.05.039.
42. Martinez de Toda I, Garrido A, Vida C, Gomez-Cabrera MC, Viña J, De la Fuente M. Frailty quantified by the “Valencia Score” as a potential predictor of lifespan in mice. *J Gerontol A Biol Sci Med Sci* 73: 1323–1329, 2018. doi:10.1093/gerona/gly064.
43. Metter EJ, Talbot LA, Schrager M, Conwit R. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. *J Gerontol A Biol Sci Med Sci* 57: B359–B365, 2002. doi:10.1093/gerona/57.10.B359.
44. Miller MG, Thangthaeng N, Shukitt-Hale B. A clinically relevant frailty index for aging rats. *J Gerontol A Biol Sci Med Sci* 72: 892–896, 2017. doi:10.1093/gerona/glw338.
45. Miller RA, Chrisp C, Atchley W. Differential longevity in mouse stocks selected for early life growth trajectory. *J Gerontol A Biol Sci Med Sci* 55: B455–B461, 2000. doi:10.1093/gerona/55.9.B455.
46. Miller RA, Harper JM, Galecki A, Burke DT. Big mice die young: early life body weight predicts longevity in genetically heterogeneous mice. *Ageing Cell* 1: 22–29, 2002. doi:10.1046/j.1474-9728.2002.00006.x.
47. Mitnitski AB, Mogilner AJ, Rockwood K. Accumulation of deficits as a proxy measure of aging. *ScientificWorldJournal* 1: 323–336, 2001. doi:10.1100/tsw.2001.58.
48. Morley JE, Vellas B, van Kan GA, Anker SD, Bauer JM, Bernabei R, Cesari M, Chumlea WC, Doehner W, Evans J, Fried LP, Guralnik JM, Katz PR, Malmstrom TK, McCarter RJ, Gutierrez Robledo LM, Rockwood K, von Haehling S, Vandewoude MF, Walston J. Frailty consensus: a call to action. *J Am Med Dir Assoc* 14: 392–397, 2013. doi:10.1016/j.jamda.2013.03.022.
49. Parks RJ, Fares E, Macdonald JK, Ernst MC, Sinal CJ, Rockwood K, Howlett SE. A procedure for creating a frailty index based on deficit accumulation in aging mice. *J Gerontol A Biol Sci Med Sci* 67: 217–227, 2012. doi:10.1093/gerona/glr193.
50. Pomatto LCD, Tower J, Davies KJA. Sexual dimorphism and aging differentially regulate adaptive homeostasis. *J Gerontol A Biol Sci Med Sci* 73: 141–149, 2018. doi:10.1093/gerona/glx083.
51. Reiman MP, Manske RC. The assessment of function: How is it measured? A clinical perspective. *J Manual Manip Ther* 19: 91–99, 2011. doi:10.1179/106698111X12973307659546.
52. Roberts BM, Lavin KM, Many GM, Thalacker-Mercer A, Merritt EK, Bickel CS, Mayhew DL, Tuggle SC, Cross JM, Kosek DJ, Petrella JK, Brown CJ, Hunter GR, Windham ST, Allman RM, Bamman MM. Human neuromuscular aging: sex differences revealed at the myocellular level. *Exp Gerontol* 106: 116–124, 2018. doi:10.1016/j.exger.2018.02.023.
53. Robertson DA, Savva GM, Kenny RA. Frailty and cognitive impairment—a review of the evidence and causal mechanisms. *Ageing Res Rev* 12: 840–851, 2013. doi:10.1016/j.arr.2013.06.004.
54. Said SA, Isedowo R, Guerin C, Nar NN, Lillie L, Bukovac S, Simone JJ, Green MR, McCormick CM, Stuart JA. Effects of long-term dietary administration of estrogen receptor-beta agonist diarylpropionitrile on ovariectomized female ICR (CD-1) mice. *Geroscience* 40: 393–403, 2018. doi:10.1007/s11357-018-0038-7.
55. Samorajski T, Delaney C, Durham L, Ordy JM, Johnson JA, Dunlap WP. Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. *Neurobiol Aging* 6: 17–24, 1985. doi:10.1016/0197-4580(85)90066-1.
56. Seldeen KL, Lasky G, Leiker MM, Pang M, Persooinus KE, Troen BR. High intensity interval training improves physical performance and frailty in aged mice. *J Gerontol A Biol Sci Med Sci* 73: 429–437, 2018. doi:10.1093/gerona/glx120.
57. Sikka G, Miller KL, Steppan J, Pandey D, Jung SM, Fraser CD III, Ellis C, Ross D, Vandegaer K, Bedja D, Gabrielson K, Walston JD, Berkowitz DE, Barouch LA. Interleukin 10 knockout frail mice develop cardiac and vascular dysfunction with increased age. *Exp Gerontol* 48: 128–135, 2013. doi:10.1016/j.exger.2012.11.001.
58. Soysal P, Veronese N, Thompson T, Kahl KG, Fernandes BS, Prina AM, Solmi M, Schofield P, Koyanagi A, Tseng PT, Lin PY, Chu CS, Cosco TD, Cesari M, Carvalho AF, Stubbs B. Relationship between depression and frailty in older adults: a systematic review and meta-analysis. *Ageing Res Rev* 36: 78–87, 2017. doi:10.1016/j.arr.2017.03.005.
59. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, Brach J, Chandler J, Cawthon P, Connor EB, Nevitt M, Visser M, Kritchevsky S, Badinelli S, Harris T, Newman AB, Cauley J, Ferrucci L, Guralnik L. Gait speed and survival in older adults. *JAMA* 305: 50–58, 2011. doi:10.1001/jama.2010.1923.

60. Todorovic S, Loncarevic-Vasiljkovic N, Jovic M, Sokanovic S, Kanazir S, Mladenovic Djordjevic A. Frailty index and phenotype frailty score: sex- and age-related differences in 5XFAD transgenic mouse model of Alzheimer's disease. *Mech Ageing Dev* 185: 111195, 2020. doi:[10.1016/j.mad.2019.111195](https://doi.org/10.1016/j.mad.2019.111195).
61. Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. *J Gerontol A Biol Sci Med Sci* 54: B492–B501, 1999. doi:[10.1093/gerona/54.11.B492](https://doi.org/10.1093/gerona/54.11.B492).
62. Wallace LMK, Theou O, Godin J, Andrew MK, Bennett DA, Rockwood K. Investigation of frailty as a moderator of the relationship between neuropathology and dementia in Alzheimer's disease: a cross-sectional analysis of data from the Rush Memory and Aging Project. *Lancet Neurol* 18: 177–184, 2019. doi:[10.1016/S1474-4422\(18\)30371-5](https://doi.org/10.1016/S1474-4422(18)30371-5).
63. Whitehead JC, Hildebrand BA, Sun M, Rockwood MR, Rose RA, Rockwood K, Howlett SE. A clinical frailty index in aging mice: comparisons with frailty index data in humans. *J Gerontol A Biol Sci Med Sci* 69: 621–632, 2014. doi:[10.1093/gerona/glt136](https://doi.org/10.1093/gerona/glt136).