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Frailty Assessment in Animal Models

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Abstract
Although frailty has been extensively investigated for the last 2 decades, preclinical models of frailty have only been developed over the past decade. Frailty is a concept that helps to explain the difference between chronologic age and biologic age and to discuss health span along with lifespan. In general, a frail individual will be more susceptible to adverse health outcomes than a healthy, nonfrail individual of the same age. However, the biology and mechanisms of frailty are still unclear. The development of preclinical models of frailty and frailty assessment tools are invaluable to geriatric research. This review briefly describes the concept of frailty and discusses the newly developed animal models of frailty, specifically the frailty phenotype- and frailty index-based models. Mouse models are the most common models for preclinical frailty research, but rat and canine models for frailty assessment have also been developed. These models can facilitate the testing of frailty-specific treatments and help to investigate the effects of various interventions on frailty. Similarities and differences between human and animal models, including sex differences in frailty, are also discussed. The availability of animal models of frailty is a valuable and welcome addition to the study of frailty, aging, or the disorders of old age and will enable a better understanding of frailty mechanisms.

Introduction to Frailty

How and why people "age" at different rates continues to elude us. It has long been known that people and animals develop health deficits at different rates as they age. Some individuals simply become frail faster than others, although the reasons for this are not well understood and may involve several mechanisms. In recent years, the interest in understanding and quantifying frailty has grown. This is, in part, due to the aging population in many developed countries. The prevalence of frail older people increases with age (i.e., >80 years) around the globe [1]. This is problematic for healthcare since frail individuals are at an increased risk of morbidity/mortality [2]. Frailty has no internationally accepted definition; however, it has been defined as an increased susceptibility to adverse health outcomes [3]. It is associated with age and disease, although neither is necessary for frailty to occur [4]. The biology of frailty is not yet well understood, although proposed mechanisms include increased oxidative stress, DNA repair malfunction, chronic inflammation, cellular senescence, endocrine dysfunction, and mitochondrial damage [4, 5]. Overall it is thought that frailty lowers physiologic reserves, which predisposes a frail person to a worse reaction to health challenges than a nonfrail person.

Frailty has been studied in humans increasingly since the early 2000s. The breakthrough into studying frailty as a clinical condition was the development of tools to quantify frailty. The 2 dominant methods to measure frailty in

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humans are the frailty phenotype (FP) proposed by Fried et al. [6] and the frailty index (FI) by Rockwood and colleagues [7]. In brief, the FP approaches frailty as a syndrome and evaluates an individual's frailty based on 5 key areas: unintended weight loss, exhaustion, weakness, slow walking speed, and low physical activity [6]. If an individual scores well on all of the categories, they are considered robust; if they score poorly on 1 or 2, they are considered prefrail; and if they score poorly on 3 or more categories, they are considered frail. On the other hand, the FI approaches frailty in a manner of deficit accumulation. It differs from the FP by grading frailty on a continuum rather than categorically. It is calculated by dividing the number of health deficits present in an individual by the total number evaluated. This yields a value between 0 and 1 to provide an "index" of frailty. Higher FI values are associated with an increased susceptibility to disease [8]. A strength of the FI approach is that there is no ceiling effect. For example, older individuals do not live long after accumulating enough deficits to achieve a score of ~0.7 [9]. This contrasts with the FP approach that categorizes individuals into broader groups and may be susceptible to a ceiling effect without recalibration [10]. In sum, several frailty assessment tools have been created to quantify frailty in people. Despite some differences in deciding who is frail, all assessments show that frailty increases nonlinearly with age, and increased frailty is associated with morbidity and mortality [11]. Both the FP and the FI approaches have strengths and limitations and are commonly used to measure frailty in humans. This review adds to the literature by giving an up-to-date assessment of frailty tools used in animals (not just rodents, which was done recently [12]) and by highlighting strengths and limitations of each assessment strategy. Finally, sex differences found in animal models of frailty are also discussed.

Assessing Frailty in Animal Models

Although the notion of frailty as a clinical concept was established in the early 2000s, the idea of measuring frailty in animals did not emerge until a decade later. Significant advancements in clinical frailty assessment with both the FP [6] and the FI [7] created questions surrounding how and why people become frail. This motivated the development of preclinical models to help answer these questions. Rodent models of aging have been extremely useful in studying aging processes due to the animals' relatively short life compared with humans and because of the ability to study their biology in detail. The growing interest in exploring frailty in preclinical models has paved the way to move the clinical FP and FI tools from the "bedside to the bench." The following sections will briefly detail the development of several preclinical methods of studying frailty in animal models [13], including FP- and FI-based approaches.

FP Approaches

The FP assessment developed by Fried et al. [6] has been modified for use in animal models. For the purposes of this review and to relate to the clinical assessment of frailty with the FP, any frailty assessment based on the human FP approach will be labelled a preclinical FP model (rather than an index, as sometimes referred to in the literature). The FP approach was first applied to 27- to 28-month-old wild-type C57BL/6 male mice by Liu et al. [14]. They based their frailty assessment on the FP approach and designed a clinically relevant FP assessment in mice. They assessed grip strength (via an inverted grip test), walking speed (rotarod test), physical activity (voluntary wheel running), and endurance (grip strength + rotarod test; see Table 1 for a summary). Animals are scored as nonfrail, mildly frail, or frail based on how many criteria are greater than 1.5 SD below the cohort's mean. This method was based on an earlier FP-based approach, developed by the same group, called the neuromuscular health span measure (Table 1). This latter more invasive approach measured muscular force production and functional performance to reflect the degree of sarcopenia [15]. Mice (C57BL/6) had their grip strength and walking speed tested using the same methods as Liu et al. [14], along with an in vitro muscle contraction test of the extensor digitorum longus. The strengths of these approaches are that there are clear cut-off values for the FP measurements and the neuromuscular health span reduces individual variability by combining the scores relative to the mean and predicted values as determined by multiple linear regression. However, weight loss is not considered in either model and, despite mathematical strengths of combining grip strength and walking speed to measure endurance, this may add measurement bias. The authors acknowledge that running to exhaustion may therefore be a better measure of endurance [14]. In addition, nonphysical frailty measurements, including blindness or deafness, are not assessed using this method. The use of within-group comparisons to derive frailty also limits its use when comparing between studies/interventions.

Table 1. Frailty assessment models or tools in animals based on the FP approach

Basis for the model	Species	Sex	Species/strain	Frailty assessment	Strengths	Limitations	Reference
IL-10 knockout to mimic the FP (weight loss, weakness, low activity, muscle changes, inflammation)	Mouse	Female	IL-$10^{tm/tm}$ on C57BL/6 background	Not conducted	Can be used to investigate biological mechanisms of frailty	Does not model frailty in natural aging; models Crohn's disease; specific housing requirements	20
Neuromuscular health span scoring system to assess physical function and sarcopenia	Mouse	Male	C57BL/6	Functional assessment based on rotarod, inverted cling grip strength and *in vitro* muscle contractility	Combined score (grip strength, rotorod and contractility) reduces individual variability within groups	Focus on physical frailty only; specialized equipent required; invasive procedure; time-consuming	15
FP (4 factors): grip strength, slow walking speed, low physical activity, endurance	Mouse	Male	C57BL/6	Inverted cling grip strength (weakness), rotarod (walking speed), voluntary wheel running (activity), grip test plus rotarod (endurance)	Evaluation is noninvasive; cut-off values to assess mice as frail or nonfrail	Focus on physical frailty; specialized equipment; no weight loss factor; time-consuming; derived endurance factor	14
FP (5 factors): grip strength, running time, weight loss, running speed, motor coordination	Mouse	Female	ICR/CDI	Grip strength (weakness), treadmill running time (endurance), weight loss, treadmill running speed (slowness), tight rope (activity)	Evaluation is noninvasive; cut-off values to assess mice as frail or nonfrail	Focus on physical frailty only; specialized equipment required	17
FP (4 factors): strength, speed, physical activity, endurance	Rat	Male	Fischer 344	Forelimb wire suspension (strength), rotorod (speed), open field (physical activity), inclined screen (endurance)	Evaluation is noninvasive; cut-off values to assess mice as frail or nonfrail	Focus on physical frailty; specialized equipment; no weight loss factor; time-consuming	18
FP (5 factors): weakness, exhaustion, chronic undernutrition, low physical activity, poor mobility	Dog	Both sexes, neutered	Mostly golden and abrador retrievers or crossbreed	Low muscle mass (weakness), fatigue/ breathlessness (exhaustion), poor body condition (undernutrition), low perceived activity (activity) gait abnormalities/joint pain (mobility)	Evaluation is noninvasive; can be completed as part of routine veterinary care	Focus on physical frailty only; subjective criteria	19

A different approach to measure frailty, called the Valencia score (Table 1) [16], has been developed based on the original human FP [6]. It measures weight loss, endurance, slowness, weakness, and activity by using a grip strength test (peak force while pulling a bar) and an incremental/graded treadmill test. The lowest 20th percentile in grip strength, running time, and running speed are considered frail based on those criteria. Mice that lost >5% body weight or failed a tightrope test were also considered frail. The Valencia score is calculated by dividing the number of failed tests by the total tests in each of the 5 categories. This frailty score is a relevant predictor of life span, and physical activity can attenuate frailty measured with this score [16, 17]. The strength of this method is that it provides clear definitions of frailty assessment and it is noninvasive, although nonphysical attributes of frailty are not assessed. As with the other FP methods, the interpretation of frailty is based on the cohort measured, which limits comparability.

The mouse FP has also been adapted for use in Fisher 344 rats [18] using the same 4 physical assessments employed by Liu et al. [14]. Rats were considered frail if they scored below the 20th percentile in 3 or more measures, mildly frail if they were below the 20th percentile in 2 measures, and nonfrail if they scored below the 20th percentile in fewer than 2 tests. Miller et al. [18] assessed 133 aged (17-month-old) rats using this FP technique and determined that 17% were mildly frail and 2% were frail. Similar to the other FP approaches, this method is useful given its straightforward cut-offs for frailty and its noninvasive nature. It is worth noting that this method also requires specialized equipment (i.e., a treadmill) and is time-consuming. As for the FP approach designed by Liu et al. [14] this technique is limited in that rats are classified as frail relevant to their own cohort, and thus comparison between studies is difficult.

The FP assessment has also branched out from rodent models. Hua et al. [19] created an FP assessment for dogs (Table 1). They followed 116 guide dogs (typically golden or Labrador retrievers or crossbreeds, all spayed or neutered) from birth to death using a 5-item FP approach. It evaluated muscular weakness, exhaustion, activity levels, nutrition, and mobility through surrogate measures, including a clinical geriatric health evaluation scoresheet. This categorized frailty-related items as either "present" or "absent" in the dog. Dogs that scored present on 2 or more of the 5 categories were considered to have a frailty-related phenotype. Dogs with 1 or fewer categories were considered not to have a frailty-related phenotype. The median follow-up time from the clinical geriatric assessment to death was 4.4 years. They showed that dogs with a frailty-related phenotype using this measurement were more likely to die than less frail dogs (adjusted HR = 3.9; 95% CI 1.4–10.9 [18]).

Overall these assessments using the FP approach are physical in nature and enable comparison with the predominantly physical frailty assessed in humans with the FP [6]. However, these measurements fail to account for other important signs or symptoms of frailty, including cognition, body composition, ocular deficits, etc. As stated by Liu et al. [14], these other aspects of frailty are important to consider. In addition, these tests require specialized equipment, and the muscle isolation for the neuromuscular health span scoring is clearly invasive. The FP approaches for animal models are therefore useful for comparison to physical frailty in humans, although they lack a multisystem approach to frailty.

FP in Genetically Altered Mice

Genetic modification has been used previously to study a variety of age-related mechanisms, and several genetic models of frailty have been developed. In 2008, Walston et al. [20] repurposed a C57BL/6-based homozygous interleukin (IL)-10 knockout mouse model (IL-$10^{tm/tm}$) to study frailty based on FP. Although it was originally developed as a model of inflammatory bowel disease, they found that IL-$10^{tm/tm}$ mice remained disease free when kept under sterile conditions and rapidly developed several characteristics of aging (i.e., decreased muscle mass and inflammation). The premise of the model is that, without IL-10, there is a subsequent increase in nuclear factor-κB, which mediates several age-associated inflammatory cytokines [20]. IL-$10^{tm/tm}$ mice have increased levels of the aging-associated cytokine IL-6 and many alterations in skeletal muscle gene expression related to mitochondrial function and cellular apoptosis at 12 months but not at 2 months of age [20]. The IL-$10^{tm/tm}$ knockout mice also declined in muscle strength faster than age- and sex-matched controls. In practice, these mice show several phenotypic signs of aging including poor muscular strength [21] and impaired cardiovascular health [22]. These findings suggest that an IL-$10^{tm/tm}$ mouse could model the multisystemic manifestations of frailty. However, some limitations exist when using this model. For example, the removal of IL-10 may lead to unforeseen differences in the biology of frailty in these mice versus naturally aging mice (i.e., apoptotic pathways [23]). Furthermore, these mice have not been characterized as frail using known frailty assessment tools (i.e., the FI). This model therefore exhibits physiologic and physical changes that accelerate frailty-like symptoms but lacks formal characterization of frailty and may not exclusively use frailty pathways associated with "natural" frailty progression. Further work with this model is needed to establish this.

Another genetically altered model that has recently been proposed for frailty research is the Cu/Zn superoxide dismutase knockout mouse [24]. This model is also based on the FP approach. Otherwise known as the Sod-1KO mouse, it exhibits several phenotypical features of frailty such as weight loss, low physical activity, weakness, and exhaustion [25]. These mice also develop increased inflammation and sarcopenia, which are related to frailty in humans. There is also evidence that Sod1KO mice may become frail through proposed mechanisms of aging, including increased oxidative stress, mitochondrial dysfunction, and cellular senescence [24]. Furthermore, caloric restriction has been shown to delay/prevent frailty in this model similarly to what has been reported in wild

type C57BL/6 mice [25]. Thus, this model provides a multisystemic approach to study physical frailty although, as with the IL-$10^{tm/tm}$ mice, it is not yet clear whether these pathways are representative of naturally aging/frail mice.

Other genetically modified mouse models of aging may provide additional insight into frailty. Although they have not been investigated, the mouse models of human Werner and Hutchinson-Gilford syndromes display premature aging characteristics [26]. However, the same mechanistic limitations presented with IL-$10^{tm/tm}$ and Sod1KO mice are present. In sum, both the IL-$10^{tm/tm}$ and the Sod1KO mice provide genetically modified mouse models for studying frailty. Both may be important in uncovering the biology of frailty, although neither may accurately represent the multiple factors that contribute to naturally occurring frailty in humans.

FI Approaches

The FI approach is unlike the FP approach in that it assesses many bodily systems to derive a whole-body measure of frailty. It rates frailty on a continuum from 0 and 1 using deficit accumulation rather than a categorical classification. The FI approach was first applied to an animal model (C57BL/6 mice) in 2012 by Howlett and colleagues using 31 possible health deficits spanning four categories (activity levels, hemodynamic measures, body composition, and basic metabolic status) (Table 2) [27]. Each variable was scored using a graded scale determined by how many SD away from the mean the mouse's score was. If the score was 1 SD different than the reference mean, it would be given a frailty value of 0.25 for that health deficit (where 0 is nonfrail). Two SD from the mean scored a value of 0.5, 3 SD scored 0.75, and >3 SD scored 1.00. All 31 health deficit measures were added and divided by the total number of deficits assessed to determine the mouse's FI score between 0 and 1. This FI tool was sensitive enough to identify frailty despite a small sample size. Furthermore, it provided a measure of frailty based on many different health parameters. The major limitations of this tool are its time-consuming nature, invasiveness (blood draws), the requirement of specialized equipment, and the lack of cognitive assessment. To simplify the procedure, an 8-item FI measure was created by the same group [27]. Seven items were activity based and the eighth item measured weight. This method was sensitive enough to detect frailty with age in male mice and it was less time consuming. However, this method only measures a small number of variables related to physical frailty and thus risks a ceiling effect. Frailty values obtained from this method were also highly variable. A limitation to both the 31- and the 8-item FI was their reliance on intragroup distributions to determine frailty. This makes between-study comparisons more difficult. Thus, further development of the mouse FI was needed.

To simplify frailty assessment, the mouse "clinical FI" was created by Howlett and colleagues (Table 2) [28]. The clinical FI is noninvasive as, along with assessing musculoskeletal health, it considers the integument, vestibulocochlear/auditory systems, ocular/nasal systems, digestive/urogenital systems, respiratory systems, and general signs of discomfort. Each deficit is assigned a 0, 0.5, or 1 based on the severity of the deficit. Using this method C57BL/6 mice demonstrated a graded increase in clinical FI scores between 5, 19, and 28 months [28]. A strength of this method is its ability to model frailty in aging humans. During a comparison study the FI scores closely modeled the FI deficit trends in older humans [9]. Another strength of the clinical FI is that it has a high interrater reliability [29, 30]. For example, 233 C57BL/6 mice aged 11.5–14 months had their frailties assessed and compared between raters. FI scores were comparable regardless of rater (p = 0.802) and differences between raters on individual deficit interpretations were ameliorated by refining techniques throughout the study (ICC = 0.77) [29]. Kane et al. [30] also found that the FI score had a high interrater reliability when assessing 45 mice across 4 people, i.e., 2 experienced and 2 inexperienced raters. They reported an ICC of 0.80 across all 4 raters, which remained high when split between rater experience (ICC = 0.76) and rater sex (ICC = 0.86) [29]. However, professional backgrounds have been identified as a major source of variation between assessors, which needs to be considered [31]. Since its inception, the clinical FI was modified slightly to create a similar 27-item clinical FI by another group [32]. The clinical FI approach therefore is adaptable, reliable between raters, noninvasive, and simple, and it considers factors outside of physical frailty, which makes it a useful tool for evaluating frailty in mice.

Interestingly, the FI and FP approaches categorize different mice as frail. Kane et al. [33] directly compared the FP [14] and FI [28] approaches in C57BL/6 mice and found that there was a 50% agreement between the 2 methods. Thus, deciding on either approach depends on the model of frailty used (i.e., naturally aging wild type or a genetic model of frailty) and the study's primary outcomes (e.g., physical fitness).

The robust nature of having many possible deficits to create an index lends itself well to adaptation. In addition to mice, the clinical FI approach has been modified for

Table 2. Use of the FI to assess frailty in animals

Species/ strain	Sex	Frailty assessment	Frailty scoring	Strengths	Limitations	Reference
Mouse, C57BL/6	Both sexes	Score 31 health-related deficits based on activity levels (open field), body composition (DEXA scan) hemodynamics (blood pressure), and metabolism (blood tests)	Deficits coded by number of SD from mean values in young adults, with >1 SD difference coded as 0.25 and >4 SD coded as 1.0; FI score calculated as deficits in an individual divided by all deficits	Assesses age-related deficit accumulation across a variety of health parameters	Does notassess cognitive function; invasive procedures (large blood samples); specialized equipment	27
Mouse, C57BL/6	Both sexes	Abbreviated 8-item frailty index from Parks et al. [27] based on activity levels (open field) only	Deficits coded by number of SD from mean values in young adults, with >1 SD difference coded as 0.25 and >4 SD coded as 1.0; FI score calculated as deficits in an individual divided by all deficits	Relatively easy to use	Focus on physical frailty only; specialized equipment required	27
Mouse, C57BL/6	Mostly female	Score 31 clinical measures across many systems including integument, ocular, nasal, musculoskeletal, digestive, urogenital, respiratory and vestibular systems	Deficits coded with a simple scale; a score of 0 indicates no deficit, a score of 0.5 indicates a mild deficit, and a score of 1 designates a severe deficit	Assesses deficits across many systems; convenient; rapid to administer; noninvasive	Does not assess cognitive function	28
Rat, Fischer 344	Male	Modified from whitehead et al. [28] and adapted to rats; score deficits based on 27 clinical measures across many systems	Deficits coded with a simple scale; a score of 0 indicates no deficit, a score of 0.5 indicates a mild deficit, and a score of 1 designates a severe deficit	Assesses deficits across many systems; convenient; rapid to administer; noninvasive	Does not assess cognitive function	34
Mouse, C57BL/6	Both sexes	Slight modification of the tool developed by Whitehead et al. [28]; score deficits based on 27 clinical measures	Deficits coded with a simple scale; a score of 0 indicates no deficit, a score of 0.5 indicates a mild deficit, and a score of 1 designates a severe deficit	Assesses deficits across many systems; convenient; rapid to administer; noninvasive	Does not assess cognitive function	32
Mouse, C57BL/6	Both sexes	Modified from Parks et al. [27]; a 23-item FI-lab was created from blood pressure, metabolism (blood tests), and echocardiography	Deficits coded based on deviation from mean values for young adult mice; values within ±1.5 SD of the mean were scored as 0 (no deficit) and values above or below the cut off were scored as 1 (deficit)	Assesses deficits across many systems	Does not assess cognitive function; focus on cardiac deficits; special equipment needed	35

DEXA, dual-energy x-ray absorptiometry.

use in male Fisher 344 rats (Table 2) [34]. It closely models the clinical FI for mice [28] and measures 27 health deficits in 9 categories (integument, physical/musculoskeletal, vestibulocochlear/auditory, ocular/nasal, neurological, digestive/urogenital, respiratory, pain/discomfort, and other [34]). Similar to mice, deficits are scored as 0, 0.5, or 1 based on the absence or presence of mild or severe deficits. Notably, this model was developed using rats aged 6–21 months. Given that Fisher 344 rats can live longer than this, the authors noted that it would be interesting to investigate frailty in even older animals [34]. Furthermore, reliability studies and studies using female rats, as have been completed for the mouse clinical FI, would strengthen its validity [29, 30]. This approach is limited in that it does not include a cognitive assessment. Nonetheless, because rats are often used in behavioral

and aging studies, this translation into Fisher 344 rats is an important step in enabling frailty research in other preclinical models.

A clinical measures/biochemical approach has also been developed whereby an FI assessment was created for C57BL/6 mice based solely on laboratory measurements of health deficits [35]. This was inspired by an FI technique in humans called the FI-lab that creates an FI based on common laboratory measurements [36]. The FI-lab in mice considers 23 items including blood pressure, metabolic markers (i.e., serum cytokine concentrations), and echocardiographic measurements [35]. Briefly, younger mice (~12 months old) are used to collect reference values from all 23 items. Older mice are considered "normal" and score a 0 when a measurement's value falls within ±1.5 SD from the younger cohort's mean, suggesting no deficit. Values greater than ±1.5 SD from the reference mean are considered to have a deficit and score a 1. Item scores are then summed and divided by the total number of assessments to calculate the FI for each mouse. Arguably the most important finding from the FI-lab is the positive sex-specific association between select proinflammatory cytokines and frailty in aging C57BL/6 mice. Similar to the original FI developed by Parks et al. [27], this method is limited in its need for specialized equipment and the lack of cognitive assessment.

Thus far the FI approach has been created for mice and rats. It is likely that the FI will be adapted for other preclinical models of aging in coming years. For example, assessment of frailty in the nematode *Caenorhabditis elegans* has been suggested for both drug screening and understanding the biology of frailty given its extensive use in science and possibility for frailty assessment [37]. Fruit flies (*Drosophila melanogaster*) are another potential candidate for frailty research, with criteria based on feeding and locomotion [38, 39]. The FI approach can arguably be created for larger animals, too, including livestock or zoo animals. Thus, the FI is a versatile tool for measuring frailty in preclinical models. Its potential for preclinical application is large and includes behavioral and pharmaceutical research aimed at the prevention of frailty [12].

Sex Differences in Frailty

In human populations females tend to live longer despite having higher frailty scores [40]. This has been referred to as the male-female health survival paradox [41]. Several explanations have been offered to solve this paradox. For example, it is possible that males exhaust physiologic reserve by better optimizing fitness, that females having fewer children than in our evolutionary past may affect physiologic reserves, or that the frailty assessments in humans are biased for females over males [41]. Regardless of the reason, it is well documented that females live longer than males, albeit with poorer health.

Sex differences in frailty are less clear in animal models; studies that have explored male-female differences in frailty in animal models are summarized in Table 3. Whitehead et al. [28] and Keller et al. [42] reported that aged (5–28 months) female C57BL/6 mice had higher clinical FI scores than males. Antoch et al. [43] also found that female Swiss mice aged 6–24 months had higher FI values than males. Similar results have been reported in older guide dogs of various breeds, where females had a higher FP compared to males [19]. However, other studies have reported no difference between the sexes. The FI scores of aged (18 months old) C57BL/6 and DBA/2J male and female mice were similar in studies by Kane et al. [25]. Parks et al. [27] also found no difference between aged (12–30 month old) C57BL/6 mice, although the sample sizes may have been too small to demonstrate sex differences with age. On the other hand, a study using 6- to 35-month-old 3Tg-AD and B6129F2 mice found that males had higher FI scores than females, at least in these strains of mice [44]. Interestingly, the female-male frailty trends reversed when using a laboratory-based FI (i.e., serum inflammatory cytokine concentrations), where aging male mice were frailer than aging females [35]. As was expected based on prior studies, female mice in the same study had higher clinical FI scores than males [35]. Thus, although most animal studies suggest that females are frailer than males, this may depend on the animal strain evaluated and on the frailty assessment tool employed. Additional work in this area may help clarify male-female differences in health span and identify underlying mechanisms.

Summary/Future Directions

Preclinical research is critical to better understand frailty. This review has highlighted and discussed current methods to quantify frailty in animals. The most common models use wild-type or genetically altered rodents. Frailty assessments in other animals, such as dogs, continue to be developed. Other preclinical models of frailty have been proposed or discussed in the literature (i.e., nematodes [37] and fruit flies [38, 39]), which may be used in

Table 3. Sex differences in frailty in animal models

Species/strain	Age	Frailty assessment tool	Sex difference	Reference
Mouse, C57BL/6	12 and 30 months	FI based on 31 health-related deficits in activity levels, body composition, hemodynamics, and metabolism	No significant sex difference was reported; small sample size	27
Mouse, C57BL/6	5, 19, and 28 months	Clinical FI based on 31 health-related deficits across many systems (e.g., integument, ocular, nasal, musculoskeletal, digestive, urogenital, respiratory, and vestibular systems, etc.)	Females had higher clinical FI scores than males, although this was not tested statistically	28
Mouse, C57BL/6 and DBA/2J	18 months	Clinical FI based on 31 health-related deficits across many systems (e.g., integument, ocular, nasal, musculoskeletal, digestive, urogenital, respiratory, and vestibular systems, etc.)	No significant difference between the sexes	25
Dogs, mostly golden and Labrador retrievers or crossbreeds	8–10 years	FP (5 factors): weakness, exhaustion, chronic undernutrition, low physical activity, and poor mobility (2 or more factors out of 5 were considered frail)	Females had significantly higher FP scores than males	19
Mouse, Swiss	6, 12, 18, and 24 months	FI based on 31 health-related deficits in activity levels, body composition, hemodynamics, and metabolism.	Females had significantly higher FI scores than males	43
Mouse, 3xTg-AD and B6129F2 wild type	6–35 months	Clinical Frailty Index based on 31 health-related deficits across many systems (e.g. integument, ocular, nasal, musculoskeletal, digestive, urogenital, respiratory and vestibular systems, etc.)	Males (both 3xTg-AD and B6129F2 wild-type) had higher clinical FI scores than females	44
Mouse, C57BL/6	17 and 23 months	Clinical FI based on 31 health-related deficits across many systems (e.g., integument, ocular, nasal, musculoskeletal, digestive, urogenital, respiratory and vestibular systems, etc.)	Females had significantly higher clinical FI scores than males	35
Mouse, C57BL/6	17 and 23 months	Modified from Parks et al. [27]; a 23-item laboratory-based FI was created from blood pressure, metabolism (blood tests), and echocardiography	Males had significantly higher laboratory-based FI scores than females	35

future frailty research. The 2 major types of frailty assessment, i.e., the FP and FI, have their own unique strengths and weaknesses. The FP remains better at identifying the physical manifestations of frailty, while the FI employs a more rounded "systems" approach that has a wide applicability. Ultimately, finding interventions that lengthen the health span, and not just their lifespan, of an individual are of the upmost importance in geriatric medicine. Frailty approaches such as the FI and FP can serve as surrogate measures to quantify the heterogeneity in aging.

These preclinical tools can allow researchers to better understand the effects of behavioral, environmental, surgical, or pharmacologic interventions on frailty [12]. In doing so, the mechanisms of frailty can be uncovered and practical therapeutic interventions to extend an individual's health span can be found. Conversely, the effects of frailty on treatment choices (i.e., drug therapies) are also important to investigate given the global risk of frailty and associated pharmacokinetic changes [45]. In addition, sex differences seen in humans are not uniformly mimicked by animal models. While human females are typically frailer than males, this does not hold true for all animal models. The underlying reasons are unclear and offer interesting research avenues, including the effects of parity on frailty in aging.

The advancement of frailty research will require further application of frailty assessments to different models and interventional studies. Contemporary challenges include the comparison between the FI and FP approaches to measuring frailty and the translation of frailty assessment into more preclinical research. To overcome these challenges researchers need to determine the most appropriate frailty assessment methods for their area of research and apply them. Different frailty measurements also need

to be directly compared to themselves to enhance our collective understanding of preclinical frailty models. Future studies should optimize known frailty tools and seek to apply them to better understand the mechanisms of frailty. The ability to quantify frailty in preclinical models enables the eventual back-translation of frailty-sparing interventions into humans and will provide insight into the mechanisms underlying the development of frailty.

Statement of Ethics

The authors have no ethical conflicts to disclose.

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S.D.H.-M, S.B., and S.E.H. contributed to the conception of this work. All of the authors also contributed to the writing of this review and approved the submission of this work for publication.

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