REVIEW ARTICLE

The biology of frailty in humans and animals: Understanding frailty and promoting translation

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Abstract

Frailty is a state of high vulnerability to adverse health outcomes. This concept is used to explain the heterogeneity in rates of aging in people of the same age. Frailty has important clinical implications, because even minor stressors can lead to adverse outcomes, including death, in frail individuals. Although frailty mechanisms are not well understood, advances in our ability to qualify frailty have encouraged efforts in this area. Quantification of frailty with both "frailty phenotype" and "frailty index" approaches has begun to highlight putative frailty mechanisms and new animal models of frailty are inspiring preclinical research. These models either adapt frailty phenotype and frailty index tools for use in animals or they use genetically manipulated mice that mimic conditions seen in frailty (eg, inflammation, sarcopenia, weakness). This review: describes commonly used tools to quantify frailty clinically, discusses potential frailty mechanisms, and describes animal models of frailty. It also highlights how these models have been used to explore frailty mechanisms and potential frailty interventions, including pharmacological treatments, diet, and exercise. These exciting new developments in the field have the potential to facilitate translational research, improve our understanding of mechanisms of frailty, and help develop new interventions to mitigate frailty in our aging population.

KEYWORDS
aging, animal models, frailty

1 | INTRODUCTION

It is projected that approximately 16% of the world's population will be 65 years of age or older by 2050, doubling the percentage from 2015 levels.[1] As world demographics shift towards an aging population, the concept of frailty is emerging as a critical area of research. This is happening because frailty increases dramatically with age, with a prevalence of 5.2% in men and 9.6% in women over the age of 65 years.[2] Although there is no generally accepted definition of *frailty*, most view it as an increased vulnerability to external stressors that leads to adverse health outcomes.[3] The idea of frailty helps explain heterogeneity in aging and provides an understanding of the difference between biological age and chronological age.

Along with the lack of a universal definition, there is no single method of quantifying frailty.[4] The two most widely used clinical methods are the "frailty phenotype" and the "frailty index" and both approaches are predictive of adverse health outcomes, such as mortality.[5] The frailty phenotype views frailty as a syndrome caused by a downward spiral of energy reserves.[6] This instrument measures five criteria: unintentional weight loss, self-reported exhaustion, low grip strength, slow walking speed, and low physical activity. An individual is considered frail if three or more of these criteria are present.[6]

By contrast, the frailty index takes a multidimensional approach and views frailty as a state characterized by the accumulation of health deficits across multiple systems.[7] This approach initially used 20 deficits[8] but can be expanded to include many more deficits.[9] Each deficit is scored as 0 if absent and 1 if present.[8] The sum is then divided by the total number of measured deficits to achieve a ratio, known as *the frailty index*. Subsequent studies have shown that the precise nature of the health deficits measured is less important than the total number of individual measured deficits, as long as at least 30 deficits are included in the index.[10,11.]

An important recent advance in the field is the translation of both frailty index and frailty phenotype approaches to animal models. This development is beneficial in terms of understanding frailty mechanisms and identifying novel strategies by which frailty can be attenuated or improved. This review will first discuss potential age/frailty mechanisms, focusing on evidence from studies in clinical populations. Newly developed animal models of frailty will then be introduced, along with recent applications of these models.

2 | MECHANISMS IMPLICATED IN FRAILTY

As age and frailty are closely linked, many of the mechanisms involved in the development of frailty are similar to those implicated in aging.[12] As in aging, there is no single cause of frailty. Instead, it appears to reflect accumulated damage across multiple systems. Putative aging and frailty mechanisms include: inflammation, loss of stem cell regeneration, DNA damage, a decline in metabolism, dysregulation of hormones, epigenetic factors, and the loss of proteostasis.[12,13] As shown in Figure 1, many of these putative frailty mechanisms may be caused by or exacerbated by environmental factors. These mechanisms are also thought to be interrelated (Figure 1).

2.1 | Inflammation

Age can give rise to chronic low-level inflammation in a process called *inflamm-aging*.[14] This state is characterized by changes in the inflammatory cytokine network that regulate the development of inflammation. The balance between pro- and anti-inflammatory cytokines is disrupted during inflamm-aging in a way that shifts the balance from anti- to pro-inflammation.[15] Clinical studies show that levels of the pro-inflammatory cytokine interleukin-6 (IL-6) increase markedly with age.[16] Interestingly, elevations in IL-6 levels are also seen in conjunction with many age-related diseases.[17] A recent meta-analysis found a number of studies that showed frailty is associated with higher inflammatory markers, including C-reactive protein and IL-6.[18] Recent work used a phenotype approach to quantify frailty in veterans aged 62-95 years and found that IL-6 levels are more closely linked to frailty than to age.[19] These studies suggest that inflammation is a key mechanism that contributes to frailty in aging individuals.
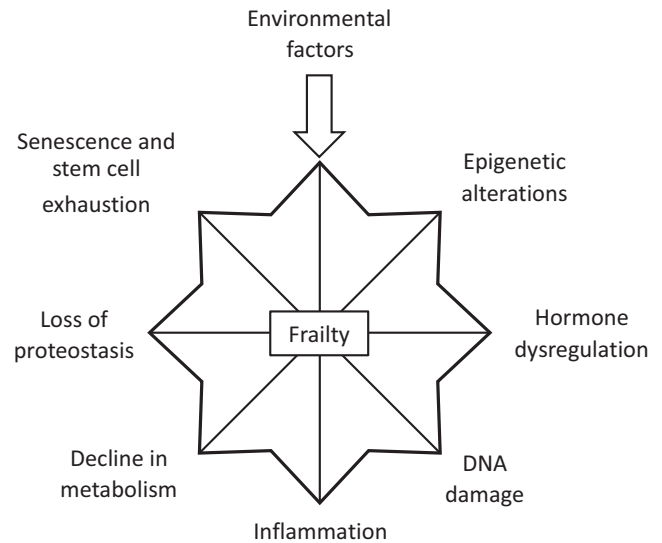
FIGURE 1 Schematic diagram that illustrates putative frailty mechanisms. Potential frailty mechanisms are interrelated and modified by environmental factors. Modified from concepts proposed as the hallmarks of aging[12] and the pillars of aging[13]

Other pro-inflammatory cytokines have a close relationship with age and may be associated with frailty. For example, elevated levels of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) are associated with higher mortality in older individuals,[20] although whether this is associated with frailty is not yet clear. It is also possible that anti-inflammatory cytokines play a role in aging and frailty. In support of this, the production of the anti-inflammatory cytokine interleukin-10 (IL-10) is increased in centenarians when compared to younger, matched controls.[21] On the other hand, high levels of IL-10 predict negative health outcomes, like cardiovascular diseases.[22] It is possible that the increase in IL-10 in these diseases may act to counter pro-inflammatory processes, although more work in this area is required. One study that explored IL-10 in frailty showed that serum levels do not change with frailty.[23] It is likely that age and frailty-dependent dysfunction reflect disruption in the balance between pro- and anti-inflammatory cytokines rather than changes in any one specific cytokine.

2.2 | Loss of stem cell regeneration and senescence

Aging is characterized by the loss of tissue regenerative properties and the accumulation of senescent cells, both of which may contribute to frailty. Stem cells are vital to tissue maintenance but they become damaged with age as a result of both intrinsic mechanisms (eg, DNA damage) and external forces.[24] Stem cells are normally quiescent but they can re-enter the cell cycle and proliferate in response to extracellular cues.[24] Mechanisms like the tumor suppressor p53 protein act to attenuate stem cell function and reduce proliferation. While this is useful in preventing cancer, stem cells may stop proliferating with time, which can lead to poor tissue repair during the aging process.

Cells, including stem cells, cannot replicate indefinitely and eventually they reach a limit (the Hayflick limit) and become senescent.[25] Senescent cells can contribute to age-related deterioration in several ways. Their abnormal morphology plus changes in gene expression can compromise tissue function and they can limit the pool of potential stem cells.[26] In addition, senescent cells develop what has been termed the *senescence-associated secretory phenotype* (SASP). In other words, they secrete cytokines, chemokines, matrix remodeling proteases, and growth factors, all elements that are implicated in aging, chronic diseases, and frailty.[26] Indeed, an exciting new development in the field of aging and frailty research is the development of so-called senolytic drugs. These biological or small molecule compounds specifically induce death in senescent cells and thereby eliminate the SASP.[27] Senolytic drugs are now being tested in early stage clinical trials to see if targeting fundamental aging mechanisms can reduce conditions such as multimorbidity, age-related loss of resilience, and frailty.[27] Such interventions could usher in a new era in the treatment of frailty and diseases of aging by targeting many diseases at once rather than one at a time.

2.3 | DNA damage

Cellular DNA is damaged by exposure to both exogenous and endogenous agents and DNA damage is thought to be a key mechanism in aging. DNA-damaging agents include reactive oxygen species (ROSs), alkylating agents, reducing sugar compounds, lipid peroxidation products, and nitric oxide metabolites.[28] There is evidence that these mechanisms play a role in aging. For example, ROSs are produced endogenously by the mitochondria as by-products of the electron transport chain.[28] External factors, such as tobacco smoke, also contain ROSs.[29] While antioxidant defense mechanisms can help attenuate ROS damage, these mechanisms do not provide complete protection and ROS damage accumulates with age.[30] Interestingly, there is recent evidence that measures of oxidative stress are more closely associated with frailty than with chronological age, which suggests that DNA damage induced by ROS may be associated with frailty.[31]

Another major cause of DNA damage is telomere shortening. Telomeres are protective, non-coding, repetitive ends of DNA that protect against shortening that arises as a result of DNA replication. Telomere shortening can be compensated by the enzyme telomerase, but even so, telomeres eventually shorten with time.[32] Short telomeres are seen in several disease states, including dyskeratosis congenita, a disease of accelerated aging, and in other age-related diseases, such as pulmonary fibrosis.[33] However, there is no evidence for a link between frailty and telomere length,[34,35] although telomere shortening might represent only a single component of the various mechanisms implicated in frailty.

2.4 | Decline in metabolism

The age-related decline in metabolism involves changes in many physiological mechanisms. Aging is characterized by the loss of lean muscle mass and the movement of adipose tissue deposits to the abdominal area. The loss of skeletal muscle in aging can be due to cachexia (muscle loss secondary to illness) or sarcopenia (degenerative loss of muscle mass and function).[36] These age-dependent changes in body composition are not solely based on low basal metabolic rate or a general decline in physical activity.[37] Muscle loss also reflects a negative protein and energy balance due to reduced food intake, inability to synthesize protein, and abnormal metabolism, as well as aging mechanisms, such as hormonal changes and inflammation.[36,38] Critically, there is growing evidence from clinical studies that sarcopenia is closely linked to frailty as well as to aging.[37-39]

As noted above, a decline in appetite in older individuals may affect metabolism. This phenomenon is termed the *anorexia of aging*[40] and it is often linked to other chronic, comorbid conditions. This loss of appetite is due to many factors, including changes in taste and smell, a reduction in energy requirements, the presence of various diseases, pain, and changes in the digestive system.[41] Loss of appetite is an important contributor to protein-energy malnutrition, a common problem in older adults that leads to sarcopenia, falls, a decline in physical function, reduced quality of life, and an increase in mortality.[42] As frail older adults often do not ingest an adequate amount of food, the loss of appetite is a potential modifiable risk factor for the development of frailty.[43]

2.5 | Dysfunctional hormone regulation

Hormones are signaling compounds that help regulate physiology and behavior. Although changes in various hormones can contribute to aging and frailty, anabolic hormones have been most clearly implicated. Anabolic hormones, such as androgens and insulin-like growth factor-1, play a key role in stimulating protein synthesis, muscle growth, and insulin secretion.[44,45] There is strong evidence that the levels of these hormones decline with age.[46] With respect to frailty, it is possible that the number of dysregulated hormones is a better predictor of frailty than any single abnormality. For example, frail women are more likely to have two or three hormone abnormalities when measuring free testosterone, insulin-like growth factor-1, and dehydroepiandrosterone sulfate levels rather than abnormal levels of a single hormone.[47] When the same hormones are measured in men, 6-year mortality is closely related to abnormal levels of all three hormones and not just one.[48] Together, these data demonstrate links between frailty and anabolic hormones, and suggest that the dysregulation of multiple hormones is a potential frailty mechanism.

2.6 | Loss of proteostasis

Proteostasis is a homeostatic process whereby the cell controls the production of properly folded proteins, and the degradation of improperly folded proteins. The aging process is associated with an overall decline in proteostasis that, when severe, can give rise to age-related diseases like Alzheimer's disease or Parkinson's

disease.[49] There are many age-related changes in proteostasis and several key examples are discussed below.

The degradation of old or damaged proteins, a process known as _clearance_, is integral to proper protein regulation. This process is partially regulated by the ubiquitin-proteasome system, where proteins are marked for degradation by ubiquitylation and degraded by proteasomes.[50] In older human cells, there is a build-up of ubiquitin-conjugated proteins.[51] This may arise as a consequence of the inability of the major mammalian proteasome (the cytosolic 26S proteasome) to deal with large numbers of abnormal proteins that accumulate during aging, which leads to aggregation of damaged proteins.[51,52] In support of this, the activity of the 20S proteasome, which is the functional core of 26S proteasome, may decline with age.[51,52] This suggests that proteasome dysfunction increases with age. There is also a proposed link between the 20S proteasome and frailty. Older individuals classified as pre-frail have lower 20S proteasome levels than those classified as frail.[53] These observations indicate that proteasome dysfunction may contribute to the accumulation of damage in the context of aging and frailty.

Individual protein "health" is also thought to contribute to detrimental changes linked to aging. For example, long-lived proteins, such as crystallin and collagen, exhibit spontaneous conformational changes as a function of age.[54] The amino acids within proteins can spontaneously move from a more stable L-form to the unstable D-form in a process known as _protein aging_.[54,55] Such changes are known to result in various age-dependent health deficits, such as the development of cataracts in the eye.[56] Although protein aging has not yet been directly associated with frailty, it is possible that such a link exists. The aging of individual proteins would be expected to affect multiple systems and thereby contribute to overall development of frailty in old age.

2.7 | Epigenetics

Epigenetics refers to the study of mechanisms that can alter gene expression without changing the genetic code itself. Epigenetic changes are potentially important in both aging and frailty, because the resultant changes in gene expression can impact underlying mechanisms, for example by silencing DNA repair genes or anti-inflammatory genes.[57,58] Although many processes fall under the term _epigenetics_, the focus here will be on DNA methylation and its relationship to age and frailty. Variations in DNA methylation patterns occur as methyl groups are either added or removed from position five of cytosine in DNA.[57] There is growing evidence for changes in DNA methylation with age, with most studies supporting the notion that advanced age is associated with global hypomethylation and local hypermethylation.[57,58] There is now some evidence that changes in DNA methylation are linked to frailty. Interestingly, one report found no correlation between global DNA methylation and age but showed that frail individuals had lower global DNA methylation levels than non-frail individuals.[59] Another study found no correlation between frailty and global DNA hypomethylation, although lower DNA methylation levels were seen at specific sites.[60]

A relatively new discovery is that DNA methylation patterns can be used to predict the functional capacity of a person (or an organ) with time, thereby providing an estimate of biological age.[61] These discrepancies between epigenetic age and chronological age have been termed _age acceleration_ and are thought to be mediated by an epigenetic clock.[62] Epigenetic age acceleration is related to the future risk of heart disease[63] and is found in many cancer cells.[62] Recent work suggests that epigenetic age acceleration is closely related to the degree of frailty. For instance, several studies have now shown that epigenetic age acceleration is associated with frailty in older individuals.[64,65] Work in this critical area is only just beginning, and new developments should improve our understanding of underlying mechanisms in frail older individuals.

2.8 | Environmental factors

Frailty is thought to be mediated by multiple systemic mechanisms, as discussed above and illustrated in Figure 1. However, it is important to recognize that frailty can be induced or exacerbated by a range of different environmental factors. A specific example is cigarette smoking, which is a predictor of worsening frailty.[66] More generally, men with lower education, lower income, and inadequate finances tend to have higher frailty scores.[67] There also is a clear inverse relationship between a country's gross domestic product and the overall mean frailty index scores.[68] While the exact reasons for these associations are not yet fully understood, frailty is clearly linked to environmental factors and additional mechanistic studies in this area would be informative.

3 | ANIMAL MODELS

It is now apparent that the heterogeneity in aging seen in humans is also present in animal models of aging. A recent approach used to quantify heterogeneity in aging animals is to measure their level of frailty. A number of laboratories have adapted tools used in humans to quantify frailty in various animal models, as discussed below. These tools have been used to provide an individualized measure of healthspan for each animal, to investigate putative frailty mechanisms and to evaluate the efficacy of interventions designed to attenuate frailty.

3.1 | Murine models of frailty

Mice are a very commonly used animal model of aging, due in part to their relatively short lifespan and to the availability of genetically manipulated models for hypothesis testing. An important recent development in the biology-of-aging field is the translation of frailty assessment tools that were originally developed in humans for use in mouse models.[69] Both the frailty index and frailty phenotype approaches have been successfully translated into mouse models.

Parks et al[70] were the first to quantify frailty in an animal model. They used naturally aging C57BL/6 mice and quantified frailty with

a frailty index based on the accumulation of health deficits in activity levels, hemodynamic parameters, body composition, and metabolism.[70] They measured 31 potential deficits, coded based on the number of standard deviations they differed from reference values in young adults and showed that frailty was significantly higher in 30-month-old mice compared with 12-month-old mice. More recently, a simplified frailty index tool was developed based on 31 clinically apparent signs of age-associated deterioration in aging mice.[71] This frailty index has a high degree of interrater reliability, even when used by investigators of different experience levels.[72,73] Major features of the murine frailty index are comparable to frailty index data in humans. For example, they have similar rates of deficit accumulation and both show a direct relationship between high frailty index scores and mortality.[74]

Mouse frailty assessment tools based on the frailty phenotype also have been developed. Liu et al[75] developed criteria for use in mice based on those used in people, including grip strength (inverted-cling grip test), maximal walking speed (rotarod), physical activity (voluntary wheel running), and endurance (rotarod plus grip strength). Interestingly, they did not include a weight loss term. Mice with three or more of these criteria are considered frail while those with two criteria are considered pre-frail and fewer than two are considered non-frail.[75] More recent models have quantified the mouse frailty phenotype with a weight loss factor included in the analysis.[76] Both studies showed that frailty increased with age in C57BL/6 mice.[75,76] When both the frailty index and frailty phenotype methods were compared in aging C57BL/6 mice, the frailty index approach identified more mice as frail than the phenotype approach.[77]

A different approach to study frailty in mouse models is to use mice that are genetically manipulated to reflect mechanisms believed to be important in frailty. As inflammation is thought to play an important role in frailty, mice with knockout of the anti-inflammatory cytokine IL-10 have been used to model frailty.[78] These animals exhibit reduced activity and muscle strength at an earlier age than C57BL/6 controls.[78,79] They also show higher overall mortality along with elevated levels of pro-inflammatory cytokines (eg, IL-6, IL-1ß, TNF-α, and interferon-γ [IFN-γ][80]). More recently, Cu/Zn superoxide dismutase knockout mice (Sod1KO) have been proposed as a model of frailty. Cu/Zn superoxide dismutase is a major antioxidant enzyme found in the mitochondria of most cells and Sod1KO mice exhibit accelerated aging.[81] These mice also show loss of muscle mass, weakness, low physical activity, and reduced endurance,[82] which is consistent with the frailty phenotype. In addition, Sod1KO mice show the same pro-inflammatory profile as the IL-10 knockout mice.[83] While both of these models are potentially interesting models of frailty, frailty itself has not been measured in either model.

3.2 | Models of frailty in other animals

Frailty assessment tools have also recently been developed for use in aging rats. For instance, the simplified mouse frailty index tool[71] has been adapted for use in aging rats.[84] This instrument measures the accumulation of age-related health deficits that are specifically seen in aging rats. Results show that frailty index scores increase with age and predict mortality in rats,[84] as seen in earlier studies in mice. The frailty phenotype method has also been validated in aging rats, using performance on four criteria: grip strength, walking speed, physical activity, and endurance.[85] This work shows that, as in the mouse model, high frailty phenotype scores predict mortality.[85] A frailty phenotype approach has also recently been developed for use in dogs based on five components: chronic undernutrition, exhaustion, low physical activity level, poor mobility, and weakness.[86] This tool was used in a large cohort of aging guide dogs and a positive relationship between signs of frailty and mortality was seen.[86] It would also be of interest to develop a frailty index tool for use in dogs.

4 | MECHANISMS OF FRAILTY IN ANIMAL MODELS

Even though the newly developed animal models of frailty are well suited to mechanistic investigations, work in this area is limited. There is some evidence that, as in frail humans, levels of the pro-inflammatory cytokine IL-6 are elevated in the IL-10 knockout model compared to control mice.[78] Several other pro-inflammatory cytokines (eg, IL-1β, tumor necrosis factor-α, and IFN-γ) are also elevated in these mice,[80] but whether this is simply a result of the absence of IL-10 in this model is not known. Other work has shown that serum levels of various pro-inflammatory cytokines (eg, IL-6, IL-9, IL-12p40, and IFN-γ) are increased in naturally aging mice with high frailty index scores.[87] Thus, there is evidence from preclinical studies that chronic inflammation may play a role in the development of frailty but more work in this area is clearly required.

5 | INTERVENTIONS TO ATTENUATE FRAILTY

The recent development of non-invasive frailty assessment tools in animal models allows cross-sectional and longitudinal testing of interventions designed to attenuate frailty. Studies conducted to date include both pharmacological and non-pharmacological interventions, as discussed in the following sections.

5.1 | Pharmacological interventions

The first study to investigate beneficial effects of drug treatment on frailty explored the effect of resveratrol treatment on frailty index scores in aging mice.[88] Resveratrol was selected as the initial pharmaceutical intervention because it prolongs lifespan in many models.[89] It also reduces risk of acute coronary artery disease,[90] acts as a chemoprotective agent,[91] and is at least partially neuroprotective.[92] Importantly, 6 months of treatment with resveratrol reduces frailty index scores in C57BL/6 mice.[88] A more recent study investigated

the effects of the angiotensin converting enzyme (ACE) inhibitor, enalapril, on frailty index scores in mice.[93] An ACE inhibitor was used because it is known to reduce inflammation as well as increase skeletal muscle strength and mass.[93] Results showed that frailty index scores were markedly reduced in aged mice treated with enalapril for up to 9 months when compared to untreated controls. These beneficial effects of ACE inhibitor treatment were mediated, at least in part, by effects on pro- and anti-inflammatory cytokines.[93]

5.2 | Non-pharmacological interventions

The influence of exercise on frailty has also been explored in animal models. Graber et al[94] were the first group to investigate the influence of voluntary exercise on frailty in aging mice. They used a frailty phenotype approach and showed that 4 months of voluntary wheel running exercise improved frailty in very old mice (28-30 months).[94] This work was extended by Gomez-Cabrera et al,[76] who used the frailty phenotype in a larger cohort of mice. They reported that long-term voluntary aerobic exercise (wheel running) attenuated the development of frailty in mice from 17 to 28 months of age.[76] Another approach explored high-intensity interval training, consisting of 10-minute uphill treadmill sessions three times per week for 16 weeks.[95] Results show that high-intensity interval training reduces frailty phenotype scores in aging mice.[95] Taken together, these studies demonstrate that exercise reduces frailty levels in aging mice and suggest that even brief periods of training may be beneficial.

Calorie restriction is another intervention that has been clearly shown to extend lifespan.[96] Therefore, the impact of calorie restriction on frailty has been investigated. Mice subjected to calorie restriction (40% of ad libitum food starting at 6 months of age) have lower frailty index scores than mice fed ad libitum, at least with respect to male animals.[88] The effect of calorie restriction on the frailty phenotype has been assessed in aging rats.[97] There, rats were subjected to calorie restriction (60% of ad libitum food starting at 6 months of age). Results clearly showed that calorie restriction also delayed the development of frailty in the rats.[97] Taken together, these studies provide convincing evidence that lifestyle modifications, namely diet and exercise, can modulate the degree of frailty in aging animals.

6 | SUMMARY AND FUTURE DIRECTIONS

The biology of frailty is an exciting emerging field of inquiry. The advent of new tools to quantify frailty in humans has begun to highlight different mechanisms that may play a role in the development of frailty in older adults. The translation of these tools to animal models promises to further advance our understanding of frailty mechanisms, as well as interventions to mitigate frailty. These new preclinical models of frailty will allow us to investigate fundamental questions about the nature of frailty and how to modify it in the setting of aging.

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