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Physiological Systems in Promoting Frailty

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Abstract

Frailty is a complex syndrome affecting a growing sector of the global population as medical developments have advanced human mortality rates across the world. Our current understanding of frailty is derived from studies conducted in the laboratory as well as the clinic, which have generated largely phenotypic information. Far fewer studies have uncovered biological underpinnings driving the onset and progression of frailty, but the stage is set to advance the field with preclinical and clinical assessment tools, multiomics approaches together with physiological and biochemical methodologies. In this article, we provide comprehensive coverage of topics regarding frailty assessment, preclinical models, interventions, and challenges as well as clinical frameworks and prevalence. We also identify central biological mechanisms that may be at play including mitochondrial dysfunction, epigenetic alterations, and oxidative stress that in turn, affect metabolism, stress responses, and endocrine and neuromuscular systems. We review the role of metabolic syndrome, insulin resistance and visceral obesity, focusing on glucose homeostasis, adenosine monophosphate-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), and nicotinamide adenine dinucleotide (NAD^+) as critical players influencing the age-related loss of health. We further focus on how immunometabolic dysfunction associates with oxidative stress in promoting sarcopenia, a key contributor to slowness, weakness, and fatigue. We explore the biological mechanisms involved in stem cell exhaustion that affect regeneration and may contribute to the frailty-associated decline in resilience and adaptation to stress. Together, an overview of the interplay of aging biology with genetic, lifestyle, and environmental factors that contribute to frailty, as well as potential therapeutic targets to lower risk and slow the progression of ongoing disease is covered.

Introduction

"Today, for the first time in history most people can expect to live into their 60s and beyond (2015)" (623). This increase in life expectancy is reflected in the current world population of approximately 7.7 billion (end of 2020) whereby approximately 1.0 billion people (13%) are over the age of 60 years. This age group, 60 years and older, is expected to grow significantly to 1.6, 2.1, and 3.1 billion in 2035, 2050, and 2100, respectively, and there

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will be a concomitant growth in many serious health concerns such as an increased risk for chronic and metabolic diseases [e.g., cardiovascular disease (CVD), cancer, and Alzheimer's disease], a decline in intrinsic capacities (e.g., mobility, cognition, psychological, vitality, hearing, and vision capacities) and a loss of resilience (i.e., the ability to resist or recover from adverse events) (https://population.un.org/wpp/) (45, 216, 453, 624). In fact, more than 80% of people older than 65 years have at least one chronic disease, which increases to at least three diseases by 72 years (74, 595). Consequently, the length and the severity of late-life multimorbidity leads to poor health (e.g., disabilities) requiring care and/or help with the activities of daily living. The burden of these conditions creates enormous clinical, social, and economic needs for healthcare systems on a worldwide level (32, 33, 342, 372).

Frailty is unquestionably one of the most serious worldwide challenges in the 21st century (253). Based on the aging demographics outlined above it is anticipated that the number of older adults with recognized frailty will significantly increase worldwide (402, 638). Over the past two decades, impressive scientific progress yielded great strides in the field of clinical frailty research; yet, many gaps remain including the lack of a universally accepted clinical definition of frailty. Nonetheless, the leaders in the field agree that frailty is a state of physiological vulnerability to stressors that results from age-related declines in biological systems, manifests clinically as greater risk of adverse health outcomes, and leads to a vicious cycle that results in further functional decline and disability (72, 87, 94, 106, 109, 249, 315, 399, 443, 597, 628, 630). Frailty is also considered a dynamic condition that occurs on a continuum from fit or robust to frail in which individuals transition in and out of the states of frailty (nonfrail, prefrail, frail) and in either direction over time (106, 137, 278, 478).

Up until now the reported prevalence of frailty from a worldwide perspective depends on many factors including operational definition of frailty, age, sex, socioeconomic status, race/ethnicity, environmental setting, and the approach to classify frailty (100, 122, 417, 418, 420, 493, 531). For instance, in one of the first published systematic reviews of frailty prevalence, the overall global prevalence of frailty was 11% (range 4%–59%, the year 2012) (109). Systematic reviews and meta-analysis found an overall frailty prevalence of 18% with the highest prevalence of frailty observed among hospital inpatients (~50%) or long-term care settings (>60%), 30% prevalence in primary care and out-patient settings, and a median rate of 10.8% in community-dwelling settings [ranging from 2% to 60% (142, 416, 417, 422)]. Another review evaluated the prevalence of frailty among community-dwelling older adults in low-income and middle-income countries and reported a 17.4% prevalence (531). To highlight the prevalence of frailty in the context of aging, the prevalence rate increases with advancing age, from 6.5% in those aged 60 to 69 years to 65% in those aged 90 or over with frailty occurring more frequently in women than in men (16% vs. 12%) (190).

Despite these staggering statistics on the prevalence of frailty, it is important to note that frailty is not an inevitable consequence of aging and, even at advanced ages, many people do not become frail. As a matter of fact, in the aging population, there are enormous inter-individual differences in terms of the decline in health, the onset of disabilities, and life expectancy (45, 453, 474). Indeed, some people die of age-related disease in their 60s, whereas there are active people at 100 years of age. Importantly, frailty is not limited to

older people: frailty and prefrailty states can exist in individuals younger than 60 years, particularly among those with multimorbidity (coexistence of two or more diseases) (266, 431).

Evidence suggests that multimorbidity is a risk factor for frailty (151, 155, 178, 589, 592). For instance, a meta-analysis examining the relationship between frailty and multimorbidity (>14,000 community-dwelling older adults, nine studies) reveals that about three-quarters of people with frailty present with multimorbidity, and that frailty is present in 16% of people with multimorbidity (589). Consistent with these findings, a prospective analysis of approximately half a million participants shows that frailty is associated with multimorbidity, reaching a frailty prevalence of 18% among participants with four or more diseases (219). A very impactful and recently published study highlights the impact of multimorbidity on the progression of frailty such that the overall decline in health (trajectories) of people with frailty associated with multiple diseases shows an early onset of frailty, a reduced period of prefrail status, and a rapid progression to frailty compared to people classified with age-associated frailty (16). Importantly, from the perspective of this comprehensive article, these findings suggest that the underlying biological mechanisms involved in the onset of frailty related to disease are different from those involved in age-related frailty (554). Considering the current clinically based frailty conceptualization it is likely age-related frailty emerges as the physiological reserves decline beyond a threshold (declines at the cellular and molecular level across multiple systems or a specific set of critical systems) and in the presence of low resilience and resistance (612). As shown in Figure 1, the cellular and molecular components that contribute to aging biology likely contribute to the overall decline in health overtime and to the increased risk in age-related frailty. From this perspective, two individuals of the same chronological age may respond to the same stressor quite differently. Further, the observed continuum of frailty and the stages of frailty (nonfrail, prefrail, frail) reflect the amount of physiological capacities (functional, intrinsic) available to react to the health stressors.

Impact of Geroscience

Geroscience, a relatively recent interdisciplinary field, is poised to play a critical role in defining the mechanisms underlying the continuum of frailty and the identified stages because it seeks to determine the molecular and cellular components at the intersection of the biology of aging, aging physiology, and the biology of age-related diseases (27, 311, 435, 508, 524). During the past decade, the field of Geroscience emerged due to significant advances in the understanding of the molecular and cellular pathways that drive the aging process and the ability to modify the rate of aging (27, 283, 300, 359, 368). For example, the rate of aging is modified by various interventions including behavioral, genetic, and pharmacological interventions (226, 227, 270–273, 375). These interventions also show remarkable improvements in aspects of health in older age groups, which is viewed as slowing the rate of aging in humans (357). From the efforts by the National Institute on Aging (NIA)-supported Geroscience Network the origins of the well-recognized Geroscience hypothesis, the Pillars of Aging, and the Hallmarks of Aging emerged (522). The Geroscience hypothesis states that, by reducing the rate of aging, it is possible to delay or slow down the appearance and progression of most age-related chronic diseases, in

parallel (311, 435, 508, 524). Whereas, the noted Pillars of Aging and Hallmarks of Aging provide a foundation to systematically investigate and understand the multitude of pathways that drive aging (300, 359).

Historically speaking and highlighted in the Geroscience hypothesis stated above, the focus of Geroscience sought to tease out the biological underpinnings for why aging is the major risk factor for disease. However, the field soon recognized health was more than just the absence of disease (523). This major shift to or the focus on health led to the idea that aging is a main driver for the general loss of functional capacities and the development of aging phenotypes, even in the absence of overt disease. In this scenario resilience, resistance, and physiological reserves play critical roles. Resilience is an established area of investigation by researchers and clinicians in many disciplines and the definition of resilience is somewhat similar across the sciences. Resilience within the discussion of health is the ability to resist or recover from adverse events after an acute or chronic health stressor (Figure 1) (216, 573, 612, 613). In contrast to resilience, resistance is the ability to prevent or counter exogenous and endogenous stressors. Resilience is reported to decline with age when there is an increased risk of health stressors (305, 335). Physiological reserve is defined as the potential capacity of a cell, tissue, or organ system to function beyond its basal level in response to alterations in physiologic demands and is consistent with the term “intrinsic capacity” introduced by World Health Organization (WHO) (613, 624). The capability to respond, resist, or adapt to stress is dependent on multiple factors including the physiological reserves present within the collective physiological systems, the extent of the stressor, and the presence of co-existing stressors or exposure to previous stressors. Thus, in the presence of low physiological reserves across multiple physiological systems, the physiological potential to respond is greatly reduced and likely contributes to frailty. Indeed, it is possible to target the understanding of frailty by examining specific characteristics of resilient profiles (e.g., nonfrail vs. frail). For instance, at the cellular and molecular level, aberrations within the deoxyribonucleic acid (DNA) repair pathways decrease the ability to recover from DNA damaging agents (e.g., chemotherapy). Imbalances in proteostasis and increased mitochondrial damage influence stress responses, whereas interruptions in stem cells (SCs) impair tissue regeneration after injury. Importantly, many of these stress-response pathways are part of a complex integrative regulatory network that becomes dysfunctional resulting in decreased resilience.

Lastly, the field of Geroscience is still in its early days; however, the potential impact in teasing out the underlying mechanisms contributing to frailty is high. Indeed, in May 2021 the National Geroscience Initiative (people and organizations from the academic, not-for-profit, industry, and philanthropy sectors) launched a White Paper with the goal to utilize the biology of aging to optimize human performance, healthspan (defined as the portion of life that is relatively healthy and free from major deficits that impair the quality of life) and lifespan, which will yield substantial benefits to the quality of life for the aging adult (51, 250).

Considering the growing worldwide aging population, the frailty prevalence rate, the close relationships between frailty, aging and chronic disease, the field of Geroscience, and the impact of physiological reserve and physical resilience on health, there are substantial

benefits to systematically evaluate the cellular and molecular factors contributing to frailty (239, 349). In this article, we provide comprehensive coverage of topics regarding what is known about factors that contribute to frailty. We base this information quite loosely on Pillars and Hallmarks of Aging; markers and processes established by leading researchers in the field of aging that are highly associated and interconnected with the aging phenotype (300, 359, 560). *These factors are not necessarily causes of aging but are more so common denominators in aging phenotypes across species*. The cause(s) of aging and frailty has not been identified at this time. Understanding the process of aging is the ultimate goal of the Geroscience field. A better appreciation of the elements underlying frailty is a necessity to move this goal forward. Before discussing the fundamental processes, we first review the literature whereby the clinical frailty assessment tools that classify people along the continuum of frailty were reverse-translated to preclinical animal models. With that information in mind, an assemblage of cellular and molecular evidence underlying aging biological mechanisms is then presented in terms of their potential contributions to frailty.

Frailty Assessments in Clinical Practice

Prompt identification of frailty is crucial, especially during the early stages, to maximize opportunities for intervention (475, 621). Within the past few decades, many clinically based frailty assessment tools emerged based on human performance measures, biomarkers, questionnaires, routine geriatric evaluations, or a combination [e.g., Frailty Phenotype, Frailty Index (FI), Clinical Frailty Scale, FRAIL scale, biomarker-based FI, Study of Osteoporotic Fractures frailty criteria, PRISMA-7, Tilburg Frailty Indicator, Groningen Frailty Indicator, Short Physical Performance Battery, Edmonton Frailty Scale] (137, 322, 394). To date, there are two popular, well-established approaches to assess frailty clinically that are validated in many populations and across multiple clinical and living settings: Physical Frailty Phenotype and FI of deficit accumulation (Table 1).

In the Physical Frailty Phenotype approach, frailty is defined as a “biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems, and causing vulnerability to adverse outcomes” (177, 179). Within this conceptualization, the biological basis of frailty is focused on altered stress response systems and energy metabolism abnormalities that drive the appearance of signs and symptoms. The Physical Frailty Phenotype consists of five clinical hallmarks (phenotypic criteria) of weight loss, weakness, poor endurance/exhaustion, slowness, and low physical activity, core features hypothesized to be proxies of manifestations of dysregulation in specific physiological domains (Table 1). Weight loss is defined as unintentional weight loss of more than 4.5 kg or 10 pounds within a year (score = 1). Weakness is identified by a grip strength test (handheld dynamometer) and is in the lowest 20% by sex and body mass index (BMI) (score = 1). Poor endurance/exhaustion is identified with self-reported positive responses to specific questions from the US Center for Epidemiologic Studies Depression Scale, 3 to 4 days/week or most of the time (score = 1) (459). Slowness is identified by a timed gait speed test (walking time/15 feet or 4.57 m) and is in the lowest 20% by sex and height (score = 1). Low physical activity is identified by the Physical Activity Scale for the elderly with energy expenditure in the lowest 20% by sex, <383 kcal/week (men) and <270 kcal/week (female) (score = 1) (605). The stages of frailty

are scored across a range from 0 to 5. Frailty is then identified when 3 or more of the five phenotypic criteria are present, which indicates diminished stress response and energetics. Prefrail is identified when 1 or 2 of the five phenotypic criteria are present, which signifies a high risk of progressing to frailty. Nonfrail is identified when 0 of the phenotypic criteria are present.

In contrast, the second approach is identified as the deficit accumulation frailty and hypothesizes that the accumulation of health and functional problems serves as an indicator of an individual's aging-related health state (395). Specifically, frailty is defined "as a continuous process characterized as a multidimensional syndrome of loss of reserves (physical ability, cognition, health, energy) that gives rise to vulnerability." Within this conceptualization, there is an established FI, which measures a wide range of health assessments (cognition, motivation, mood, communication, mobility, balance, activities of daily living, nutrition, bowel and bladder function, comorbidities, laboratory abnormalities, as well as social resources) with more deficits conferring greater risk of mortality (Table 1). Each deficit is scored as 0 if absent and 1 if present, and a ratio is calculated by the actual number of health deficits in an individual divided by the total number of potential health deficits that were measured. The FI provides a score on a scale from 0 (no deficits) to 1 (all items exhibit deficits). Importantly, the FI is focused on the number of deficits (a minimum of 30) rather than the specific type of the health deficit (473, 536).

Both approaches to assess frailty (Physical Frailty Phenotype, FI) are useful for identifying vulnerable adults at higher risk for mortality and have been used extensively since conception (28, 601, 629). It is worth noticing that comparisons between these two clinically based frailty assessment tools show predictive validity (adverse outcomes) even in the presence of a high degree of heterogeneity with respect to the selection of tests used to meet criteria and to the inclusion of reference standards and their thresholds to determine cut-off values. In fact, these two well-established frailty assessment tools classify different groups of older adults and mice as frail, prefrail, or nonfrail indicating a discordance (104, 292, 629). It is reasonable to assume this reported discordance in specific assignment to frailty subgroups aligns with the assessment tool's theoretical construct (physical frailty vs. health deficits). From the perspective of teasing out the underlying biology contributing to the continuum of frailty, it is now imperative to closely align theoretical construct (e.g., physical frailty) with the corresponding assessment tool (e.g., Physical Frailty Phenotype) when classifying frail, prefrail, and nonfrail individuals (20, 629).

In the 21st century to successfully prevent or treat frailty and increase healthspan, the recognition of its intrinsically complex underlying biological processes is the first step. It is not surprising to hypothesize that frailty involves a *cumulative* decline in physiological, cellular, and molecular functions and frailty is apparent at multiple levels of biological organization: genome, epigenome, tissues, organs, and the organism (Figure 1). Practically speaking, studying the burden of frailty in humans is challenging particularly due to the ethical (complicated, high-risk), logistical (cost, labor-intensiveness), and biological complications (genetic diversity, lifestyle) associated with working with older adults. In this regard, it is an exciting time for researchers interested in the study of the biology of frailty at the preclinical research level together with the interdisciplinary field of Geroscience.

Frailty Assessments in Preclinical Research

It is well-established that mouse models are developed (genetically), characterized, and tested to advance biomedical research in human aging and disease (71, 442, 579). The major advantages of mouse models in representing a human disease and/or aging are the investigation of the underlying biological mechanism(s), the identification of potential cellular targets for developing therapies, and the opportunity for translational bi-directional approaches. Bi-directional translational science facilitates iterative changes when additional new information is available, either preclinically or clinically, for offering the greatest opportunity for the diagnosis, prognosis, and treatment of disease or aging. The increased use of health assessments in preclinical animal aging models is an excellent example of successful translational science (3, 47, 164, 280, 470, 549).

For the study of frailty, mice are suitable because the lifespans of 1 to 3 years (strain-dependent) facilitate longitudinal lifespan research designs in both sexes (54). Mice exhibit many of the visible signs associated with humans such as hair graying, kyphosis, deafness, and baldness as well as cognitive decline and display physical performance declines such as balance, coordination, gait speed, strength, and endurance (34, 153, 183, 208–212, 280). Utilizing mice can also reduce and/or isolate factors that contribute to frailty such as lifestyle and address the possibility of detecting a frailty state before disability. As mammals, the physiology of mice resembles that of humans in many aspects. Most importantly, preclinical models enable in parallel tissue-to-tissue examination of mechanisms contributing to frailty and of the impact of genetic, pharmacological, and behavioral interventions.

Development of the mouse Frailty Phenotype

Liu et al. (351), developed a preclinical mouse Frailty Phenotype that followed the clinical criteria used by Fried et al. (179), which included measures of strength (inverted grip hang), walking speed (rotarod), physical activity (voluntary wheel running), and an endurance score (inverted grip hang plus rotarod). Each criterion was scored (score = 1) based on a selected cutoff percentile corresponding to 1.5 standard deviations below the cohort mean (i.e., the lowest seventh percentile of the group). Mice with 3 or more positive frailty markers were identified as frail, with 2 positive markers as prefrail, and with 1 or no positive frailty markers were identified nonfrail. This initial mouse Frailty Phenotype was further improved and validated in two rigorous studies that assessed cohorts of male and female mice across the lifespan (42, 321) (Table 2). In these two studies, the mouse Frailty Phenotype was redesigned to include body weight, reliable and quantitative measures of endurance/exhaustion (treadmill fatigue test) and strength (an electronic grip meter test), as well as the original walking speed (rotarod), and physical activity (voluntary wheel running) measures. Importantly, the evaluation of frailty markers in a longitudinal lifespan research design permitted the evaluation and identification of a reference group and cut-off values for each measure. Mice that fell in the bottom 20% for strength, walking speed, exhaustion, and activity were considered to be positive for the frailty measure (score = 1). In contrast, mice with the highest 20% body weight are considered positive for frailty. Designation of frail, prefrail, and nonfrail was defined with the same number of positive frailty markers as in the original Liu et al. report (179, 351). Importantly, because the measures were evaluated

across the lifespan it was possible to establish that the mouse Frailty Phenotype identifies the onset of frailty, progression and prevalence of frailty, and mortality risk (41, 42, 321).

In addition to the mouse Frailty Phenotype described above, there are four other frailty assessment tools reverse-translated from the criteria within the clinical Physical Frailty Phenotype (physical frailty, Valencia Score, Comprehensive Functional Assessment Battery, Neuromuscular Healthspan Scoring System), which adapt similar criteria with modified approaches and similar cut-off values (204, 209, 212, 374, 511). Given the focus on measures of physical function within the mouse Frailty Phenotype and other assessment tools listed above, the importance of skeletal muscle arises (sarcopenia) as a major contributing factor for frailty.

Development of the mouse clinical Frailty Index

The first mouse FI selected 31 health-related variables to provide health information highlighting four categories: activity (distance moved, velocity of movement, rearing frequency); hemodynamic factors (systolic and diastolic blood pressures, heart rate, blood volume); body composition (body mineral content, percent body fat, percent lean tissue); and metabolic status (electrolytes, hematocrit, and urea (Table 2) (434). A graded scale was used to determine frailty, based on how many standard deviations the measured value differed from the mean reference values (adult mice). Because the conceptual framework of the FI is grounded on the number of deficits (a minimum of 30) rather than the specific nature of the health deficit, the mouse FI was redesigned to be noninvasive and simple to implement in the research laboratory (473, 536). The noninvasive 31-selected variables (index) provide health information across several physiological systems including the integument, musculoskeletal, vestibulocochlear/auditory, ocular, nasal, digestive, urogenital, respiratory, plus sign of discomfort, body weight, and body surface temperature measures. A severity of each deficit was rated on a simple scale of 0 = absent, 0.5 = mild, and 1 = severe. In 2017, Antoch et al. (18) defined the physiological Frailty Index (PFI) with the aim of including parameters to be (i) diverse to reflect the status of different health-related physiological systems, (ii) objective and quantitative, and (iii) minimally invasive. Using 29 variables reflective of physical fitness, the cardiovascular system, total blood cell composition, plasma concentration of chemokine C-X-C motif ligand/keratinocytes-derived chemokine (Cxcl1/Kc), triglycerides, and glucose, the PFI showed a gradual age-associated increase in frailty in a cross-sectional study with sex-specific differences (females more rapid and higher than males).

In mouse indices of frailty, the number of health-related variables and the inter-rater reliability when assessing these variables are important for experimental and data fidelity (156, 291, 611). An 8-item mouse FI shows an increase with age; however, the results lacked sensitivity to detect frailty between age groups, exhibited high variability, and there was greater test-to-test variability compared to a mouse FI with 31-items (611). Note, it is possible to achieve inter-rater reliability with careful selection and training of the raters when using the FI (156, 291). In addition to the mouse FI identified above, others are developing frailty indices in mice based on common laboratory tests (blood pressure, basic metabolic status, echocardiography, and blood-based biomarkers) (293).

Preclinical frailty research

With the development of the preclinical frailty assessment tools and the emerging interests in health and in the biology of frailty described above, more attention to assessing frailty status (phenotype) as an experimental outcome variable is taking place. To date, there are reports with the widely used C57Bl/6 mouse in both cross-sectional lifespan research (cohorts of mice at different ages) and in rigorous, prospective longitudinal lifespan research (one cohort tested across the lifespan) (41, 42, 204, 321, 351, 434, 445, 472, 611). Longitudinal lifespan studies are considered more rigorous because survival bias influences the results in studies using a cross-sectional lifespan design. In addition to the C57Bl/6 mouse, short-lived and long-lived, accelerated aging and inbred/outbred mice and mouse models of Alzheimer's, oxidative stress, and inflammation have been assessed for frailty status (18, 25, 262, 264, 290, 295, 374, 471, 502, 609). To date, there are several studies evaluating frailty in rats, dogs, nonhuman primates, and in *Caenorhabditis elegans* models (30, 246, 386, 410, 537, 565, 631, 635, 646).

Given the multidimensional nature of frailty (Figure 1), it is likely the development of therapeutic interventions that target several cellular systems linked to multiple aspects of health will have the greatest beneficial effects. Indeed, several lines of evidence now point to the potential to modify frailty in preclinical animal models (mice, rats, nonhuman primates) by targeting global physiological systems (e.g., inflammation, oxidative stress) or signaling pathways [e.g., mammalian target of rapamycin complex 1 (mTORC1)] (471, 516). The well-established longevity-modulating interventions such as caloric restriction (CR), intermittent fasting, and treatment with antioxidants or mammalian target of rapamycin (mTOR) inhibitors and others reduce frailty (25, 227, 231, 270, 290, 516, 563). It is possible to reverse frailty with healthy-lifestyle interventions including defined exercise training (e.g., high-intensity interval training), physical activity, diet [e.g., reduced branched-chain amino acid (BCAA) diet], and Vitamin D supplementation (204, 210, 471, 509–512). Specific pharmacological therapies (e.g., antihypertensive agents; anti-inflammatory agents) attenuate frailty, too (299). In contrast to strategies shown to improve the status of frailty, premature or enhanced frailty is reported when testing approaches known to be detrimental to health such as polypharmacy, high-drug burden, high-fat feeding, and irradiation (18, 162, 251, 294, 366). Conceptually, these interventions converge to improve cell physiology, homeostatic functions, and boost protective cellular pathways.

Challenges

In the previous section, we describe the increasing use of the frailty assessment tools in preclinical research studies focused as an outcome variable when describing phenotypes and when testing interventions. While being informative, the evidence to support the reversal of frailty is limited, at times contradictory, inconclusive, and incomplete. For instance, in some reports, only one sex was investigated (563). Investigating both sexes is critical because there is controversy within the mouse literature indicating that either older females exhibit greater frailty than males or vice versa or no sex differences at all (18, 290, 295, 434, 611). In our work, we show sex differences in mice at a specific age within the lifespan (41). More research is definitely indicated to further expand these findings and elucidate the reasons for the varied reports (e.g., strain, cross-sectional vs. longitudinal lifespan

study, frailty assessment tool). Following up on these sex-difference observations, studies evaluating therapeutics or interventions to delay frailty also show sex-specific responses whereby the sex-specific response is intervention-dependent (471). For instance, treatment with alpha-ketoglutarate reduced frailty in both sexes; whereas interventions by which there is a restriction of dietary BCAAs or supplementation with Vitamin D reduced frailty in males, but not females (471, 509, 516). With these concerns, future studies require close examination of sex-specific responses, aspects of intervention (e.g., age of initiation, dosing, toxicity testing), and comprehensive, standardized research designs to clearly understand the mechanistic details underlying frailty.

The challenges noted above bring to the forefront three important points for discussion: the multidimensional aspects of the frailty condition (domains of frailty), selection of the most appropriate frailty assessment tool, and the manner in which age is described in experimental design. To date, the assessment of frailty in preclinical models focuses on loss of physical functions (physical frailty) or as accumulation of multiple health deficits; however, in humans, there are multiple domains of frailty (cognitive, social, psychological which includes motivation and mood), that coexist, have potential to influence each other, and have specific assessment tools (215, 378). For instance, there is an association between cognitive frailty and physical frailty, and cognitive frailty is identified as a determinant of resilience to stressors (15, 215). At this time, preclinical assessments for frailty identification do not emphasize measures for cognition, depression, motivation, etc.; yet it is important to determine whether the presence of multiple frailty domains increases the risk for negative outcomes of frailty and to elucidate the biological underpinnings to develop multidomain interventions. In regards to the second point, because assessment tools to identify frailty status in preclinical animal models are in their infancy, selecting or developing a frailty assessment tool for animals requires adherence to general principles such as theoretical basis and validity of the constructs (discriminant validity, construct validity and reliability, high sensitivity and specificity), matching the assessment tool to the intended purpose (domain or domains captured), feasibility and implementation (quick and easy, testing and housing environment, time of day), past use, and degree of invasiveness, etc. Observing these principles has potential to propel frailty research in preclinical animals in a positive trajectory toward impactful discoveries. Lastly, in consideration to the third point, most studies compare organisms of the same chronological age (i.e., 18-month-old control and 18-month-old treated mice). However, our understanding of aging biology as well as clinical presentations suggest that: (i) aging rates amongst individuals differ; and (ii) various interventions can alter this rate (delay or accelerate). These observations have been verified using clock-based assessments (epigenetic, metabolomic, transcriptomic among others) that indicate the variability in biological age between individuals of the same chronological age. In other words, chronological age is a time-based description while biological age reflects differences in the rate of aging between organisms within a species as well as between cells, tissues, and organs. Thus, in this comprehensive article, when we describe the epigenetic clocks for instance, these measures are utilized as predictors of biological age and mortality and are often compared to chronological age to indicate health.

Collectively, these research studies show remarkable progress in preclinical frailty research (e.g., increase with age, predict adverse outcomes, reversible or delayed, in agreement

with human populations); however, it is clear the next generation of preclinical frailty work and human frailty research can inform each other and be more integrated going forward. Very recently, a new international public-private venture emerged called the INSPIRE Research Initiative. This INSPIRE Research Initiative is dedicated to biological and healthy aging with the ultimate goal of preventing adverse health consequences of aging and delaying their onset or reducing their severity (134, 492). The INSPIRE Research Initiative is unique and has potential to be impactful for the field of frailty because it aims to create a bio-resource platform spanning from animals to humans, from cells to individuals, and from research to clinical care. INSPIRE brings together internationally recognized experts from basic and translational science, clinical gerontology, primary care, and public health with the objectives of identifying biomarkers and implementing a function-centered healthcare pathway. Importantly, INSPIRE applies the principles of Gerosciences to foster discoveries by including comprehensive phenotyping and extensive biobanking of a human translational cohort, an animal cohort (outbred Swiss mice), and the accelerated aging model Nothobranchius Furzeri (African Killifish) within the program. Considering the heterogeneity of the frailty condition, the variety of assessment tools, and experimental designs, it is fundamental to merge all potential molecular mechanisms and pathophysiological consequences into a systemic approach that facilitates advances in the field.

Investigating the Biology of Frailty

Given the multiplicity of mechanisms underlying frailty, one potential productive approach to uncover these biological mechanisms is to develop a construct composed of common pathways that become dysfunctional with time. One of the first conceptual clinical frameworks for frailty emphasized an organization of the biological connections between age-associated molecular alterations, physiological decline, and clinical signs and symptoms (Figure 2) (599). The neuro-immuno-endocrine systems formed the basis, which were theorized to be less effective in individuals with frailty, because of (or in part due to) the presence of low-grade inflammation (inflammaging) and excessive and unopposed oxidative stress. This clinical framework for frailty laid the groundwork for the current conceptualization for clinical frailty discussed below (160, 450, 597). As a significant outcome of the emerging field of Geroscience, there is potentially great overlap between the framework of aging (hallmarks/pillars) and the current, clinical conceptual framework for frailty.

Hallmarks

Aiming to understand the mechanisms underlying frailty a focus on the identification and categorization of the cellular and molecular hallmarks is valuable. The concept of hallmarks is not new, and in fact, to date, there are two well-established conceptual frameworks for understanding the development and progression of human cancers and aging (Hallmarks of Cancer, Hallmarks of Aging, Pillars of Aging) (218, 300, 359). Because frailty likely arises from the failure of multiple mechanisms associated with the described Hallmarks/Pillars of Aging to sustain health, these suggested pathophysiological mechanistic pathways provide an initial scientific roadmap to drive preclinical frailty investigations. Briefly, the Hallmarks

of Aging represent fundamental and interconnected biological pathways which are divided into three broad categories: primary, antagonistic, and integrative (359) (Figure 3). Genomic instability, epigenetic alterations, telomere attrition, and loss of proteostasis are described as primary hallmarks, which are the drivers or triggers of the aging process leading to damage. Thus, in the context of clinical symptoms of frailty, these make sense as underlying processes that initiate and/or propagate widespread dysfunction among multiple cell and tissue types (or organ systems). The antagonistic hallmarks include deregulated nutrient-sensing, mitochondrial dysfunction, and cellular senescence, which represent protective compensatory mechanisms. Key to the concept of compensatory mechanisms is that these mechanisms are initially protective (function to preserve homeostasis and biochemical balance); however, beyond a certain threshold and/or over prolonged time periods these compensatory mechanisms lead to severe detrimental adaptations or outcomes. Currently, it is hypothesized that these compensatory mechanisms contribute to the reported variability in survival rates and importantly, to the presence of diverse phenotypes within chronological aging (160). Because frailty is dynamic and exists on a continuum from robust (fit) to frail (or stages nonfrail, prefrail, and frail), it is logical to hypothesize the continuum of frailty is the manifestation of compensatory mechanisms within specific cells and specific tissues reaching thresholds and beyond, yielding detrimental adaptations (478). SC exhaustion and altered intercellular communication (integrative hallmarks) represent the final outcomes of the damage caused by both the primary and antagonistic hallmarks, leading to dysfunction within the various tissues and to age-related chronic diseases. The collective physiological dysfunctions potentially result in frailty, a clinical term that describes the combined deficits of many systems.

The seven Pillars of Aging are consistent with the Hallmarks of Aging and include adaptation to stress, epigenetics, inflammation, macromolecular damage, metabolism, proteostasis, and SCs and regeneration (Figure 3) (300). Although the contribution of each of these hallmarks or pillars towards the biology of frailty is unknown, the processes are certainly interwoven influencing physiological potential, physical resilience, and intrinsic capacity within tissues. Clinically, it is worth noting the detectable changes currently utilized to characterize frail individuals (independent of the frailty assessment tool) are only apparent when compensatory mechanisms begin to fail and results in detrimental adaptations. In this context, the nature of the drivers or triggers, the compensatory mechanisms, and their maladaptations are not well understood. It is becoming clear that the pillars and hallmarks provide guiding principles for preclinical frailty research. Arguably, targeting the compensatory mechanisms (pathways) within specific tissues and organs in lifespan longitudinal studies currently holds the strongest impact for its usefulness as a strategy to understand the underpinnings inducing frailty.

Linking the biology to the clinic

Progress in basic aging research during the last decade influenced the current clinically based frailty framework, which now states that frailty is caused by an overt age-associated dysregulation of multiple homeostatic systems or a loss of harmonic interactions between multiple domains (genetic, biological, functional, cognitive, psychological, and socioeconomic) that lead to homeostatic instability (160, 450, 597) (Figure 4). This general

framework is based on a hierarchical organization of three different levels of complexity (biological mechanisms, pathophysiological mechanisms, manifestations of frailty) (159). Two of the layers (inner and intermediate) take clear advantage of the pillars and hallmarks discussed above. The inner layers focus on the biological mechanisms involved in frailty at the subcellular level [e.g., mitochondrial dysfunction, oxidative stress, DNA damage, shortening of telomere length, maladaptive DNA methylation (DNAm)]. The intermediate layers consist of potential pathophysiological mechanisms leading to frailty (chronic low-grade inflammation, energetic imbalance, anabolic deficiency, neurodegeneration). The outer layers comprise the clinical consequences and the manifestations of frailty (e.g., functional deficits, reduced mobility, cognitive impairment, loss of independence, multiple chronic diseases). At present, our understanding of the interplay of the components within each layer and between each layer is very rudimentary and remains a distant prospect. Nonetheless, these gaps in knowledge can be filled by rigorous preclinical animal research and new informatics technologies, which enable the processing and interpretation of complex constellations among interacting biological parameters.

Our preclinical mouse longitudinal lifespan investigations are initial steps to address the three layers within the hierarchical organization discussed above. In these studies, one of the outer layers representing manifestations of frailty (physical functional deficits) identified frailty onset, progression, and mortality risk (40–42, 321). The value of identifying the onset of frailty lies in the opportunity to tease out the factors triggering frailty. The intermediate layer focused on the metabolic demand or energetic imbalance (e.g., aerobic vs. anaerobic) is associated with the specific functional tests. For instance, the treadmill run to exhaustion yields information primarily about the cardiorespiratory system response to stress; whereas the grip meter strength test yields information about the neuromuscular system. The value of these individual functional tests within the mouse Frailty Phenotype is certainly acknowledged in providing direction in identifying mechanisms of frailty associated with muscle function. Yet arguably the biggest value lies in the observations that these physical function measures do not decline at the same rate, mice demonstrate frailty with different functional measures, and not every mouse becomes frail (40–42, 321).

Now that we introduced the general frailty framework and its development, we will expound on specific mechanisms believed to be critical to set-up or contribute to the frail phenotype described in both preclinical and clinical studies. Indeed, the current conceptual framework for frailty, distinctive and complementary, constitutes an organizing principle for rationalizing the complexities of frailty, as investigations from both preclinical and clinical research progress. With the biological and pathophysiological mechanisms of this current framework in mind, in this article (the following sections), available reports related to potential biological underpinnings of frailty are presented and include the layers, pillars, hallmarks, and other potentially related topics.

Epigenetics, Genomic Instability, and Frailty

Decades of research indicate that both genetic and environmental factors influence aging and the propensity to become frail. Appreciating that these factors also drive methylation processes suggests that epigenetics may play a role in the development or progression

of frailty. We understand that frailty is strongly associated with age-related phenotypes, reduced longevity, and has been used as a measure of biological aging. Therefore, studying epigenetic alterations represents another avenue of biological research to better understand mechanisms that promote aging and potentially frailty.

Epigenetic modifications refer to chemical and structural alterations to the genome that have been shown to significantly change gene expression and phenotype without altering the underlying DNA sequence. DNAm, histone modifications as well as microRNAs (miRNAs) contribute to the epigenomic landscape with DNAm being the most common epigenetic modification studied (53, 259). It is clear that DNAm levels are modifiable, and the effects can be cumulative, thus their role in aging and age-related pathologies and disease is under intense investigation (172). In aging, a global (whole genome) decline in DNAm (172) has been observed along with an increase in variability (184, 525, 641). However, results differ as to whether lower global DNAm is associated with people considered frail compared to those that are nonfrail. These differences are due in part to a variety of challenges that face the field, including the type of frailty assessment, be it the FI or the Frailty Phenotype (described earlier in this article) as well as the type of methylation analysis performed among others (67, 110, 191). All of which takes place with the understanding that DNAm *patterns* likely differ in frail people.

By far, the most well-studied aspect of DNAm in its association with aging has been in the development of “clocks” which are algorithms based on DNAm status at sets of specific 5′-C-phosp-G-3″ (CpG) sites that vary with age. These DNAm clocks have been used to predict mortality and the influence of external factors by estimating biological age via predicted DNAm age (220, 241, 242). DNAm age may be considered a biomarker of aging as chronological age is not the best measure of aging processes nor mortality (as the rate of aging varies between individuals). The differences between DNAm age and chronological age are predictive of health and longevity [termed Epigenetic age acceleration; reviewed in Horvath and Raj (243), Levine et al. (343), and Lu et al. (360)]. This estimation of biological age is predicted from samples of blood (primarily) and tissue analyzed for methylation status at the sites that vary with age.

A recent report performed a meta-analysis of 61 studies (over 50,000 participants) that examined associations between chronological age and DNAm age (mostly blood-based) using either the Hannum or Horvath clocks (486). These investigators found that 56 studies showed associations with known risk factors for chronic disease and increased DNAm age. Forty-eight of these studies found a relatively strong correlation between chronological age and DNAm age. There were three frailty studies included consisting of 3092 individuals each associating frailty with increased methylation age (67, 191, 304). Two other studies supportive of the frailty and methylation age data showed decreased strength positively correlated with increased DNAm age (373, 528). Additional studies have refined the concept of the initial clocks by incorporating age-related health outcomes and training. The resulting clocks, DNAm PhenoAGE (phenotypic age) and GrimAge, act as highly predictive biomarkers of morbidity and mortality outcomes such as time to death, time to cancer, and time to coronary heart disease (343, 360). Important for the discussion of frailty, the Frailty Inferred Geriatric Health Timeline clock and a second model, the Analysis of Frailty

and Death clock have been generated for use in mice and incorporate frailty indices in the prediction of chronological age and life expectancy (504, 505). Overall, these association studies predict that biologically older adults as determined by methylation age are more likely to exhibit co-morbidities and potentially to be physically frail but much more data is needed.

It is difficult to determine the cause of frailty as the biological drivers of multisystem dysregulation are many and likely to be interconnected. Few studies have focused on the biology underlying the contribution of epigenetic processes such as methylation status to the development or progression of frailty. One such report focused on understanding fatigue and muscle weakness by investigating hyperhomocysteinemia (HHcy) which has been implicated in frailty as it appears to augment the age-associated decline in physical function (584, 585). $CBS^{+/-}$ mice (cystathionine beta-synthase), a model of HHcy, are produced by creating a deficiency in the enzyme that metabolizes homocysteine resulting in high homocysteine. These mice are more fatigable, and exhibit reduced contraction force but their skeletal muscles exhibit no changes in muscle morphology (fiber type composition), only fewer large muscle fibers and more medium size fibers compared to wild type mice. It is known that fatigue and muscle weakness encompass both structural (decreased muscle mass, dystrophin complex assembly deficiency) as well as energy imbalances and that exercise intolerance and fatigue occur in frailty (56, 130, 163, 407, 610). Results of this study indicated that the excess fatigability was partly due to lower adenosine triphosphate (ATP) levels in skeletal muscle fibers. They also observed altered miRNAs (mir-31, mir-494) involved in dystrophin regulation, lower dystrophin levels, and decreased mitochondrial transcription factor A (MtTFA) and nuclear respiratory factor 1 (NRF-1). In contrast, no changes in enzymes regulating muscle metabolism nor changes in creatine kinase were detected, thus, an energy imbalance was not considered. Four weeks of exercise increased ATP, reversed low MtTFA, and decreased miRNAs. C2C12 myoblast cells treated with homocysteine exhibited increased mir-494, Dnmt3a-3b levels and global methylation while MtTFA and ATP decreased, supporting the animal studies. Thus, one mechanism linking epigenetics with frailty may be through enhanced DNAm. This may, in turn, change gene expression directly by downregulating MtTFA or indirectly by upregulating miRNAs resulting in epigenetic changes induced by HHcy that undermine skeletal muscle function. However, as stated above, studies directly linking epigenetic alterations to a biological outcome and then, to frailty are scarce.

Genetic outcomes have been analyzed in terms of frailty biomarkers (from blood) categorizing people into nonfrail, prefrail, and frail cohorts by investigating associations with mutagenicity, DNA repair competence, and genetic damage (572). This particular study found that genomic instability and frailty are linked but that a combination of markers would provide key information on frailty severity and assist with potential health care strategies in frail individuals.

Histone modifications represent an additional mechanism that can mediate changes in gene expression and phenotype through silencing transcription and regulating genome stability among other means (61). The patterns of histone marks have been shown to change with age at specific loci as well as globally (50). Changes in the activities of enzymes that place and

remove histone marks play a large role in the outcomes of each mark and its patterning. For example, histone deacetylases have been investigated extensively in the aging field with the sirtuins capturing the most attention. In general, sirtuin activation improves skeletal muscle metabolism and protects against sarcopenia thus likely plays a role in frailty (196, 203, 608). However, a discussion of sirtuins and their modulation is beyond the focus of this article. Aging has been shown to trigger chromatin changes in skeletal muscles of mice, humans, and more recently, in killifish (84, 607). This species of fish exhibits a progressive loss of muscle function with age sometimes leading to sarcopenia that is characterized by weakening muscle strength and impaired mobility. The killifish was identified earlier in this comprehensive as a model organism to be examined in the current ongoing INSPRIRE Program. The combination of increased tri-methylation of lysine 27 on histone H3 protein (H3K27me3), heterochromatin protein 1a (HP1a), polycomb complex subunits, and senescence-associated heterochromatic foci along with reduced H3K9ac results in an accumulation of heterochromatin that is thought to contribute to the loss of muscle mass, decreased cell proliferation and mitochondrial function, and increased inflammation in old skeletal muscle. Similar findings have been reported in mice and humans (21, 607). Thus, reports regarding altered histone marks with aging are emerging but changes with frailty specifically are underexplored at this time.

Small ribonucleic acid (RNA) molecules such as miRNA impact mRNA processing and multiple processes (617). A number of miRNAs have been associated with aging as well as physiological processes in muscle and a review of miRNAs involvement in frailty is nicely presented by Rusanova and coworkers (484). Many of the studies indicate associations between specific miRNAs in older subjects and inflammation (147, 240, 426). In serum, frail individuals exhibit higher mir-21 compared to nonfrail while mir-223 and mir-483 increase in robust and frail aged participants to similar extents (483). In skeletal muscles, several laboratories have studied age-related miRNAs and found increased mir-146a, -155, -185, -206, -215, and -223 and decreased mir-148a, and -434 in mice, monkeys, and humans (141, 217, 303, 383). These miRNAs are known to modulate aspects of muscle physiology. Sarcopenic-associated miRNA changes have also been described, but results of this study rely on small numbers and warrant further examination (649).

There is an essential need to understand molecular mechanisms leading to the onset and progression of frailty. Epigenetic mechanisms are known to influence a variety of processes in aging and skeletal muscle physiology with strong associations with frailty. However, the challenges involved are many and discussed earlier in this article. The type of frailty assessment impacts the associations observed, assaying global versus specific loci, serum/plasma versus muscle tissue, direct measures versus associative studies (and use of algorithms like methylation clocks) as well as the context (patterning of histone marks and methylation, aging, frail, nonfrail) in which the studies are conducted, and the lack of animal models together impact the outcomes and determine the strength of the conclusions. Beyond the associations and presence of biomarkers lies the understanding of the biology which represents the biggest challenge to ascertain therapeutic interventions to slow, delay, reverse or prevent frailty.

Stress-response System in Frailty

The loss of physical and cognitive reserve and decreased function that often occurs with advanced age may also be accompanied by an increased vulnerability to stressors and parallel physiological dysregulation. Terminology employed within this topic includes physiological reserve, robustness, resilience (described earlier in this article), coping mechanisms, and homeostasis disruption among others. Fried and coworkers (177) describe frailty as a high-risk physical state with decreased reserves and increased vulnerability to stress and suggest that the key driver is energetic imbalance. Others suggest that this inability to generate an optimal response to stressful stimuli is the underlying mechanism that leads to frailty (68). Together, based on what is known currently, it appears that the mechanisms that contribute to frailty are multifactorial.

The pathways involved in an organisms' response to stressors depend in part on the exposure type, strength, length, and the state of health. There is general agreement, however, that with advanced age the ability to adapt to or resist stress is lower than at younger ages resulting in heightened vulnerability (432). Another way to think about this vulnerability is that physiological systems decline in efficiency and cellular communication deteriorates with time resulting in dysregulation. This physiological dysregulation may not be apparent initially (or in the resting state) but is observed when the system is challenged. Responses to acute stress vary but can include changes in heart rate, respiratory rate, glucose availability, digestive tract activity, and muscle tension among others. In turn, chronic stress can be more detrimental and impair growth, reproduction, immune competence, bone quality, and physical functioning. Thus, the dysregulation that occurs over time that results in altered responsiveness to acute and chronic stress may contribute to the development and progression of frailty.

The key biological systems that respond to stress and impact daily activities include the nervous (sympathetic), endocrine, and immune systems, leading to downstream physiological/metabolic adaptations to short- or long-term conditions (Figure 4). What dictates a "stressor" or stressful situation is beyond the scope of this article, but in general terms, the physiological response to a stressor involves coordination of events in both the brain and periphery. Physiological systems activated by a stressor are many and range from molecular to organismal. Activation of the hypothalamic-pituitary-adrenal (HPA) axis is one component of the systemic response assisting the organism in coping with stress. The HPA axis is primarily involved in energy mobilization but has evolved in the literature as a biomarker of stress (a discussion that is also beyond the scope of this article and in many cases is truly integral to the overall systemic response) (365).

HPA axis and frailty

We think of the HPA axis as a primary coordinator generating behavioral responses but also the adaptive responsiveness in intermediary metabolism and immunity as well as reproduction and feeding (102, 103). As such, upon a stressful event activation of the HPA axis includes corticotropin-releasing hormone (CRH) release from the hypothalamus and subsequent stimulation of adrenocorticotropic hormone (ACTH) release from the anterior pituitary. ACTH propagates the signal by stimulating the adrenal gland to

release glucocorticoids (cortisol in humans, corticosterone in rodents). In acute situations, glucocorticoids rise within minutes to hours and impact neuronal activities, glucose stores, and immune cell distribution, among other events (glucocorticoids impact thousands of genes). As with other endocrine factors, negative feedback is in place to maintain homeostasis with glucocorticoids downregulating the release of CRH and ACTH. Thus, in the context of physiological dysregulation and the development and progression of frailty, maladaptive or unrestrained responses of the HPA axis may be considered one of the main drivers of a physically frail state interacting with metabolism and the musculoskeletal system (Figure 4) (177, 187, 432).

Accordingly, an increased vulnerability to stressors is documented as neurons age that in turn, impacts HPA hormone production and release (180). Plasma cortisol levels vary with time of day (diurnal variation) but are typically high in the morning and lower in the evening. Although differences in morning cortisol levels in prefrail, frail, and nonfrail individuals varied between studies, all the studies found that physical frailty was associated with higher evening cortisol levels and an overall blunted diurnal variation in cortisol. This blunted or loss of a dynamic cortisol response (via altered negative feedback of ACTH) results in prolonged exposure to higher overall cortisol levels in these older adults and likely contributes to vulnerability and the clinical presentation of frailty (195, 238, 267, 334, 415, 581). This low reactivity of the HPA axis has been previously correlated with negative health outcomes (447). Furthermore, the physical characteristics of gait speed and grip strength, which are two tests within the Frailty Phenotype assessment tool, are correlated with morning to evening cortisol ratios (194, 195, 267, 546). Walking speed and chair rise time (measure of strength) are also associated with impaired diurnal cortisol (194). These findings suggest a link between disrupted cortisol and muscle atrophy underlying physical frailty. Consistent with HPA axis dysregulation and prolonged exposure to cortisol there is evidence that these changes also contribute to altered stress responsiveness and deterioration including neurodegeneration and cognitive decline (157, 494, 495, 553).

There are several examples of stimulus-response experiments that have been conducted to characterize responses to stress that strongly support the hypothesis that this dysregulation in community-dwelling older adults contributes to frailty [as reviewed in Fried et al. (177)]. Within the HPA axis, an ACTH challenge elicited exaggerated dehydroepiandrosterone (DHEA) responses associated with increasing frailty from nonfrail to prefrail and frail suggesting inappropriate negative feedback (334). In women challenged with lower extremity isometric exercise, skeletal muscle phosphocreatine recovery was slower in frail when compared to prefrail and nonfrail individuals (580). Consistent with the previous study, Lewsey and coworkers (345) also showed that exercised frail persons exhibited significant declines in skeletal muscle energetics compared to nonfrail older adults. When considering glucose metabolism, in nondiabetic older women subjected to an oral glucose tolerance test (stress challenge), those categorized as physically frail exhibited an exaggerated and prolonged increase in mean insulin and glucose levels compared to nonfrail and prefrail women (285–287). In addition, the frail women displayed dysregulated ghrelin following this glucose tolerance test (614). Though glucose dysregulation was not uncommon in these individuals, it was remarkably dysregulated in the frail women. Finally, when considering responses within the cardiovascular system orthostatic hypotension was

significantly more prevalent in community-dwelling older adults considered frail compared to nonfrail when challenged by an orthostatic blood pressure test (lying to standing) (614). In individuals categorized as frail, each of these altered responses to a stressful event provides evidence of increased susceptibility to stress that is tied to physiological dysregulation across many physiological systems.

In view of the discussion of stress adaptation and frailty, it is important to recognize the role glucocorticoids play in skeletal muscle glucose and protein metabolism. Indeed, glucocorticoids inhibit insulin-stimulated glucose uptake and glycolysis as well as by decreasing protein synthesis and enhancing proteolysis (320). In the presence of chronic glucocorticoid-mediated protein degradation by the ubiquitin-proteasome system and autophagy-lysosome system, there is significant skeletal muscle atrophy and weakness (66, 143, 320, 497). We also know that dehydroepiandrosterone sulfate (DHEAS), another adrenal-derived hormone, exerts anabolic functions in muscle and is decreased with aging. Moreover, the serum cortisol/DHEAS ratio (≥0.2) from older adult patients aged ≥65 years with type 2 diabetes (T2D) was identified as the strongest risk factor for sarcopenia and was associated with increased odds of frailty in a 10-year longitudinal study (44, 632). Thus, the concomitant increase in cortisol levels and decrease in DHEA likely contribute to physical frailty and sarcopenia (PF&S) (632).

Taken together, while the evidence is still incomplete and with the contributions of the many cellular mechanisms that regulate glucocorticoid levels unknown, the inability to maintain homeostatic control and the resulting “persistent high cortisol levels” are likely playing a role in triggering frailty onset and frailty progression within multiple tissues (105).

Somatotropic axis in frailty

Other components of the hypothalamic-pituitary (HP) axis have also been linked to aging and frailty. The somatotropic axis, in particular, has been investigated for its anabolic role in muscle and as a major player in longevity (69, 70, 586). The somatotropic axis consists of growth hormone (GH), upstream hypothalamic hormones, the insulin-like growth factors (IGFs), and downstream signaling molecules. The balance of two hypothalamic factors, growth hormone-releasing hormone (GHRH) and somatostatin (SS) determines the rate of GH secretion from the anterior pituitary. Plasma GH directly stimulates IGF-1 production and secretion by the liver in addition to exerting direct effects on other tissues. Local tissue production of GH or IGF-1 also occurs, suggesting the importance of autocrine and paracrine actions of these hormones. GH and IGF-1 have both somatic effects stimulating the growth of tissues and metabolic effects that play a role in protein, carbohydrate, and lipid metabolism. Alterations in these interrelated pathways can thus lead to both growth retardation or tissue proliferation and a variety of metabolic disturbances.

In mammals, there is a natural age-related decline in plasma GH levels and a concomitant decrease in IGF-1 that likely act as protective mechanisms to decrease metabolic activity and cellular division (255). Many studies have shown that GH secretion patterns, GH receptor deletions, IGF-1 receptor (IGF1R) mutations, and low circulating IGF-1 levels are associated with longevity and survival in nonagenarians and centenarians (49, 58, 390, 548, 575, 576). Yet, the role of the IGF-1 pathway in relation to aging and longevity in mammals

is inconclusive, which may be related to ethnicity, sex, age, and dietary differences among cohorts and the fact that GH is driving much of the IGF-1 expression (95, 391, 461, 645). Briefly, the field began to focus on IGF-1 instead of GH because invertebrate longevity data pointed to the insulin-IGF signaling pathway as integral to lifespan determination. Invertebrates (nematodes, flies) do not have GH or an upstream master regulator and function associated with GH driving insulin/IGF activities (at least not identified at this point in time). Thus, the translation from invertebrate systems to mammalian signaling is not direct, misinterpreted, and perhaps misguided (35). That said, while GH/IGF-1 pathway declines with aging in mammals, intriguingly, from a frailty biomarker perspective, low IGF-1 levels increase the odds of frailty and symptoms of frailty (strength, physical performance) (139, 338, 578). These reported associations involving the GH/IGF-1 signaling pathway are likely due to its role as a major player in metabolism, whereby its decline leads to a multitude of physiological consequences (e.g., frailty). For instance, IGF-1 promotes a major role in regulating skeletal and cardiac muscle growth by increasing myocyte number, activating muscle cell hypertrophy, anti-apoptotic properties, or inhibiting muscle protein breakdown (36, 446, 567). Similar to the insulin pathway, the IGF-1 signaling cascade is centrally regulated by Akt (protein kinase B), that controls protein synthesis via the kinases mTOR and glycogen synthase kinase 3β (GSK3β), while protein degradation is mediated by forkhead box protein O (FoxO) transcription factors (503). Indeed, variants in *AKT1* and *FOXO3A* genes were identified in 567 nonagenarians/centenarians as important to the aging phenotype (229, 616). Overall, the somatotropic system plays a role in the maintenance of muscle and its function as well as in aging and longevity and therefore, is key to our understanding the hormonal contributions to frailty.

The physiological response to stress changes with age. With advanced age, key biological systems such as the HPA axis and the somatotropic signaling axis respond less optimally resulting in a decreased ability to adapt to stress and heightened vulnerability compared to younger organisms. It is this loss of resilience that may lead to prefrail and frail states in older organisms.

Inflammation and Frailty

An area under current intense investigation focuses on the role of the immune system as an underlying cause of aging processes, age-related disease, and frailty. Chronic nonre-solved inflammation is a shared clinical condition among many immunometabolic disorders, including age-related diseases and frailty (186, 515). The contribution of chronic inflammation to the pathogenesis of age-related disorders has been termed *inflammaging*. Conceptually, when the resolution phase of inflammatory process is delayed or fails, other severe detrimental conditions ensue such as chronic secretion of proinflammatory cytokines and glucocorticoids, inappropriate initiation of inflammatory stimuli, misplaced molecules, misfolded molecules, and oxidized proteins which may promote inflammaging (119, 173, 174, 577). The overproduction and/or extended exposure to high levels of proinflammatory cytokines may lead to a loss of homeostasis and an exacerbated catabolic state within tissues that are especially vulnerable [e.g., muscle, (4, 186, 518)]. Figure 5 highlights potential triggers of inflammation, mediators, and the consequences of inflammaging.

Over the last decade, there is an abundance of analyses investigating inflammatory markers associated with aging and frailty from a biomarker perspective to identify populations at risk for poor outcomes (80, 281). Briefly, the most commonly studied inflammatory markers are C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, and interferon-gamma (IFN-γ) with CRP, IL-1-receptor antagonist, IL-6, IL-18, and TNF-α receptor-1 [nuclear factor kappa B (NF-κB)-mediated pathway markers of inflammation] associated with adverse health outcomes and mortality in older adults (22, 119, 173, 455, 582).

There is a preponderance of cross-sectional studies reporting that high levels of IL-6 and CRP are associated with frailty, frailty severity, poor physical performance (gait speed, strength, physical activity), and poor cognitive performance (23, 277, 337, 449, 455, 498, 539, 600). Prefrail and frail older adults also show elevation in white blood cells and fibrinogen compared to robust adults (111, 339, 340, 539). Importantly, many of the clinical frailty-associated inflammatory markers identified above and other biomarkers (e.g., transferrin) are also confirmed with advanced technologies such as proteomics-based screening (128). In contrast, the analyses from four large prospective studies (longitudinal design) failed to confirm these findings and other studies do not always report elevation of these classical inflammatory biomarkers (328, 633). Differences between studies are due, in part, to a variety of challenges that face the field, including study design, and conditions that increase inflammatory markers such as medical conditions and presence of obesity (328, 533, 534, 539, 587). Collectively, although evidence is emerging that greater inflammatory activity is associated with frailty, it is not true of all inflammatory markers. These studies are promising but much more research is needed to identify best practices (e.g., inclusion criteria of participants, selection and analyses of biomarkers) to yield the most useful information. We foresee longitudinal research studies further delineating the role of inflammation at the onset of frailty and transitions between frailty severity, delineating inflammatory sources and their targets, and development of targeted interventions.

Interestingly, only a few of these inflammatory biomarker studies are designed to report sex differences. For instance, high concentrations of CRP and fibrinogen are more strongly predictive of incident frailty in women than in men (188). In older institutionalized men with multiple comorbidities, a higher IL-6 level is positively associated with the Frailty Phenotype, while no significant correlations are noted for TNF-α and CRP levels (324). Recently, while investigating the immunological aspects of frailty higher numbers of myeloid-derived neutrophils and monocytes, but not lymphoid-derived T-, B-, or NK(natural killer)-cell numbers, were associated with frailty in both women and men (491).

Although much of the research is focused on inflammatory biomarkers, this approach has identified associations between frailty and with distinct metabolic/hormonal pathways (IGF-1, triiodothyronine, CRP, erythrocyte sedimentation rate, white blood cell, and lymphocyte counts), between frailty and reactive oxygen metabolites and between slower gait speeds (a frailty symptom) and isoprostanes, lipoprotein phospholipase A2 and osteoprotegerin (350, 498). Collectively, these associations demonstrate the loss of homeostasis across many cellular systems and tissues (169, 539). However, the underlying biological explanations for these changes in terms of initiation have yet to be uncovered.

The biomarker approach is useful for demonstrating that inflammatory activity is related to frailty and frailty risk, but there is a dearth of studies focused on mechanistic information needed to combat frailty or focused on the anti-inflammatory regulatory pathways. One example of a mechanistic study comparing sixteen pairs of frail and age-, race-, and sex-matched nonfrail participants, found that pro-inflammatory C-X-C motif chemokine ligand 10 (*CXCL10*) expression as well as serum IL-6 levels positively correlated with frailty status, suggesting CXCL10 as a possible biological target in preventing frailty (456). Future research in the field of frailty and inflammation will take advantage of molecular, transcriptional, and proteomic biomarkers as well as analyses to integrate information of inflammatory activity and immune regulation and dysregulation (131).

Given that preclinical animal investigations focused on frailty and inflammation are in their infancy, there are a few studies implicating chronic inflammation as an underlying mechanism to frailty. For example, treating frail mice with Enalapril, an angiotensin-converting enzyme (ACE) inhibitor, alleviated symptoms of physical function while reducing proinflammatory cytokines IL-1α, monocyte-recruiting chemokine monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1a and up-regulating the anti-inflammatory IL-10 cytokine (299). Paralleling the development of preclinical assessment tools to classify frailty, the use of frailty as an outcome variable, and the studies focused on frailty intervention, there's a growing body of evidence using preclinical rodent models that mimic specific aspects of immune dysfunction that are proposed to contribute to frailty.

IL-10KO mouse and IL-10/6DKO mouse:

The IL-10KO (*Il10*^{tm1Cgn}/J) mouse develops a chronic inflammatory bowel disease and is a model proposed to study the biological basis of frailty (598). Briefly, IL-10 is a cytokine with anti-inflammatory properties and maintains the balance of the immune response by allowing the clearance of infection while minimizing damage (496). Using the deletion of the *IL10* gene as a comparable model to human frailty, skeletal muscle weakness paralleled enhanced serum levels of proinflammatory cytokines such as IL-6, IL-1β, TNF-α, IFN-γ, CXCL 1 (308). Because of these findings a series of studies determined whether the IL-10KO mice display a Frailty Phenotype and further sought to examine cellular pathways closely associated with the Frailty Phenotype. In this regard, the IL-10KO mice exhibit phenotypic frailty characteristics in onset of muscle weakness, fat mass and resting metabolic rate, and activation of low-grade inflammatory pathways (526). Other effects associated with IL-10 KO mice include dysregulated adipokine and hormone levels (leptin, adiponectin), differential expression of skeletal muscle genes related to mitochondrial function and apoptosis, and altered mitophagy pathways suggesting failure to clear abnormal mitochondria (8, 307, 308, 598, 609). With respect to mitochondrial dysfunction, the IL-10KO mice present with low rates of ATP synthesis, reduced energy release from ATP hydrolysis and mitochondrial death signaling, and high levels of damaged mitochondria (8, 307). The disruption in mitochondrial homeostasis likely contributes, in part, to increased oxidative stress damage and further triggers apoptosis, and the observed performance impairment and phenotype (strength and exhaustion). Lastly, these mice show cardiovascular changes such as stiffer blood vessels, impaired vascular relaxation, cardiac

hypertrophy, and contractile dysfunction consistent with widespread metabolic changes. Indeed, these extensive properties support the hypothesis of inflammation and tissue dysfunction; however, this mouse model also has limitations that are associated with IL-10 deficiency and require attention when interpreting results (e.g., altered lymphocyte and myeloid profiles, increased cancers, altered responses to inflammatory stimuli).

IL-10$^{tm/tm}$/IL-6$^{tm/tm}$ mouse:

To identify the precise role of IL-6 on chronic inflammation and mitochondrial impairment, a double knockout (DKO) mouse deficient in both IL-10 and IL-6 was created, IL-10$^{tm/tm}$/IL-6$^{tm/tm}$ (364). Briefly, IL-6 is a pleiotropic cytokine with a central role in the integrated immune defense network in response to tissue damage and infections (480, 556). The biological consequences of IL-6 production are associated with both pro- and anti-inflammatory effects, highlighting IL-6's pivotal role in the activation and regulation of the immune response (501). Phenotypic characteristics, serum measurements (cytokine, lipid metabolite, and mitochondrial energetics), cardiac oxidative metabolism and mitochondrial energetics, treadmill testing, and survival were determined in the DKO mice and compared to age- and gender-matched IL-10 KO and WT mice. The overall findings demonstrate that selective knock-down of IL-6 in a frail mouse with chronic inflammation results in the reversal of some of the chronic inflammation-related alterations. The DKO mice had increased protective mitochondrial-associated lipid metabolites (serum), improved myocardial oxidative metabolism, and a transitory improvement in functional performance. However, these mice also had higher mortality.

Inducible IL-6 expression (IL-6$^{TET-ON/+}$) mouse:

The Inducible IL-6 expression (IL-6$^{TET-ON/+}$) mouse was developed to determine to what extent a single cytokine in isolation, recapitulates features of frailty in mice (264). IL-6 was selected because serum IL-6 is consistently found to be elevated in frail individuals and was suggested to be a causal driver. In this model, IL-6 induction was doxycycline dose-dependent and increased independently of other inflammatory cytokines and to levels observed in old mice. Importantly, increased IL-6 levels lead to increased frailty and disrupted muscle mitochondrial homeostasis. These results suggest a direct causal relationship between IL-6 and frailty.

Lastly, to provide a more complete picture of the inflammatory state during frailty and to the connectedness of the pillars, cellular senescence will be discussed briefly. Cellular senescence is a complex process, which is characterized by the inability of cells to proliferate, leading to over-production of proinflammatory factors (cytokines, chemokines, and other pro-inflammatory molecules) by senescent cells (senescence-associated secretory phenotype, SASP) (117). This process is favored by aging, was reported in multiple tissues, has a wide range of effects, and can promote chronic health diseases including insulin resistance, CVD, chronic obstructive pulmonary disease (COPD), neurological disorders, cancer, and osteoarthritis (302, 651). Recently SASP proteins [a panel of seven SASP factors composed of growth differentiation factor 15 (GDF15), TNF receptor superfamily member 6, osteopontin, tumor necrosis factor receptor 1 (TNFR1), Activin A, chemokine (C-C motif) ligand 3 (CCL3), and IL-15] were positively associated with age, frailty, and

adverse postsurgery outcomes (500). In addition to these seven SASP proteins which are closely associated with inflammation; increased cell senescence due to enhanced autocrine and paracrine feedback mediated by NF-κB, cycloxygenase-2 (COX-2), and reactive oxygen species (ROS), results in enhanced telomere dysfunction (279). Evidence suggests it is possible to suppress the elevated SASP by targeting the Janus kinase (JAK) pathway and observing a wide range of effects. In fact, JAK inhibition in frail mice alleviated both adipose tissue and systemic inflammation while enhancing physical function and improving many symptoms of frailty (627). Equally interesting is the fact that targeting cellular senescence using multiple approaches to reduce inflammation (e.g., senolytics: Dasatinib and Quercetin) in old mice and mouse models of accelerated aging improved physical function and reversed aspects of frailty, suggesting cellular senescence is a driver of the diminished skeletal muscle function (626, 652). It is important to note that the field identifies and provides evidence for many potential causes of SASP including DNA damage, dysfunctional telomeres, epigenomic disruption, mitogenic signals and oxidative stress, infections, lifestyle, and environment; hence, interventions to prevent or attenuate cellular senescence will likely be multifactorial (78, 144, 545, 639).

Complex and deeply integrated physiological systems work together to maintain homeostasis and function. Thus, an imbalance or disruption in one system has multiple downstream effects. So much that, the aging-related chronic physiological stimulation of the innate immune system may arise from metabolic regulation at the same time that immune molecules and cells may also impact metabolism in turn. Dysfunctional immunometabolism increases susceptibility to age-related disorders and physical frailty.

Metabolism and Frailty

The major contributor to aging and mechanism by which biological changes are induced with age is *metabolism* (Figure 3). There is compelling evidence that a multitude of metabolic-associated genes and major signaling pathways compress the period of morbidity including target of rapamycin (TOR), adenosine monophosphate-activated protein kinase (AMPK), and the nicotinamide adenine dinucleotide (NAD^+)-dependent sirtuin deacylases among others. It is believed that these pathways sense and respond to the nutritional environment and promote cellular defense mechanisms in the face of stress with the overall goal of maintaining homeostasis. Collectively, metabolic processes have been shown to change over time in multiple organs and represent an underlying cause of aging that likely contributes to the onset and progression of frailty.

Many theories describe the metabolic features that drive aging and potentially frailty. Two of them are complementary, associating energy expenditure and oxidative stress to physiological and physical decline. The *Rate of Living Theory* postulates that the metabolic rate is inversely related to lifespan (i.e., lower metabolic rate associates with longer lifespan). Similarly, *the Free Radical Theory* links the excessive mitochondrial ATP production from high metabolic rate to oxidative stress damage and lifespan shortening (165, 468). In this sense, limiting energy consumption could potentially cause metabolic rate to decline, delaying frailty. In fact, since 1935, evidence consistently highlights CR as the only intervention able to extend lifespan (Figure 6) in many species, including humans

and rhesus monkeys (113, 114, 532). It is worth noting that the rhesus monkey provides important insights on the health benefits of CR to humans given that the rhesus monkey shares nearly 93% of sequence identity with the human genome and that, similarly as in humans, CR increases survival and lowers age-related morbidity (113, 114, 379, 393).

Population-based studies, randomized controlled trials, and intervention studies clearly provide evidence that CR (15%–30%) for extended periods of time (1–2 years) in healthy individuals decreases mortality rate, slows metabolic rate, and reduces oxidative stress (48, 235, 468). Results of these studies support both the rate of living and free radical theories discussed above. Other physiological adaptations associated with CR include reductions in body weight, central obesity (visceral fat mass), daily energy expenditure, inflammation, and cardiometabolic risk, as well as increases in insulin sensitivity (230, 261, 269, 384, 401, 458, 467). Similarly, these physiological adaptations to CR are reported in individuals with obesity following CR regimens (269, 331).

Next, the question arises as to whether CR reduces frailty. Although there is an emerging number of small studies the findings are very promising. One such example is a study in mice by Kane and colleagues (290) whereby reduced calorie intake led to an improvement in frailty status as determined by the FI. In another rodent model, dietary restriction in rats led to a decrease in frailty incidence, improvement in animal activity, and spatial memory (563). Likewise, genetically long-lived hypopituitary Ames dwarf mice subject to 30% CR show a protection in some of the clinical features of the frailty syndrome (i.e., grip strength and fatigue test) (24). Consistent with the observed improved behavioral performances, CR delays sarcopenia by favoring protein synthesis, regulating mitochondrial function, and promoting SC regeneration in several animal models [reviewed in Xie et al. (625)].

To date, it is unknown whether intermittent fasting reduces frailty per se; however, there is evidence that there are beneficial effects in frailty features [physical activity and survival in older mice (24-month age)] (485). It is also important to point out that there is evidence that the gut microbiome shows a shift in metabolic and taxonomic properties with increasing frailty, demonstrating changes in availability of specific nutrients (330). In nonhuman primates, a long-term study clearly indicates that CR reduces the incidence of frailty and increases healthy lifespan (631). In a close view of these limited number of studies, the results suggest that interventions of longer duration and/or initiated earlier in the lifespan have potential to be most effective for frailty (563).

With the noted benefits of CR, reduced levels of certain macronutrients primarily in human studies raise questions of whether longevity and more recently healthspan are affected by the calories per se, or if they are related to proteins specifically (22, 107, 108, 433, 535). There are numerous studies highlighting important concepts related to diet (e.g., single essential amino acid, methionine, specific BCAAs, ratio of macronutrients, sources of proteins and timeframes) supporting longevity, multiple metabolic benefits, and healthspan (170, 310, 344, 428, 471, 636). For example, we understand that restricting dietary proteins, such as branch-chain amino acids (important mTOR regulators) extends lifespan and promotes metabolic health in mice (471). It is also logical to see the potential impact to prevent, delay or reverse physical frailty given the importance of amino acid availability to alleviate

sarcopenia through skeletal muscle protein synthesis (333). However, the literature in the field of macronutrients reveals great complexity. Clinically, in patients with cachexia, protein supplementation is not always successful on preserving lean mass as fat mass is the tissue depot that often prevails [reviewed in Evans et al. (152)]. Moreover, the effectiveness of dietary protein supplementation against muscle mass depletion and poor physical function might be dependent upon many factors such as physical stimulation (85, 233, 555). While being promising and exciting at this time more research is indicated to evaluate the cellular mechanisms with preclinical frailty models based on findings relating restricted protein intake to sarcopenia and frailty (108). Regardless, to date, the role of CR in human longevity remains to be elucidated.

Within the discussion of CR, it is unclear whether CR-related weight loss might be detrimental to overweight older individuals [reviewed in Locher et al. (355)]. Epidemiological data indicate that overweight older adults have lower risk of mortality than normal-weight older adults (97, 166). There are potential detrimental effects to muscle and bone health with CR-induced weight loss because the weight loss parallels reduced lean mass and bone mineral density in humans [reviewed in Locher et al. (355)].

Taken together, metabolism is significantly impacted by diet (amount, composition, feeding regimen, etc.) which in turn, modulates aging processes likely resulting in changes in susceptibility to the development and progression of frailty. Yet, given the heterogeneity of frailty, the efficacy of dietary interventions might depend on age, research model, interventional strategy (e.g., % of calories, % of macronutrients, length of intervention), stage of frailty, presence of comorbidities, etc. In the following paragraphs, some of the key factors involved in metabolism will be introduced and discussed as they relate to aging. However, these descriptions are not meant to be all encompassing but are focused on their relationship to frailty.

mTOR

At the center of aging and metabolism is the mTOR pathway. The mTOR signaling network is a major nutrient-sensing system which ultimately through downstream effects regulates metabolism, mRNA translation, and protein turnover (Figure 6) (499). Within the aging research community, there is extensive effort to understand this signaling network because disruption is reported in many diseases, including cancer, T2D, and frailty. From an aging perspective, mTOR inhibition (e.g., Rapamycin) is a well-established mechanism that is subject to genetic and drug manipulation to influence healthspan and longevity (55). Of note, rapamycin is an Food and Drug Administration (FDA)-approved immunosuppressive drug to which clinical relevance is applied to organ allograft rejection and more recently in clinical practice for immunological, physical performance, and cognitive outcomes among older adults (317, 368, 369).

Indeed, in a range of animal models, mTOR is a key modulator of lifespan and healthspan acting through various mechanisms (227, 270, 387, 388). These mechanisms include extension of lifespan while promoting gut homeostasis via SC function (inhibiting mTORC-1 activation) (361), microbiome remodeling, as well as via inhibition of senescence

and SASP by suppressing translation of IL-1α and suppression of carcinogenesis (17, 323). Moreover, feeding mice rapamycin reduces resting metabolic rate, delayed death, and development of pathological lesions while improving motor function in both sexes (647).

Loss of skeletal muscle mass and strength is central to the phenotypic criteria or clinical hallmarks used to classify physical frailty (Figure 8). Regulation of muscle mass is thus a critical biological step in triggering frailty onset and frailty progression. To date, animal studies are the main contributors to the current knowledge on the physiological aspects leading to muscle atrophy. These studies demonstrate the activation of the mTOR network by IGF-1 and phosphoinositide-3-kinase (PI3K)/Akt promotes protein synthesis via S6 kinase phosphorylation (488, 499, 503). The importance of S6 kinase is further reported in studies whereby mice lacking S6 kinase show extension of lifespan along with a resistance to age-related pathologies (513). Important to this comprehensive article, with aging the chronic activation of mTORC1 stimulates increased numbers of abnormal skeletal muscle mitochondria leading to oxidative stress, fiber damage, and fiber loss over the lifespan (557). The pro-oxidative mitochondrial effect is likely associated with alterations in expression of GDFs, including GDF-15 (557). Whereas, inhibiting mTORC1 (rapamycin) alleviates oxidative stress and reduces muscle fiber loss in old mice (557). The preservation of muscle fiber size and muscle mass is also associated with decreasing gene expression of cellular senescence markers (276). In principle, in the presence of chronic activation or by the inhibition of mTORC1 in skeletal muscle it is logical to predict a significant impact on muscle function leading to changes in physical performance.

In a pilot study with a focus on frailty, rapamycin administration did not improve frailty status even in the presence of reduced inflammation (530). In contrast, in an accelerated aging mouse model [genetically enhanced NF-κB activity ($nf\kappa b1^{-/-}$)], rapamycin reduces frailty and improves long-term memory, as well as neuromuscular coordination and tissue architecture (118). Additional positive outcomes indicative of a protective role against frailty are reported in IL-10 homozygous knockout mice treated with rapamycin and/or maraviroc —[the only specific C-C chemokine receptor type 5 (CCR5) antagonist approved for clinical use] (438). Overall, the evidence supporting the role of inhibiting mTOR to combat frailty is promising; but at this time the identification of a specific target tissue is lacking which is critical for a significant impact. Taken together, in the next generation of preclinical frailty research, understanding the mTOR signaling pathway and its downstream effects may be critical to preserve muscle function and prevent the onset of frailty.

AMPK

AMPK represents an additional promising metabolic target in the quest to prevent, delay or reverse frailty (Figure 6). The rationale to target this molecule is not based on substantial evidence assessing frailty but is based on investigations in the fields of aging (longevity) and pharmacological strategies to manage chronic diseases (e.g., diabetes T2D). The beneficial effects of AMPK are via metabolic modulation through a multitude of pathways in many tissues. Briefly, AMPK is a highly conserved molecule, acting as an energy sensor such that upon low intracellular ATP levels, AMPK stimulates catabolic pathways while regulating mitochondrial homeostasis through complex processes that aim

to switch off anabolic pathways (AMPK-dependent transcriptional reprogramming) (232). In fact, in skeletal muscle, AMPK regulates energy metabolism in a NAD^+- and SIRT1(NAD-dependent deacetylase sirtuin-1)-dependent manner, leading to activation of the peroxisome proliferator-activated receptor (PPAR)-gamma and the FOXO1 and FOXO3 transcription factors discussed earlier in this article. Energy metabolism is also influenced as AMPK modulates metabolic enzymes, which are part of the fatty-acid and sterol synthesis network [e.g., acetyl-CoA carboxylase (ACC) and β-hydroxy β-methylglutaryl-coenzyme A (HMG-CoA) reductase] (185).

Critical to the discussion of metabolism, aging, and the development of frailty interventions, AMPK regulates glucose uptake in muscle and adipose tissues by stimulating glucose transporter type 4 (GLUT4) trafficking (182). To date, there are well-established means to regulate glucose uptake through AMPK activation such as physical exercise and insulin sensitizers (thiazolidinediones); however, other classical glucose uptake regulators (e.g., anti-T2D interventions) are also emerging as exciting global therapeutics for health (31, 116, 171, 643). Since optimal glucose and insulin levels are integral to health and disease prevention, past and present studies utilizing metformin, in particular, are gaining attention. Indeed, as a key regulator of many metabolic pathways that are also involved in age-related diseases, metformin has also emerged as a potential anti-age player with effects that mimics those observed in CR. It is well-established that metformin attenuates microvascular and macrovascular complications in T2D in addition to its antihyperglycemic actions (via SIRT1/LKB1/AMPK pathway) (98, 519, 650). Metformin treatment also results in decreased hepatic gluconeogenesis and mitochondrial redox state, inhibition of mTORC signaling and Akt phosphorylation, and down-regulation of lipogenic pathways (356, 408, 439). Collectively, these metformin-associated changes have potential to influence the homeostasis within tissues. A compelling link to understanding frailty and potential cellular mechanisms is the increasing evidence supporting the role of AMPK to combat inflammation (demonstrated as NF-kB dependent via both AMPK dependent and independent pathways) (371, 489, 490). Understandably, metformin has been studied by many investigators as an intervention to delay aging in part because it is already FDA-approved for use in humans. Regarding AMPK as a target of metformin, the field of Geroscience and others are convinced that metformin has a geroprotective effect because it reduces all-cause mortality as well as age-related diseases (77, 347). The mortality benefits of metformin are more easily observed when comparing patients with T2D to individuals without T2D. Indeed, these two groups have similar mortality rates, even though they are more obese and exhibit co-morbidities (29).

To date, there are a small number of studies investigating frailty with metformin treatment as an intervention. In one study, two clinical features of frailty are differentially affected in healthy older adults, gait speed performance improved with metformin treatment whereas there was no change in grip strength (325). The protective effect of metformin against frailty and symptoms of frailty is also observed in older diabetic patients (decreased odds of frailty; improved muscle strength and body balance) (550, 603). Yet, the efficacy of metformin against frailty is inconclusive. For instance, one recent study indicates no correlation between metformin consumers and frailty prevalence (228). Because there are a small number of studies with differing outcomes, at this time the efficacy of metformin as

a therapeutic strategy against frailty is unknown. We will await corroboration, as ongoing clinical trials (e.g., preventive nature of metformin against frailty in prediabetic adults aged more than 65 years old; TAME study) are completed (150).

There is a plethora of studies investigating metformin treatment in preclinical animal studies (e.g., *C. elegans,* rodents) that are beyond the focus of this article. Collectively, however, metformin-induced benefits, acting through AMPK activation, encompass extension of lifespan, improvement in physical performance and insulin sensitivity, and reductions in oxidative stress and inflammatory damage (73, 375, 506). These findings suggest a role for AMPK in triggering the onset of physical frailty, which is consistent with reports indicating an attenuation or suppression of AMPK activation in muscles from older rats with contractile dysfunction (222). Overall, there is promising evidence to pursue AMPK as a target to alter the onset and progression of physical features of frailty in both aging individuals and preclinical animal studies.

NAD^+

A reduction in NAD^+, a cofactor of key enzymatic reactions in many metabolic pathways and plays a pivotal role in maintaining the integrity of the mitochondrial electron transport chain, is likely involved in frailty. Yet there is a dearth of specific investigations focused in this area (Figures 4, 6, 7). Many studies in the aging field suggest that an increase in $NAD(^+)$ catabolism (down-regulation of NAD^+) due to DNA oxidative damage occurs through SIRT3 pathways and impairs normal cellular function (76, 377). Because of the multiple cellular roles of $NAD(^+)$ a reduction has a widespread impact including disruption of the peroxisome proliferative activated receptor, gamma, coactivator (PGC)-1α/β-independent nuclear-mitochondrial communication (203). Hence, in view of the importance of energetics for cellular metabolism, there are substantial efforts to design therapeutics that target this pathway with the goal to increase, regulate or maintain NAD^+ at youthful levels. For instance, elevating NAD^+ with nicotinamide riboside (NAD^+ precursor) in a placebo-controlled, randomized, double-blind, crossover trial, elevates muscle NAD^+-related factors as determined in the metabolome while reducing systemic inflammation. Transcriptional expression via RNA sequencing further revealed downregulation of energy metabolism and mitochondrial pathways in muscle in these aged men (146). Promising results are also reported in patients with T2D (treatment with nicotinic acid derivative acipimox, an NAD^+ precursor) revealing reduced skeletal muscle lipid content, increased insulin sensitivity, and further enhanced *ex vivo* mitochondrial respiration likely through activation of the mitochondrial unfolded protein response (UPR) in skeletal muscle (574).

Investigations in preclinical animal models (naturally aging, accelerated aging, mitochondrial and diabetic disease models, genetically engineered models to overexpress *SIRT1* or *SIRT6*) and encompassing a broad range of outcome measures provide very promising support for targeting this pathway, too (60, 86, 133, 389, 558, 637). Foremost, investigations specifically focused on NAD^+ therapeutic intervention and frailty are limited; however, the improvements associated with frailty symptoms in the following studies suggest regulating NAD^+ levels would have positive outcomes to prevent, delay, or reverse frailty. Pharmacological prevention of age-related NAD^+ decline (using 78c, a

thiazoloquin(az)olin(on)e CD38 inhibitor) improves glucose tolerance, muscle function, exercise capacity, cardiac function along with enhanced expression of pro-longevity factors such as AMPK (558). Age-associated physiological decline is mitigated with nicotinamide mononucleotide (NMN) administration, a key NAD^+ intermediate, as revealed by enhanced energy metabolism, physical activity, along with improved insulin sensitivity and plasma lipid profile (389). In old mice, NMN supplementation reverses the age-derived decline in endothelial function while upregulating NAD^+, restoring vascular SIRT1 activity and increasing manganese superoxide dismutase (MnSOD) levels (133). Lastly, muscle SCs respond to nicotinamide riboside by inducing the mitochondrial UPR and synthesis of prohibitin proteins, causing the SCs to rejuvenate (644). Indeed, collectively these experiments uncover not only the multitude of effects of this pathway but demonstrate the wide range of benefits.

From a muscle perspective and its importance in physical frailty, depleting skeletal muscle of an essential enzyme in the NAD^+ pathway results in fiber degeneration and progressive loss of two of the frailty symptoms, strength, and treadmill endurance in mice (175). In view of the positive outcomes of targeting this pathway in aging studies noted above, it is not surprising these functional deficits and muscle morphological changes are reversed with nicotinamide riboside supplementation (175). As evidenced in muscle disease studies, the Duchenne's muscular dystrophy mouse model exhibits reduced NAD^+ levels, decreased mitochondrial function, and impairment of tissue energetics. Targeting this pathway to replenish NAD^+ levels results in significant improvements in mitochondrial function and structural protein expression as well as significant reductions in general poly (ADP)-ribosylation, inflammation, and fibrosis. These morphological changes are associated with improvements in skeletal muscle function and heart pathology (487). This cofactor plays a significant role in metabolism and specifically in metabolic pathways integral to aging and disease processes. Looking forward the NAD^+ pathway will be pivotal in future studies identifying targets to prevent onset and progression of physical frailty.

Metabolites and frailty

Metabolites are substrates and products of metabolism or "proxies of metabolism" which have far-reaching cellular effects. Several of the cellular functions closely associated with this comprehensive review include regulation of epigenetic and SC mechanisms; cellular responses and signal transduction; and metabolism. Importantly, given that metabolites have effects within the local environment in which they are produced, they also have potential to impact and/or control homeostasis (268, 296). Notably, the homeostatic controls are likely compromised with age, leading to a failure to return to steady state and ultimately to a functional decline.

With the opportunities to profile metabolites in biofluids, cells, and tissues and to the advances in bioinformatics and analytical technologies, understanding tissue- and system-level effects of metabolites is emerging (268, 296). Due to the accuracy, specificity, and sensitivity of metabolomics, there is the possibility of detecting subtle alterations in biological pathways to provide insight into the multiple mechanisms underlying frailty and the progression of frailty and then integrate this knowledge with functional and mechanistic

biological studies (112, 348). Although investigations focused on metabolites in their infancy in the field of frailty, evidence is emerging of differential expression of metabolites in individuals with frailty and in individuals at risk of becoming frail (prefrail) (161, 288, 316, 454, 466). As expected, these initial metabolomic studies identify diminished antioxidative defenses (e.g., carnitine shuttle, peroxisomal degradation, kynurenine pathway, vitamin E metabolism), decreases in radical scavengers (methionine, proline, tryptophan), disruptions in protein-amino acid, lipid and nitrogen metabolism, aminoacyl-transfer RNA biosynthesis, and citric acid cycle, and in the metabolome of frail individuals (288, 466). Equally interesting, there is a discriminating profile for prefrailty, which is sex-dependent (changes in 2,4-diaminobutyric acid for both genders, dimethyloxazole for men, and threonine, phenylalanine, and fructose for women). Thus, these metabolites form molecular signatures of frail and prefrail phenotypes, suggest the involvement of underlying biological mechanisms, and importantly have potential to tease out mechanisms that trigger frailty onset (354, 454).

Many of the metabolites identified above are consistent with the metabolome of skeletal muscle in frail individuals, correlate with physical performance (e.g., gait speed), and suggest dysregulation or decline in skeletal muscle mass and quality (sarcopenia) (75, 154, 363, 370, 396, 405). Furthermore, several of the metabolites overlap with metabolites that decrease with aging and cognition, demonstrating the connectedness of the physiological systems (288, 316, 454). Not only do these findings align well with the hallmarks and pillars (e.g., overwhelmed compensatory mechanisms: oxidative stress resulting from diminished antioxidant levels) the overlapping metabolic profiles support the idea that frailty is a dynamic condition involving multiple and integrated physiological systems.

The overall goal of maintaining homeostasis involves multiple metabolic processes and pathways that have been shown to change over time and represent an underlying cause of aging that likely contributes to the onset and progression of frailty. Interpreting the responses of multiple pathways suggests metabolic and neuroendocrine changes occur to conserve metabolic energy. The imbalance or dysregulation in overall energy metabolism likely influences physiological reserve within tissues. As noted earlier in this article, physiological reserve and resilience are key players in clinical frailty.

Mitochondrial Function and Frailty

Mitochondrial oxidative phosphorylation is the major source of energy production for cellular functions (423, 424). There is substantial evidence supporting the involvement of impaired mitochondrial function in the development of diseases, including manifestations of aging (65, 123–125, 214). Briefly, mitochondrial health is dependent on many fundamental mitochondrial processes such as biogenesis, fission, fusion, autophagy/mitophagy, proteostasis, and pathways associated with the regulation of quality control, metabolism, and oxidative stress as well as the crosstalk between tissues and organs that influence inflammation, the senescence of distant tissues, and the whole-body metabolic homeostasis. Indeed, it is well-known that dysfunctional mitochondria produce an excessive amount of ROS, which can trigger inflammation (341, 451, 552). There is evidence that

mitochondrial dysfunction is associated with chronic inflammation likely leading to a loss in cellular homeostasis in many tissues (358).

The maintenance of mitochondrial health or a functional mitochondrial network is paramount for preserving skeletal muscle homeostasis, whereby a disruption in the pathways controlling mitochondrial quality is a mechanism triggering sarcopenia and has potential to impact physical frailty (158, 319). Prevailing evidence supports the association between a reduction in mitochondrial oxidative capacity and physical performance such as walking speed, strength, and physical activity, symptoms of frailty and between oxidative protein damage and low grip strength (5, 101, 244, 527, 642). In principle, the loss of a functional mitochondrial network has far-reaching results such as alterations in ATP production, proteostasis, calcium handling, oxidative stress, and inflammation, all with the potential to impact frailty. An example of this complex cascade is supported by the decline in ATP production which is accompanied by enhanced ROS production, leading to further mitochondrial DNA (mtDNA) damage and electron transport chain dysfunction that amplifies the energetic deficit (89, 521). Moreover, a transcriptional signature of mitochondrial bioenergetic dysfunction in skeletal muscle is defined with (205) low PGC-1α/estrogen-related receptor (ERR) signaling, as well as downregulation of oxidative phosphorylation (385). In the functional perspective, such transcriptional modulations are translated into fewer mitochondria, reduced mitochondrial respiratory complex activity, and perturbed NAD^+ biosynthesis resulting in low NAD^+ levels in sarcopenic muscle (385). With alterations in the mitochondrial quality control mechanisms, there is abnormal organelle accumulation reducing the mitochondrial ability to adapt (or compensate) to challenging conditions (stress, increased vulnerability).

A growing body of evidence supports the contribution of mitochondrial dysfunction as an early biological mechanism triggering the onset of frailty (prefrail status) and a biological mechanism in the progression of frailty (Figure 3). Evidence of impaired function (reduced phosphocreatine recovery along with declined mitochondrial respiratory complex protein and activity) and down-regulation of mitochondrial genes in prefrail individuals, highlights the key role of mitochondrial function in frailty development (14). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that mediates antioxidant responses contributing to the regulation of mitochondrial function (257). While investigating the role of Nrf2 in frailty and sarcopenia, Huang et al. (247) demonstrate the contribution of Nrf2 as a regulator of mitochondrial biogenesis and dynamics in promoting muscle mass and maintaining physical function, where Nrf2 deficiency exacerbates frailty in a time-dependent manner. There is evidence of lower mtDNA copy number associated with frail individuals indicating processes such as mitochondrial depletion, energy reserves, and oxidative stress are playing a role in the progression or continuum of frailty (26).

Mimicking mitochondrial dysfunction by boosting oxidative stress in preclinical rodent models of frailty allows important advances for investigating the mechanisms underlying this syndrome. The SOD1 knockout (SOD1KO) mice, which lack the antioxidant enzyme copper- and zinc-containing superoxide dismutase (Cu/ZnSOD), is an animal model proposed to study frailty. Briefly, Cu/ZnSOD is the major antioxidant enzyme responsible for removing superoxide anions (conversion of superoxide anions to hydrogen peroxide) in

the cytosol and intermembrane space of the mitochondria and protecting cells from oxidative stress/damage (425). The Sod1KO mice display signs of accelerated aging such as hearing loss, cataracts, skin thinning and impaired wound healing, muscle atrophy, and a 30% reduction in lifespan (145, 258, 298, 403, 427). The Sod1KO mice exhibit characteristics of the Frailty Phenotype: weight loss, weakness, low physical activity, and exhaustion, and the skeletal muscle of Sod1KO mice show a dramatic increase in oxidative damage (135, 403). Frailty in Sod1KO mice is attenuated by dietary restriction. Sixty percent of ad libitum fed dietary restriction reversed the loss of muscle mass and function, improved mitochondrial function, and attenuated the increase in oxidative damage, cell senescence, and circulating levels of IL-6 (262, 648). As such, it is suggested that the Sod1KO mouse model may assist in investigating the biology of frailty and therapeutic strategies, specifically focused on oxidative stress, mitochondrial dysfunction, inflammation, and cell senescence.

A transgenic mouse model, with overexpression of the antioxidant glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme responsible for nicotinamide adenine dinucleotide phosphate (NADPH) protection against oxidative damage, was developed to evaluate if improved ROS detoxification improved healthspan (414). This mouse model exhibits increased resilience in response to age-associated decline of muscular and brain function suggesting that a lower accumulation of oxidative damage is beneficial for healthspan (414). Because these transgenic mice show partial protection from age-related functional declines that depend on several metabolic processes (e.g., glucose tolerance, insulin sensitivity, neuromuscular function) they may be a model for future frailty investigations targeted at the role of metabolism. In this respect, previously generated geroprotected animal models are available and could readily be utilized to study metabolic or other physiological aspects that contribute to prevented/delayed frailty.

In summary, most studies concur that mitochondrial dysfunction is a major player in frailty through multiple mechanisms. The beginning or early stages of frailty include unresolved inflammation and increased oxidative stress triggering a myriad of metabolic changes that involve many tissues, especially skeletal muscle. Initially, the compensatory mechanisms within cells, tissues, and organs are activated to maintain homeostasis and structure/function. However, at some point, these compensatory mechanisms become overwhelmed resulting in metabolic imbalance and frailty progression.

Stem Cells and Frailty

SCs are characterized by their multipotency and capacity to self-renew, providing progeny with important SC properties to ensure the SC pool and progeny that differentiate to repair injured tissues. Advancements in the study of SCs over the past two decades provide novel paradigms for the development of therapeutic strategies aimed at addressing multiple diseases. Importantly, there is an abundance of research highlighting the detrimental effects of aging on all types of SCs [e.g., hematopoietic SCs, bone-marrow mesenchymal stem cells (MSCs), umbilical cord SCs, adipose-derived SCs, skeletal SCs, muscle satellite cells] and the subsequent influence of these effects to further accelerate tissue deterioration (392, 463, 464, 594). Prevailing evidence suggests that the negative impact of aging is all encompassing with every biological characteristic of the SC being affected including

capacity for self-renewal, proliferative activity, differentiation potential, regenerative and repair capacity, immunomodulatory potential, anti-inflammatory capacity, stimulatory capacity, interaction potential with the microenvironment (paracrine action), and others (79, 297). Whether these biological changes are driven by SC-intrinsic and/or extrinsic alterations, these molecular, functional, and phenotypical changes collectively contribute to a loss of tissue homeostasis, to physiological systems failure, and to a decline in overall health, including frailty.

Similar to the various discussions presented earlier in this article specific changes occur in the genomic and epigenomic landscapes of aged SCs with respect to DNA modifications (e.g., methylation), specific histone posttranslational modifications, chromatin architecture, and epi-polarity (46, 167, 551). There is evidence of transcriptional changes, reductions in the DNA damage response and repair and dysregulation of quiescence (429, 520, 538, 544). Many of these intrinsic SC changes in the genomic and epigenomic landscapes lead to permanent dysfunction (591). SC dysfunction is also driven by dysregulation of metabolic pathways (421). With aging, the basal metabolism of SCs transitions to oxidative metabolism, which increases the production of ROS leading to SC metabolic related-stress and loss of SC mitochondrial homeostasis. Indeed, these consequences contribute to many of the age-associated SC phenotypes such as abnormal SC proliferation, compromised SC self-renewal, disruption of quiescence, and SC apoptosis (52, 92, 421, 566, 588). The specialized microenvironments, SC niches, promote SC maintenance and regulate many of the SC functions. Most studies suggest that there is breakdown of the interactions between SCs, their niches, the molecular feedback loops, and signaling pathways with aging [e.g., extracellular matrix (ECM) components, fibronectin, Notch signaling; TGF1-β, (FGF)-extracellular-signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK), NF-κB or wingless-related integration site (Wnt)] (57, 64, 90, 91, 167, 168, 329, 362, 457). Just as the SC niche is critical for SC function, maintenance of the SC proteome is equally important to prevent further SC cellular damage and dysfunction (381). Loss of autophagy, lower levels of proteasomal degradation, and inactive UPR endoplasmic reticulum (ER) in SCs lead to protein and metabolic stress, which impair self-renewal activity and regenerative potential (237, 400, 604).

Chronic inflammation also creates a detrimental environment for SCs (460). Foremost, SCs adopt an immunoregulatory phenotype in response to inflammatory factors such as IFN-γ and TNF-α through paracrine effects (e.g., metabolic regulation) and exosomes, and these immunomodulatory properties are reduced in aged SCs (306, 318). Skeletal muscle studies provide evidence indicating upon injury a “temporally-regulated, acute and transient immune response” is necessary for regeneration (562). With aging, chronic accumulation of pro-inflammatory mediators disrupts this tightly regulated immune response resulting in altered cytokine composition in the SC niche (62). Accordingly, the exposure of the SCs to this altered niche environment results in many detrimental consequences to the delicate balance of regulatory networks necessary to regulate tissue remodeling such as miscommunication between immune cells and SCs, SC exhaustion, impaired regeneration, and favored adipogenic differentiation (538). Taken together, these studies on age-related changes in SCs culminate in challenges to maintain cellular homeostasis, to preserve healthy tissue function, and to prevent frailty.

Frailty and stem cells

During the last decade, it was suggested that alterations in the production (numbers) and differentiation capacity of MSC were contributing to physical frailty in older adults (198, 199, 201, 202, 223–225). Because the physical performance measures described in the Physical Frailty Phenotype involve tissues (e.g., muscle, bone) from mesenchymal origin they share the same precursor or progenitor cell, the MSC (136, 179). Thus, in principle, it is logical to hypothesize that alterations in MSC function may play a potential role in frailty by aggravating muscle contractility (e.g., strength, endurance) as well as contributing to degeneration of other critical components required for optimal muscle contractility (e.g., neuromuscular junctions) (352). Yet, to date, there is a dearth of published investigations to support this hypothesis. The lack of investigations is likely due to the many challenges in evaluating the contributions of MSC to the development and progression of frailty.

One challenge identified in the frailty literature is a reliable source of MSC even though they can be obtained from almost any tissue within the human body. With the goal to investigate the role of SCs and frailty, circulating osteogenic progenitor (COP) cells were proposed and evaluated as a surrogate marker of the MSC population within the bone marrow. Using this approach, significant associations between COP cells and frailty and disability were reported with a confirmatory study that included a role for lamin A (a factor critical for muscle and bone function) (9). Although these initial studies incorporating this novel methodology are encouraging, there is still a vast amount of missing information surrounding SCs and frailty. A recent pilot study noted that circulating hematopoietic stem cells (cHSC) from frail seniors show the highest total DNA damage, compared to fit seniors and young controls providing initial evidence linking SCs with frailty (380).

Regenerative medicine and frailty

Currently, there are multiple clinical trials using MSCs to test therapeutic interventions in a large number of clinical conditions in almost every organ system (192, 223–225, 476, 541, 568). Many of these clinical conditions are associated with frailty and provide a rationale to consider SCs as a therapeutic to fight frailty. These organ systems include cardiac (dilated cardiomyopathy, heart failure); bone (nonunion bone fracture); eye (glaucoma, macular degeneration, retinitis pigmentosa); kidney (acute kidney injury); lung (COPD, pulmonary fibrosis); immune (rheumatoid arthritis); nervous (multiple sclerosis); and endocrine systems (diabetes type 1) (192, 568, 606). Collectively and important for this comprehensive article, the results from multiple clinical trials show that allogeneic human mesenchymal stem cells (hMSCs) are safe, irrespective of age (200). Equally interesting is the fact that for older adults allogeneic SCs harvested from younger donors are preferable because age-related changes in SCs make them less efficient for transplantation (559).

In 2017, the first human clinical trials were designed to determine the effects of allogeneic MSCs as an intervention to fight frailty. Results of these early-phase trials (www.clinicaltrials.gov: #NCT02065245) identified as CRATUS, allogeneiC human mesenchymal stem cells (allo-hMSCs) in Patients With Aging FRAilTy Via IntravenoUS delivery were encouraging demonstrating significant improvements in physical performance measures closely associated with the clinical manifestations of frailty. Outcomes

of a Phase II randomized, double-blinded, placebo-controlled trial of allo-hMSC (www.clinicaltrials.gov: #NCT02065245) were consistent with the phase I safety study indicating that infusion of allo-hMSCs was safe in older individuals with frailty and produced benefits in multiple outcome measures of physical performance as well inflammatory biomarkers (564). Together, these early studies suggest that allo-hMSCs may be an effective biological modifier of frailty and support ongoing investigation of allo-hMSCs. At this time, a review of ClinicalTrials.gov for clinical trials focused on key words "frailty and stem cells" highlights 14 registered trials from around the world indicating the excitement and evidence supporting a role for SCs in treating aspects of frailty in older adults. Importantly, these clinical trials include assessments of frailty with well-established assessment tools such as the Physical Frailty Phenotype (179).

Most recent efforts in preclinical animal studies demonstrate the utility of SCs to fight physical dysfunction boosting the evidence for both the understanding of molecular underpinnings and treatment potential. A series of studies tested whether transplanting adipose-derived mesenchymal stem cells (ADSCs) from young and aged donors caused detrimental physical effects in middle-aged mice (301, 353, 602, 634). Intriguingly, ADSCs from old donors significantly impairs walking speed, grip strength, endurance, and daily activity in the middle-aged mice posttransplantation. Whereas the middle-aged mice transplanted with the same number of ADSCs from the young donors do not show these impairments. Overall, these findings suggest that ADSCs from old donors can induce physical frailty, which is highly associated with morbidity and loss of independence (148). Thus, regenerative approaches entailing transplantation of ADSCs from aged donors might generate previously unrecognized risks.

The transcriptomes of the ADSC isolated from the young and old donors show that several SASP-related genes are up-regulated in the ADSC isolated from old donors. However, it is also worth noting that the study identifies $p21^{high}$ cells (identified as an expression level greater than 97% of cells from young donors) with transcriptomic signatures similar to *in vitro*-generated senescent cells, which have altered signaling pathways closely associated with muscle dysfunction; hence, these specific cells ($p21^{high}$ cells) may be a culprit contributing to the physical dysfunction (626).

In principle, the future is bright in the use of SCs as a strategy to combat frailty. However, the field faces several hurdles from the understanding of the SCs precise molecular underpinnings to specific clinical protocols that will improve clinical outcomes. For instance, if chronic inflammation within SCs is one of the main contributors to the progression of frailty, repeated SC administration over time will be required to maintain low levels of inflammation. Important considerations in repeated SC administration are cell dose, time intervals between administration, and route of delivery.

Age-related Frailty and Disease

Knowing that multiple physiological systems contribute to the susceptibility to frailty, it would be remiss not to present known associations of chronic disease with frailty. Thus, we present some of the major morbidities that are strongly associated with tissue level

dysfunction and states of frailty. Importantly and noted earlier in this comprehensive article the underlying biological mechanisms involved in the onset and progression of frailty related to disease are different from those involved in age-related frailty (16, 554).

Sarcopenia

The definition of sarcopenia is evolving with the increased emphasis on aging research. "Sarcopenia" was first described as the decline in muscle mass caused by aging in 1989 (481, 482). In the following decade, the definition of sarcopenia was changed to include low muscle mass as identified as lean appendicular mass/height^2 with specific cut-off points (being <2 SDs below the sex-specific mean of a young reference group) (43). In 2010, the definition of sarcopenia was further modified by the European Working Group on Sarcopenia for Older Persons (EWGSOP) (121). The EWGSOP defined sarcopenia as generalized loss of skeletal muscle mass together with low muscle function (a measure of strength or performance) and also recommended sex-specific cut-off points for sarcopenia diagnosis. Recently, the EWGSOP2 refined the definition of sarcopenia (120). The refined sarcopenia definition includes documentation of both low muscle strength and low muscle mass and includes recommendations of new cut-off points for sarcopenia diagnosis. In this refined definition, physical performance is used to categorize the severity of sarcopenia (120). The evolvement of the definitions for sarcopenia reflects the high-quality research over the past three decades and the complex nature of skeletal muscle health—the combination of muscle tissue (quantity and quality) and neuromuscular function translating into muscle strength and physical performance.

Sarcopenia and frailty are distinct entities. One of the most compelling and impactful observation contributing to our understanding of these two entities emerges from the Toledo Study of Healthy Aging (≥65 years) whereby sarcopenia correlates with frailty; yet, the results clearly establish these two terms cannot be used interchangeably. The major take-home message for the field is sarcopenia is not a useful clinical biomarker of frailty, but an individual's sarcopenia status (specifical absence of sarcopenia) is useful to exclude the presence of frailty or a frailty diagnosis (129). Nonetheless, both are associated with similar adverse health outcomes and most likely share pathophysiological similarities including inflammation, oxidative stress, and hormonal and energy imbalances (120, 179, 327). Conceptually, in this article focused on frailty, sarcopenia contributes to the decline of physical function (Figure 7) through pathophysiological processes when frailty is identified with the Physical Frailty Phenotype assessment tool. Indeed, physical performance or function is defined as an objectively measured whole-body function related to locomotion, involving muscles and nervous functions (central and peripheral) (120). Within this conceptualization, sarcopenia may precede frailty or predispose an individual to physical frailty due to multisystemic dysfunctions (120). Because a comprehensive review of sarcopenia is beyond the scope of this article, here we briefly describe the evidence for the relationship between sarcopenia and frailty and an overview of the established mechanisms underlying sarcopenia that perturb cellular homeostasis potentially leading to the onset and progression of frailty.

There are several cross-sectional observational studies describing positive correlations between sarcopenia and frailty, and as expected, there are studies demonstrating relationships between sarcopenia and individual symptoms of frailty (99, 129, 152, 176, 181, 376, 542). For instance, analysis with older adults from the Berlin Aging Study II shows that lower appendicular lean mass related to BMI has higher odds (1.4–2.8 times) of difficulties in physical activity (540). Importantly, because of the decades of research aimed at sarcopenia, longitudinal studies are now concluding that sarcopenia is associated with increased risk of incident disability, institutionalization, and mortality (236).

In the last decade, PF&S and the International Conference on Frailty and Sarcopenia Research (ICFSR) Task Force (internationally recognized scientists and clinicians) emerged to recognize the strong relationship between PF&S and to accelerate discoveries in treatment and prevention to combat sarcopenia and frailty (88, 477). One initiative of the ICFSR was the identification of promising biomarkers of frailty and sarcopenia resulting in one study identifying an association between GDF-15 and slower gait speed, increased walking time for 400 m (increased exhaustion/poor endurance), and lower physical performance in older adults (Baltimore Longitudinal Study of Aging) (514). The importance of GDF-15 in frailty, a member of the transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) superfamily, is first based in its involvement with sarcopenia-related outcomes (muscle wasting and cachexia) and second in the consequences associated with dysregulated metabolism (mitochondrial dysfunction and energy imbalance) (115, 436, 569). Inflammation and oxidative stress activate transcription factors (e.g., p53, hypoxia-inducible factor-1α, nuclear factor-κB) to upregulate *GDF15* expression (569) increasing skeletal muscle's vulnerability to metabolic changes within the microenvironment. Although this report is promising, caution is advised because of the complex pathophysiology of frailty and other skeletal muscle markers, such as protein quality control markers, that are associated with frailty (140). Moreover, it is unlikely that there will be a single biomarker identifying frailty.

Along similar lines altered energy metabolism is present within skeletal muscle of individuals with prefrailty and frailty, and this altered energy metabolism is related to the level of physical performance. For instance, in older adults, the postrecovery rate of phosphocreatine is lower and is correlated with poorer performance in fast and long walking tasks (101). The energetic cost of muscle contraction (measured during maximal intermittent and maximal contractions in the quadriceps) reveals a higher ATP cost potentially contributing to the observed age-related decline in muscle efficiency (332). These energy-related alterations or changes in bioenergetics are further exposed under conditions of stress such that frail adults have faster reductions in skeletal muscle energetics during fatigue tests compared to nonfrail adults (e.g., fatigue, postexercise) (14, 101, 275, 345). Based on the altered energy metabolism present in the pre-frail state, an interpretation of the findings it that skeletal muscle mitochondrial impairment is a hallmark of prefrailty development and the onset of decline in muscle function (14). Indeed, research incorporating the preclinical assessment tools to classify frailty or physical function supports this conclusion. Mice exhibiting frail phenotypes exhibit reductions in skeletal muscle ATP kinetics, high-energy phosphate levels, and energy release from ATP hydrolysis (8). The decreases of physical function and muscle mass are associated with reduced expression levels of genes involved

in mitochondrial biogenesis and dynamics, as well as reductions in mitochondrial number and content, mtDNA copy number, and abnormal mitochondria morphology (247). These reported aberrations in skeletal muscle metabolism and energetics are also influenced by the reduction in glucose metabolism and cell membrane phospholipids and an increase in small extracellular vesicles (448). Overall, the rapid decline, delayed recovery, altered kinetics and energy cost, and aberrations in mitochondrial structure suggest that changes in skeletal muscle metabolism (including microenvironment) disrupts the delicate balance between muscle structure and function, increasing vulnerability which may lead to frailty.

Much work in the field of sarcopenia shows evidence of increased muscle fat infiltration/content (a.k.a. myosteatosis), decreased protein synthesis, and enhanced proteolysis as causal factors contributing to aberrations within the microenvironment, loss of muscle quality (structure and composition), and muscle atrophy (fiber size and number) (Figure 7) (13, 121, 409, 640). The triggers for these events arise from many sources including inflammation, oxidative stress, lipid stress, and senescence (82, 409, 413).

Considering fat infiltration and frailty, fat accumulation within skeletal muscles is consistently reported in frail/prefrail individuals and in individuals where performance measures are used as proxy for frailty (12, 234, 382, 441, 570, 571). The consequences of fat accumulation within skeletal muscle are certainly detrimental, as observed in metabolic syndromes (MetSs) and high-fat diets. Indeed, frail individuals show increased fat infiltration (intermuscular adipose tissue) and inflammation (IL-6) and low functioning individuals show an abundance of senescent-like cells and intermuscular adipose tissue compared to nonfrail or highly functional individuals (4, 282). The consequences of these changes within the skeletal muscle microenvironment impact other components such as motor units and the structure of neuromuscular junctions, leading to impaired innervation and altered physical performance (81, 583).

There is evidence of deficits in protein quality control (e.g., increased levels of heat shock proteins, protein modifications, lipofuscin, misfolded proteins, aggregation, impaired mitophagy/autophagy), which contribute to reductions in muscle quantity and quality with aging and MetSs (Figure 7) (1, 11, 206, 430). To date the number of studies investigating both skeletal muscle protein quality control in the presence of frailty is limited; however, the results point toward impairment with the protein quality control network and the involvement of several physiological systems (1, 140, 254, 367). For instance, autophagy and mitophagy gene expression is downregulated in inactive frail older adults suggesting some degree of mitochondrial dysfunction; the presence of autophagy markers denotes the processes of autophagosome formation and autophagosome-lysosome fusion are affected in frail adults; and the low expression of E3 ubiquitin ligases suggests impaired proteolytic systems in frail adults (1, 140, 254). At this time the role of these changes in protein quality control per se in sarcopenia, frailty, and even in health is not fully understood; however, changes in the protein quality control network are now recognized in the pathophysiology of neurodegenerative disease and afford potential directions for further investigations in skeletal muscle (274, 618, 619). Thus, loss in proteostasis likely contributes to frailty through protein quality control disruptions in skeletal muscle.

Equally interesting is the impairment in skeletal muscle's anabolic response to stimuli (e.g., exercise, diet), defined as anabolic resistance, observed in older adults and in frail individuals (7, 127). It is suggested that the presence of anabolic resistance to stimuli predisposes protein synthesis to decrease, while protein degradation is facilitated (615). Indeed, older men require a higher dose of dietary proteins than younger men to stimulate similar postprandial activation of muscle protein synthesis (398). Likewise, postprandial muscle protein synthesis rates are reduced in older individuals (596). Anabolic resistance is observed in individuals with frailty and in those in the earlier stages of frailty following various exercise interventions programs (7, 561). Several cell-signaling pathways contribute to this impaired response such as inhibition of IGF-1/PI3K/Akt1/mTORC1 signaling pathway in the presence of inflammation, oxidative stress, and others [reviewed in Bonaldo and coworkers (59, 207, 406)].

Together these results suggest there is potential overlap in the pathophysiological mechanisms underlying sarcopenia and frailty. Although mechanistic details are still not understood, it is becoming clear that cellular homeostasis is disrupted in aging skeletal muscle through the interactions of metabolism, inflammation, adaptation to stress, and proteostasis that lead to physical frailty. Even though remarkable progress is noted in interplay between sarcopenia and frailty, formidable challenges lie ahead in the understanding of the critical mechanisms triggering the onset of frailty.

Metabolic syndrome

Here, we discuss the features of MetS because their pathophysiology such as reduced insulin secretion, insulin resistance, poor glucose homeostasis, chronic inflammation, oxidative stress, mitochondrial dysfunction, and dyslipidemia within many tissues, potentially play a role in triggering frailty and its progression (Figure 8) (96, 193). Foremost, MetS is a cluster of risk factors for diabetes and CVD featured by increased visceral obesity, insulin resistance, sustained hyperglycemia, and hypertension (10). To date, the studies investigating associations between MetS and frailty produce varied results. MetS is associated with frailty risk and prefrailty; whereas, there are reports indicating no association between MetS and frailty (37, 221, 289). These mixed findings bring to the forefront the impact of study designs, including definition of MetS and selection of frailty assessment tools and populations, etc. Even so, individuals with MetS are more likely to have frailty (593).

Visceral obesity

Several aspects of body composition, in particular the distribution and amount of body fat, and lean body mass (muscle) play an important role in overall health and likely frailty (Figure 8). In fact, abdominal obesity is identified as a driver of muscle dysfunction, supported by population-based investigations (265). Given the importance of this topic, the concept of sarcopenic obesity emerged whereby the accumulation of intramuscular lipid leads to an enhanced catabolic state (309, 312, 507). The negative metabolic consequences of visceral obesity together with skeletal muscle dysfunctions contribute to the development and progression of frailty in this population (2, 138, 252). In particular, accumulation of adipose tissue or the presence of adipocyte-infiltrating macrophages leads to increased secretion of pro-inflammatory cytokines, and production of pro-inflammatory adipokines

promoting lipotoxicity in skeletal muscle, thereby contributing to further pathophysiology of muscle and impaired function (e.g., loss of homeostasis, defects in protein quality control) (39, 256, 326). Indeed, in large population-based studies, abdominal obesity predicts frailty incidence or is associated with frailty (193, 248, 346, 462, 469, 543).

Insulin resistance

Insulin resistance has a myriad of consequences because insulin action is involved in various functions in a multitude of tissues. In general, there is evidence that insulin resistance (in the presence or absence of diabetes) is a risk factor for, is associated with, and predicts frailty, demonstrating the importance of regulating insulin and glucose homeostasis (Figure 8) (149, 284, 286, 287, 437, 440, 529). In skeletal muscle, insulin resistance leads to autophagy, protein degradation, and mitochondrial dysfunction resulting in muscle atrophy and weakness (Figure 7). The loss of muscle mass subsequently impacts glucose transport and further exacerbates insulin resistance (336, 622). Within the cellular signaling pathways, insulin resistance triggers the downregulation of the PI3K/Akt pathway, decreasing protein synthesis as well as FoxO phosphorylation. There is stimulation of atrogin-1 and muscle RING-finger protein-1 (MuRF1), both E3 enzymes, and the ubiquitin-proteasome proteolytic pathway is enhanced (Figure 7). Kalyani and collaborators report that the enhanced expression of E3 enzymes in insulin-resistant individuals is the mechanistic link contributing to skeletal muscle protein degradation (284). Importantly, this reduction in muscle mass further impacts blood glucose homeostasis through lower peripheral glucose uptake causing hyperinsulinemia and insulin resistance, a vicious cycle with detrimental outcomes (126, 132, 517). Further, metabolic changes in lipid metabolism associated with fat accumulation also affect skeletal muscle synergizing within this vicious cycle (444).

Overall, individuals with MetS including visceral obesity and insulin resistance experience considerable alterations in metabolism in key organs including adipose tissue, liver, and skeletal muscle. Because of the tightly coordinated cellular processes within these tissues and the crosstalk between these tissues, the loss in cellular homeostasis facilitates a dangerous cycle. These dysregulated systems create an imbalance between anabolism-catabolism, which will overwhelm the compensatory mechanisms and decrease physiological reserve, potentially leading to the onset and progression of frailty. Moving forward, more research is needed to rigorously differentiate frailty from metabolic disorders. Even though there is noted considerable overlap in pathological mechanisms (e.g., systemic inflammation, oxidative stress) between these, there may be subtle differences in the ability to mitigate frailty in these groups with interventions. It will be important to determine under which conditions metabolic disorders precede frailty and when the presence of frailty induces these diseases.

Cardiovascular disease

CVD and frailty may share several common underlying pathophysiologies (e.g., endocrine and immunologic systems) such as elevated levels of CRP, IL-6 (6, 106, 600). These pathophysiological manifestations underlie the increased systemic arteriolopathy (e.g., arterial stiffness) that may be found in both CVD and sarcopenia (260, 313, 419). Thus, it

is not surprising that there are observational studies and systematic reviews (cross-sectional and longitudinal) reporting associations of CVD risk factors with frailty (Figure 8) (63, 93, 189, 197, 213, 412, 547, 620).

Individuals with hypertension are highly heterogeneous, with variability in their physiological capacity, physiological reserve, and vulnerability to stress (404). Yet, it is suggested that long-term hypertension and poor control of blood pressure contribute to the observed systemic arteriolopathy noted above, causing ischemia, tissue damage, and dysfunction. These outcomes could potentially impair physiological reserve, increase vulnerability, and trigger frailty in this clinical population (452). Indeed, frailty is associated with hypertension (38, 83, 314, 411). In particular, a recent meta-analysis based on six cohort studies and one cross-sectional study demonstrates a significant association between frailty status (frail > prefrail > robust) and risk of falls and all-cause hospitalization among patients with hypertension (245). Whereas it is important to point out, there are reports that do not support a relationship between frailty and hypertension (19, 465, 479, 590). Vetrano and coworkers (590) investigated the prevalence of frailty in hypertensive individuals by reviewing 27 articles from longitudinal and cross-sectional studies with mixed results. The reasons underlying the inconsistencies within the reports are many including the status of the interrelationships between the physiological systems within individuals and the varied components with the designs of the studies (e.g., presence, types, and duration of co-morbidities). to pinpoint the role of hypertension and frailty, the dissection of the multiple pathways contributing to the metabolic perturbations may provide insights. Currently, the limited number of preclinical animal models investigating cardiac physiology and frailty provide evidence of associations between frailty and atrial dysfunction (electrophysiology, fibrosis, myocyte morphology) (263, 397, 434).

Collectively, we understand that molecular, cellular, and tissue level pathophysiology contribute to the susceptibility to frailty. The consideration of multiple co-morbid states increases the complexity of potential therapeutic development (Figure 8).

Conclusion

Considering the remarkably complex nature of the biological processes that underlie frailty, it is not surprising that the biological/clinical framework for frailty is stated in broad terms and many of the proposed biological processes lack sound, rigorous scientific evidence. Thus, it is challenging to unify all the relevant aspects. Yet, significant progress is obvious due to the synergy between the fields of Geroscience and clinical frailty, the reverse-translation of the two-well established frailty assessment tools into preclinical animal studies, and the development of advanced “omic” technologies providing a window into molecular and cellular processes and critical transition events in the presence or absence of frailty. Although reviewing the literature clearly demonstrates phenotypic parameters are available in both human and preclinical animal studies and cellular homeostasis is disrupted through a multitude of mechanisms within many tissues, the limited knowledge about the compensatory mechanisms and when they can no longer compensate hampers forward progress to fully understand frailty development. As recent findings from preclinical animal studies increase the palette of possibilities for mechanisms (and potential therapeutics),

making almost all of the “pillars or hallmarks of aging” targets as mechanisms, growing interest is expected. Consequently, more and more attention will be given to preclinical models in the field of frailty. It is important to emphasize that pinpointing the molecular and cellular pathways along the frailty continuum is crucial (frailty onset, transitions between robust, prefrail, frail) and will not only answer biological scientific questions but will also impact healthspan and lead to improvements in quality of life. The multitude of “omics” studies is important in identifying affected pathways but remains descriptive of the Frailty Phenotype versus understanding the biology of risk and onset. It will be critical to continue to identify genetic, lifestyle and environmental risk factors for frailty knowing that frailty is not an inevitable consequence of aging. Equally important is the development of therapeutic targets to lower risk, prevent frailty onset, and slow progression of ongoing disease. In turn, accomplishing these aims will assist in removing the stigma of advanced age, create opportunities and allow us to continue productive lives.

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Didactic Synopsis

Major teaching points

- Frailty is emerging as a serious global public health challenge.
- Two well-established clinical frailty assessment tools, the Frailty Phenotype and Frailty Index, have been reverse-translated successfully in preclinical animal studies.
- The conceptual frameworks, Hallmarks/Pillars of Aging, provide a roadmap of the biological areas contributing to the aging phenotype and to the pathophysiology of frailty. Key biological areas include genetics/epigenetics, adaptation to stress, inflammation, metabolism, proteostasis, and stem cells and regeneration.
- There is a growing body of evidence indicating that frailty occurs when compensatory mechanisms can no longer maintain homeostasis and is characterized by physiological dysregulation and increased vulnerability to stressors.
- There is overlap in the pathophysiological mechanisms underlying sarcopenia and physical frailty.
- There is a need for design standards, multiomics approaches coupled with physiological methods, and development of preclinical animal models that closely mimic frailty in humans to test specific mechanisms that contribute to this complex syndrome.

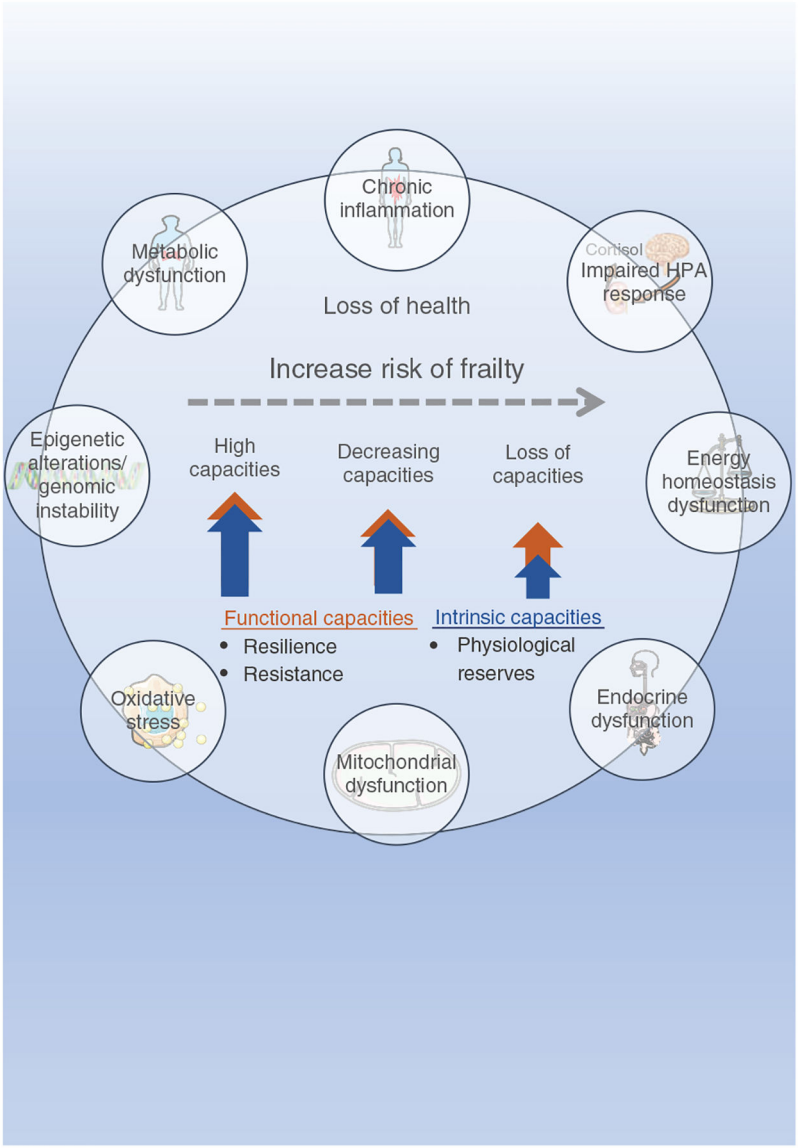

Figure 1. Health, frailty, and aging.

Frailty is characterized by a loss of health and is classified as an age-related medical syndrome that features the progressive reduction of health-promoting capacities. The health-promoting capacities are determined by *functional capacities,* when referring to both resilience and resistance abilities, and *intrinsic capacities,* when referring to physiological reserves. The substantial loss of these capacities increases the risk of frailty via dysregulation of multiple physiological systems. At the molecular level, epigenetic alterations, genomic instability, mitochondrial dysfunction, and oxidative stress are great contributors to impaired physiology that includes metabolic, energy homeostasis and endocrine dysfunction, chronic inflammation as well as impaired hypothalamic-pituitary-adrenal (HPA) axis response. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

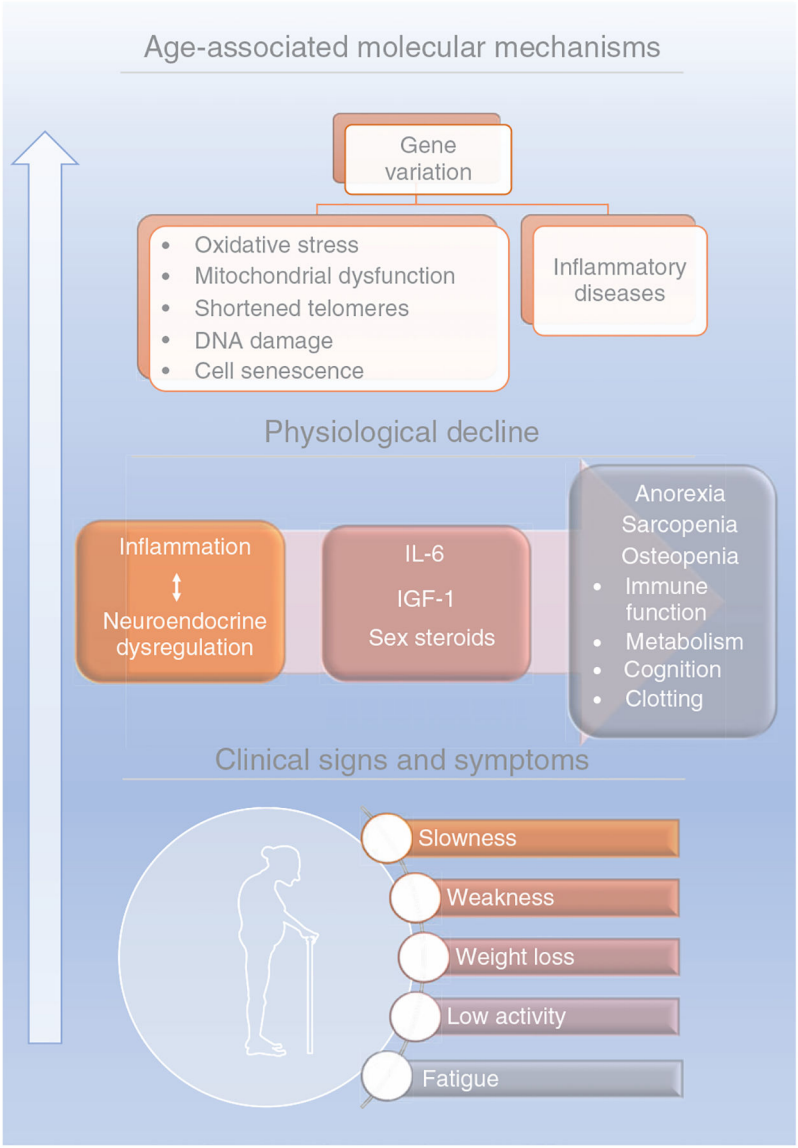

Figure 2. Conceptualization of physical frailty during the first decade of the 21st century (599).
Because the clinical signs and symptoms were known to be physiologically related to one another, in theory, they provided possible connections between molecular alterations associated with aging, physiological decline, and clinical systems. These biological connections were organized conceptually. In aging, the combination of gene variation, DNA damage, and telomere shortening contribute to oxidative stress, mitochondrial dysfunction, cell senescence, and inflammation that in turn, promotes a decline in the physiological functioning of the organism. The aging-related physiological decline occurs following chronic unresolved inflammation along with neuroendocrine dysregulation triggering anorexia, sarcopenia, and osteopenia, which are conditions related to body, muscle, and bone mass loss. This systemic loss and tissue dysfunction as well as the associated cognitive decline lead to the clinical signs of frailty: slowness, weakness, weight loss, low activity, and fatigue. This conceptualization emphasized the complexity of the multiple systems and

visually suggested the manifestations of frailty were a cumulative outcome of dysregulation of these multiple systems. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

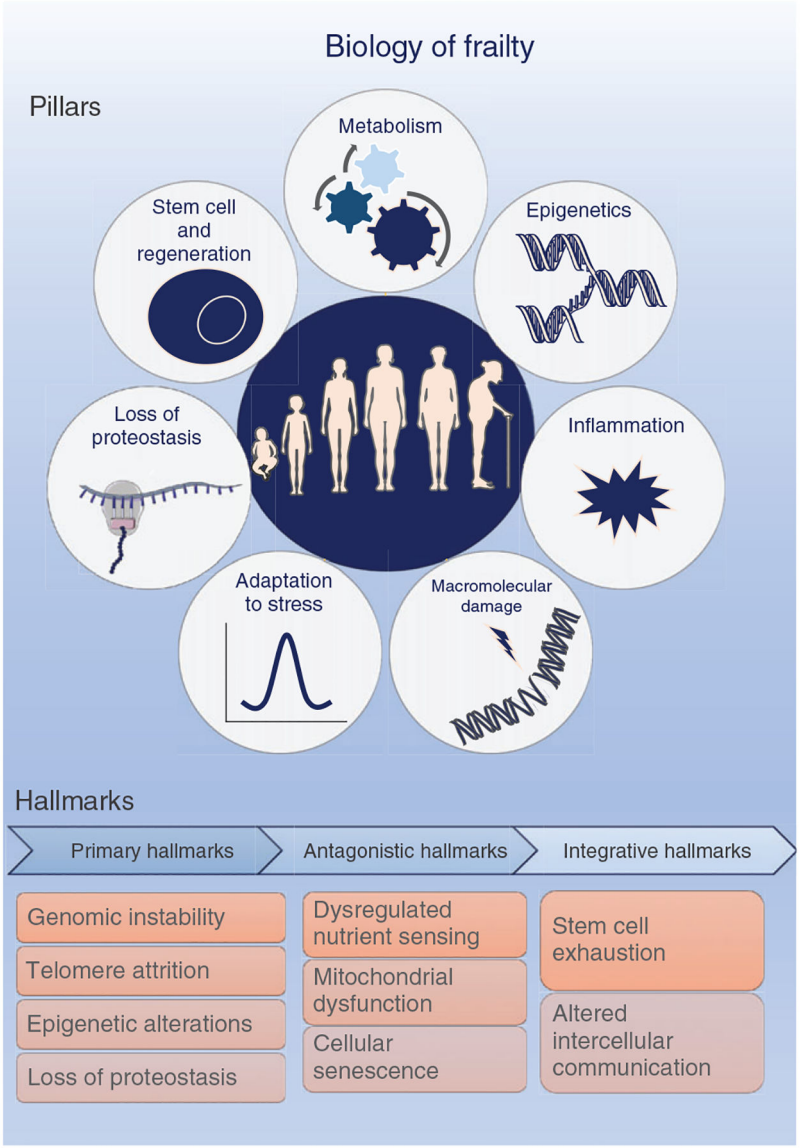

Figure 3. Biology of frailty.

The two well-established conceptual frameworks defining the biology of aging are the Seven Pillars of Aging proposed by Kennedy et al. in (300) and the Hallmarks of Aging proposed by López-Otin et al. in (359) The Seven Pillars define the biological areas that likely contribute to the pathophysiology of aging and include metabolism, epigenetics, inflammation, macro-molecular damage, adaptation to stress, loss of proteostasis, and stem cells and regeneration. Similarly, the Hallmarks of Aging categorize the cellular and molecular processes that may lead to the aging phenotype as (i) the primary hallmarks—genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis; (ii) the antagonistic hallmarks—dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence; and (iii) the integrative hallmarks—stem cell exhaustion and altered intercellular communication. Together, these two concepts identify potential routes to be targeted to extend healthspan and prevent or reduce frailty. The pillar of metabolism defines the

signal transition pathways linked to the metabolism of aging, such as impaired glucose homeostasis and dysregulated nutrient sensing, whereas the epigenetics pillar links age-related environmental pressures altering the gene function, which might trigger genomic instability. The macro-molecular damage pillar is also illustrated as a primary hallmark as genomic instability and telomere attrition, which are all considered *the causes of damage* that might evolve to antagonistic hallmarks that are *the response to damage* and includes mitochondrial dysfunction and cellular senescence. The adaptation to stress illustrates the loss of resilience and resistance or how well the organism can combat and recover from a stressor, which might be molecular (loss of proteostasis, genomic instability), cellular (macromolecular damage accumulated in stem cells, stem cell function decline) or physiological (altered intercellular communication). Once the organism reaches the integrative hallmark level, a systemic dysfunction is reached, culminating in the Frailty Phenotype. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATO-RIES SERVIER, SAS.

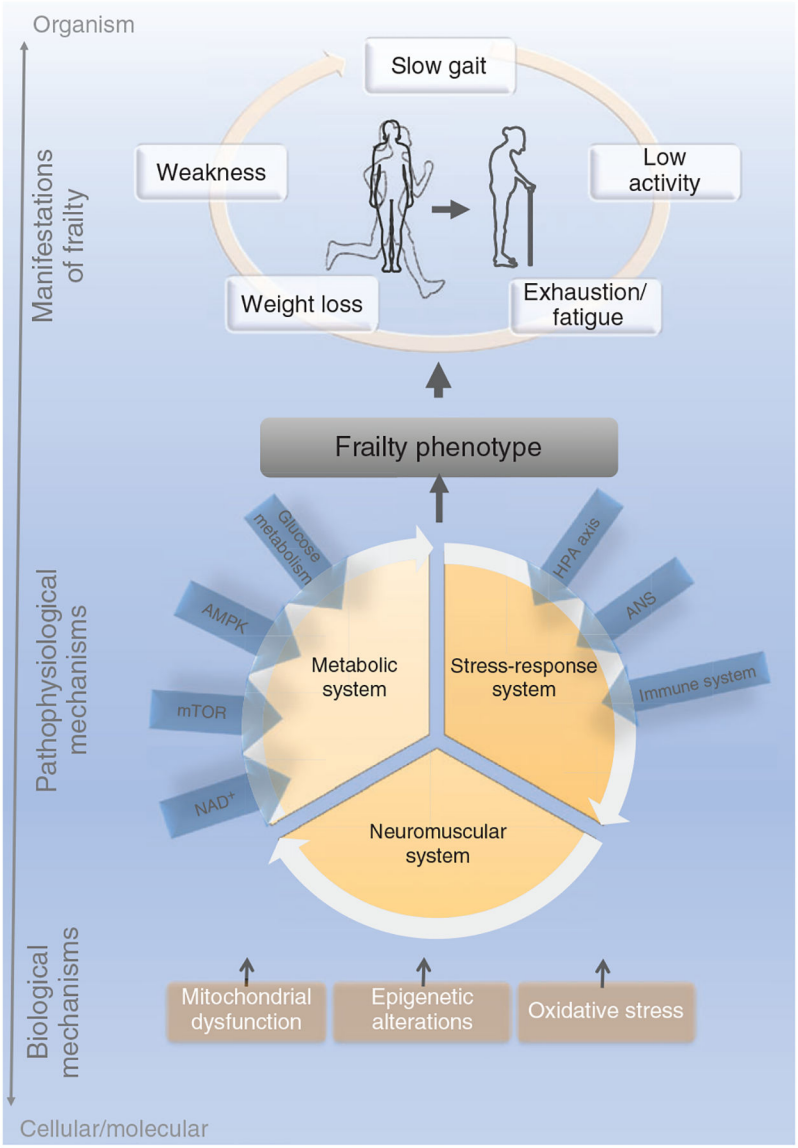

Figure 4. The current clinically Based conceptualization of frailty (160, 450, 597).

Integrating the clinical manifestations of frailty with the hallmarks/pillars of aging results in the current conceptualization. Mitochondrial dysfunction, epigenetic alterations, and oxidative stress represent cellular/molecular factors that contribute to three central physiological systems that promote the Frailty Phenotype. The mitochondrial dysfunction accounts for a reduction in the efficiency of oxidative phosphorylation and a reduction in the energy production generating long-term exhaustion/fatigue. Epigenetic alterations such as DNA methylation and histone modifications are triggered by chronological aging and environmental factors, influencing pathways of health and longevity. Lastly, oxidative stress refers to excessive production of reactive oxygen species (ROS) that leads to cell and tissue damage. The metabolic system represents pathways that are centrally mediated by nutrient-sensing mechanisms, in which the glucose metabolism, insulin signaling cascade as well as AMP-activated protein kinase (AMPK) and nicotinamide adenine dinucleotide (NAD^+)

are pivotal players. The stress-response system is mainly influenced by the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system, and by the immune system. The cognitive and muscular declines, here illustrated by the neuromuscular category, are driven by tissue waste and dysfunction, leading to weight loss, weakness, fatigue, low activity, and slow gait at the organismal level. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

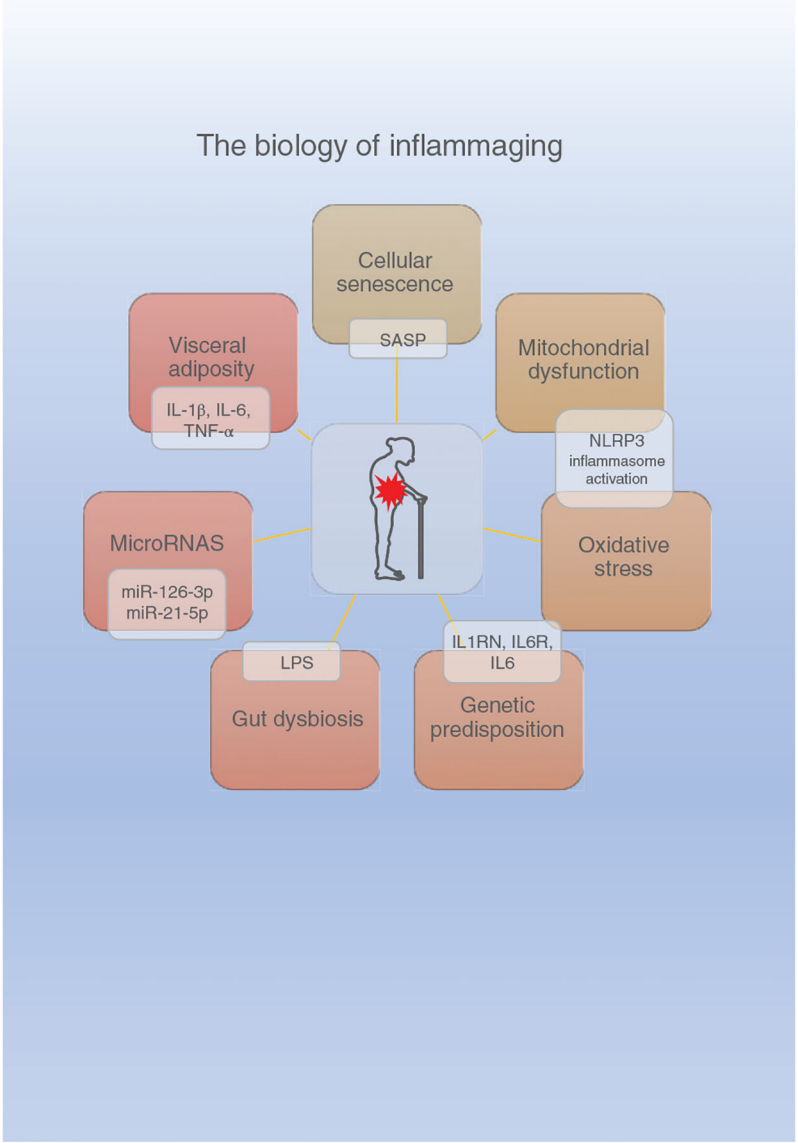

Figure 5. Inflammaging.

The biology underlying inflammaging is multifactorial. The mechanisms that contribute to inappropriate inflammatory responses and ultimately low-level chronic inflammaging include cellular senescence, mitochondrial dysfunction, oxidative stress, visceral adiposity, gut dysbiosis, genetic predisposition, and epigenetics factors such as microRNAs. Potential mediators contributing to the chronic inflammation have both local and systemic impacts that likely promote physical decline. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

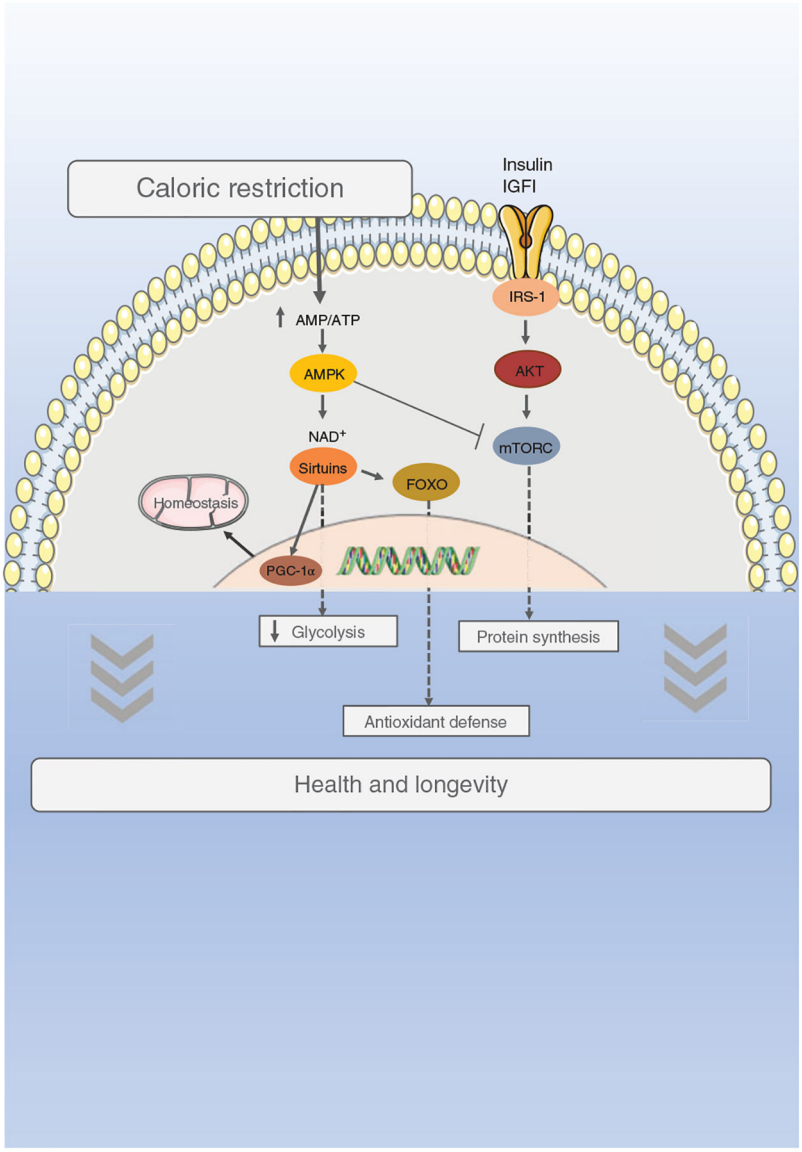

Figure 6. Caloric restriction.

Caloric restriction is the most well-established longevity-modulating intervention. Importantly, dietary restriction whether caloric (protein, carbohydrates, fat), intermittent feeding, or fasting improves health by decreasing morbidities that are associated with aging including frailty. It does so through alterations in energy restriction pathways. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

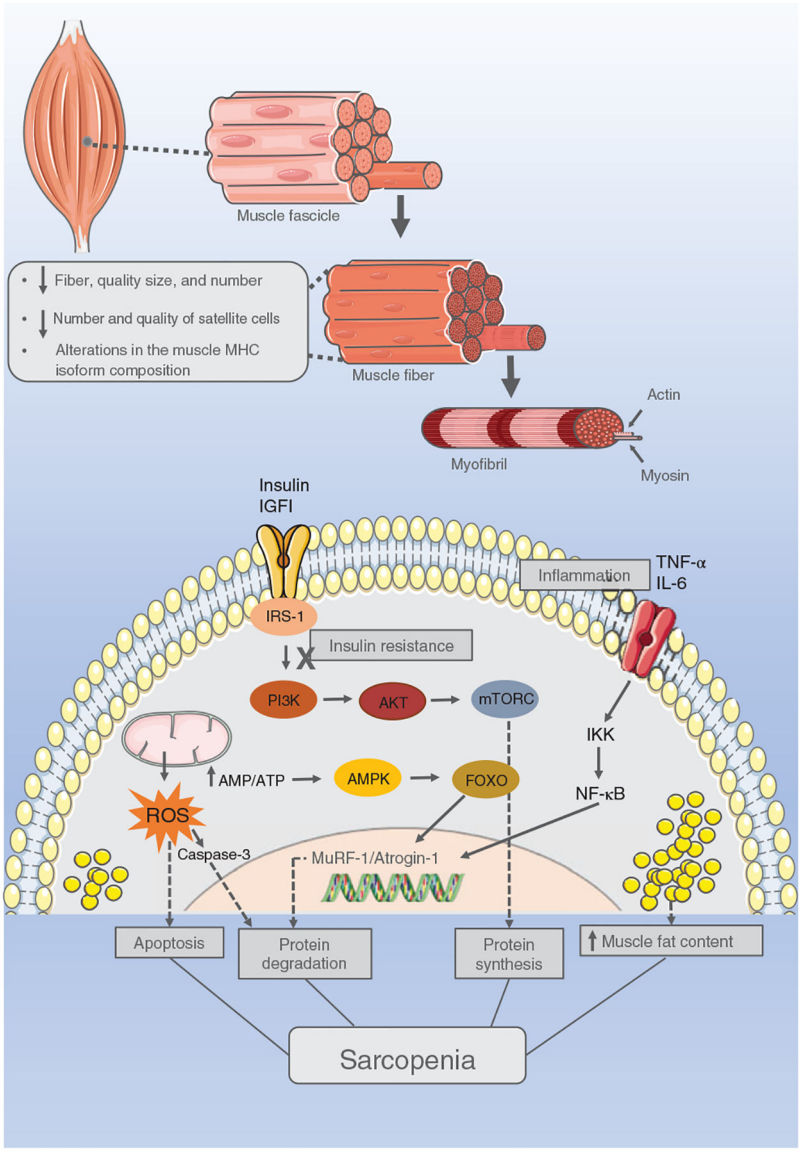

Figure 7. Sarcopenia.

Sarcopenia is the natural event that is characterized by muscle loss and function. At the muscle fiber level, it is observed as a reduction in the fiber quality, size, and number. There is also a reduction in the number and quality of satellite cells, which are stem cells that promote skeletal muscle homeostasis and repair. In the muscle cell, the sarcopenic process is not only driven by increased protein degradation and decreased synthesis, but also by oxidative stress, insulin resistance, ectopic fat accumulation, and inflammation. Multiple signaling pathways provide avenues for therapeutic intervention. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

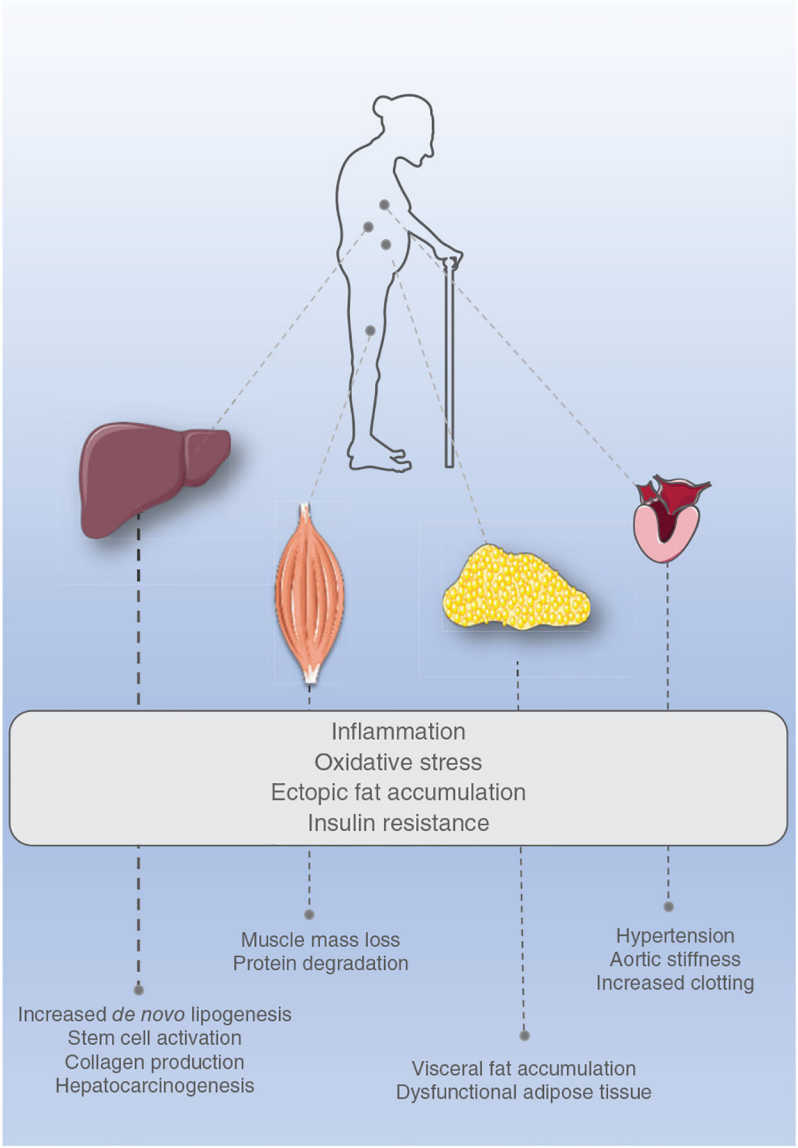

Figure 8. Physiological systems promoting frailty.

Frailty involves a multiple organ network that deteriorates with age and features a decline in functional reserves of many physiological systems. There are common impaired responses observed in many organs of the individual with frailty including inflammation, oxidative stress, ectopic fat accumulation, and insulin resistance. The liver is a central player in metabolism and has thus a key role in the aging process. In frailty, there is an increase in *de novo* lipogenesis that refers to the biochemical synthesis of fatty acids from the carbohydrate catabolism, boosting ectopic fat accumulation. The fatty liver, combined with inflammation and oxidative stress, promotes hepatocyte injury, facilitating fibrosis (collagen production), stem cell activation, and even cancer development. Muscle is also central to the biology of frailty and is the main organ system contributing to the Frailty Phenotype as muscle mass loss and protein degradation trigger weakness, slowness, and weight loss. As compared to subcutaneous adiposity, visceral adiposity is the most detrimental to health

due to its pro-inflammatory profile. The increased inflammation, ectopic fat accumulation, and oxidative stress are all risk factors to cardiovascular events by facilitating endothelial dysfunction, aortic stiffness, and clotting. On top of that, increased visceral adiposity and hepatic *de novo* lipogenesis promote dyslipidemia, which also contributes to cardiovascular dysfunction. Illustrations were obtained on https://smart.servier.com, Published by LES LABORATORIES SERVIER, SAS.

Table 1

Two Common, Well-established Clinical Frailty Assessment Tools

Physical Frailty Phenotype
Evaluates five clinical hallmarks (phenotypic criteria of signs and symptoms)
Criteria
• Weakness: weak grip strength, lowest quintile stratified by sex and body-mass index • Slow gait speed: lowest quintile of gait speed (*m*/*s*) stratified by sex and height • Low physical activity: low energy expenditure, based on physical activity questionnaire • Exhaustion: self-reported, based on two items from the Center for Epidemiological Studies Depression scale • Unintentional weight loss: self-reported weight loss of measured weight loss of ≧5% in the past year
Frailty states: nonfrail (0 criteria present), prefrail (1–2 criteria present), and frail (≧3 criteria present)
Frailty Index of accumulative deficits
Counts health deficits (at least 30), such as signs, symptoms, diseases, disabilities
Health deficits should meet these criteria:
• Represent multiple domains of functioning or multiple organ systems • The prevalence must increase with age • Not be too common before the age of 65 • The prevalence should not be lower than 1%
Frailty score: sum of health deficits present divided by total number of deficits measured
Continuous score between 0 and 1, higher scores indicate higher degree of frailty, with ≧0.25 indicating frailty

The Physical Frailty Phenotype consists of clinical hallmarks of weight loss, weakness, poor endurance/exhaustion, slowness, and low physical activity, core features hypothesized to be proxies of manifestations of dysregulation in specific physiologic domains (179). The Frailty Index hypothesizes that the accumulation of health and functional problems serves as an indicator of an individual's aging-related health state and consists of a minimum of 30 items (the number of deficits rather than the specific type of health deficit) (395). Both frailty assessment tools are useful for identifying vulnerable adults at higher risk for adverse health outcomes.

Based on, with permission, Fried LP, et al., 2001 (179); Mitnitski AB, et al., 2001 (395).

Table 2

Reverse-translated Mouse Frailty Assessment Tools

Mouse Physical Frailty Phenotype		
Criteria	**Measure**	**Equipment**
Strength	Grip strength (g)	Electronic grip meter
Walking speed	Time (s)	Rota-rod
Endurance/exhaustion	Fatigue test (s)	Treadmill
Activity level	Daily running distance (km/day)	Running wheel
Bodyweight	Weight (g)	Scale

Mouse Clinical Frailty Index			
Activity levels	**Hemodynamic measures**	**Body composition**	**Basic metabolic status**
Distance moved (total and maximal, cm)	Systolic pressure (mmHg)	Weight	Na (mmol/L)
Velocity (cm/s)	Diastolic pressure (mmHg)	BMD and BMC (g/cm^2)	K (mmol/L)
Meander (degree/cm)	Pulse pressure (mmHg)	Body surface area (cm^2)	Cl (mmol/L)
Duration of movement (s; % total activity)	Average BP (mmHg)	Lean and fat mass (g)	pH
Rearing frequency (per 5 min)	Heart rate (beats/min)	Percent body fat	Glucose (mmol/L)
	Tail blood flow and volume (uL)	Total body tissue	Hematocrit (%) HCO_3 (mmol/L) Hb (g/L) Urea (mmol/L)

The mouse Physical Frailty Phenotype includes measures of strength, walking speed, endurance, physical activity, and body weight reverse-translated from the clinical Physical Frailty Phenotype (Table 1). Each criterion is scored (score = 1) based on a selected cutoff percentile corresponding to the bottom 20%, with the exception of body weight (dependent on research design). Mice with three or more positive frailty markers are identified as frail, with two positive markers as prefrail, and with one or no positive frailty marker are identified nonfrail (40–42, 321, 351). The mouse clinical Frailty Index selected 31 health-related variables to provide health information highlighting four categories: activity (distance moved, velocity of movement, rearing frequency); hemodynamic factors (systolic and diastolic blood pressures, heart rate, blood volume); body composition (body mineral content, percent body fat, percent lean tissue); and metabolic status (electrolytes, hematocrit, and urea). A graded scale is used to determine frailty, based on how many standard deviations the measured value differed from the mean reference values (adult mice) (434).

Based on, with permission, Liu H, et al., 2014 (351); Baumann CW, et al., 2018 (42); Kwak D, et al., 2019 (321); Baumann CW, et al., 2020 (40); Parks RJ, et al., 2012 (434).

Table 3

List of Abbreviations

ACC	acetyl-CoA carboxylase
ACE	angiotensin-converting enzyme
ACTH	adrenocorticotropic hormone
ADSCs	adipose-derived mesenchymal stem cells
Akt	protein kinase B
AMPK	adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
BCAA	branched-chain amino acid
BMI	body mass index
BMP	bone morphogenetic protein
CBS	cystathionine beta-synthase
CCR5	C-C chemokine receptor type 5
CD	cluster of differentiation
cHSC	circulating hematopoietic stem cells
COP	circulating osteogenic progenitor
COPD	chronic obstructive pulmonary disease
COX-2	cycloxygenase-2
CpG	5′-C-phosp-G-3″
CR	caloric restriction
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
Cu/ZnSOD	copper- and zinc-containing superoxide dismutase
CVD	cardiovascular disease
CXCL	chemokine C-X-C motif ligand
Cxcl1/Kc	chemokine C-X-C motif ligand/keratinocytes-derived chemokine
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DKO	double knockout
DNA	deoxyribonucleic acid
DNAm	DNA methylation
ECM	extracellular matrix
ER	endoplasmic reticulum
ERK	extracellular-signal-regulated kinase
ERR	estrogen-related receptor
FDA	Food and Drug Administration
FI	Frailty Index
FoxO	forkhead box protein O
G6PDH	glucose-6-phosphate dehydrogenase
GDF	growth differentiation factor
GH	growth hormone

GHRH	growth hormone-releasing hormone
GLUT4	glucose transporter type 4
GSK3β	glycogen synthase kinase 3β
H3K27me3	tri-methylation of lysine 27 on histone H3 protein
HMG-CoA	β-hydroxy β-methylglutaryl-coenzyme A
HP	hypothalamic-pituitary
HPA	hypothalamic-pituitary-adrenal
HP1a	heterochromatin protein 1a
HHcy	hyperhomocysteinemia
ICFSR	International Conference on Frailty and Sarcopenia Research
IFN-γ	interferon-gamma
IGF	insulin-like growth factor
IGF1R	IGF-1 receptor
IL	interleukin
JAK	Janus kinase
LKB1	liver kinase B1
LMNA	lamin A/C
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MetS	metabolic syndrome
miRNA	microRNA
MnSOD	manganese superoxide dismutase
MSC	mesenchymal stem cell
mtDNA	mitochondrial DNA
MtTFA	mitochondrial transcription factor A
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
MuRF1	muscle RING-finger protein-1
NAD^+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor kappa B
NIA	National Institute on Aging
NK	natural killer
NMN	nicotinamide mononucleotide
NRF-1	nuclear respiratory factor 1
Nrf2	nuclear factor erythroid 2-related factor 2
PFI	Physiological Frailty Index
PGC	peroxisome proliferative activated receptor, gamma, coactivator
PhenoAGE	phenotypic age
PI3K	phosphoinositide-3-kinase
PF&S	physical frailty and sarcopenia
PPAR	peroxisome proliferator-activated receptor
RNA	ribonucleic acid

ROS	reactive oxygen species
SASP	senescence-associated secretory phenotype
SIRT	NAD-dependent deacetylase sirtuin
SOD	superoxide dismutase
Sod1KO	SOD knockout
SS	somatostatin
SC	stem cell
T2D	type 2 diabetes mellitus
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-alpha
TNFR1	tumor necrosis factor receptor 1
UPR	unfolded protein response
WHO	World Health Organization
Wnt	wingless-related integration site