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Beyond the hallmarks of aging: Rethinking what aging is and how we measure it

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Aging is frequently assessed through lifespan extension and proxy biomarkers, yet these approaches may not fully capture the complexity of biological aging. Here, we propose refinements to discovery and evaluation strategies in aging research. Drawing on cross-species data, from humans to invertebrate models, we show mortality is often driven by a narrow set of life-limiting pathologies rather than a uniform systemic decline. This suggests lifespan extension can result from delayed disease onset without broadly slowing aging. Similarly, while tools like DNA methylation clocks and frailty indices offer value for stratification and prediction, their largely correlational nature limits mechanistic insight. Our systematic review exposes a key limitation in the widely cited "hallmarks of aging" framework: many supporting studies conflate baseline physiological shifts with genuine changes in aging rate. We advocate for study designs that enable differentiation of symptomatic effects from alterations to the trajectory of age-related phenotypic change. By integrating these refinements, the field can move toward a more mechanistic, nuanced understanding of aging, one that supports identifying causal regulators and developing interventions that truly modify aging trajectories.

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Introduction

Aging is one of the most profound and complex puzzles in science, captivating researchers from diverse fields, including philosophy, social science, biology (spanning subfields like evolutionary biology, genetics, and physiology), and medical research (1–8). Together, these disciplines contribute unique perspectives, addressing fundamental questions about the mechanisms, causes, and consequences of aging while offering insights into how we might mitigate its effects on human health and longevity. This diversity of perspectives is also reflected in how aging is defined across fields, with each discipline emphasizing different facets of the process. Some definitions focus on the accumulation of biological damage that gradually undermines the body's systems (9–15), while others highlight the progressive decline in function and fertility with advancing age (16–19), or the gradual impairment of repair mechanisms that disrupts the balance between damage and resilience (20–22). These views are not mutually exclusive; most contemporary definitions integrate elements of damage accumulation and functional decline, portraying aging as a gradual erosion of the body's ability to maintain homeostasis (23–25). Although a rise in mortality risk with advancing age has often been treated as a universal feature of aging, comparative demographic analyses show the pattern is not universal. Several species, especially certain invertebrates, reptiles, and plants, exhibit negligible or even declining age-specific mortality (26). These cases indicate that while mortality acceleration is common, it is not a necessary property of aging across taxa.

Despite some differences in perspectives (outlined above), aging is broadly recognized as a time-dependent series of *phenotypic changes* observable within populations over the course of an average lifespan (27–30). These changes increase the likelihood of age-related diseases and mortality, with some patterns appearing universally across species and others shaped by specific genetic or biological contexts. A key distinction among definitions of aging lies in whether they incorporate presumed causes of age-related changes or focus solely on observable aging outcomes. Nonetheless, there is consensus that aging fundamentally represents the progressive *alteration of phenotypes* over time, ultimately contributing to an individual's death. Understanding this progression provides a foundation for aging research, which aims to identify the underlying drivers of these changes across biological levels, from molecular mechanisms to organismal processes. These insights are essential for developing interventions that target the root causes of aging, with the goal of reducing the burden of age-related diseases and improving overall health in old age in humans.

This paper reflects on current discovery and intervention strategies in aging research, highlighting their limitations and suggesting ways to develop more comprehensive approaches for advancing our understanding of aging. We begin by exploring whether lifespan extension truly reflects slower aging, or whether it often results from delaying specific life-limiting diseases. Drawing on cross-species pathology data, we highlight that mortality frequently stems from a narrow range of conditions rather than from generalized systemic decline, suggesting that lifespan alone is an incomplete proxy for aging.

We next to examine widely used aging clocks and frailty indices, two major classes of composite biomarkers proposed to quantify biological aging. Aging clocks, built from molecular, cellular, or physiological data, are trained on features linked to chronological age and interpreted as an organism's "biological age." Among these, epigenetic clocks based on DNA methylation patterns are the most established and widely applied examples. While such measures provide useful predictions of age-related outcomes, they primarily capture statistical correlations rather than direct information about causal mechanisms of aging.

To test whether an intervention truly slows aging, that is, reduces the rate of age-dependent change in age-sensitive phenotypes (ASPs), it is essential to distinguish rate effects from baseline effects. Baseline effects are age-independent shifts in phenotype levels that alter function without changing how fast those phenotypes evolve with age. Thus, if an intervention produces comparable improvements in young individuals (before detectable age-related change) and in old individuals (after such change has emerged), it reflects a baseline shift rather than a slowing of aging itself.

Together, these sections follow a coherent progression: from clarifying whether lifespan extension reflects slower aging, to assessing biomarker validity, reevaluating the hallmarks framework, and concluding

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with methodological recommendations for identifying genuine modulators of aging.

Does lifespan truly reflect aging?

While extended lifespan is often taken as evidence of slowed aging (31, 32), it may instead reflect the delayed onset of specific life-limiting pathologies, rather than broad modulation of the biological processes that drive age-related change (30, 33).

In humans, for instance, the relative importance of specific life-limiting pathologies has shifted dramatically in recent history. Infectious diseases once dominated as primary causes of death, with pandemics like the bubonic plague, smallpox, and tuberculosis claiming millions of lives due to poor sanitation, limited medical knowledge, and the absence of effective treatments (34–38). However, scientific advancements, including vaccines, antibiotics, and improved public health measures, have dramatically reduced mortality from infectious diseases. Smallpox was eradicated, and diseases like cholera and typhoid were controlled through better sanitation and hygiene (39–42). As infectious diseases were mitigated, the causes of death shifted toward chronic noncommunicable diseases such as heart disease, cancer, and diabetes, particularly in developed nations (43). This transition, known as the "epidemiologic transition," marks a shift from high mortality due to infectious diseases to lower overall mortality, accompanied by new health challenges (44). Crucially, this transition reflects a shift in the dominant causes of death, not a fundamental slowing of aging itself: reduced mortality from infections primarily delayed the occurrence of death but did not alter the underlying biological rate of aging.

This historical transition illustrates a broader principle: lifespan is often limited by the dominant pathologies of a given era rather than by a universal, systemic decline in function. As infectious diseases were brought under control, other conditions, such as cardiovascular disease (CVD), cancer, and neurodegeneration, emerged as the primary life-limiting factors. This pattern illustrates that interventions targeting specific pathologies can extend lifespan by addressing critical bottlenecks to survival, but they do not *necessarily* slow the overall aging process. To fully understand current drivers of mortality and their implications for aging, it is crucial to examine the specific life-limiting pathologies observed today. Recent autopsy analyses in humans and animal models provide valuable insights into life-limiting pathologies, underscoring species-specific factors that shape mortality and suggesting that lifespan is frequently constrained by a limited subset of age-associated diseases, rather than by a uniform, systemic physiological decline.

Although late-life mortality is precipitated by discrete pathologies on an aged background (e.g., cancers in mice), removing one such pathology would mostly defer death until another becomes limiting, typically at a later age. Human epidemiology mirrors this: CVD, cancer, and neurodegeneration have distinct age-incidence curves (45, 46), so individuals who outlive one risk window are increasingly likely to die of another pathology in the next. Consequently, when lifespan is used as the primary readout of "effects on aging" (e.g., for genetic, dietary, or pharmacological interventions), observed gains may simply reflect delaying one or a few pathologies rather than more broadly slowing aging *per se*. Sound interpretation of pro-longevity effects therefore requires knowing which pathologies limit lifespan in the species and context under study.

Humans

CVD consistently ranks as the leading cause of death among older adults, accounting for 35%–70% of cases across studies (47–54). In U.S. death certificate data, heart disease represents about 35% of deaths among older adults, followed by cancer (22%) and stroke (10%) (53). However, autopsy studies provide a more accurate picture by revealing undiagnosed cardiovascular conditions often missed clinically. In one study of centenarians who were considered healthy prior to death, all individuals died from identifiable diseases and none from "old age." Acute organ failure was present in every case, with CVD responsible for 68% of deaths, followed by respiratory (25%), gastrointestinal (5%), and cerebrovascular (2%) causes. Notably, while 60% were perceived as healthy before death, autopsy findings contradicted this impression (47). Similarly, a Danish study reported chronic CVD in 72% of living centenarians (54). Large-scale autopsy analyses confirm this pattern: among 2410 examined cases, CVD was the predominant pathological cause of death, including myocardial infarction (39%), cardiopulmonary failure (38%), cerebrovascular lesions (17.9%), pulmonary embolism (10%), and aortic rupture or cardiac tamponade (9.9%), often in combination. Myocardial infarction was the most common yet frequently undiagnosed cause, underscoring the hidden burden of lethal cardiac pathology (52). A study of unexpected out-of-hospital deaths in individuals aged 85 years and older found cardiovascular events, mainly acute coronary syndromes and arrhythmias, responsible for about 77% of cases (51). Even among the oldest-old (97–106 years), vascular conditions, including stroke, pulmonary embolism, and myocardial infarction, remained leading causes of death. Although pneumonia was the single most frequent cause, cardiovascular and other vascular diseases collectively represented a substantial proportion, emphasizing that extreme longevity rarely ends in "old age" alone but in specific pathological processes (49). Together, these findings show that death, even in individuals perceived as healthy, is rarely due to "pure old age." It almost always results from identifiable diseases, with cardiovascular conditions leading (Figure 1). Autopsy analyses are crucial for correcting misperceptions by relatives and physicians, offering a more accurate assessment of mortality and exposing the hidden burden of lethal disease.

Nonhuman primates

As in humans, the prevalence of age-related diseases increases markedly as captive nonhuman primates live longer (55–58). In rhesus macaques aged 20–37 years, detailed autopsies of 175 animals revealed CVD, particularly coronary sclerosis, as the leading cause of death, accounting for over 60% of cases (55). None died without an identifiable disease, underscoring the role of age-related pathologies in mortality (55). In older chimpanzees, CVDs similarly dominate: cardiac issues caused 57% of deaths in zoo-housed individuals, while infections accounted for 26% (59). Another study of geriatric female chimpanzees found chronic age-related disease in 81% of cases, with cardiovascular conditions present in 88% (60). Together, these studies show that, like humans, captive nonhuman primates rarely die of "old age" alone, with CVD consistently representing the primary cause of death (Figure 1).

Rodents

Cancer is the leading cause of death in mice across multiple studies (61–66). In genetically diverse populations, neoplasia accounted for 84% of identified age-related deaths (63). In C57BL/6J mice, tumor incidence (proportion of animals that developed one or more neoplasms during their lifespan) reached 89% in males and 86% in females under normal feeding, and dietary restriction reduced this to 64% in both sexes (61). Similarly, our previous work found that 86% of *ad libitum*–fed C57BL/6J males died from cancers, while every-other-day fasting lowered this to 70% (65). Even with rapamycin treatment, 74% of mice still succumbed to cancer (66), but did so at an older age, consistent with rapamycin's anticancer effects. In rats, tumors also predominate among the causes of age-associated deaths (67, 68). A large carcinogenicity study of 2400 OFA (Outbred French Albino, Sprague–Dawley–derived) and Wistar rats showed that approximately 63% died from tumors, underscoring neoplasia as the principal cause of death (67). Another study in Outbred French Albino, Sprague–Dawley–derived rats identified pituitary adenomas as the main cause of death across all dietary groups (68). Overall, neoplasia is the dominant cause of death in both mice and rats, with high consistency across strains, feeding conditions, and interventions, underscoring its central role in determining lifespan in rodent models (Figure 1).

Dogs

Several studies indicate that neoplasia accounts for nearly half of deaths among older dogs (69–71). In Bahia, Brazil, neoplasia accounted for 42% of canine deaths, with no cases attributed solely to "old age," underscoring that mortality in older dogs is linked to identifiable diseases (69). Another study reported similar findings, with neoplasia causing nearly 50% of deaths, followed by cardiovascular failure (17%) and inflammatory conditions (15%) (70). An analysis of canine mortality in North America (1984–2004) also identified neoplasia as the most common

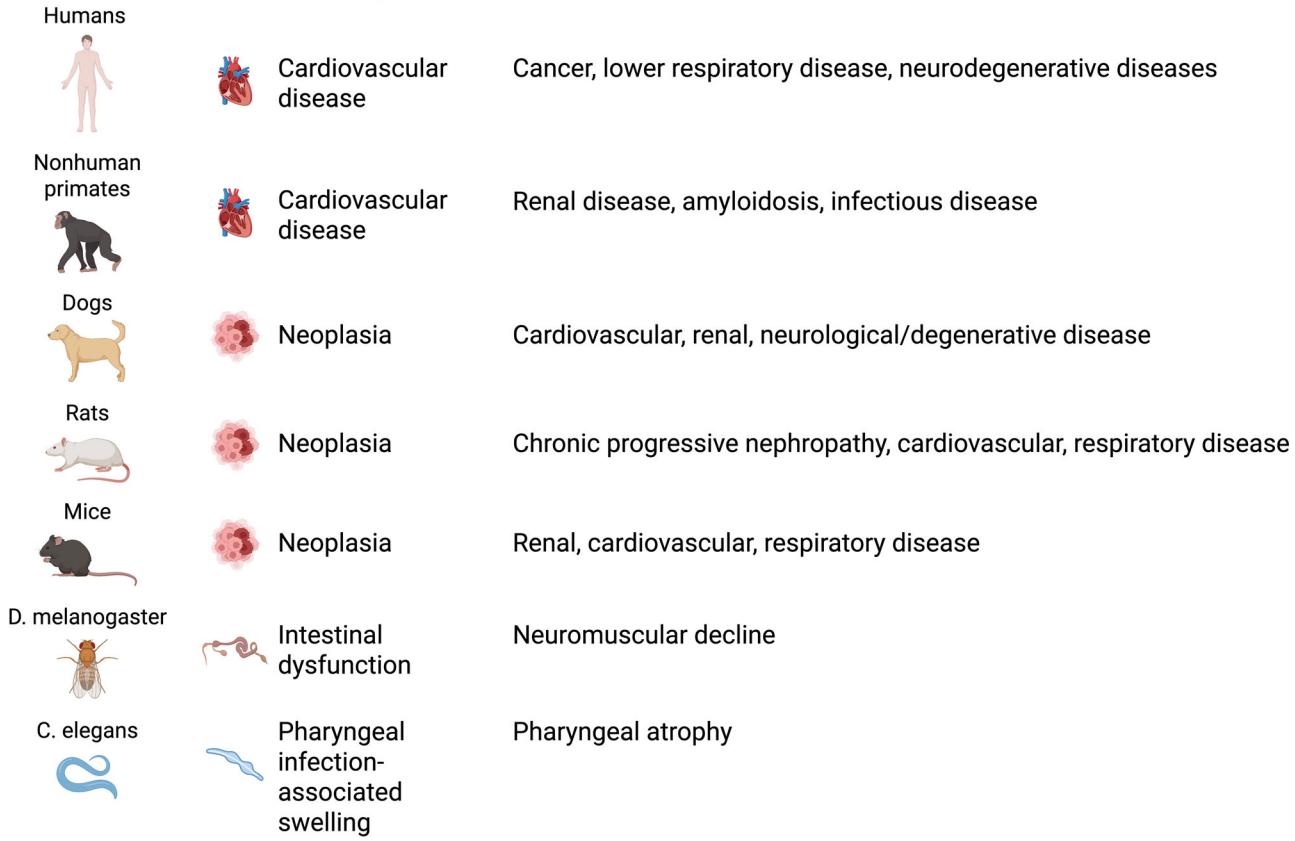

Figure 1. Main causes of death in selected animals: highlighting the role of pathology in limiting lifespan. This figure illustrates leading causes of death across different species, emphasizing that lifespan is often limited by specific pathologies rather than a generalized decline in physiological function. In humans, nonhuman primates, rodents, and dogs, age-related mortality is predominantly driven by identifiable diseases, most notably cardiovascular conditions and neoplasia, suggesting that lifespan is largely shaped by a limited set of age-related pathologies. Figure created with BioRender.com.

cause of death, particularly in large breeds, again finding no deaths attributed to "old age" (71). Therefore, neoplasia stands out as the leading cause of death in older dogs across multiple studies (Figure 1).

Fish

Killifish and zebrafish are increasingly used in aging research because of their short lifespans, genetic tractability, and relevance to vertebrate biology (72, 73). Despite their popularity, the main causes of death in these species remain poorly defined. In farmed fish, infections are the predominant life-limiting factor, with bacterial, viral, and parasitic pathogens representing major causes of mortality. For example, the bacterial pathogens *Lactococcus garvieae* (74) and *Aeromonas salmonicida* (75) can cause systemic disease and organ failure, while viral pathogens such as Infectious Salmon Anemia Virus (ISAV) cause severe anemia and high mortality in farmed Atlantic salmon (76). Parasitic infections, such as sea lice (*Lepeophtheirus salmonis*) in salmon (77) and *Ichthyophthirius multifiliis* in other farmed fish (78), damage gills and skin and increase susceptibility to secondary infections. By contrast, autopsy-based data on killifish and zebrafish are scarce, making it unclear whether infections similarly drive mortality in laboratory settings. Some studies have identified age-related pathologies such as cancer and neurodegeneration in killifish (72, 79, 80), suggesting these may contribute to mortality under controlled conditions, but their prevalence remains unknown. Comprehensive necropsy protocols for fish provide detailed methodologies for identifying tissue abnormalities and underlying causes of death (81). Understanding the life-limiting pathologies in killifish and zebrafish is critical for interpreting pro-longevity effects in these species.

Drosophila melanogaster

In *Drosophila*, the intestinal epithelium acts as a key barrier against microorganisms and environmental toxins (82, 83). With age, its structure and function deteriorate markedly, making intestinal failure a major life-limiting factor (82). A characteristic pathology of the aging gut is epithelial dysplasia, caused by excessive proliferation of intestinal stem cells (ISCs), leading to progenitor cell accumulation and abnormal differentiation (Figure 1) (82, 84–88). Several pro-longevity interventions, including rapamycin, caloric restriction, and reduced insulin/insulin-like growth factor signaling, slow ISC proliferation, delay dysplasia, and extend lifespan (84, 88–91). Moreover, direct genetic modulation of ISCs alone has been sufficient to prolong lifespan (92–97), underscoring that intestinal dysplasia is a critical life-limiting pathology. Consistently, intestinal barrier dysfunction ("Smurf" phenotype) predicts imminent death in aging flies, supporting the idea that specific tissue failures, rather than generalized aging, determine lifespan (98). Recent single-fly transcriptomic analyses further demonstrate that death in Drosophila follows a defined molecular progression distinct from aging, with gut barrier breakdown emerging as part of a coordinated late-stage "dying program" rather than stochastic physiological collapse (99).

Beyond intestinal failure, neuromuscular decline is another major contributor to mortality. Aging flies frequently develop progressive motor impairments, manifested as reduced locomotion, postural instability, and erratic movement, that precede death (98, 100). Longitudinal studies show that climbing deficits and terminal immobility strongly correlate with survival, and some flies display a brief phase of hyperactivity before death (100).

Together, these findings demonstrate that lifespan extension in flies can be achieved by targeting single, life-limiting age-associated pathologies, such as intestinal or neuromuscular failure, without necessarily altering other aspects of aging.

Caenorhabditis elegans

The nematode *Caenorhabditis elegans* is a key model organism in aging research, with many genetic and environmental factors known to extend lifespan. However, the life-limiting pathologies that naturally determine its death remain relatively understudied. A longitudinal study revealed a positive correlation between adult lifespan and the pharyngeal pumping span (the duration of pharyngeal activity) (101). Subsequent work identified pharyngeal infections and deterioration as principal life-limiting pathologies, revealing two distinct death types: early death with a swollen, infected pharynx, and late death with pharyngeal atrophy (Figure 1) (102). Notably, long-lived mutants such as *glp-1*, *eat-2*, *ced-1*, and *daf-2* alter the timing or frequency of these death types (102–104), supporting the hypothesis that targeting specific life-limiting pathologies can extend lifespan in *C. elegans.*

These findings collectively challenge the notion that lifespan is a reliable and comprehensive marker of the aging process. Across the species examined in our analysis, mortality is more commonly driven by a relatively narrow set of identifiable pathologies, rather than by a uniform, systemic decline typically associated with aging. Historical shifts in causes of death, from infectious diseases to chronic noncommunicable conditions, illustrate that lifespan primarily reflects survival constraints and our capacity to manage specific pathologies. This distinction highlights the importance of disentangling lifespan from aging when interpreting mortality patterns and evaluating aging-related interventions.

Limitations and considerations in the use of aging clocks

Aging clocks have emerged as useful tools for estimating "biological age" based on a range of molecular features (105, 106). These models have shown value in predicting health outcomes, mortality risk, and "age acceleration" across populations, and have become increasingly popular in both basic and translational aging research. However, as discussed below, certain limitations may constrain their ability to provide deeper insight into the biological mechanisms of aging.

Aging clocks can be constructed using diverse types of biological data, including epigenomic, transcriptomic, proteomic, and metabolomic profiles (107, 108). Among these, DNA methylation–based clocks have received the most attention due to their high predictive accuracy for chronological age (109). These clocks range from simplified models using only a few CpG sites to more complex approaches involving large-scale methylation datasets (110–112). Nonetheless, studies have shown that their predictive performance is often driven by a small subset of informative CpGs (113). This reflects the effects of regularization techniques, such as elastic net regression, which assign nonzero weights to only the most informative sites while down-weighting or excluding others. In addition, due to high correlations among methylation changes across nearby CpG sites, many included features may be functionally redundant, capturing overlapping biological signals without adding new information (114). Notably, even large-scale clocks often retain predictive accuracy when many CpGs are removed, reinforcing the idea that these models may functionally rely on a relatively small number of key sites (113, 114).

An important conceptual consideration is that aging clocks are fundamentally correlational. They are trained on age-associated changes but may not distinguish whether these changes are causally involved in aging or are downstream consequences (115). This may be akin to estimating age based on facial images: while such image features can be predictive, they offer limited insight into the biological processes driving aging. Supporting this concern, a recent study using epigenome-wide Mendelian randomization found that traditional aging clocks are not significantly enriched for CpG sites with causal roles in aging, suggesting that many of these models may reflect correlation rather than causation (115). Still, it is worth noting that correlation-based tools can offer considerable value for stratification, early risk detection, and longitudinal tracking, even if they fall short of providing direct mechanistic insight.

Another challenge is that most clocks offer only a static snapshot of biological age. As such, it can be difficult to determine whether observed changes, such as those following an intervention, reflect a genuine slowing of aging or simply a shift in biomarker baselines. Recent developments, such as DunedinPACE, aim to estimate the rate of aging rather than absolute biological age, representing a meaningful step forward (116). However, even these newer models often rely on biomarkers that correlate with age-related phenotypes, without necessarily identifying the mechanisms underlying those changes. This makes it challenging to infer whether an intervention is modifying the aging process itself or simply altering short-term biomarker trajectories (see also discussion below in section "Study design considerations to facilitate the identification of regulators of aging").

In addition, many aging clocks are based on a single class of biomarkers (typically DNA methylation) despite the fact that aging is a complex, multilayered process involving coordinated changes across molecular, cellular, and physiological levels. While DNA methylation clocks can accurately estimate chronological age, they may not fully reflect functional changes in other biological domains, such as proteomics or metabolomics. This could limit their interpretive value when assessing the efficacy of interventions. In response to this limitation, some researchers have begun developing multiomics clocks that integrate data across different layers, including epigenomics, transcriptomics, metabolomics, and proteomics (117). However, while promising, such integrative approaches present new challenges. Collapsing diverse biological signals into a single composite score may obscure important tissue-specific effects. For example, improvements in one tissue or omics layer could mask adverse effects in another. Given the heterogeneous nature of aging across organs and systems (118–120), a single unified score may risk oversimplifying the nuanced and context-dependent effects of aging and interventions.

To address these issues, a more layered and systematic approach may be beneficial. Rather than collapsing all biological signals into a single metric, it may be more informative to analyze each omics layer independently, evaluating positive, neutral, and negative changes, before integration. Likewise, tissue-specific data should be examined separately to account for the variability of aging and intervention effects across different organs. By preserving this biological complexity, researchers may be better positioned to develop more mechanistically informative biomarkers and move beyond correlation-based models toward a deeper understanding of the aging process.

In conclusion, aging clocks estimate age from a (sometimes small) set of age-sensitive features. Although changes in these features track chronological age, the relationship is correlational and, without further evidence, does not imply a mechanistic role in aging (i.e., that the feature causally influences the rate of aging). Aging clocks can serve as readouts in experiments testing factors that slow or accelerate aging (for example, genetic manipulations) but the resulting insights generally apply only to the aspects of aging captured by the specific clock.

Frailty indices as aging measures: limitations and considerations

Frailty indices are gaining traction as biomarkers of aging and as tools for assessing the impact of antiaging interventions (121). While they are complementary to traditional lifespan-based assessments, it is important to consider their limitations when interpreting results in the context of aging biology.

Frailty indices do not usually comprehensively measure the aging process, as they typically rely on a limited set of easily observable health markers (122). In many preclinical studies, these indices are constructed from a small number of semiquantitative traits, such as fur condition, kyphosis, or tumor presence, often scored on simple categorical scales (e.g., 0–0.5–1) (123, 124). Even when more phenotypes are included, the measures often remain focused on inspection-based phenotypes rather than broadly capturing molecular or functional biomarkers of aging (122, 125). As a result, frailty indices typically capture only a narrow subset of age-related phenotypic changes, largely limited to easily observable indicators of general health.

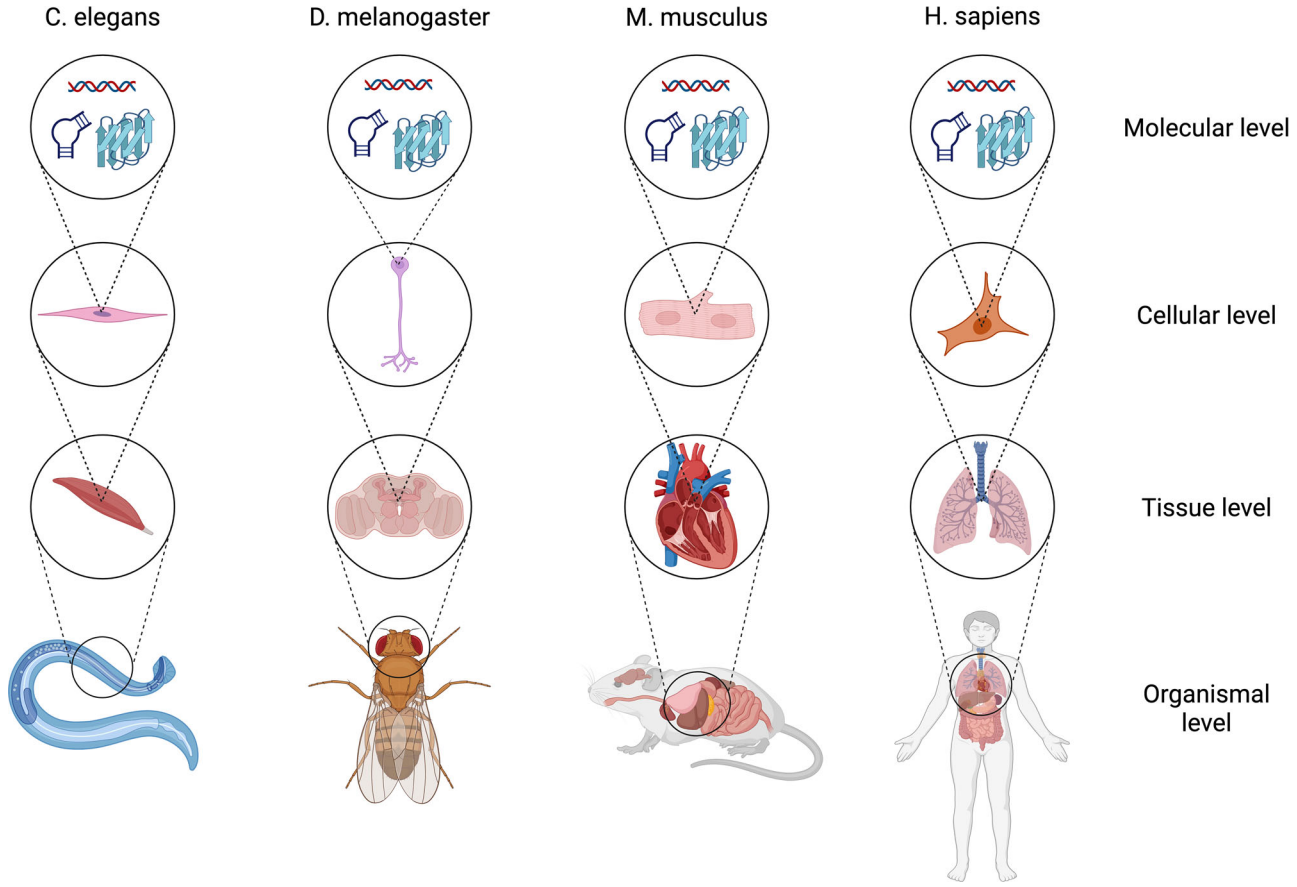

Figure 2. Multidimensional nature of aging: phenotypic changes across levels of biological complexity. The figure illustrates time-dependent phenotypic changes across molecular, cellular, tissue, and organismal scales in multiple species. As one illustrative brain-focused example, aging is accompanied at the molecular level by declining neuronal proteostasis with accumulation of misfolded proteins; at the cellular level by synaptic dysfunction with reduced neurotransmission and spine loss; at the tissue level by circuit remodeling characterized by chronic neuroinflammation and degraded connectivity/white matter; and at the organismal level by cognitive slowing and memory impairment, with species-specific readouts. This example is not exhaustive; icons are illustrative. Figure adapted from Keshavarz *et al*. (2023) (license: http://creativecommons.org/licenses/by/4.0/) and created with BioRender.com.

Additionally, by summing diverse deficits into a single score, frailty indices implicitly assign equal biological weight to each component. This means that improvements in one or two areas, such as reduced tumor burden or improved coat condition, can lower the overall frailty score, potentially giving the impression of a broader antiaging effect. However, such changes might reflect improvements in specific pathologies rather than broader modifications of aging phenotypes. In this respect, frailty indices may face challenges similar to those associated with using lifespan alone as an aging metric; namely, the risk of oversimplifying complex biological processes into a single summary measure.

Study design considerations to facilitate the identification of regulators of aging

Aging is a complex process involving time-dependent phenotypic changes that emerge across molecular, cellular, tissue, and organismal levels of analysis (27, 30, 126–130) (Figure 2). These changes manifest over the lifespan and entail a wide array of ASPs (27). While substantial progress has been made in identifying ASPs across various species, including humans, many of the existing research strategies were not primarily developed to uncover underlying drivers or modifiers of these changes.

Currently, the identification of potential regulators of ASPs often relies on cross-sectional data, derived from aged individuals, to infer relationships between variables of interest, such as genes, transcripts, proteins, metabolites, environmental exposures, and lifestyle factors like diet and exercise, and phenotypic measurements observed in older individuals (Figure 3). For example, studies on brain aging often analyze the relationship between cognitive scores, brain connectivity, or brain volume in older individuals and potential contributing factors (131–139). However, individuals with the lowest scores on these measures, such as reduced cognitive ability, smaller brain volumes, or weaker neural connectivity, may not necessarily have undergone the greatest age-related decline; rather, they may have started from a lower baseline earlier in life. Directly analyzing the factors that influence age-related decline over time may therefore provide more meaningful insights into the underlying biology of aging.

Indeed, substantial evidence highlights significant individual variability in brain structure, function, and connectivity from early adulthood (140–142). Variability in phenotypes such as cognitive performance, brain volume, or neural connectivity is influenced by numerous factors independent of aging. Genetic predispositions play a key role, with specific genetic variations shaping brain networks and contributing to differences in structural and functional phenotypes (143–145). In addition, environmental factors, including education, lifestyle, and psychosocial influences, can shape these phenotypes throughout life (146, 147). Early life experiences, prenatal conditions, and developmental trajectories further contribute to individual differences that can persist into later life (148, 149). These findings emphasize the need to account for baseline individual differences across the lifespan to avoid incorrectly attributing low scores in old age to aging-related changes. True regulators of aging can be defined by explicitly quantifying within-individual changes, comparing younger and older timepoints, rather than relying on isolated late-life measures (Figure 3). Although longitudinal study designs may be particularly well-suited for identifying factors that influence age-dependent phenotypic change, insights into such mechanisms can also be obtained

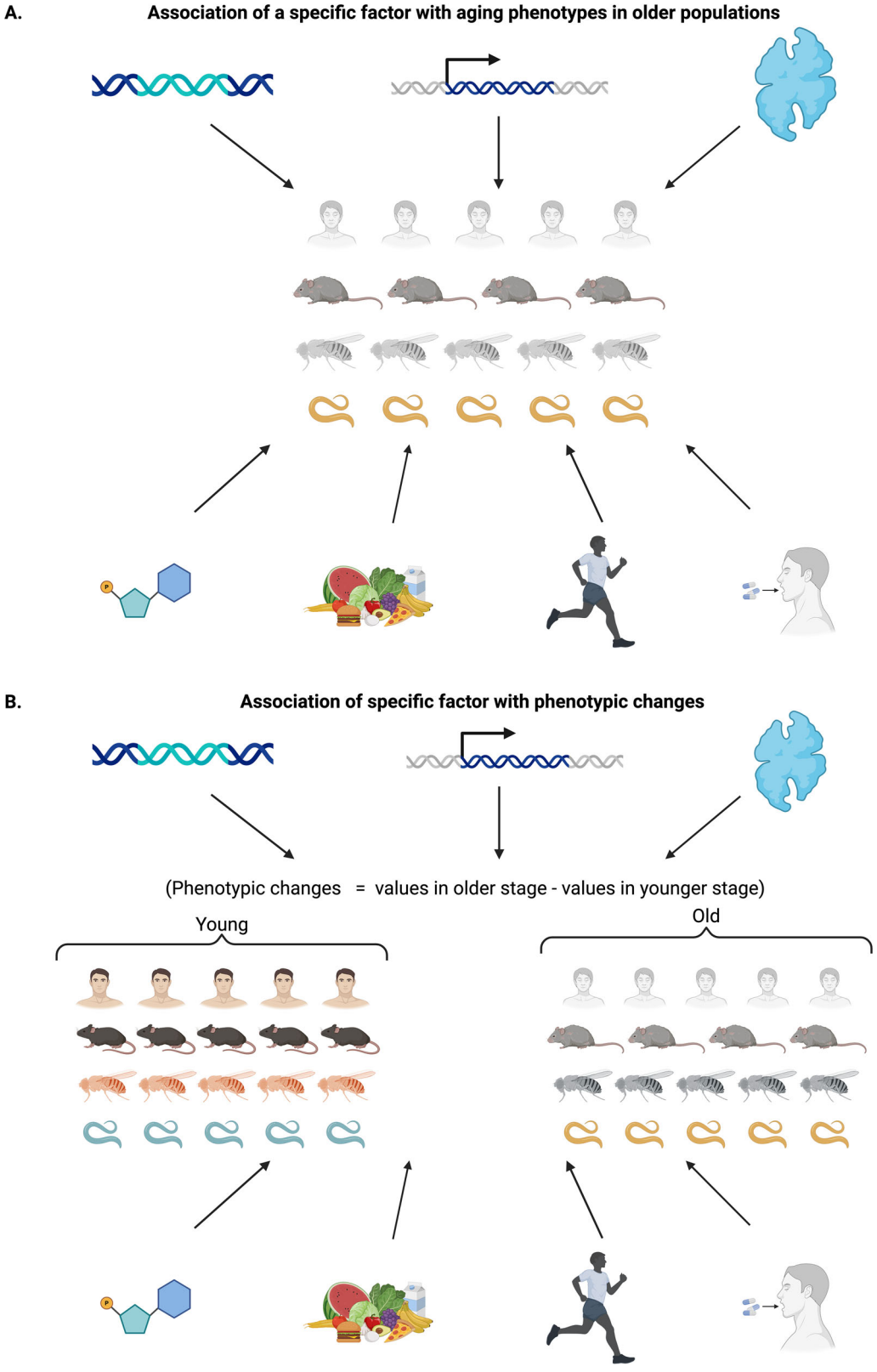

Figure 3. How to identify regulators of aging? (A) This panel illustrates a commonly used strategy in aging research, where experimental variables, such as genetic, pharmacological, or lifestyle factors, are tested for their influence on phenotypes measured primarily in older populations. While this can yield useful relationships, it often assumes that phenotypic states in old age reflect aging-related change, without accounting for preexisting individual differences or baseline variability. (B) A more refined approach entails explicitly tracking within-subject phenotypic changes over time, from younger to older ages. By directly quantifying how experimental variables influence the rate or trajectory of age-related change, this design enables more accurate attribution of effects to aging processes. Such frameworks can improve mechanistic insight and help distinguish between interventions that truly modify aging versus those that merely influence phenotype levels independent of age. Figure created with BioRender.com.

from cross-sectional approaches, for example, by comparing the treatment effect sizes in aged animals exposed to chronic interventions with those in young animals treated prior to the onset of age-related changes in phenotypes (65, 150) (as described in more detail below).

Similarly, current evaluation strategies for putative antiaging interventions (PAAIs) face notable challenges in capturing genuine aging-related effects. Many existing approaches tend to assess PAAIs using a limited set of ASPs, often emphasizing a narrow range of measurable outcomes (33). This focus can inadvertently overlook the broader and more complex nature of aging-related changes. A key refinement would be to expand these assessments to encompass a more comprehensive array of ASPs, ideally spanning multiple biological systems and levels of analysis. Extensive phenotyping studies across model organisms, including mice (129), flies (127), worms (128), and humans (130), have documented a diverse landscape of ASPs that reflect the multifaceted character of aging (Figure 2). Importantly, ASPs often vary between tissues, highlighting the necessity of tissue-specific analyses to more accurately characterize the impact of an intervention (118, 119). Given this heterogeneity, improvements in a single phenotype within one tissue should not be assumed to generalize across other phenotypes or organ systems. Nonetheless, it is not uncommon for conclusions about a PAAI's effects on aging to be drawn from changes observed in only a small subset of outcomes (33). While this may offer initial insights, such interpretations risk oversimplifying the intricate and tissue-specific nature of the aging process. This tissue-specific variability in ASPs raises a broader question: why do tissues age at different rates, and to what extent is aging systemically coordinated across organs? Although tissue-specific aging trajectories are well documented, their causes remain unclear. They likely reflect, in part, developmental patterning and lifelong differences in turnover, metabolic demand, and exposure to stressors. At the same time, cross-tissue coupling via endocrine, immune, neural, and circulatory signals suggests partial systemic coordination. Whether aging is driven chiefly by central, non–cell-autonomous "pacemakers" or by predominantly cell-autonomous processes (stochastic or programmed) remains an open question that will require integrated, multitissue studies.

In general, individual ASPs can be shaped by diverse biological processes, underscoring the importance of disentangling the specific contributions of aging-related mechanisms from other influencing factors. For instance, reduced bone density in aged individuals may be linked to age-associated osteoblast dysfunction or hormonal changes, such as decreased estrogen levels (151, 152). However, similar phenotypic outcomes can also arise from non-aging-related causes, including dietary deficiencies (e.g., insufficient calcium or vitamin D intake) (153) or prolonged immobility due to injury (154). If a PAAI is observed to improve bone density in older animals, it is essential to determine whether the observed effect reflects a direct impact on age-dependent processes, such as the cellular mechanisms governing osteoblast function, or whether it is instead due to correction of extrinsic, non-aging-related factors. Without such mechanistic resolution, conclusions regarding the intervention's relevance to aging remain uncertain and risk being overstated. Relying solely on the observation of phenotypic improvement without accounting for baseline variability or age-independent effects may lead to misinterpretations about the aging-modulatory potential of a given intervention.

As a concrete example of cross-tissue coupling, the immune system shows age-related change across organs: immunosenescence (reduced naïve lymphocyte output/diversity, weaker pathogen/vaccine responses) coexists with inflammaging (chronic low-grade inflammation), and both patterns track late-life morbidity (155–159). These features should be included among ASP panels when evaluating interventions. Crucially, for immune-targeted PAAIs, the key question is whether the trajectory of immune phenotypes changes with age (e.g., naïve–memory balance, repertoire diversity, vaccine responsiveness), because trajectory-based analyses place marker shifts in biological context and reveal mechanisms underlying immunosenescence/inflammaging. These are insights that single-timepoint reductions in cytokines (baseline shifts) cannot provide.

Testing PAAIs only in aged organisms can obscure whether they target aging-related mechanisms or merely influence ASPs through other pathways. To distinguish these possibilities, interventions should be evaluated in both young and old subjects while quantifying ASP trajectories to determine whether treatments alter the rate of age-dependent change or simply shift baseline values. Building on analytical models that separate age-independent main effects from age × treatment interactions, and also on our earlier studies (33, 65, 66, 150, 160, 161), we classify intervention effects on ASPs into three categories (Figure 4): (1) rate effects, in which an antiaging treatment reduces the slope of age-dependent change, consistent with targeting processes underlying phenotypic aging (causal treatment effect); (2) baseline effects, in which similar changes in young and old animals indicate age-independent, symptomatic action; and (3) mixed effects, in which ASPs change in both young and old animals but more strongly in older animals. Operationally, we detect baseline effects by including young treated cohorts to reveal age-independent shifts and identify rate effects by testing for age × treatment interactions (150); mixed patterns are more challenging to interpret, as they may reflect combined age-independent and age-dependent mechanisms or differences in treatment duration.

Recent experimental findings illustrate the value of this distinction. Studies examining well-known pro-longevity interventions, such as intermittent fasting (65), rapamycin (66), and genetic interventions that modulate key longevity pathways, such as hypomorphic $mTOR^{KI/KI}$ mice (162) (which exhibit attenuated mTOR signaling) and $Ghrhr^{lit/lit}$ mice (163) (which lack functional growth hormone signaling), have applied this approach to evaluate whether these interventions truly modulate aging trajectories or merely induce age-independent changes. Deep phenotyping of both young and old cohorts treated with these interventions revealed that, despite their established lifespan-extending effects, their influence on many ASPs was predominantly characterized by baseline shifts rather than changes in the rate of age-dependent progression (150). That is, the interventions altered phenotype values at both young (before the manifestation of aging-associated changes in phenotypes) and old (after the manifestation of aging-associated changes in phenotype) ages similarly, rather than slowing the rate of age-dependent change. For instance, although rapamycin and intermittent fasting reliably extend lifespan (and have many effects on a wide range of ASPs), detailed multidimensional analyses showed that they do not consistently slow the rate of age-dependent changes in ASPs across a broad range of physiological systems (65, 66). These findings suggest that while such interventions can robustly affect specific phenotypes, they may not broadly modulate the underlying processes that drive organismal aging. Collectively, these observations highlight the need for systematic evaluation of PAAIs using study designs that can distinguish true antiaging effects from aging-independent, symptomatic influences on phenotypes.

Reassessing the evidence for the "hallmarks of aging" as modifiers of aging rate

The paper by López-Otín *et al*. (2013) (32) introduced the nine hallmarks of aging, which were later expanded to 12 in an updated review published in 2023 (31). These hallmarks, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, dysbiosis, and disabled macroautophagy, have since become a widely accepted framework for aging research.

The notion that the so-called "hallmarks of aging" causally determine the rate of aging has become deeply embedded in the field. This framework has strongly influenced research priorities, funding allocation, and intervention strategies. However, despite its widespread adoption, the extent to which these hallmarks truly govern aging trajectories, as opposed to merely modulating physiological phenotypes in an age-independent manner, has not been systematically evaluated.

To critically assess this assumption, we examined the primary studies cited in the López-Otín *et al*. 2023 paper, focusing specifically on those used to support causal relationships between each hallmark and aging (Supplementary File S1). Our objective was to determine how much of the cited evidence supports the claim that targeting these hallmarks can

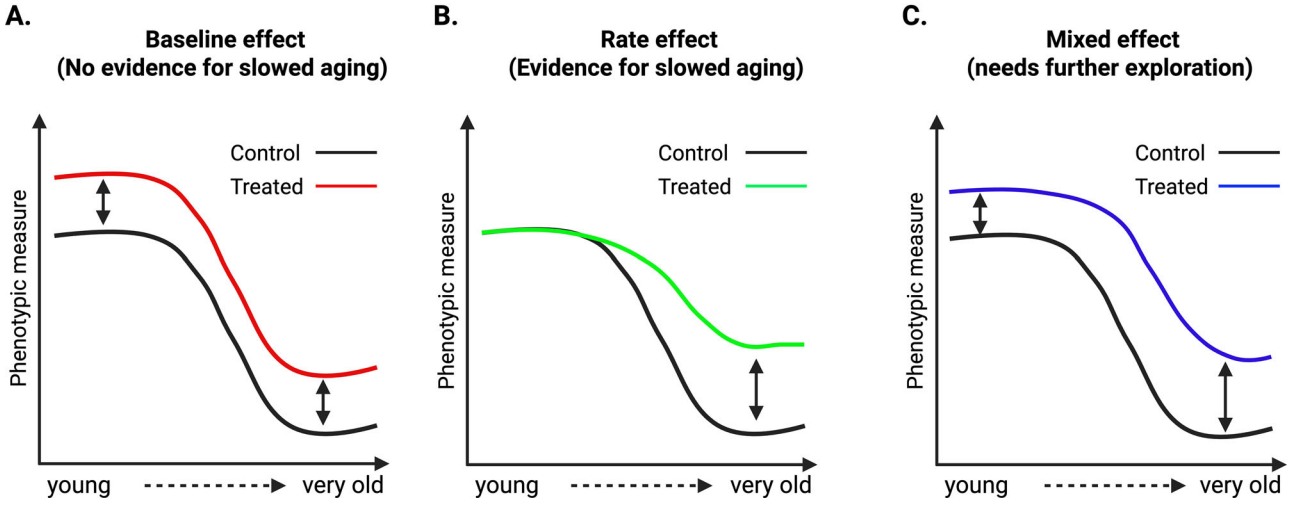

Figure 4. Distinguishing intervention effects on aging: baseline shifts versus changes in aging rate. The effects of a PAAI on ASPs can be explained by three possible models: (1) the baseline model, (2) the rate model, or (3) a combination of both. In the baseline model (A), a short-term treatment in young animals (prior to the emergence of age-dependent phenotypic changes) has the same impact on ASPs as a long-term treatment in older animals (after the emergence of aging-associated phenotypic changes), indicating that treatment effects are unrelated to influences on aging. In the rate model (B), an antiaging treatment slows the progression of an ASP but does not affect the ASP before the onset of age-related changes. This suggests that the treatment slows the aging process itself and targets the mechanisms underlying age-dependent changes in ASPs. Alternatively, a treatment may influence ASPs in both young and old animals, with stronger effects in older animals (C). This scenario is more complex to interpret, as it could result from a combination of age-independent effects and changes in the rate of aging, or it could be purely age-independent, depending on how treatment duration affects the size of the treatment effect (with long-term treatments in older animals potentially yielding larger effects than short-term treatments in younger animals). Figure adapted from Keshavarz *et al*. (2023) (license: http://creativecommons.org/licenses/by/4.0/) and created with BioRender.com.

modify the rate of aging, rather than merely altering phenotype levels irrespective of age.

As detailed in Supplementary File S1, the majority of studies cited reported intervention effects only in older animals, without including parallel assessments in young treated cohorts. In fact, our analysis, reviewing evidence for each hallmark separately, showed that between 56.86% and 99.96% of the supporting phenotypes were examined solely in aged animals (Supplementary File S1; Table 1); therefore, these studies lacked the design needed to distinguish between baseline effects and changes in aging rate. Where young groups were included, effects were frequently observed in both young and old animals (Supplementary File S1; Table 1), suggesting that the interventions induced age-independent baseline shifts rather than slowing age-dependent change. Across all studies cited in support of the hallmarks of aging (31), we identified 602 phenotypes that included assessments in young animals (Supplementary File S1). A total of 436 out of these (corresponding to 72.4%) showed intervention effects in young groups of animals (Supplementary File S1), indicating that baseline effects accounted for the majority of cases.

Table 1. To what extent is the evidence supporting the hallmarks of aging's role in aging based on baseline versus rate effects on aging phenotypes? Note that, in many cases, it is not possible to make this distinction (category "Not clear") given that the supporting evidence was generated using study designs that do not allow to estimate intervention effects on aging rate (because young animals subjected to intervention were not included in the respective studies).

Hallmark	Rate effect (%)	Baseline effect (%)	Not clear (%)
Genomic instability	0	100	0
Telomere attrition	3.92	3.92	92.16
Epigenetic alteration	1.2	3.19	95.61
Loss of proteostasis	25.49	17.65	56.86
Disabled macroautophagy	2	3.84	94.16
Deregulated nutrient-sensing	0.11	1.35	98.55
Mitochondrial dysfunction	0.26	27.18	72.56
Cellular senescence	0	4.65	95.34
Stem cell exhaustion	0.04	0.0	99.96
Altered intercellular communication	0.09	0.1	99.81
Chronic inflammation	3.28	5.56	91.16
Dysbiosis	2.14	0.53	97.33

Consequently, the evidence cited for most hallmarks supports the presence of general physiological effects rather than true antiaging mechanisms. These observations align with findings from large-scale phenotyping analyses, such as Xie *et al*. (2022) (150), which further show that interventions targeting pathways like nutrient sensing often produce similar effects in young animals, reinforcing the predominance of baseline over rate effects. Specifically, in a large-scale study of three major pro-longevity interventions; namely, genetic targeting of mTOR signaling, genetic targeting of growth hormone signaling, and intermittent fasting, Xie *et al*. found that 145 out of 180 ASPs also showed an intervention effect in young animals that was equal to or greater than that observed in aged animals (corresponding to 80.56% of cases) (150).

This distinction is more than semantic. Geroscience aims to uncover mechanisms that influence age-related phenotypic change, not merely those that regulate phenotypes per se, which are already addressed by established fields like endocrinology, neuroscience, and immunology. For instance, while a treatment that enhances cognitive performance generally (i.e., at any age) may certainly have very useful applications, it cannot be said to target cognitive aging unless it demonstrably alters the rate of cognitive decline over time. Failing to distinguish baseline effects from true age-dependent changes risks attributing general physiological modulation to mechanisms that regulate the rate of aging.

Therefore, identifying true regulators of aging demands that we explicitly test whether interventions alter the trajectory of age-related phenotypic change. This requires study designs that include both young and old cohorts, with sufficient resolution to distinguish between

age-independent and age-dependent effects. Without such methodological rigor, the field risks building its foundational models on untested assumptions. Given the influence of the hallmark framework on the direction of aging research, a systematic reevaluation of the evidence base is overdue. To distinguish genuine modulators of aging from interventions that merely influence phenotype expression, treatments should be evaluated across multiple life stages, using longitudinal within-subject analyses and/or age-stratified cross-sectional comparisons, to account for baseline variability and the timing of age-related changes.

Conclusion

Aging research has long been shaped by assumptions that may not fully account for the complexity and heterogeneity of the aging process. One of the most persistent assumptions is that extending lifespan equates to slowing aging. However, as shown in our cross-species analysis above, age-related mortality is often determined by a narrow set of life-limiting pathologies rather than by a generalized, systemic aging process. As a result, lifespan extension frequently reflects the delayed onset of specific diseases rather than a slowing of aging *per se*. Consequently, when lifespan is used as the primary readout of "effects on aging" for genetic, dietary, or pharmacological interventions, observed gains may simply reflect delaying one or a few pathologies rather than broadly slowing aging *per se*. One might argue that even when mortality in late life is precipitated by a specific pathology, it ultimately reflects an organism-wide deterioration: in other words, had one system not failed, another soon would have. In this view, "old age" functions as a diffuse background cause that indirectly leads to death. Aging indeed alters multiple systems simultaneously, reducing physiological resilience and thereby increasing the likelihood that a particular pathology will become life-limiting. However, these systemic changes are permissive rather than causative: each major age-related disease follows its own discrete mechanistic trajectory: atherosclerosis through lipid deposition and inflammation, cancer through somatic mutation and clonal selection, neurodegeneration through protein misfolding and glial activation. Thus, while aging shapes the vulnerability landscape on which such events occur, mortality itself arises from specific mechanistic failures, not from a generalized, uniform process of decline.

Beyond assumptions about lifespan, oversimplified approaches also appear in how aging is quantified. Widely used biomarkers like epigenetic clocks and frailty indices, while valuable for stratification and risk prediction, have significant limitations when used to infer aging mechanisms or evaluate antiaging interventions. These tools are fundamentally correlational and often reflect only very limited aspects of the changes associated with aging. For example, DNA methylation clocks frequently rely on a small subset of CpG sites with unclear functional relevance. Similarly, frailty indices aggregate diverse traits into a single score, where improvements in isolated features may give a misleading impression of broad antiaging effects. Both types of biomarkers risk oversimplifying aging's complexity by collapsing heterogeneous biological signals into summary measures, which may obscure mechanistic insights and tissue-specific effects. Thus, while useful for tracking and stratifying individuals, these tools are limited in their capacity to reveal whether an intervention genuinely slows the biological aging process.

Though widely cited, the "hallmarks of aging" framework also warrants closer examination. Many of the studies underpinning this model lack rigorous evidence that targeting individual hallmarks slows age-related change across tissues or systems. In numerous cases, interventions were tested only in aged cohorts, or effects in young and old animals were comparable (Supplementary File S1), suggesting baseline shifts rather than changes in the rate of aging. Such findings challenge the idea that these hallmarks causally regulate the trajectory of aging.

To advance the field, both discovery pipelines and intervention assessment strategies should be refined. In discovery studies, phenotypic measurements from aged individuals should not be used in isolation to infer intervention effects on aging. Instead, studies must explicitly quantify within-individual or within-cohort changes over time to distinguish aging-related progression from variation unrelated to aging. When evaluating interventions, deep phenotyping across multiple biological levels and organ systems is essential. Claims about systemic aging modulation must be grounded in evidence spanning a wide array of ASPs. If an intervention affects a single ASP or tissue, conclusions should be limited accordingly. Moreover, interventions should be tested in both young and old groups to determine whether observed effects reflect aging rate modulation or general physiological shifts. To facilitate experimental translation, we outline practical design principles for multitissue ASP assessment in Box 1, drawing on deep-phenotyping studies and analysis pipelines from our prior work (33, 150).

Refining both discovery pipelines and intervention testing frameworks will support a more mechanistic understanding of aging by enabling researchers to distinguish between interventions that simply extend lifespan or improve isolated ASPs, and those that fundamentally modify the biological processes driving age-related decline.

Implementing such refined frameworks also depends on recognizing that aging manifests differently across species. As discussed earlier, lifespan alone is an incomplete proxy for aging, and this limitation

Box 1. How to operationalize multitissue age-sensitive phenotype (ASP) assessment: high-level study design principles

(1) Build and harmonize a multitissue ASP panel
Assemble ASPs that span levels of biological organization (molecular, cellular, tissue, and organismal levels) and multiple organ systems (e.g., hematology, immunology, metabolism, cardiovascular, neurobehavior, and sensory) (Figure 2). Use deep phenotyping catalogs as a starting point and adapt to the species/strain at hand (127–129, 130, 150). Use standardized pipelines and centralized assays where possible; a multitissue histopathology block helps link ASPs to life-limiting pathology and cause of death (65). Deep phenotyping enables detection of heterogeneous intervention modes across systems, rather than overgeneralizing from a narrow marker set.

(2) Choose ages and map ASP trajectories to avoid survival bias
Map when each ASP first departs from the young-adult baseline (Figure 4); these ASPs' trajectories are essential for interpreting whether an intervention changes the rate vs. baseline (150) (Figure 4). In mice, assess "old" cohorts at ages with widespread ASP changes but before appreciable population attrition [e.g., ~20 months in C57BL/6J (150)], to minimize differential survival bias while enabling rich aging signal. Adjust for other strains/species based on ASP trajectory maps.

(3) Include young-treated and old-treated groups
To distinguish slowed aging (rate effects) from age-independent shifts (baseline effects), test putative antiaging interventions both before ASPs begin to change (young-treated) and after change is evident (old-treated) (Figure 4). Compare effect sizes across ages and test for intervention × age interactions (150). Prior work shows many "antiaging" effects manifest similarly in young animals, arguing for baseline rather than rate effects, unless an age-interaction is demonstrated (65, 66, 150).

(4) Analysis: quantify rate vs. baseline effects
For each ASP, estimate age effects, intervention main effects, and intervention × age interactions (two-way models), then compare effect sizes in young vs old; based on results, classify ASPs into rate, baseline, or mixed patterns (150) (Figure 4). Interpret "mixed" cases cautiously (could reflect longer exposure in old groups rather than true rate modulation) (150).

(5) Study design choices and power
Cross-sectional designs are efficient for large, multitissue ASP batteries (including terminal measures); longitudinal elements can be added selectively (129, 150). Plan for larger sample sizes at older ages because variability increases with age; base power on effect sizes from comparable age groups/strains (129, 164).

becomes even more apparent when comparing across species. The limited translational success of findings from model organisms to humans likely reflects conceptual and biological mismatches in how aging is studied across species. Most animal studies have relied on lifespan as a proxy for aging, which does not necessarily capture the underlying biological processes or life-limiting pathologies relevant to humans. As discussed here, the leading causes of death differ fundamentally across species, with CVD dominating in humans, neoplasia in mice, infections in fish, intestinal or neuromuscular failure in flies, and bacterial infection in worms, making direct translation inherently difficult. This divergence underscores that aging is not a single, universal process, but a mosaic of species- and tissue-specific mechanisms shaped by evolutionary history and environmental context. This limitation also reflects the constraints of reductionist paradigms that treat aging as a unitary process, overlooking the species-specific nature of life-limiting pathologies.

At the same time, studying aging exclusively in humans also cannot resolve its underlying mechanisms, as many hypotheses cannot be tested experimentally for ethical and practical reasons. Human studies provide valuable correlative and associative insights but are limited in establishing causality. Therefore, a balanced approach is essential, recognizing both the strengths and the limitations of model organisms. Animal studies remain indispensable for mechanistic discovery, but their interpretive power depends on a clear understanding of what aspect of aging is being tested, delayed, or accelerated within each model system. Without such clarity, even well-designed interventions risk being misinterpreted. Progress in the field will depend on integrating mechanistic insights from model organisms with deep human phenotyping in a bidirectional framework, where experimental precision and translational relevance continually inform one another.

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Author contributions

MK and DE jointly conceived and wrote the review. MK conducted the literature analysis. Both authors contributed equally to the conceptual development, figure preparation, and revision of the manuscript, and approved the final version.

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