

Review

A *C. elegans*-to-Mouse Discovery Framework for Prioritizing Sarcopenia Interventions

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ABSTRACT: Sarcopenia is a manifestation of musculoskeletal aging, yet no approved therapy is available. A major challenge is not the lack of potential interventions, but the difficulty of efficiently identifying which candidates should advance to aged-mammal validation. Sarcopenia is driven by multiple biological processes, including mitochondrial dysfunction, impaired proteostasis, redox dysregulation, inflammaging, and altered nutrient sensing. Consequently, candidate interventions encompass not only conventional drug candidates but also natural products, dietary compounds, food-derived metabolites, and multi-component formulations. This diversity creates a candidate space that is difficult to systematically evaluate using aged-mouse models alone. Here, we propose a *C. elegans*-to-mouse discovery framework for sarcopenia intervention development. *C. elegans* enables rapid organism-level assessment of locomotor function, muscle integrity, toxicity, genetic dependency, and conserved aging mechanisms within a single in vivo system. These features allow large candidate pools, including diverse compounds, doses, and combinations, to be screened and prioritized before resource-intensive mammalian studies. Within this framework, preservation of age-related function serves as the primary selection criterion, whereas conserved biological mechanisms provide additional support for candidate advancement. Mitochondrial quality control, redox resilience, proteostasis, and gut-muscle communication are discussed as representative domains that facilitate cross-species interpretation. Examples including urolithin A, norharmane, and spermidine illustrate how worm-based functional screening can be integrated with mechanistic evidence and aged-mouse validation. Collectively, this review proposes a practical framework that integrates function-centered screening with conserved mechanistic interpretation to improve early-stage prioritization of sarcopenia interventions. This framework provides a conceptual strategy for accelerating the discovery of function-preserving therapeutics for skeletal muscle aging.

Keywords: sarcopenia, *Caenorhabditis elegans*, aging, muscle function, candidate prioritization

1. Introduction

With the global aging of the population, sarcopenia has emerged as a significant clinical challenge. Sarcopenia is a disease assigned an International Classification of Diseases (ICD) code in 2016 and is closely associated with frailty, falls, disability, metabolic vulnerability, reduced quality of life, and increased mortality in older adults. However, despite these considerable clinical and societal burdens, no pharmacological treatments have yet received regulatory approval [1-7]. Despite intense

research efforts, the translation of promising preclinical candidates into effective sarcopenia therapies has remained limited.

One important contributing factor is that existing therapeutic development strategies have placed excessive emphasis on increasing muscle mass, rather than on muscle strength and physical performance capacity — the core clinical outcomes of sarcopenia. Indeed, many sarcopenia treatment strategies have set muscle mass augmentation as their primary endpoint. Hypertrophy-oriented approaches, including myostatin/activin axis

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inhibitors and selective androgen receptor modulators (SARMs), have demonstrated the capacity to increase lean body mass. However, these gains in muscle mass have not consistently translated into meaningful recovery of muscle strength, mobility, or physical performance capacity [8-16]. These findings demonstrate that therapeutic candidates for sarcopenia should not be selected solely on the basis of whether they increase muscle mass. Clinically, sarcopenia manifests primarily as a decline in function rather than a deficit in muscle size. Accordingly, the development of sarcopenia therapeutics must prioritize the preservation of function over the augmentation of mass, and must evaluate whether functional benefits are underpinned by biological mechanisms that sustain this function.

A further bottleneck in the development of sarcopenia therapeutics is the complexity of the candidate space, compounding the inherent biological complexity of the disease. Sarcopenia is driven by multiple interacting processes, including mitochondrial decline, impaired proteostasis, redox dysregulation, inflammaging, anabolic resistance, and altered nutrient sensing. Therefore, many candidate interventions are not developed as single agents but as combinations of nutritional, dietary, or geroprotective components designed to target complementary aging mechanisms. Examples from the broader aging field, such as dasatinib plus quercetin [17], illustrate how combination strategies can enhance biological effects by targeting complementary pathways. Unlike single-target small-molecule drugs, natural products, dietary materials, functional food ingredients, and multi-component formulations generate a combinatorial space defined by diverse substances, dosages, treatment durations, and combination conditions. This candidate space is vast, and its systematic exploration in aging mammalian models is practically infeasible. Although aged mice remain indispensable for final preclinical validation of sarcopenia candidates, they are poorly suited as a first-line platform for broad candidate exploration, owing to their high cost, extended experimental timelines, substantial animal usage, and low throughput. The discovery of sarcopenia therapeutic candidates therefore necessitates a stepwise decision-making strategy in which the candidate space is first substantially narrowed, and only candidates supported by functional and mechanistic evidence are advanced to mammalian validation.

This review proposes a *Caenorhabditis elegans*–mouse preclinical workflow for the discovery of function-preserving therapeutic candidates for sarcopenia. *C. elegans* possesses a short lifespan, tractability to genetic manipulation, optical transparency, a well-defined body wall muscle structure, and scalable functional assays [18]. These characteristics enable the simultaneous evaluation

of age-related motor decline, muscle integrity, pathway activity, toxicity, and biological aging at the individual level within a single in vivo system. Furthermore, the nematode platform permits rapid, parallel comparison of not only individual candidates but also diverse substances, dosage ranges, treatment durations, and combination conditions across aging individuals. Therefore, it is well suited for narrowing a broad and complex candidate pool prior to mammalian validation.

Importantly, this framework does not attempt to encompass all biological processes involved in sarcopenia. Instead, it focuses on conserved mechanisms that satisfy two criteria: they make direct contributions to the preservation of muscle function during aging and can be experimentally interrogated in *C. elegans*. Accordingly, mitochondrial quality control, mitophagy, redox resilience, and proteostasis are discussed because they represent muscle-intrinsic determinants of contractile performance that can be linked to functional phenotypes across species. Inflammaging and gut–muscle communication are further considered as systemic modifiers that influence whether these muscle-intrinsic benefits are maintained within the aged organism. Other important components of sarcopenia biology, including satellite cell-mediated regeneration, vertebrate neuromuscular junction remodeling, endocrine regulation, and additional vertebrate-specific processes, are discussed as limitations of worm-based screening and are regarded as essential targets for subsequent mammalian validation rather than initial candidate prioritization. Through this structure, worm-based discovery, mechanistic support, and mammalian validation are integrated into a practical stepwise prioritization strategy.

This framework is further supported by several reported cases of aging-modulatory materials. Urolithin A [19] represents a paradigmatic example in which improvements in *C. elegans* motor function, mammalian muscle function, and human-relevant evidence are connected through a mechanistic axis centered on mitochondrial quality control and mitophagy. Norharmane [20] illustrates how functional improvement in *C. elegans* can be linked to redox resilience via the SKN-1/NRF2 pathway, with subsequent evidence suggesting attenuation of muscle aging in mammalian models. Spermidine [21] demonstrates that conserved aging biology and functional maintenance can be bridged through the regulation of autophagy and protein homeostasis. Although these examples involve distinct biological mechanisms, they collectively illustrate how *C. elegans* can be used to identify, prioritize, and interpret candidate interventions before mammalian validation. Their value lies not only in mechanism-based interpretation, but also in demonstrating how worm-stage

functional screening can efficiently reduce candidate complexity while retaining translational relevance. Therefore, this review reframes early sarcopenia intervention discovery as a process of candidate-space reduction, functional prioritization, and stepwise cross-species validation. On this basis, we present a practical framework that positions *C. elegans* as a scalable discovery platform for sarcopenia intervention development.

2. Clinical Functional Endpoints Define the Target of Sarcopenia Discovery

Human sarcopenia is defined through functional indicators. Current diagnostic frameworks, including the European Working Group on Sarcopenia in Older People

2 (EWGSOP2) and the Asian Working Group for Sarcopenia 2019 (AWGS 2019), define sarcopenia through three related domains: muscle strength, muscle mass, and physical performance [22]. This clinical structure is important for identifying intervention strategies because the diagnosis of sarcopenia itself follows a stepwise functional logic. EWGSOP2 uses reduced muscle strength as the primary indicator of probable sarcopenia, reduced muscle mass for diagnostic confirmation, and reduced physical performance to define severity. AWGS 2019 also includes muscle strength, muscle mass, and physical performance, with emphasis on screening approaches suitable for community and primary-care settings. Therefore, these frameworks do not regard sarcopenia simply as a decrease in muscle mass. Instead, they evaluate sarcopenia in stages.

Table 1. Clinical methods for assessing muscle mass, muscle strength, and physical performance in sarcopenia.

Device/Method	Measured Parameter	Features	Criteria (EWGSOP2 & AWGS)	Advantages	Disadvantages	Ref.
CT	Muscle Mass	High-resolution cross-sectional imaging	No specific cutoff provided	High accuracy	Costly and involves radiation	[93]
MRI	Muscle Mass	High-resolution 3D imaging	No specific cutoff provided	No radiation exposure	Expensive and time-consuming	[94]
DXA	Muscle Mass	Measures bone, fat, and muscle	<7.0 kg/m ² (Men) <5.5 kg/m ² (Women, EWGSOP2)	Quick and straightforward	Involves radiation exposure	[95-97]
			<7.0 kg/m ² (Men) <5.4 kg/m ² (Women, AWGS)			
BIA	Muscle Mass	Analyzes body composition using bioelectrical impedance	<7.0 kg/m ² (Men) <5.7 kg/m ² (Women, AWGS)	Cost-effective	Sensitive to hydration status	[98]
Grip Strength	Muscle Strength	Simple strength assessment tool	<27 kg (Men) <16 kg (Women, EWGSOP2)	Easy to use and widely adopted	Reflects localized strength only	[96, 97, 99]
			<28 kg (Men) <18 kg (Women, AWGS)			
SPPB	Physical Performance	Evaluates mobility, balance, and strength	≤8 points (EWGSOP2) ≤9 points (AWGS)	Enables functional evaluation	Time-consuming	[100]
TUG	Physical Performance	Assesses mobility and fall risk	≥20 seconds (EWGSOP2)	Simple and quick	Requires controlled environment	[101]
6m Gait Speed	Physical Performance	Measures walking and mobility ability	≤0.8 m/s (EWGSOP2) ≤1.0 m/s (AWGS)	Simple and reliable	Requires sufficient space	[96, 97, 102]
5 time Chair Stand Test	Physical Performance	Assesses lower limb strength and functionality	≥12 seconds (AWGS)	Evaluates lower limb strength effectively	Requires minimal equipment	[96, 97, 103, 104]

Cutoff values are presented according to EWGSOP2 and AWGS recommendations where available. CT, computed tomography; MRI, magnetic resonance imaging; DXA, dual-energy X-ray absorptiometry; BIA, bioelectrical impedance analysis; SPPB, Short Physical Performance Battery; TUG, Timed Up and Go; EWGSOP2, European Working Group on Sarcopenia in Older People 2; AWGS, Asian Working Group for Sarcopenia.

Functional decline indicates clinical risk, reduced muscle mass provides anatomical confirmation, and impaired physical performance defines the clinical consequence of disease progression. This stepwise

clinical logic directly influences the development of preventive and therapeutic strategies. Candidate substances that increase muscle mass but fail to maintain muscle strength or physical performance do not meet the

clinical endpoint structure of sarcopenia. Muscle mass provides anatomical evidence, whereas muscle strength and physical performance determine whether muscle aging has reached a clinically meaningful level. Therefore, candidate substances for sarcopenia prevention should be evaluated based on whether they maintain the functional domains that define sarcopenia in older adults.

The clinical measurement tools used to evaluate these domains are summarized in Table 1. The purpose of this table is not merely to list diagnostic equipment, but to show why muscle mass, muscle strength, and physical function must be considered together when seeking interventions for sarcopenia. Muscle mass can be measured using DXA, BIA, CT, or MRI. These methods provide structural information, but they do not demonstrate functional benefit by themselves. Muscle strength is typically measured by grip strength and chair stand performance, which provide clinically meaningful indicators of force generation and lower-limb function. Physical function is evaluated using gait speed, the Short Physical Performance Battery, the Timed Up and Go test,

and the Sit-to-Stand test. These measurements connect muscle biology to mobility, disability risk, fall risk, and mortality. Table 1 defines the scope of clinical evaluation indicators that should ultimately be addressed in preclinical studies. This point is crucial for designing screening strategies. A useful preclinical platform must identify candidate substances that preserve function as well as morphology.

Therefore, animal-based testing is meaningful when it measures age-related functional decline, strength-related output, muscle structure, and conserved pathway activity. Based on this clinical logic, this review proposes a worm-to-mouse framework for sarcopenia intervention discovery. *C. elegans* is used at the early stage because it allows age-related functional decline, muscle integrity, pathway activity, toxicity, and organismal aging to be evaluated together in a scalable living system. Mammalian validation is required to determine whether the same candidate preserves muscle strength, physical performance, and tissue-level muscle quality in an aged vertebrate system.

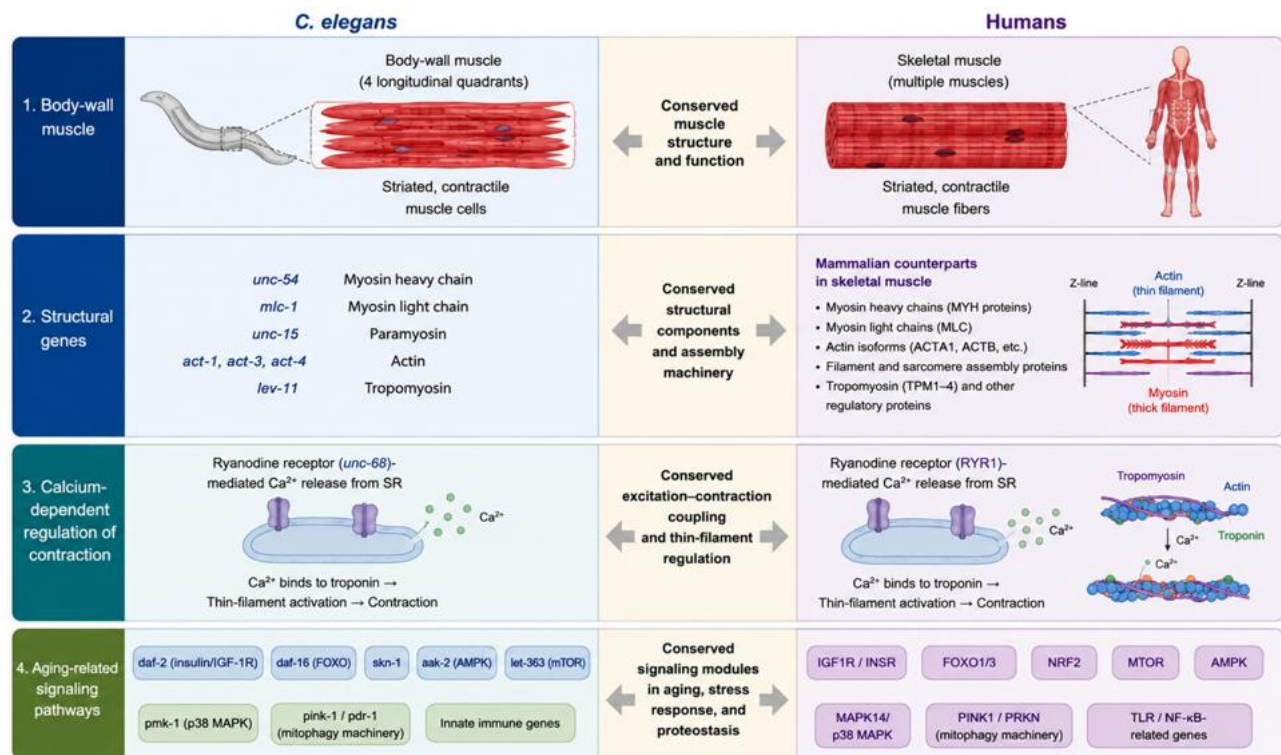


Figure 1. Conserved muscle biology between *C. elegans* and mammals. *C. elegans* body-wall muscle and human skeletal muscle share core structural and functional features relevant to muscle aging. These include striated contractile organization, actin–myosin-based force generation, sarcomere-associated structural components, calcium-dependent excitation–contraction coupling, and conserved aging-related signaling pathways. Worm muscle genes such as *unc-54*, *mhc-1*, *unc-15*, *act-1/3/4*, and *lev-11* correspond to mammalian contractile and filament-regulatory systems, while *unc-68* represents the ryanodine receptor-mediated calcium-release machinery. Conserved signaling modules, including insulin/IGF-1–FOXO, mTOR, AMPK, p38 MAPK, SKN-1/NRF2, mitophagy machinery, and innate immune pathways, provide mechanistic links between worm functional phenotypes and mammalian muscle-aging biology. These shared features support the use of *C. elegans* as an early functional and mechanistic screening platform before mammalian validation.

3. Cross-Species Conservation of Muscle Aging Biology

3.1 Conserved muscle biology between *C. elegans* and mammals

C. elegans provides a conserved biological basis for muscle-aging discovery. Its value comes from the fact that core processes required for muscle contraction, maintenance, and age-related functional decline are shared with mammals (Fig. 1). *C. elegans* body-wall muscle is arranged in four longitudinal quadrants and contains key contractile components that parallel mammalian skeletal muscle, including actin thin filaments, myosin thick filaments, and sarcomere-like organization that generates force through cross-bridge

cycling [18, 23, 24]. Several structural genes also have mammalian counterparts. *unc-54* encodes a myosin heavy chain related to mammalian MYH proteins. *mhc-1* encodes myosin light chain. *act-1*, *act-3*, and *act-4* encode actin isoforms. Other genes, including *unc-15*, *unc-45*, *unc-60*, *unc-73*, and *lev-11*, regulate sarcomere assembly, filament dynamics, myosin folding, and tropomyosin-mediated contraction [25]. Calcium-dependent regulation of contraction is also conserved. Ryanodine receptor-mediated calcium release controls excitation–contraction coupling in both worms and mammals, and thin-filament regulation follows related principles [18, 26]. Detailed information on *C. elegans* muscle genes relevant to conserved muscle structure and function is provided in Table 2.

Table 2. Representative *C. elegans* muscle genes relevant to conserved muscle structure and function.

Functional Category	Gene	Protein	Localization/Structure	Primary Function
Contractile apparatus	<i>myo-3</i>	Myosin heavy chain A	Thick filament (body wall muscle)	Core component of thick filaments; force generation
	<i>unc-54</i>	Myosin heavy chain B	Thick filament (body wall muscle)	Muscle contraction and filament assembly
	<i>mhc-1</i>	Myosin light chain	Thick filament	Regulation and stabilization of myosin
	<i>mhc-2</i>	Myosin light chain	Thick filament	Modulation of myosin activity
	<i>unc-15</i>	Paramyosin	Thick filament core	Structural support of thick filaments
Thin filament	<i>act-1</i> , <i>act-3</i> , <i>act-4</i>	Actin	Thin filament	Formation of thin filaments
	<i>lev-11</i>	Tropomyosin	Thin filament	Stabilization of actin filaments; regulation of contraction
	<i>unc-27</i>	Troponin I	Thin filament	Ca ²⁺ -dependent inhibition of contraction
	<i>tnt-1</i> / <i>tnt-2</i>	Troponin T	Thin filament	Anchoring troponin complex to tropomyosin
	<i>pat-10</i>	Troponin C	Thin filament	Ca ²⁺ binding and activation of contraction
Giant sarcomeric proteins	<i>unc-22</i>	Twitchin (titin-like kinase)	A/I band boundary	Regulation of sarcomere elasticity and contraction
	<i>unc-89</i>	Obscurin-like protein	M-line	Organization of M-line structure
	<i>ketn-1</i>	Kettin	Z-disc / dense body	Filament anchoring and sarcomere stability
Z-disc/dense body	<i>atn-1</i>	α -Actinin	Dense body (Z-disc analog)	Actin anchoring
	<i>deb-1</i>	Vinculin	Dense body	Integrin-associated adhesion complex
	<i>tln-1</i>	Talin	Dense body	Integrin binding and force transmission
	<i>unc-112</i>	Kindlin	Dense body	Integrin activation
	<i>pat-4</i>	Integrin-linked kinase (ILK)	Dense body	Adhesion signaling
	<i>pat-6</i>	Parvin	Dense body	ILK complex stabilization
M-line	<i>unc-97</i>	PINCH	Dense body	ILK-associated adaptor
	<i>unc-98</i> , <i>unc-96</i>	M-line adaptors	M-line	Myosin–cytoskeleton linkage
	<i>unc-95</i>	LIM-domain protein	M-line / Z-line	Structural organization
Sarcolemma/costamere	<i>pat-2</i> / <i>pat-3</i>	Integrin α / β	Muscle–ECM junction	Muscle attachment to extracellular matrix
	<i>zyx-1</i>	Zyxin	Costamere	Mechanical stress signaling
Dystrophin complex	<i>dys-1</i>	Dystrophin	Sarcolemma	Sarcolemma stabilization
	<i>dyb-1</i>	Dystrobrevin	Sarcolemma	Dystrophin-associated complex
	<i>sgn-1</i>	Sarcoglycan-like proteins	Sarcolemma	Membrane integrity

Basement membrane / ECM	<i>unc-52</i>	Perlecan	Basement membrane	ECM–integrin ligand
	<i>him-4</i>	Hemicentin	ECM	Cell–cell and cell–matrix adhesion
Protein folding / assembly	<i>unc-45</i>	Myosin chaperone	Cytosol / sarcomere	Myosin folding and assembly
Myogenic regulation	<i>hlh-1</i>	MyoD homolog	Nucleus	Muscle cell differentiation
Excitation–contraction coupling	<i>egl-19</i>	L-type Ca ²⁺ channel	Sarcolemma	Ca ²⁺ influx for contraction
	<i>unc-68</i>	Ryanodine receptor	Sarcoplasmic reticulum	Ca ²⁺ release
	<i>sca-1</i>	SERCA	Sarcoplasmic reticulum	Ca ²⁺ reuptake
	<i>itr-1</i>	IP ₃ receptor	ER / SR	Ca ²⁺ signaling

The table lists worm genes associated with contractile filaments, sarcomere-like organization, dense bodies, M-lines, sarcolemma stability, extracellular matrix attachment, protein folding, myogenic regulation, and excitation–contraction coupling. These representative genes illustrate the conserved structural and functional components that support the use of *C. elegans* for investigating mechanisms underlying muscle aging across species. ECM, extracellular matrix; ER, endoplasmic reticulum; SR, sarcoplasmic reticulum.

This conservation extends to aging-related signaling pathways. Insulin/IGF-1 signaling and its downstream FOXO transcription factor are represented by the DAF-2–DAF-16 pathway in *C. elegans*, a central pathway in lifespan and healthspan regulation [27]. In mammals, FOXO factors regulate atrophy-related genes, including MuRF1 and atrogin-1, linking this conserved pathway to muscle proteolysis and remodeling [28]. Other shared modules include mTOR, AMPK, and p38 MAPK, which regulate nutrient sensing, autophagy, mitochondrial adaptation, stress responses, and immune signaling. The SKN-1/NRF2 axis, mitophagy machinery, and innate immune pathways provide additional conserved entry points into redox resilience, mitochondrial quality control, and inflammation-related aging biology.

3.2 Functional and mechanistic readouts for cross-species interpretation

Cross-species interpretation requires a link between worm phenotypes and mammalian endpoints. Several worm assays can measure age-associated changes in movement and muscle function. Locomotor decline can be quantified using body-bend frequency, thrashing or swimming assays, and maximum velocity. Maximum velocity is a sensitive measure of peak movement capacity and healthspan [29, 30]. These assays are not identical to mouse rotarod, treadmill endurance, grip strength, or open-field activity. However, they measure related aspects of age-associated decline in coordinated movement and physical performance [31] (Fig. 2).

Strength-related phenotypes can also be measured in worms. The NemaFlex microfluidic platform estimates force generation by measuring the displacement of deformable micropillars. This provides a quantitative proxy for muscle strength that can be conceptually compared with grip strength testing in mice [32, 33]. Pharyngeal pumping also declines with age and provides

another muscle-dependent functional readout [34, 35] (Fig. 2). Structural and mechanistic readouts provide additional interpretation. Muscle-specific fluorescent reporters such as *myo-3::GFP* can be used to visualize sarcomere disorganization during aging [36, 37]. Mitochondrial morphology can be monitored using MitoTracker or mitochondrially targeted GFP. Respiratory capacity can be assessed through oxygen consumption measurements [38–40] (Fig. 2).

3.3 Positioning *C. elegans* within the sarcopenia discovery pipeline

Although several experimental platforms contribute to sarcopenia research, they occupy complementary rather than interchangeable positions within the discovery pipeline (Table 3). Cultured myotubes provide molecular precision but cannot evaluate organism-level aging or functional performance. Zebrafish offer vertebrate physiology but are less suited for large-scale aging-based prioritization, whereas aged mice remain indispensable for physiological validation despite their high cost and limited throughput. Therefore, the principal value of *C. elegans* does not lie simply in the conservation of aging pathways, which is shared across multiple model organisms. Rather, its unique advantage is the integration of organism-level aging, longitudinal functional phenotyping, conserved genetics, mechanistic interrogation, and scalable pharmacological screening within a single experimental system, making it particularly well suited for early-stage candidate prioritization before mammalian validation (Table 3).

Beyond conserved biology (3.1) and cross-species interpretable readouts (3.2), *C. elegans* offers a practical advantage in handling the complexity of the candidate space. Natural products, dietary materials, functional food ingredients, and multi-component formulations generate a vast combinatorial space defined by the multiplication of

substance type, dose, treatment duration, and combination condition. Unlike single-target small molecules, this space is difficult to narrow a priori and is impractical to explore directly in aged mice owing to constraints of cost, time, animal use, and throughput. Because of its short lifespan, small size, and compatibility with 96-well liquid culture, *C. elegans* allows many conditions to be compared in parallel within aging animals. It enables

simultaneous evaluation—on the same aging axis—of not only single-candidate effects but also dose–response relationships, the timing and duration of treatment, and the interactions (additive, synergistic, or antagonistic) that arise when two or more materials are combined [41]. This is particularly useful for deconvoluting which component or combination within a formulation actually contributes to functional preservation.

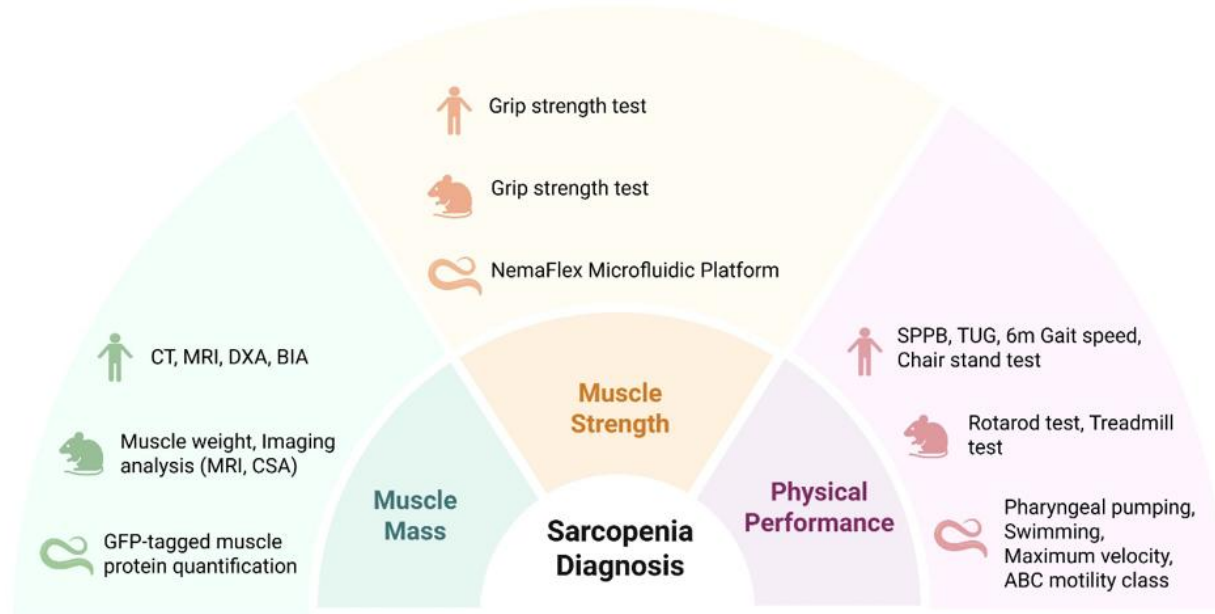


Figure 2. Cross-species alignment of functional readouts for sarcopenia-oriented preclinical screening. Age-related muscle decline in *C. elegans* can be assessed using locomotor readouts such as body bends, thrashing or swimming movement, and maximum velocity, as well as strength-related assays such as NemaFlex and muscle-dependent measures such as pharyngeal pumping. These functional readouts can be combined with structural and mechanistic assays, including body-wall muscle morphology, mitochondrial morphology, oxygen consumption, mitophagy reporters, stress-response reporters, and genetic pathway tests. This integrated readout structure supports the prioritization of worm-stage candidates for aged-mouse validation through integrated functional and mechanistic evaluation. Importantly, these worm-based readouts should be interpreted as candidate-prioritization evidence rather than direct evidence of sarcopenia efficacy. Improvements in locomotor performance, muscle morphology, or pathway activity indicate preservation of age-related function within the worm model but do not, by themselves, establish therapeutic benefit in mammals. Consequently, worm-stage findings should be integrated with orthogonal muscle-specific assays and subsequently validated in naturally aged mammalian models before translational conclusions are drawn.

Importantly, this throughput advantage becomes meaningful only when coupled with function-centric readouts. Quantitative functional measures such as maximum velocity, body-bend frequency, swimming locomotion, and NemaFlex-based strength proxies allow many conditions to be ranked on a common scale, and—when paired with structural reporters such as *myo-3::GFP* or pathway reporters—permit combinatorial effects to be interpreted mechanistically as well. Therefore, the nematode platform functions not merely as a high-throughput filtering system, but as a biologically informed decision layer in which organism-level functional phenotypes, conserved mechanisms, and genetic

dependency are evaluated together to prioritize candidates for subsequent mammalian validation.

3.4 Boundaries of worm-based inference

The proposed framework is intentionally restricted to biological processes that satisfy two conditions. First, they make direct contributions to age-related decline in muscle function. Second, they can be experimentally interrogated within the *C. elegans* system while maintaining mechanistic interpretability across species. Consequently, this framework is not intended to encompass every aspect of sarcopenia biology. Rather, it focuses on conserved muscle-intrinsic mechanisms that

can serve as practical entry points for early-stage candidate prioritization before mammalian validation. Thus, *C. elegans* serves as an early screening system for mechanism-guided sarcopenia intervention discovery.

However, a worm phenotype should be used to prioritize candidate interventions, not as direct evidence for treating mammalian sarcopenia. Improved locomotion in *C. elegans* is not sufficient to define a sarcopenia intervention candidate because movement can increase through altered feeding, delayed development, reduced

toxicity, nonspecific stress resistance, or general vigor. Accordingly, functional improvement should be supported by conserved mechanistic evidence before progression to mammalian validation. Based on this logic, the following sections focus on three muscle-intrinsic mechanisms that directly support functional maintenance during aging: mitochondrial quality control and mitophagy, redox resilience, and proteostasis. Inflammaging and gut–muscle communication are then discussed as systemic modifiers of muscle aging.

Table 3. Complementary roles of experimental models in the sarcopenia intervention discovery pipeline.

Feature	Cultured myotubes	Drosophila	Zebrafish	C. elegans	Aged mouse
Organism-level aging	X	✓	△	✓	✓
Longitudinal functional phenotyping	X	△	✓	✓	✓✓
Muscle integrity assessment	△	△	✓	✓	✓✓
Genetic manipulation	✓	✓✓	✓	✓✓	△
Mechanistic pathway interrogation	✓	✓	✓	✓✓	✓
High-throughput pharmacological screening	✓✓	✓	△	✓✓	X
Multi-dose and combination screening	△	✓	△	✓✓	X
Time and cost efficiency	✓✓	✓	△	✓✓	X
Integrated function + mechanism in the same aging organism	X	△	△	✓✓	✓
Primary role in the discovery pipeline	Mechanistic validation	Genetic discovery	Vertebrate biology	Early candidate prioritization	Final preclinical validation

Relative strengths of each experimental model are indicated as ✓✓ (strong), ✓ (moderate), △ (limited), and X (not suitable). The table highlights the complementary rather than competitive roles of each model within the sarcopenia intervention discovery pipeline.

Note that some mammalian-specific features of sarcopenia cannot be resolved in nematodes. Candidate interventions that act mainly through satellite cell-mediated regeneration, vertebrate neuromuscular junction remodeling, endocrine or sex-steroid signaling, adaptive immunity, pharmacokinetics, polypharmacy, frailty, or chronic disease-related biology require mammalian or human-relevant systems for proper evaluation. These biological domains remain indispensable for understanding sarcopenia progression and therapeutic efficacy. However, because they cannot be adequately modeled in *C. elegans*, they are positioned as essential components of the mammalian validation stage rather than the worm-based discovery stage. Accordingly, these mammalian-specific biological domains should be evaluated during the mammalian validation stage, preferably using naturally aged animals whenever feasible. Compared with accelerated or injury-based models, naturally aged mice more faithfully recapitulate the multifactorial physiological changes underlying sarcopenia and therefore provide a more appropriate

setting for validating candidates prioritized through the worm-based discovery stage.

4. Mechanistic Axis I: Mitochondrial Quality Control and Mitophagy

4.1 Mitochondrial quality as a functional bottleneck in aged muscle

The mass–function disconnection directs attention to mechanisms that influence muscle performance beyond fiber size. Among the conserved mechanisms that directly influence age-related muscle function, mitochondrial quality control represents one of the most experimentally tractable and translationally interpretable biological axes. In aged skeletal muscle, reduced mitochondrial oxidative capacity is associated with slower gait speed, greater fatigability, lower aerobic fitness, and reduced grip strength, even after accounting for lean mass [42–44]. Therefore, mitochondrial decline is a biochemical feature of aged muscle and it is linked to functional impairment. Mitochondrial dysfunction also amplifies other aging-

related changes in muscle. Excess mitochondrial reactive oxygen species can damage mitochondrial DNA, lipids, and proteins [45–47]. Altered mitochondrial dynamics can impair calcium handling, proteostasis, and regenerative capacity [48, 49]. Damaged mitochondria also increase the burden on autophagic and stress-response systems. Thus, mitochondrial quality control is positioned upstream of several mechanisms that determine muscle quality. Mitophagy provides one route to maintain mitochondrial quality [49]. Damaged mitochondria must be removed, but removal alone is not sufficient. Mitochondrial renewal also requires adequate biogenesis. Therefore, the key question is whether mitochondrial clearance and replacement are coordinated in a way that preserves muscle function.

4.2 Why mitophagy is testable across worms and mammals

C. elegans is useful for this axis because mitochondrial structure, turnover, and functional decline can be examined in the same aging organism. Mitochondria in body-wall muscle can be visualized using mitochondrially targeted GFP, MitoTracker dyes, or related reporters [38, 40]. During aging, the mitochondrial network changes from elongated structures to fragmented and rounded forms. This transition is associated with locomotor decline [38]. Worm studies also show that coordinated activation of mitophagy and mitochondrial biogenesis is required for healthspan extension during aging [38]. The core mitophagy machinery is sufficiently conserved to support cross-species interpretation. The worm PINK-1/PDR-1 pathway corresponds to the mammalian PINK1/Parkin system [38]. However, increased mtGFP or MitoTracker intensity alone may reflect mitochondrial biogenesis, reduced turnover, or altered dye uptake. Static mitochondrial signals are therefore not sufficient to support mitophagy claims. Flux-sensitive mitophagy readouts are required, for example mitochondria-targeted dual-fluorescent, pH-sensitive mitophagy reporters (such as mtRosella-type systems in *C. elegans*) or analogous tandem fluorescent reporters in mammalian models [50].

4.3 Urolithin A as a prototype of mechanism-concordant translation

Urolithin A is one of the strongest examples of mitophagy-axis translation from worms to mammals. It is a gut microbial metabolite derived from ellagitannins in foods such as pomegranates and some nuts. In *C. elegans*, urolithin A induced mitophagy, preserved mitochondrial network morphology, improved locomotor function, and extended lifespan [19]. The key point is not the positive worm phenotype alone. The stronger point is that

functional rescue was linked to mitochondrial quality-control biology. The mammalian evidence follows the same direction. In aged mice, urolithin A improved grip strength, increased voluntary running activity, and increased muscle ATP and NAD⁺ levels, together with evidence of enhanced mitophagy [19, 51]. In human primary skeletal muscle cells, it delayed senescence-associated changes and improved mitochondrial respiratory capacity [52]. Human studies also support oral bioavailability and pharmacodynamic signatures consistent with improved mitochondrial health [53]. Therefore, urolithin A is a strong translational prototype.

4.4 Interpretive checkpoint for the mitophagy axis

Mitophagy-axis candidates should be prioritized only when functional rescue is accompanied by evidence of improved mitochondrial quality control. Improved locomotion alone is not sufficient. Static mitochondrial labeling alone is also not sufficient. Stronger evidence requires flux-sensitive mitophagy readouts, support from mitophagy-related genes such as *pink-1* or *pdr-1*, and preservation of function in mammalian muscle. Furthermore, mitophagy activation should not be interpreted as uniformly beneficial. Excessive clearance may reduce mitochondrial content when biogenesis is insufficient. Basal mitophagy differs by species, muscle type, and fiber type. Therefore, priority should be given to candidates that restore mitochondrial turnover while preserving strength, endurance, or physical performance.

5. Mechanistic Axis II: Redox Resilience through SKN-1/NRF2

5.1 From oxidative damage to adaptive redox resilience

Redox balance is essential for muscle function during aging [47]. Reactive oxygen species are not only damaging by-products. They also support contraction-induced adaptation, mitochondrial remodeling, and stress-response signaling when properly controlled. In aged muscle, ROS production often exceeds antioxidant buffering capacity. This shift promotes oxidative damage to proteins, lipids, and DNA and contributes to impaired muscle performance [47, 54, 55]. Redox dysregulation can reduce force generation without proportional loss of muscle mass. Oxidative modification of contractile proteins can impair sarcomere function. Redox-sensitive regulators of calcium handling, including ryanodine receptors and SERCA, are also altered during aging [47]. These changes compromise excitation–contraction coupling and help explain why aged muscle can become weak without large changes in size. This biology also

explains why generic antioxidant strategies have often shown limited efficacy. Broad ROS scavenging may reduce oxidative damage, but it can also suppress physiological redox signaling. Therefore, the more relevant strategy is not simple antioxidant supplementation. It is reinforcement of endogenous stress-response capacity. The SKN-1/NRF2 axis provides a conserved entry point into this adaptive form of redox resilience.

5.2 Conserved stress-response logic from SKN-1 to NRF2

SKN-1 is the major *C. elegans* transcriptional regulator of cytoprotective responses to oxidative and electrophilic stress. It activates antioxidant, detoxification, and stress-response genes, including glutathione S-transferases, UDP-glucuronosyltransferases, cytochrome P450 enzymes, and thioredoxin- and glutathione-related genes. SKN-1 activity is also connected to several stress- and aging-related pathways, including p38 MAPK/PMK-1 signaling, insulin/IGF-1 signaling, and nutrient-responsive signaling [56]. Therefore, SKN-1 does not simply indicate oxidative stress. It provides an integrated transcriptional response that links environmental stress, metabolic state, host defence, and organismal aging.

SKN-1 and mammalian NRF2 are not structurally identical, but they perform related functions in adaptive stress resistance. In mammals, NRF2 is regulated mainly through KEAP1-dependent degradation under basal conditions. Oxidative or electrophilic stress allows NRF2 to accumulate, enter the nucleus, and activate antioxidant response element-driven genes [57]. These genes support glutathione metabolism, detoxification, redox buffering, and cytoprotective repair. In skeletal muscle, NRF2 contributes to antioxidant defence, mitochondrial homeostasis, proteostatic support, and functional maintenance during aging. Thus, the conserved logic from SKN-1 to NRF2 is not structural identity, but adaptive cytoprotection. In both systems, stress-responsive transcription increases cellular resilience by coordinating detoxification, redox buffering, mitochondrial maintenance, and repair capacity. This logic is important for sarcopenia-oriented discovery because aged muscle does not only suffer from oxidative damage. It also loses the ability to adapt to repeated metabolic, contractile, and inflammatory stress. Therefore, the SKN-1/NRF2 axis provides a conserved entry point for identifying candidates that preserve muscle function by reinforcing endogenous stress-response capacity.

In worms, this logic can be examined using *gst-4::GFP* [56], SKN-1 target-gene expression, oxidative stress resistance, and genetic or RNAi-based pathway testing. These readouts are most informative when they

are interpreted together with age-related functional outcomes, such as locomotor preservation, muscle integrity, or resistance to redox-linked functional decline [58]. In mammalian muscle cells or aged muscle, NRF2 nuclear localisation and target-gene expression should be connected to muscle-relevant outcomes, including mitochondrial function, redox damage, myotube integrity, or muscle performance [59]. Therefore, SKN-1/NRF2 signaling is useful not as a simple marker of stress activation, but as a conserved mechanism that can link stress adaptation to functional preservation.

5.3 Norharmane as a redox-resilience candidate

Norharmane is a useful example of a redox-resilience candidate identified through *C. elegans* muscle-aging assays [20]. In aged worms, norharmane improved locomotor capacity and preserved muscle structure. Its effect depended on SKN-1. SKN-1 depletion abolished the protective phenotype, and norharmane induced canonical SKN-1 target genes, including cytochrome P450s, UDP-glucuronosyltransferases, and glutathione S-transferases [20]. Norharmane also activated SKN-1 through the p38 MAPK/PMK-1 pathway. The mammalian data followed the same biological direction. In mammalian muscle cells, norharmane activated NRF2 by disrupting the NRF2-KEAP1 interaction and promoting KEAP1 degradation [20]. In aged mice, it preserved muscle mass, strength, and endurance. It also reduced markers of cellular senescence and improved mitochondrial respiratory capacity. In human primary skeletal muscle cells, it delayed replicative senescence and enhanced myogenic differentiation [20].

A related SKN-1/NRF2-axis example is citrinin, a fungal secondary metabolite historically studied primarily in the context of nephrotoxicity but reported at low, non-toxic doses to engage the SKN-1 stress-response pathway [60]. In *C. elegans*, citrinin treatment attenuated age-related decline in locomotor function and extended lifespan, with SKN-1 activity implicated as the underlying mechanism [60]. Comparable protective effects were reported in C2C12 mammalian myoblasts, where citrinin treatment reduced markers of cellular senescence through SKN-1/NRF2 pathway engagement [60]. However, citrinin has not yet been evaluated in aged-mouse muscle or in human-relevant systems. Therefore, citrinin is presented as an emerging, SKN-1/NRF2-linked candidate at an earlier stage of translational maturity, illustrating the worm-based screening logic described in this framework, rather than as an established example of cross-species translation.

5.4 Interpretive checkpoint for the redox-resilience axis

Redox-resilience candidates should be prioritized when functional rescue is linked to SKN-1/NRF2 pathway engagement and reduce redox-linked damage. SKN-1 reporter activation alone is not enough. Reduced oxidative markers alone are also not enough. Stronger evidence requires functional rescue, target-gene induction, and loss or reduction of benefit when SKN-1/NRF2 signaling is suppressed. Therefore, the key point is not whether a compound activates SKN-1 or NRF2, but whether SKN-1/NRF2 activity is required for the functional benefit.

Several points require caution. SKN-1/NRF2 activation is not automatically beneficial. Many toxic or nonspecific stressors can activate this pathway [61]. Transient activation may support adaptation, whereas sustained or excessive activation may produce maladaptive effects. Therefore, the timing, intensity, and duration of pathway activation matter. Broad stress-response activation may reflect toxicity rather than adaptive resilience. NRF2 activation may also differ by dosing duration, age, tissue context, and sex [62].

For sarcopenia-oriented discovery, redox-resilience candidates should therefore be evaluated through a function-first logic. In worms, a candidate is stronger when improved movement or muscle integrity is accompanied by SKN-1 target induction and reduced benefit after *skn-1* suppression. In mammalian systems, NRF2 activation should be interpreted together with muscle function, mitochondrial function, redox-linked damage, and safety under repeated exposure. A compound that activates NRF2 but does not preserve strength, endurance, or muscle quality should not be considered a strong sarcopenia intervention candidate. Conversely, a compound that improves function but lacks evidence of SKN-1/NRF2 dependency requires additional mechanistic clarification before advancement.

6. Mechanistic Axis III: Proteostasis and the Integrated Stress Response (ISR)

6.1 Proteostasis as a broad quality-control axis

Skeletal muscle depends on continuous protein synthesis, folding, assembly, repair, and clearance. During aging, several parts of this network become impaired. Chaperone capacity declines, the ubiquitin–proteasome system loses efficiency or selectivity, and autophagy flux decreases. These changes promote the accumulation of misfolded, oxidized, or aggregated proteins in aged muscle fibers. This burden can impair sarcomere organization, calcium handling, metabolic enzyme function, and contractile quality without a major change in fiber size [47]. Therefore, proteostasis is directly linked to the maintenance of muscle quality and function during aging.

Proteostasis is broader than a single degradation pathway. It includes cytosolic heat-shock responses, ER stress responses, mitochondrial unfolded protein responses, macroautophagy, proteasome activity, and aggregate clearance. These systems operate together to preserve protein quality in long-lived muscle cells. They also interact with mitochondrial function and redox balance because damaged proteins, oxidized proteins, and dysfunctional mitochondria can reinforce one another during aging. Therefore, proteostasis should be understood as a broad quality-control network that supports contractile integrity and functional resilience in aged muscle.

In the context of muscle aging, this functional diversity is important. A decline in proteostasis can affect several levels of muscle biology at the same time, including sarcomere stability, excitation–contraction coupling, mitochondrial enzyme function, and stress-response capacity. Thus, proteostasis provides a mechanistic basis for understanding why muscle function can decline even when muscle size is not markedly reduced. This makes proteostasis a key muscle-intrinsic axis for function-preserving sarcopenia intervention discovery.

6.2 Conserved proteostasis mechanisms across worms and mammals

Proteostasis is included in the proposed framework because maintenance of contractile protein quality is essential for preserving muscle function during aging and can be experimentally interrogated through conserved stress-response pathways in *C. elegans* [63]. Rather than representing a general hallmark of aging, proteostasis is discussed here as a muscle-intrinsic determinant of contractile integrity that provides mechanistic support for candidate prioritization across species.

C. elegans is useful for examining conserved proteostasis mechanisms during aging. Protein quality and stress-response phenotypes can be monitored in vivo across the lifespan. Several proteostasis-related pathways are experimentally accessible in worms. These include the HSF-1-mediated cytosolic heat-shock response [64], the IRE-1/XBP-1-mediated ER stress response [65], the ATFS-1-mediated mitochondrial unfolded protein response [66], SKN-1-dependent stress responses, and DAF-16/FOXO signaling. These pathways provide entry points for evaluating how aged organisms maintain protein quality under stress.

Reporter strains also allow pathway activity to be followed in living animals. For example, *hsp-16.2p::GFP* can be used to monitor cytosolic heat-shock responses. *hsp-4::GFP* reports ER stress responses. *hsp-6::GFP* or *hsp-60::GFP* can be used to monitor mitochondrial stress

responses [67]. The effector side of proteostasis can also be examined. Autophagy flux can be monitored using *lgg-1* reporters and tandem fluorescent autophagy systems [68]. Proteasome activity can be measured using biochemical or reporter-based assays. Muscle-expressed aggregation models, including polyQ, A β , and α -synuclein transgenes, provide direct readouts of aggregate burden in body-wall muscle [69].

These worm-based tools are relevant because mammalian skeletal muscle uses related protein quality-control systems. HSF1 contributes to muscle force maintenance and protects against proteotoxic stress. IRE1 α /XBP1, PERK/ATF4, and ATF6 regulate ER stress responses in mammalian cells [70]. ATF5-related mitochondrial stress signaling links mitochondrial proteostasis to mitochondrial quality control [71]. Small heat-shock proteins, including HSPB1, HSPB5/ α B-crystallin, and HSPB8, help protect contractile proteins from misfolding and aggregation [72]. The ubiquitin–proteasome system also includes muscle-relevant E3 ligases such as MuRF1, atrogin-1, and TRIM32 [28]. Therefore, the value of *C. elegans* in this axis is that it allows conserved proteostasis pathways to be monitored together with organismal aging and muscle-related function. Worm readouts do not reproduce the full complexity of mammalian skeletal muscle proteostasis. However, they provide an early way to determine whether a candidate affects protein quality-control pathways that have interpretable counterparts in mammalian muscle.

6.3 Spermidine as a proteostasis-related candidate

Spermidine is a representative proteostasis-related candidate. It is a naturally occurring polyamine obtained from diet and endogenous synthesis. Its relevance to this axis comes mainly from its ability to induce macroautophagy. Mechanistically, spermidine promotes autophagy in part through inhibition of EP300-mediated acetylation of autophagy-related machinery. This route is conserved across species and can operate independently of canonical mTOR or AMPK activation.

In *C. elegans*, spermidine extends lifespan and improves healthspan-related phenotypes [3]. Autophagy involvement is supported by *lgg-1*-related readouts and by the loss of lifespan extension in autophagy-deficient backgrounds. Muscle-related benefits, including preserved locomotor capacity and reduced sarcopenia-like phenotypes during aging, have also been reported. These findings support spermidine as a proteostasis-related candidate based on conserved aging biology. Mammalian evidence also supports the relevance of spermidine to muscle aging. Aged-mouse studies have reported effects on endurance, grip strength, and autophagy-related markers [73]. Human-cell studies also

suggest reduced senescence-associated features and enhanced autophagy flux following spermidine treatment [3]. Therefore, spermidine provides an example in which conserved autophagy and protein quality-control biology are linked to age-related functional maintenance across species.

6.4 Interpretive checkpoint for the proteostasis axis

Proteostasis candidates should be prioritized when functional rescue is accompanied by branch-resolved evidence of improved protein quality control. Proteostasis and the Integrated Stress Response (ISR) reporter induction alone is not sufficient. Aggregate reduction, autophagy activation, or proteasome changes are also insufficient if they are not linked to functional benefit. Therefore, the key question is not whether a candidate activates a proteostasis-related marker, but whether it restores protein quality-control capacity in a way that supports age-related functional maintenance.

Stronger evidence requires three elements: (1) improved age-related muscle function, (2) a defined proteostasis readout, and (3) pathway support through regulators such as HSF-1/HSF1, IRE-1/XBP-1, ATFS-1/ATF5, autophagy genes, or proteasome-related components. These elements help distinguish beneficial protein quality-control restoration from nonspecific stress activation. Mammalian validation should use flux-based autophagy assays, branch-specific ISR markers, proteasome activity measurements, aggregate or protein-damage readouts, and functional endpoints. Chronic activation should be interpreted carefully because sustained stress signaling may not always be adaptive. In particular, prolonged PERK/ATF4 activity may suppress protein synthesis and impair muscle adaptation [74]. Therefore, proteostasis-based candidates should be advanced when improved protein quality-control readouts are connected to preserved muscle function, rather than when stress-response markers are activated alone.

7. Inflammaging and the Gut–Muscle Interface as a Systemic Modifier of Muscle Aging

7.1 Inflammaging as a systemic modifier of muscle function

Inflammatory aging and the gut-muscle interface must be distinguished from the intrinsic muscle axis discussed above. Mitochondrial quality control, redox recovery, and protein homeostasis can be studied as relatively direct and conserved mechanisms of aging-related functional decline in *C. elegans*. In contrast, inflammatory aging is a systemic state formed by immune cells, intestinal barrier tissues, microbial signaling, metabolic organs, and aged

skeletal muscle. Therefore, it should not be treated as the same as the major classification axis of the worm stage.

The key implication of inflammatory aging is that it defines a systemic environment in which intrinsic muscle interventions in the mammalian aging process either maintain or lose their effects. Age-related muscle loss is influenced by interactions between skeletal muscle, immune cells, adipose tissue, the liver, the gut, and the microbiome. Chronic low-intensity inflammation is one of the most consistent systemic features associated with sarcopenia [75]. Elevated levels of IL-6, TNF- α , and CRP are associated with decreased muscle mass, reduced muscle strength, and reduced physical activity capacity in older adults [76]. Inflammatory signals can affect muscle function through various pathways. This can promote anabolic resistance, activate NF- κ B-dependent catabolic pathways, impair mitochondrial function, and reduce regenerative capacity [28, 77]. Additionally, it can exacerbate redox and protein homeostatic stress in aged muscle. Therefore, inflammatory aging does not simply act outside the muscle. It alters the biological environment in which aging mechanisms within the muscle operate. Consequently, even compounds that improve mitochondrial, redox, or protein homeostatic biology within the muscle may have limited effects if systemic inflammation caused by aging continuously induces catabolic stress. For this reason, inflammatory aging is positioned as a systemic regulator of sarcopenia biology. While this does not replace the intrinsic muscle axis used for early worm stage selection, it helps determine whether such muscle-oriented effects can be effectively maintained in an aged mammalian environment.

7.2 Gut–muscle communication in worms and mammals

The gut–muscle interface is an important component of systemic muscle aging. In mammals, the intestine affects skeletal muscle through microbial metabolites, inflammatory mediators, nutrient availability, endocrine signals, and barrier integrity. Aging is associated with changes in gut microbial composition and reduced intestinal barrier function. These changes can increase exposure to microbial products such as lipopolysaccharide and peptidoglycan fragments [78, 79]. These signals activate innate immune pathways, increase systemic inflammatory tone, and contribute to anabolic resistance in skeletal muscle [77]. Therefore, gut aging can influence muscle function even when the primary defect is not located within muscle itself.

Several mechanisms link the mammalian gut to age-related muscle decline. Microbiota-derived metabolites, including short-chain fatty acids, bile acid derivatives, indole metabolites, and urolithins, can influence

mitochondrial metabolism, inflammation, and muscle quality [80, 81]. Reduced intestinal barrier integrity can increase microbial product leakage and promote chronic low-grade inflammation [81, 82]. Inflammatory cytokines such as IL-6 and TNF- α can impair protein synthesis, promote catabolic signaling, and reduce muscle regenerative capacity [80]. Thus, the gut–muscle axis provides a systemic route through which diet, microbiota, and intestinal aging can modify sarcopenia biology.

C. elegans provides a useful system for studying selected aspects of gut–muscle communication. The worm has a functional intestine, conserved innate immune signaling, and experimentally tractable interactions between microbial exposure, intestinal aging, and organismal function [83]. In standard culture, *E. coli* OP50 serves both as a food source and as a persistent microbial exposure. During aging, intestinal bacterial accumulation increases and contributes to organismal decline [84]. This makes the worm useful for examining how age-related changes in intestinal microbial burden and host defence are associated with functional deterioration.

Innate immune signaling provides one point of conservation between worms and mammals. The p38 MAPK/PMK-1 pathway in *C. elegans* regulates host defence and responses to microbial stress [85]. This pathway is functionally related to mammalian stress and innate immune signaling pathways that respond to microbial and inflammatory cues. Therefore, worm studies can connect microbial exposure, intestinal aging, innate immune activation, and organismal function in a single living system. However, the worm does not reproduce the full mammalian gut–muscle axis. It lacks adaptive immunity, vertebrate endocrine regulation, complex microbiota ecology, and the multi-organ inflammatory network that shapes sarcopenia in aged mammals. Thus, while worm gut readouts can reveal conserved host–microbe mechanisms, mammalian validation is required to determine whether those mechanisms influence muscle strength, physical performance, and sarcopenia progression in an aged vertebrate system.

7.3 Translational validation of gut–muscle candidates

Short-chain fatty acids affect host–microbe biology in worms and mammals and have been linked to muscle outcomes [86]. Polyphenols can influence microbial composition, barrier integrity, oxidative stress, and inflammation [87]. Other diet-derived or microbiota-modifying interventions may also influence muscle aging through barrier function, microbial metabolites, or inflammatory tone. These examples support the biological importance of gut–muscle communication. However,

they do not yet establish a single, well-defined worm-to-mammal prototype comparable to the leading examples discussed for the muscle-intrinsic axes.

This distinction defines how worm data should be interpreted. *C. elegans* lacks adaptive immunity, mammalian cytokine networks, adipose–muscle endocrine crosstalk, comorbidity-driven inflammation, and complex mammalian microbiota. Therefore, worm data in this area should be interpreted as evidence for host–microbe and innate-immune modulation, not as direct evidence for modulation of mammalian inflammaging.

In worms, functional rescue should be interpreted together with host–microbe and innate-immune readouts. These may include PMK-1/p38 MAPK activity, pathogen resistance, intestinal bacterial burden, barrier-related phenotypes, and bacterial-food-source dependence. These readouts can identify candidates whose functional effects may involve microbial or barrier-related biology. Mammalian validation should then determine whether the

candidate improves muscle function under aged systemic conditions. This requires assessment of circulating inflammatory markers, intestinal barrier integrity, microbiota composition or microbial-metabolite profiles, muscle-level inflammatory signaling, and functional endpoints such as strength, endurance, and physical performance. The strongest translational support would come from candidates that preserve muscle function while also reducing systemic inflammatory burden or maintaining barriers and microbial homeostasis in aged mammals. By contrast, changes in immune or microbial markers without functional benefit should be interpreted as mechanistic signals, not as sufficient evidence for sarcopenia-relevant efficacy. Together, these points define how worm-stage functional hits should be advanced to mammalian validation. Table 4 summarises the required worm-stage evidence, mammalian validation endpoints, main interpretive cautions, and advancement criteria for each mechanistic axis and systemic modifier.

Table 4. Operational decision framework for advancing candidate interventions from *C. elegans* screening to aged-mouse validation.

Stage	Evaluation	Decision criterion
Primary functional screening	Maximum velocity, body bends, thrashing, swimming, NemaFlex	Significant attenuation of age-associated functional decline
Safety assessment	Toxicity, lifespan, development	Functional benefit without overt toxicity or developmental delay
Dose optimization	Dose–response relationship	Reproducible functional improvement within an effective dose range
Muscle-specific confirmation	myo-3::GFP, body-wall muscle morphology, NemaFlex	Functional improvement supported by preservation of muscle structure or strength-related phenotypes
Mechanistic validation	Genetic dependency, pathway reporters, mitochondrial/redox/proteostasis assays	Functional rescue supported by conserved mechanistic evidence
Aged-mouse validation	Grip strength, rotarod/treadmill, muscle mass, mitochondrial function	Preservation of muscle strength and/or physical performance in naturally aged mice

Candidate advancement is based on concordant evidence obtained through sequential evaluation of age-related functional preservation, safety, dose optimization, muscle-specific confirmation, mechanistic support, and validation in naturally aged mice. This framework is intended as a conceptual decision guide for candidate prioritization rather than a prescriptive experimental workflow. Progression to mammalian studies should be supported by both functional improvement and appropriate mechanistic evidence whenever feasible.

7.4. Integration of Muscle-Intrinsic Mechanisms and Systemic Context

Although these four mechanistic domains are discussed separately for clarity, they are not expected to operate independently during aging. Rather, they interact to determine whether muscle function can be maintained under physiological aging conditions. This integrated perspective provides the mechanistic basis for the proposed discovery framework. The purpose of axis-based classification is not to assign each candidate to one rigid mechanism, but to identify the dominant mechanism that best explains functional recovery and to determine

whether changes in secondary pathways support that interpretation.

Mitochondrial dysfunction, redox imbalance, proteostatic stress, and inflammatory signaling are biologically interconnected in aging muscle. Mitochondrial damage increases oxidative burden. Oxidative stress disrupts protein homeostasis and amplifies inflammatory signaling. Proteostatic dysfunction further impairs mitochondrial integrity and contractile function. Inflammatory signaling can then exacerbate metabolic, redox, and proteostatic stress in muscle. Therefore, candidate prioritization should be based on evidence coherence rather than on axis labels

alone. A strong candidate should not be defined by the largest change in a single molecular marker. Instead, it should be defined by concordance among functional recovery, pathway activation, genetic or pharmacological dependency, and mammalian validation. Collectively,

these mechanistic axes constitute the mechanistic foundation of the proposed workflow (Fig. 3), providing biological support for interpreting functional phenotypes and prioritizing candidates before mammalian validation.

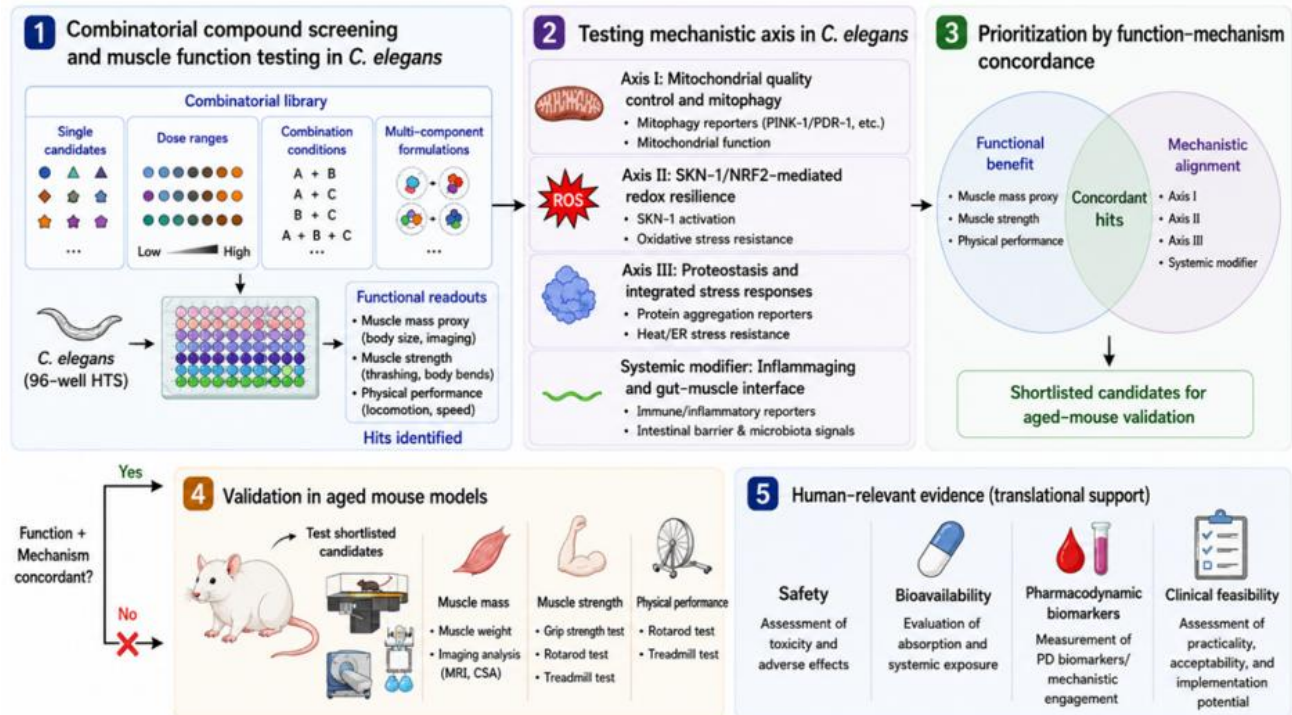


Figure 3. Function-Mechanism concordance Framework for Sarcopenia intervention discovery. Broad candidate pools (Natural products, dietary fibers, functional food ingredients, and multi-component formulations) are first screened in *C. elegans* using muscle-related functional readouts, including locomotor capacity, maximum velocity, body bends, swimming movement, and strength-related proxy assays. Functional hits are then evaluated across conserved mechanistic axes, including mitochondrial quality control and mitophagy, SKN-1/NRF2-mediated redox resilience, proteostasis, and systemic modifiers such as inflammaging and gut-muscle communication. Candidates showing concordance between functional rescue and mechanistic support are advanced to aged-mouse validation, where muscle strength, physical performance, muscle quality, and pathway-relevant tissue outcomes are assessed. This workflow prioritizes candidates based on function-mechanism concordance rather than muscle mass gain or isolated biomarker changes. Human-relevant evidence, including safety, bioavailability, pharmacodynamic biomarkers, and clinical feasibility, may then support later translational assessment, but it is not used as the primary discovery layer in this preclinical workflow.

7.5 Why worm-positive findings still require mammalian validation

The examples discussed above illustrate how worm-derived functional phenotypes can be integrated with conserved biological mechanisms and mammalian evidence. However, positive findings in *C. elegans* should not be interpreted as direct evidence of efficacy in mammals [88, 89]. Differences in physiology, tissue complexity, and species-specific biology mean that phenotypes observed in worms may not necessarily translate to mammalian systems.

In contrast to urolithin A, norharmane, and spermidine, for which worm-stage functional phenotypes were followed by muscle-specific mammalian validation prior to human translation, EGCG illustrates the outcome

when this staged sequence is bypassed. In *C. elegans*, EGCG extends healthy lifespan through a mitohormetic mechanism involving transient mitochondrial ROS production and compensatory mitochondrial biogenesis, dependent on AMPK/AAK-2, SIRT1/SIR-2.1, and DAF-16/FOXO signaling [90]. However, this worm-stage evidence was derived from organismal lifespan and general stress-resistance readouts rather than from muscle-specific functional or structural assays such as locomotion, NemaFlex-based strength, or *myo-3::GFP*-based structural confirmation, and a dedicated aged-mouse validation stage assessing muscle mass, strength, or physical performance has not been established for EGCG. Despite this incomplete worm-to-mouse evidentiary chain, a recent community-based, cluster-randomized, placebo-controlled trial combining catechin-

enriched green tea with exercise training in older adults found no significant improvement in physical performance or muscle mass on intention-to-treat analysis [91]. This example illustrates that positive worm phenotypes alone are insufficient to justify advancement toward clinical translation.

Therefore, the predictive value of *C. elegans* lies in candidate prioritization rather than in forecasting clinical efficacy. Positive worm phenotypes should be interpreted as early prioritization evidence, not as proof of therapeutic efficacy. Advancement to mammalian studies should require concordance among functional improvement, mechanistic support, genetic dependency where applicable, and validation in aged mammalian systems. This staged decision process is designed to improve the efficiency and biological interpretability of candidate selection while recognizing the inherent limitations of worm-based discovery.

8. Function–Mechanism Concordance as a Translational Decision Principle

The proposed discovery workflow is built around one principle. Early screening should identify candidates that preserve muscle-related function during aging through conserved aging mechanisms. It should not focus only on compounds that change muscle mass or isolated biomarkers. *C. elegans* is useful at the entry stage because it allows functional and mechanistic screening in an intact aging organism at a scale that is difficult to achieve in aged rodents. The value of the workflow lies in staged concordance. A candidate should retain functional and mechanistic coherence as it moves from worms to aged mice, and ultimately to human-relevant systems.

8.1 Principles of candidate selection

Three principles guide candidate selection (Fig. 3 and Table 4). First, function is the primary gate. A compound should not advance solely because it induces mitophagy, activates SKN-1/NRF2, reduces inflammatory markers, or stimulates autophagy. These changes are meaningful for sarcopenia intervention discovery only when they accompany preservation of locomotor capacity, strength-related readouts, endurance, or other function-linked phenotypes during aging. Second, concordance is more important than effect size alone. A modest but reproducible delay in age-associated locomotor decline, supported by structural and mechanistic evidence, is more informative than a large single-endpoint effect without pathway support. Large isolated effects often reflect toxicity, altered feeding, altered general vigor, developmental delay, or stress hormesis rather than true modification of muscle aging. Third, mechanistic

narrowing should become stricter at each validation step. At the worm screening step, broad pathway engagement may be acceptable. At the aged-mouse step, mechanistic evidence should align with clinically relevant outcomes, including muscle mass, strength, and physical performance. Before human translation, pharmacokinetics, safety, and tissue distribution should be addressed.

8.2 Worm screening and mammalian validation steps

The first step uses *C. elegans* to identify candidates that preserve muscle-related function or delay functional decline during aging. The minimum requirement is a measurable attenuation of age-associated decline in at least one quantitative functional readout. These readouts may include maximum velocity [30], body-bend frequency, swimming locomotion, thrashing, or a strength-proxy assay such as NemaFlex [32, 33]. Where possible, locomotor measures should be paired with body-wall muscle structure using reporters such as *myo-3::GFP* or equivalent sarcomere markers. Preservation of function without muscle-structure or pathway evidence should be interpreted cautiously.

This identification step need not be limited to single candidates. The throughput of *C. elegans* and its amenability to parallel comparison within aging animals allow many materials, dose ranges, treatment durations, and combinations of two or more materials to be tested together against the same functional readouts. This is especially useful when the candidate space is large—as with natural products, dietary materials, and multi-component formulations—and can be used to deconvolute and prioritize which single component or combination actually contributes to functional preservation during aging. However, functional benefits observed under combination conditions should be interpreted alongside appropriate single-material controls and systematic dose-combination comparisons to distinguish additive, synergistic, and antagonistic interactions, thereby facilitating deconvolution of the components responsible for functional preservation.

Functional preservation hits are then profiled across the four mechanistic axes. Mitochondrial quality control can be assessed by mitochondrial network morphology and, ideally, flux-based mitophagy reporters rather than static mitochondrial intensity alone. Redox resilience can be examined using SKN-1 target reporters such as *gst-4::GFP*, oxidative stress resistance, and epistasis with *skn-1*. Proteostasis candidates should be evaluated using stress-response reporters, autophagy markers, aggregation models, and epistasis with relevant regulators such as *hsf-1*, *ire-1/xbp-1*, *atfs-1*, or autophagy components. Inflammation-related candidates require particular

caution because worm immune biology does not fully capture mammalian chronic inflammation. Pathogen resistance, innate immune reporters, and microbiota-dependent effects can be informative, but they should be interpreted as selected host–microbe and innate-immune readouts rather than full models of mammalian inflammaging.

The preclinical step is aged-mouse validation. This step tests whether the candidate preserves function in a mammalian organism with systemic aging biology. Mouse validation should include muscle mass, strength, and physical performance. Muscle mass can be measured by muscle weight, imaging, or fiber cross-sectional area. Strength should include grip strength and, where feasible, ex vivo or in situ contractility. Physical performance should include treadmill endurance, voluntary wheel running, rotarod, or related assays. Depending on the

proposed mechanism of action, additional endpoints such as mitochondrial respiration, neuromuscular junction integrity, muscle fiber-type composition, fatigue resistance, or inflammatory markers may further strengthen biological interpretation.

A candidate that increases muscle mass without preserving strength or performance should not be considered successful in this workflow. Sex-stratified analysis is also important because skeletal muscle aging and responses to interventions differ between males and females [92]. In addition to functional efficacy, successful translation requires confirmation that candidate compounds achieve biologically relevant exposure in mammalian tissues. Pharmacokinetic evaluation, including bioavailability, metabolism, tissue distribution, tolerability, and dose–exposure relationships, should be incorporated into the mammalian validation stage.

Table 5. Representative examples illustrating translational evidence across the proposed worm-to-mouse discovery framework.

Evidence	Candidate	Worm function	Worm mechanism	Mammalian cell	Aged mouse	Human	Function-mechanism concordance	Interpretation
Advanced	Urolithin A	✓	Mitophagy	✓	✓	✓	Advanced evidence support	Best validated prototype
Intermediate	Norharmane	✓	SKN-1/PMK-1	—	✓	—	Intermediate evidence support	Strong preclinical example
Intermediate	Spermidine	✓	Autophagy	✓	✓	Observational	Intermediate evidence support	Conserved aging biology
Intermediate	Citrinin	✓	SKN-1	—	—	—	Intermediate evidence support	Emerging candidate
Mechanistic	Vitamin A	Lifespan	SKN-1	✓	—	—	Mechanistic-marker support	Mechanistic example
Mechanistic	Polyphenols	Variable	DAF-16/SKN-1	Variable	Limited	Limited	Mechanistic-marker support	Mechanistic support only
Preliminary	Quercetin	✓	Partial	—	Limited	—	Function-only preliminary support	Function only
Preliminary	Sulforaphane	✓	Partial	✓	Limited	—	Function-only preliminary support	General healthspan
Preliminary	SCFA(short-chain fatty acids)	✓	Gut–muscle communication	✓	Limited	Observational	Function–mechanism preliminary support	Representative microbiota-derived metabolites

Representative intervention examples are categorized according to the level of translational evidence supporting progression from *C. elegans* to mammalian studies. Function–mechanism concordance reflects the extent to which age-related functional improvement is supported by conserved biological mechanisms across experimental systems, rather than the overall therapeutic efficacy of each intervention.

Within this scope, the central value of the workflow is straightforward. It provides a practical route for selecting candidates whose worm-stage functional benefit is supported by a conserved mechanistic signal and therefore justifies progression to mammalian testing. The operational decision process underlying this framework is summarized in Table 4, which defines the minimum criteria for advancing candidates through sequential

evaluation of functional preservation, toxicity exclusion, dose–response relationships, mechanistic support, muscle-specific assessment, and aged-mouse validation. Note that this framework is not intended to rank the intrinsic efficacy or therapeutic superiority of individual candidates. Rather, it describes the degree to which functional rescue, mechanistic support, and cross-species validation are aligned. Candidates with advanced

evidence support show worm functional rescue, mechanism dependency, aged-mouse functional benefit, and human-relevant signal. Candidates with intermediate support show worm functional rescue, mechanistic support, and partial validation in mice. Candidates with mechanism-marker support show pathway activation without sufficient functional validation. Candidates with function-only preliminary support improve worm function but lack a clearly defined mechanistic anchor. Representative examples are shown in Table 5.

9. Future Perspectives and Conclusions

The absence of approved pharmacological treatments for sarcopenia does not simply reflect a lack of research effort or experimental models. Rather, the field has relied too heavily on a limited question: how to increase muscle mass. Considerable progress has been made towards this objective, particularly through hypertrophy-oriented strategies such as myostatin/activin-axis inhibition and anabolic modulation. However, clinical experience has shown that increased lean mass alone is not sufficient to treat sarcopenia. For older adults, the most important outcome is not muscle size itself, but preserved function. This includes the ability to rise from a chair, walk safely, maintain mobility, and avoid disabling falls. Therefore, sarcopenia intervention discovery requires a shift from muscle enlargement towards the biology of functional preservation.

This review proposes that sarcopenia intervention discovery should be organized around biological systems that can connect muscle function, pathway activity, tissue-level phenotypes, toxicity, and organismal aging. In this context, *C. elegans* provides a useful discovery platform. Its value lies in its capacity to act as a rigorous and scalable genetic and pharmacological filter. The worm allows age-related functional decline, stress-response biology, muscle integrity, toxicity, and organismal aging to be examined together at a speed, scale, and cost that are difficult to achieve routinely in mammalian systems. Used in this way, *C. elegans* does not replace rodent or human-cell validation. Rather, it helps define which candidates have sufficient functional and mechanistic support to justify advancement into mammalian studies.

The central principle of this framework is function–mechanism concordance. A candidate should not advance simply because it improves worm movement or activates a stress-response reporter. Improved function is necessary, but it is not sufficient. The functional benefit should be supported by an interpretable and conserved mechanism that can be tested further in mammalian systems. In this framework, worm data are not treated as direct therapeutic evidence. They are used as decision

evidence. The purpose of the worm stage is to determine whether a candidate deserves further validation, not to prove mammalian efficacy. Accordingly, the proposed framework is not intended to encompass every aspect of sarcopenia biology. Rather, it focuses on conserved determinants of age-related muscle function that can be efficiently interrogated in *C. elegans* and subsequently validated in aged mammalian models. Within this framework, mitochondrial quality control and mitophagy, SKN-1/NRF2-mediated redox resilience, and proteostasis are treated as conserved muscle-intrinsic mechanisms that directly support functional maintenance during aging. Inflammaging and gut–muscle communication are considered systemic modifiers that influence whether these muscle-intrinsic benefits are maintained in aged mammals. This structure provides a biologically interpretable basis for candidate prioritization by linking functional outcomes to conserved mechanisms of muscle aging.

The reported cross-species examples discussed in this review illustrate this logic. Urolithin A shows how mitophagy-linked mitochondrial quality control can be connected to functional benefit across models. Norharmane provides an example in which SKN-1/NRF2-related redox resilience is linked to muscle-aging phenotypes. Spermidine illustrates how conserved autophagy and proteostasis biology can contribute to age-related functional maintenance. These examples do not imply that one mechanism is sufficient to solve sarcopenia. Rather, they show how functional rescue, mechanistic support, and mammalian validation can be aligned to improve candidate interpretation.

Given that several sarcopenia-related clinical programs have increased lean mass without producing consistent functional recovery, the field may benefit from moving beyond muscle-mass-centered discovery strategies towards function-preserving approaches grounded in conserved aging biology. From this perspective, the primary value of *C. elegans* is not that it models every aspect of sarcopenia, but that it enables functional phenotypes, genetic dependency, and conserved mechanisms of aging to be evaluated within a single organism. Overall, this review proposes function–mechanism concordance as a decision principle for sarcopenia intervention discovery. By integrating functional preservation with conserved mechanisms of muscle aging, this framework provides a practical strategy for prioritizing candidates with stronger biological and translational rationale before mammalian validation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Two of the cross-species case studies discussed in this review (norharmane, citrinin) are based on research conducted by the authors' own group. These examples are presented as illustrative applications of the proposed framework and have not yet been independently replicated by other laboratories. This should be considered when weighing their evidentiary status relative to urolithin A, which has been validated across multiple independent groups and human trials.

Author contributions

Y.C and J.H.H. conceived and designed the study and wrote the manuscript. Y.C, H.D.S., C.H.J., J.A. and J.H.H., edited the manuscript.

Data Availability

Data will be made available on request.

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