

Review

Lysosomal Competence in Skeletal Muscle Aging: Autophagic Flux, Measurement Gaps, and Human Translation

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ABSTRACT: Age-related deterioration of skeletal muscle contributes to loss of mobility, frailty, and disability. Altered autophagy-lysosome competence has been proposed as a mechanism linking impaired proteostasis, mitochondrial damage, inflammation, and regenerative failure to sarcopenia. Chronological muscle aging and clinically defined sarcopenia overlap, but evidence from healthy aging, frailty, disuse, cachexia, obesity, or chronic disease cannot be treated as interchangeable. This narrative review examines how lysosomal acidification, degradative capacity, biogenesis, and turnover influences autophagic flux in aging muscle and evaluates the strength of evidence across experimental models and human studies. Genetic and pharmacological studies show that severe disruption of autophagy or specific lysosomal functions can cause myofiber damage, mitochondrial impairment, defective regeneration, and weakness. However, direct evidence that lysosomal acidification, fusion, hydrolase activity, membrane integrity, recycling, cargo degradation, or completed autophagic flux is impaired in aging human muscle remains limited. Much of the human literature relies on static abundance of LC3-II, SQSTM1/p62, lysosomal proteins, or transcriptional signatures, which cannot distinguish increased autophagosome formation from impaired degradation. We therefore separate causal experimental evidence from human associations and focus on V-ATPase, TMEM175, LAMP proteins, TFEB/TFE3, lysophagy, and crosstalk with mitophagy and the ubiquitin-proteasome system. Exercise improves clinically relevant outcomes in older adults, but mediation through completed autophagic flux has not been established. Recent trials of metformin and once-weekly sirolimus did not improve prespecified functional outcomes in the tested populations and regimens, whereas urolithin A produced endpoint-specific signals while missing key primary outcomes.

Keywords: skeletal muscle aging, sarcopenia, lysosome, autophagic flux, mitophagy, clinical translation

1. Introduction

Sarcopenia is a progressive skeletal muscle disorder characterized by reduced muscle strength, with low

muscle quantity or quality used to confirm the diagnosis and poor physical performance used to indicate severity in the European Working Group framework. The Asian Working Group uses related but population-specific

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algorithms and cut points. These definitions matter because muscle mass, strength, and physical performance are biologically related but not interchangeable outcomes. Sarcopenia has also been assigned an ICD-10-CM code, reinforcing its recognition as a clinical condition rather than an inevitable descriptor of chronological aging [1, 2, 3]. Loss of muscle strength and mobility increases the likelihood of falls, loss of independence, hospitalization, and death, although the magnitude of risk depends on the population, diagnostic definition, and covariate adjustment [4, 5].

Terminology is used conservatively throughout. ‘Aging muscle’ denotes age-associated observations unless a clinical sarcopenia definition is reported. ‘Sarcopenia’ is reserved for cohorts meeting consensus diagnostic criteria or for an explicitly labeled experimental sarcopenic phenotype. Frailty is a multidomain syndrome, whereas disuse or denervation, cachexia, obesity, and chronic disease introduce distinct catabolic, neural, inflammatory, or metabolic contexts. Findings are therefore labeled by population and model and are not transferred across these contexts without qualification [1, 2, 6, 7].

The biology of skeletal muscle aging is multifactorial. Motor-unit remodeling, endocrine and immune changes, reduced physical activity, altered nutrient sensing, mitochondrial dysfunction, impaired regeneration, and loss of proteostasis all contribute [6, 7]. Protein quality control is especially relevant because long-lived myofibers must preserve contractile structures and organelles while repeatedly adapting to mechanical and metabolic stress. The ubiquitin–proteasome system mainly processes short-lived or soluble proteins, whereas the autophagy–lysosome system can remove protein aggregates, membrane structures, and entire organelles. Neither pathway acts independently; failure or overload in one arm changes demand on the other.

Lysosomes are both terminal degradative organelles and signaling platforms. Their acidic lumen enables hydrolase activity, while their membranes coordinate amino-acid sensing, mammalian target of rapamycin complex 1 (mTORC1) activity, calcium signaling, and transcriptional programs that regulate autophagy and lysosomal biogenesis [8, 9]. These functions make lysosomal competence a plausible point of convergence for proteostatic, mitochondrial, and inflammatory disturbances in aging muscle. Plausibility, however, should not be confused with demonstration. Many studies infer “autophagic flux” from a single measurement of LC3-II, SQSTM1/p62, LAMP1/2, cathepsins, or pathway transcripts. Such measurements cannot by themselves

determine whether cargo delivery increased, degradation slowed, or both occurred.

Recent reviews have summarized autophagy in skeletal muscle homeostasis and wasting disorders and the regulation of muscle lysosomes during exercise, disuse, and aging [10, 11]. The present review is therefore not positioned as another pathway overview. Instead, it addresses three unresolved questions: (i) whether aging muscle fails at lysosomal acidification and terminal degradation rather than at autophagosome formation alone; (ii) which assays can distinguish dynamic flux from static marker abundance in human muscle; and (iii) why mechanistically plausible interventions have produced mixed trial-level functional and safety outcomes. Evidence is organized by species, muscle context, study design, assay, and endpoint, with explicit separation of causal experimental studies from human associations.

Our central interpretation is deliberately bounded: impaired lysosomal competence and altered autophagic flux are mechanistically plausible contributors to skeletal muscle aging, but their presence, magnitude, causal importance, and therapeutic tractability in human sarcopenia remain uncertain. Accordingly, the review calibrates mechanistic findings, biomarkers, and intervention studies to the species, tissue, assay, and endpoint from which they were derived.

1.1. Review approach and evidence framework

This narrative review was informed by searches of PubMed/MEDLINE, Scopus, and Web of Science from database inception through 11 August 2026 using combinations of terms related to skeletal muscle aging, sarcopenia, lysosome, acidification, autophagic flux, mitophagy, chaperone-mediated autophagy, exercise, and clinical trials. English-language peer-reviewed articles were considered without a publication-date restriction. Potentially relevant records were screened by title and abstract and, when appropriate, by full text. We prioritized human studies, primary experimental studies that directly assessed skeletal muscle, current consensus statements, and randomized trials. Evidence was organized by species, muscle context, study design, assay, and endpoint. Non-muscle and non-mammalian studies were included only to explain candidate mechanisms and are identified as such. Reference lists of key reviews were screened to identify additional studies. Because the objective was a mechanism-focused narrative synthesis rather than a systematic review, formal meta-analytic pooling and a PRISMA flow diagram were not undertaken. Reporting and interpretation of autophagy assays were aligned with current consensus guidance [12].

The principal PubMed/MEDLINE search was: ("skeletal muscle" [Title/Abstract] OR myofiber* [Title/Abstract] OR sarcopenia [Title/Abstract]) AND (lysosome* [Title/Abstract] OR autophagy [Title/Abstract] OR "autophagic flux" [Title/Abstract] OR mitophagy [Title/Abstract] OR "chaperone-mediated autophagy" [Title/Abstract]) AND (aging [Title/Abstract] OR ageing [Title/Abstract] OR older [Title/Abstract] OR sarcopenia [Title/Abstract]). The principal intervention search was: (sarcopenia OR "older adults" OR "skeletal muscle") AND (exercise OR metformin OR sirolimus OR rapamycin OR "urolithin A" OR spermidine OR TFEB OR TFE3) AND (trial OR random* OR biopsy OR autophagy OR mitophagy). Syntax and field tags were adapted for Scopus and Web of Science, and target-specific searches were used for V-ATPase, TMEM175, LAMP2A/CMA, lysophagy, *Atg7*, TP53INP2, and BNIP3. The search was last updated on 11 August 2026. Because selection was narrative rather than systematic, the review was not registered and did not use dual independent screening or a formal study-level risk-of-bias instrument.

For interpretation, we use four evidence tiers. Tier 1 comprises muscle-specific causal perturbation or a dynamic assay that directly measures cargo processing or a defined lysosomal function. Tier 2 comprises muscle-relevant experiments with paired perturbation and functional outcomes but incomplete resolution of the full flux pathway. Tier 3 comprises human muscle associations, static protein abundance, transcriptional signatures, or single-time-point imaging. Tier 4 comprises findings from non-muscle cells, invertebrate models, or broad reviews that support biological plausibility but cannot establish what occurs in aging human muscle. This hierarchy is not a formal study-quality grade; it prevents extrapolation across species, tissue, assay, endpoint, and clinical context.

Tiers are assigned at the claim or endpoint level, not once for an entire study. A single report may therefore provide Tier 1 evidence that a perturbation causes a mouse-muscle phenotype but only Tier 3 evidence that a related human marker is associated with age or performance. For example, the CMA study provides Tier 1 evidence for loss and rescue in muscle-specific mouse models and Tier 3 evidence for age-associated human-muscle measures; the LAMP–TMEM175 mechanism is Tier 4 for an aging-human-muscle claim because it was resolved in non-muscle human cell systems. Borderline evidence is labeled by its measured component, and the more conservative tier is used for the target claim. Tiers are neither averaged across endpoints nor transferred from a model-specific mechanism to clinical efficacy.

2. Lysosomal competence and the meaning of autophagic flux

2.1. Acidification, degradation, and recycling

Lysosomes receive substrates through endocytosis, phagocytosis, macroautophagy, chaperone-mediated autophagy (CMA), and organelle-selective pathways. Their luminal acidity, commonly approximated at pH 4.5–5.0, is maintained principally by vacuolar H⁺-ATPase (V-ATPase). The proton gradient supports the activity and maturation of acid hydrolases, metabolite export, membrane trafficking, and signaling [13, 14]. In skeletal muscle, lysosomal proteolysis participates in basal turnover, adaptation to fasting and contraction, and the removal of structures too large for the proteasome [15].

Acidification is not controlled by V-ATPase alone. Counter-ion conductance, proton leak, membrane composition, organelle position, and ion channels influence pH and degradative function. In general cell systems, LAMP1 and LAMP2 inhibited the lysosomal channel TMEM175 and thereby facilitated acidification, defining a candidate LAMP–TMEM175 pH-control mechanism [16]. Loss of V-ATPase function has coupled impaired acidification to cellular iron deficiency, mitochondrial disturbance, and inflammation in experimental systems [17]. Whether either mechanism contributes to muscle aging depends on tissue and disease context; neither has been established as an age-dependent lesion in human skeletal muscle.

Terminal degradation also requires delivery and fusion, hydrolase activity, export of degradation products, and regeneration of functional lysosomes. Thus, “lysosomal dysfunction” should specify the measured defect: altered pH, reduced enzyme activity, impaired fusion, membrane damage, defective cargo degradation, or failed recycling. An increase in LAMP1-positive structures can represent biogenesis, impaired turnover, organelle enlargement, or a shift in cellular composition. It is not synonymous with greater degradative capacity.

Lysosomes also coordinate nutrient signaling. In nutrient-replete conditions, mTORC1 on the lysosomal surface phosphorylates transcription factor EB (TFEB) and related MiT/TFE factors, limiting their nuclear localization. During nutrient or energetic stress, calcineurin- and AMPK-linked signals can favor nuclear translocation of TFEB/TFE3 and transcription of the Coordinated Lysosomal Expression and Regulation (CLEAR) network [18, 19, 20]. These pathways connect lysosomal biogenesis to metabolic adaptation, but increased expression of CLEAR-network genes does not guarantee completion of cargo degradation. Biogenesis

can be adaptive, insufficient, or disconnected from organelle quality.

2.2. Macroautophagy, CMA, mitophagy, and lysophagy

Macroautophagy sequesters cytoplasmic cargo within double-membrane autophagosomes that fuse with late endosomes or lysosomes. Basal macroautophagy is required for muscle homeostasis, while starvation, exercise, denervation, and disease can change different steps of the pathway. CMA is mechanistically distinct: HSC70 recognizes proteins containing KFERQ-like motifs and delivers them to the LAMP2A translocation complex at the lysosomal membrane [21]. Recent skeletal muscle work indicates that CMA responds to exercise and tissue repair, declines with age and obesity, and contributes to calcium handling and myofiber integrity. However, the strongest causal evidence comes from muscle-specific mouse models; age-associated changes in human tissue remain observational [22].

Mitophagy denotes selective delivery of mitochondria to lysosomes. PINK1–Parkin and receptor-mediated pathways involving BNIP3 and NIX are well described, but their relative importance varies by developmental stage, metabolic state, damage severity, and model [23, 24]. In muscle, mitochondrial fission, transport, and local lysosomal availability influence whether a damaged mitochondrial segment can be removed. A change in a mitophagy-related protein is therefore not equivalent to measured mitochondrial turnover.

Lysophagy, the selective removal of damaged lysosomes, is another potentially important but understudied layer. Membrane permeabilization exposes luminal glycans, recruits damage sensors and ubiquitin-linked machinery and promotes sequestration of irreparable lysosomes. Conversely, limited damage may be repaired without organelle removal. These pathways could explain how chronic oxidative or mechanical stress reduces the pool of competent lysosomes; however, direct evidence for age-dependent lysophagy failure in human skeletal muscle is lacking, so lysophagy remains a research hypothesis in sarcopenia.

2.3. Dynamic flux versus static marker abundance

In this review, impaired autophagic flux refers to reduced cargo processing through autophagosome–lysosome fusion, lysosomal degradation, and recycling as demonstrated by a dynamic assay. Changes in LC3-II or

SQSTM1/p62 at a single time point are described only as changes in marker abundance and are not interpreted as proof of flux blockade. This definition follows the principle that autophagosome abundance is determined by both formation and clearance [12].

In animal muscle, flux can be estimated by comparing LC3-II or cargo turnover in the presence and absence of a lysosomal or trafficking block. Colchicine-based approaches, tandem fluorescent reporters, pulse–chase designs, organelle-specific reporters, and direct assays of isolated lysosomes each address different steps and introduce different perturbations. A mature-muscle method using colchicine demonstrated why LC3-II measured without a block can miss an induced autophagic response [25]. Another study using a pharmacological block found higher mitophagy flux in aged rat muscle and a reduction after chronic contractile activity, illustrating that aging does not invariably produce a simple decrease in every form of flux [26].

Human skeletal muscle presents a harder problem. Repeated biopsies can quantify signaling, transcript abundance, ultrastructure, and selected turnover processes, but systemic lysosomal inhibition is generally not ethically suitable solely to measure autophagy. Ex vivo incubation, stable-isotope approaches, reporter strategies, and organelle isolation may improve inference, yet tissue viability, ischemic and transport time, biopsy composition, perturbation specificity, and between-laboratory reproducibility require explicit validation. A method should be benchmarked against in vivo biology in an appropriate animal reporter model and then related to human muscle function rather than accepted as an in vivo flux surrogate by assumption. Table 1 distinguishes dynamic measurements from static abundance and identifies the inference limit of representative studies.

3. What changes with age in skeletal muscle?

Age-related studies interrogate different levels of the pathway—from organelle abundance and marker expression to pH, enzyme activity, cargo turnover, and functional rescue. The following sections therefore move from direct functional measurements to human associations and organelle-quality mechanisms, while preserving the distinction between the measured endpoint and the broader interpretation.

3.1. Direct evidence for altered lysosomal degradative capacity

Studies of aging muscle report changes in lysosome number or size, LAMP and cathepsin abundance, autophagosome content, luminal pH, hydrolase activity, and cargo turnover. These variables can change in different directions and are not interchangeable: more

lysosomal structures may indicate compensation or stalled clearance, and higher LC3-II may indicate increased formation or reduced degradation. We therefore organize the evidence by what was actually measured and treat species, muscle group, fiber composition, sex, habitual activity, feeding state, and sampling time as modifiers rather than assuming a uniform age-related lesion. Representative studies are mapped to their main-text sections in Table 1.

Table 1. Claim-level evidence-tier and assay map for lysosomal and autophagy findings in aging skeletal muscle.

Pathway or claim	Model/context	Assay	Evidence tier and type	Principal finding	Inference limit
LAMP–TMEM175 pH control	Human cell systems; non-muscle	Proteomics, cryo-EM, electrophysiology, pH imaging	Tier 4 for aging human muscle; direct non-muscle organelle mechanism	LAMP1/2 inhibited TMEM175 and facilitated acidification (16)	Not tested as an age-dependent lesion in skeletal muscle
Mitophagy with age and contractile activity	Young and aged rat skeletal muscle	Colchicine block with mitophagy-related measurements	Tier 1 for dynamic animal-muscle flux estimate	Flux was higher with age and fell after chronic contraction (26)	Drug block and species limit translation; not a simple decline model
Reduced isolated-lysosome degradation with age	Young versus aged mouse skeletal muscle	Isolation of intact lysosomes; enzyme and degradation assays	Tier 1 for direct organelle function; not whole-pathway flux	Lower degradative activity in aged muscle lysosomes (27)	Responsible step and human functional mediation remain unknown
Muscle-type heterogeneity	Young/old mouse soleus and extensor digitorum longus	Autophagy proteins and lysosomal activity	Tier 2: static markers plus organelle-function readouts	Age pattern differed by oxidative/glycolytic muscle type (28)	Whole-muscle averages; limited sex and human generalizability
V-ATPase disassembly during aging	Replicatively aged yeast	Vacuolar V-ATPase assembly and function	Tier 4 for mammalian muscle; non-mammalian mechanism	Disassembly reduced vacuolar acidification and function (29)	Cannot establish mammalian muscle acidification failure
miR-1 regulation of V-ATPase	Aging <i>Caenorhabditis elegans</i> muscle	Genetic manipulation and proteotoxicity/function outcomes	Tier 4 for human muscle; causal invertebrate evidence	miR-1 coordinated V-ATPase and lysosomal biogenesis (30)	Mammalian and human muscle relevance untested
TSC1mKO/4E-BP1 rescue	Ckmm-Cre TSC1mKO and 4EBP1mt-TSC1mKO male mice	Genetic rescue, protein synthesis, flux, protease activity, mass and force	Tier 1 for causal rescue in a defined mouse model	4E-BP1 activation restored flux/protease activity and preserved mass/strength (31)	Chronic genetic mTORC1 activation is not natural aging or human therapy
Human autophagy-related expression	575 older SOMMA participants	Muscle RNA sequencing linked to respiration and performance	Tier 3: human association; static transcripts	Multidirectional modules associated with phenotypes (32)	No pH, cargo degradation, or completed flux
TP53INP2 as an autophagy regulator	Mouse muscle perturbation plus human association	Overexpression/rescue, markers, strength association	Tier 1 mouse mechanism; Tier 3 human association	Mouse benefit; higher human TP53INP2 associated with strength (33)	Human efficacy and mediation untested
Age-related CMA change	Muscle-specific <i>Lamp2a</i> mouse models and human biopsies	Loss/rescue, proteomics, calcium handling, force; human markers	Tier 1 mouse causality; Tier 3 human association	CMA loss caused myopathy; rescue partly improved old mice (22)	No causal or completed-flux test in human sarcopenia

Necessity of basal macroautophagy	<i>Atg7</i> fl/fl; <i>Mlc1</i> f-Cre skeletal-myofiber knockout mice	Genetic deletion, morphology, mass, and force	Tier 1 for pathway necessity in the knockout model	<i>Atg7</i> loss caused organelle damage, atrophy, and weakness (34)	Severe ablation does not prove benefit of modest enhancement in humans
BNIP3 mitophagy–lysosome–inflammation circuit	C2C12 knockdown; adenoviral repression in mouse gastrocnemius; human association	Bafilomycin-sensitive flux, lysosomal activity, TLR9/NLRP3, atrophy	Tier 1 experimental circuit; Tier 3 human association	BNIP3 loss reduced flux and activated inflammatory signaling; old-mouse atrophy worsened (37)	Human findings are associative; circuit is not universal

Abbreviations: CMA, chaperone-mediated autophagy; SOMMA, Study of Muscle, Mobility and Aging.

A recent method for isolating intact lysosomes from small amounts of mouse skeletal muscle preserved membrane integrity and enabled direct enzymatic testing. The investigators reported lower degradative activity in lysosomes isolated from aging muscle [27]. This is Tier 1 evidence for an age-associated reduction in isolated-lysosome degradative activity in mouse muscle. It does not identify whether pH, hydrolase maturation, membrane transport, or another step is responsible, and it does not establish the temporal relation to strength loss or the presence of the same defect in human muscle (Table 1, Section 3.1).

Muscle type also matters. In young and old mouse soleus and extensor digitorum longus, age-related changes in autophagy-related proteins and lysosomal activity differed between predominantly oxidative and glycolytic muscles [28]. Such heterogeneity may help explain apparently conflicting reports of unchanged, increased, or decreased LC3-II, SQSTM1/p62, lysosomal proteins, and mitophagy markers. Sex, fasting status, activity, time of sampling, fiber composition, and the use of whole-muscle homogenates further complicate comparison.

Several mechanistic observations often used to support acidification failure originate outside mammalian muscle. Age-related V-ATPase disassembly was demonstrated at the yeast vacuole, and miR-1 regulation of V-ATPase and lysosomal biogenesis was studied in *Caenorhabditis elegans* [29, 30]. These models can reveal conserved principles, but they should be labeled as non-mammalian evidence. Similarly, the LAMP–TMEM175 interaction was resolved in human cell systems, not in aging muscle [16]. Together, these data justify testing V-ATPase, TMEM175, and LAMP regulation in muscle; they do not justify stating that this axis is already demonstrated causal lesion in human sarcopenia.

A model-specific rescue experiment provides a complementary line of evidence. In myofiber-specific *Tsc1*-knockout mice generated with *Ckmm*-Cre (TSC1mKO), chronic mTORC1 activation was combined with muscle expression of an mTORC1-resistant 4E-BP1

mutant transgene (4EBP1mt-TSC1mKO). In 12-month-old male mice, 4E-BP1 activation preserved muscle mass and strength, reduced aberrant protein synthesis, restored autophagic flux, and increased lysosomal protease activity despite persistent upstream ULK1 inhibition [31]. This is Tier 1 evidence for rescue within that genetic model; it is not evidence that 4E-BP1 activation corrects natural aging or human sarcopenia, and its effects need not be lysosome-specific (Table 1, Sections 3.1 and 4.1).

3.2. Human evidence: associations, not yet direct flux measurements

The Study of Muscle, Mobility and Aging (SOMMA) analyzed expression of 281 autophagy-, mitophagy-, and mTOR-related genes in muscle biopsies from 575 older adults. Distinct expression patterns were associated with mitochondrial respiration, peak oxygen uptake, walking speed, leg power, and thigh muscle volume [32]. The sample size and phenotypic depth are strengths, but the cross-sectional transcriptomic design does not measure lysosomal pH, hydrolase activity, cargo degradation, or autophagic flux. The direction of association also varied across regulatory modules, arguing against a single “low-autophagy” signature.

TP53INP2 provides another instructive example. Muscle-specific overexpression in mice prevented or improved age-related muscle phenotypes and enhanced autophagy or mitophagy-related outcomes. In human muscle, higher TP53INP2 abundance was associated with greater strength and healthy aging [33]. The mouse perturbation supports causality within that model, whereas the human result supports association. The combined evidence nominates TP53INP2 for mechanistic and pharmacodynamic study but does not establish clinical efficacy.

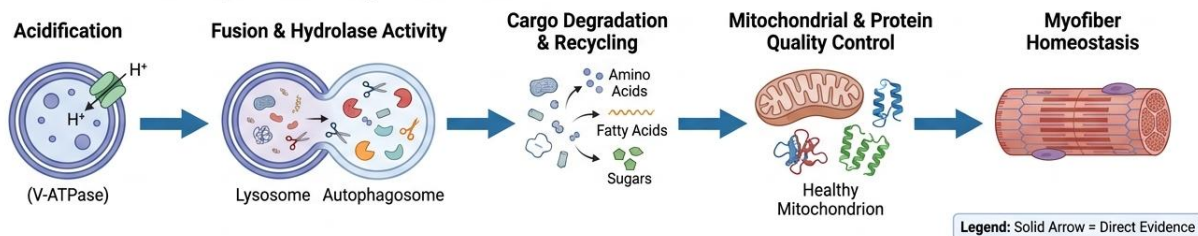
CMA evidence also differs sharply by species and endpoint. Muscle-specific *Lamp2a*-deficient mice developed progressive myopathy, altered mitochondrial proteostasis, impaired SERCA turnover and calcium handling, and reduced force; genetic enhancement of

CMA partially improved phenotypes in old mice (22). These perturbation and rescue results are Tier 1 for mouse-muscle causality. Human biopsies showed age-associated reductions in CMA-related measures, but did not include a causal perturbation or a validated completed-flux assay and are therefore Tier 3 for human translation. The magnitude, timing, and functional mediation of CMA decline in clinically defined human sarcopenia remain unresolved (Table 1, Section 3.2).

Overall, mouse evidence includes direct organelle assays, genetic perturbation, and rescue, whereas human

evidence remains predominantly observational. Direct measurements of lysosomal pH, cargo degradation, and completed autophagic flux in aging human muscle are sparse; most human studies provide Tier 3 evidence from transcripts, protein abundance, morphology, or associations with performance. The literature therefore supports age-associated changes in autophagy–lysosome regulation and defines consequences of severe or targeted disruption in experimental muscle, while leaving a generalized terminal-degradation defect in clinically defined human sarcopenia as a testable hypothesis.

A. Resilient Reference Sequence in Young Skeletal Muscle



B. Domains & Contextual Modifiers in Aging Skeletal Muscle

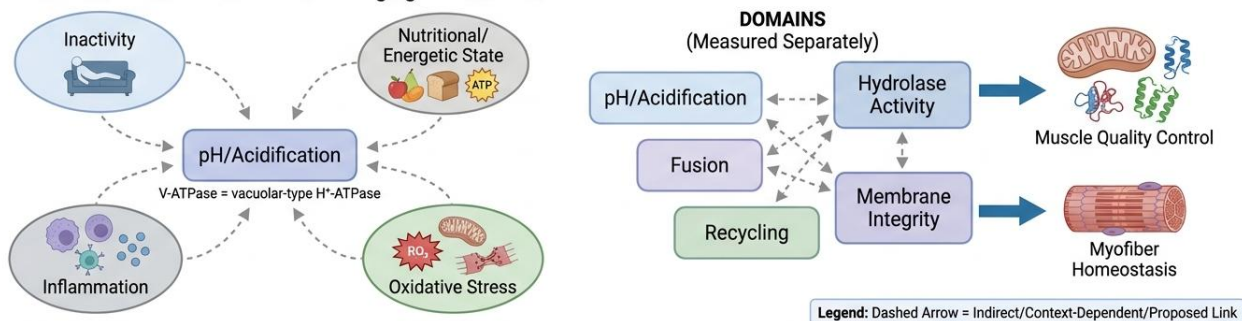


Figure 1. Evidence-calibrated model of lysosomal competence in young and aging skeletal muscle. Panel A shows a resilient reference sequence linking acidification, fusion and hydrolase activity, cargo degradation and recycling, mitochondrial and protein quality control, and myofiber homeostasis. Panel B groups the domains that must be measured separately in aging muscle—pH/acidification, fusion, hydrolase activity, membrane integrity, and recycling—and places inactivity, nutritional or energetic state, inflammation, and oxidative stress as context modifiers. Solid arrows indicate muscle-specific causal or dynamic evidence; dashed arrows indicate indirect, proposed, or context-dependent links. The figure does not assume a universal lesion, and direct dynamic evidence in aging human skeletal muscle remains sparse. Abbreviations: V-ATPase, vacuolar-type H⁺-ATPase.

3.3. Biogenesis, recycling, and organelle quality

TFEB/TFE3-dependent biogenesis can compensate for increased degradative demand, but more lysosomes do not necessarily mean more degradation. Persistent stress may produce enlarged, substrate-loaded, or damaged organelles; conversely, increased lysosome number can reflect successful adaptation. Static measures of TFEB localization, LAMP proteins, cathepsins, or lysosomal area should therefore be interpreted alongside pH, enzyme activity, membrane integrity, cargo turnover, and recycling.

Autophagic lysosome reformation restores lysosomes after prolonged autophagy, while lysophagy removes irreparably damaged organelles. Both processes are plausible bottlenecks in long-lived myofibers exposed to repeated contraction and oxidative stress. Yet age-, sex-, and muscle-specific data are insufficient to define whether biogenesis, repair, removal, or reformation is the dominant lesion. The field would benefit from experiments that quantify competent lysosomes rather than total lysosomal structures and that pair organelle measurements with force, fatigue, or mobility outcomes.

The evidence-calibrated model is summarized in Figure 1 using larger, simplified labels. The reference

panel separates acidification, fusion and hydrolase activity, cargo degradation, recycling, and downstream quality control. The aging panel lists measured domains rather than a generic ‘lysosomal dysfunction’ label and shows context modifiers as dashed, non-universal links.

4. Consequences for muscle quality control

This section separates direct myofiber evidence from downstream or contextual domains. Demonstrating pathway necessity after severe genetic disruption does not establish that a modest age-associated change is the dominant cause of sarcopenia or that pathway enhancement will be beneficial.

4.1. Myofiber proteostasis and contractile function

The relevant model was an *Atg7*^{fl/fl}; *Mlc1f*-Cre conditional knockout, in which *Atg7* was deleted in differentiated skeletal myofibers [34]. These mice accumulated abnormal mitochondria, showed sarcoplasmic-reticulum distension and disorganized sarcomeres, and developed weakness and atrophy. The model establishes that basal macroautophagy is necessary for muscle integrity. However, severe, constitutive pathway ablation is not equivalent to the smaller and heterogeneous changes of natural aging and does not prove that modest autophagy enhancement in naturally aging humans will improve clinically defined sarcopenia (Table 1, Section 4.1).

Protein synthesis and degradation are coupled. Chronic mTORC1 activation may simultaneously drive translation and restrict autophagy, producing proteotoxic load that exceeds lysosomal capacity. In the *Ckmm*-Cre *TSC1*^{mKO} background described in Section 3.1, the *4EBP1*^{mt} transgene reduced aberrant translation, restored autophagic flux and lysosomal protease activity, and rescued muscle mass and force [31]. The result demonstrates model-specific rescue rather than a generic *4E-BP1* effect. In natural aging, anabolic resistance, inactivity, inflammation, denervation, and nutrition also influence net protein balance, so a model focused only on accelerated degradation is incomplete.

Static accumulation of ubiquitinated proteins, LC3-II, SQSTM1/p62, or lipofuscin is compatible with impaired clearance but can also reflect greater substrate production, altered synthesis, cell-type composition, or compensatory pathway activation. Mechanistic inference is strongest when marker changes are linked to a block-and-release design, cargo turnover, direct lysosomal assays, or rescue of a functional phenotype.

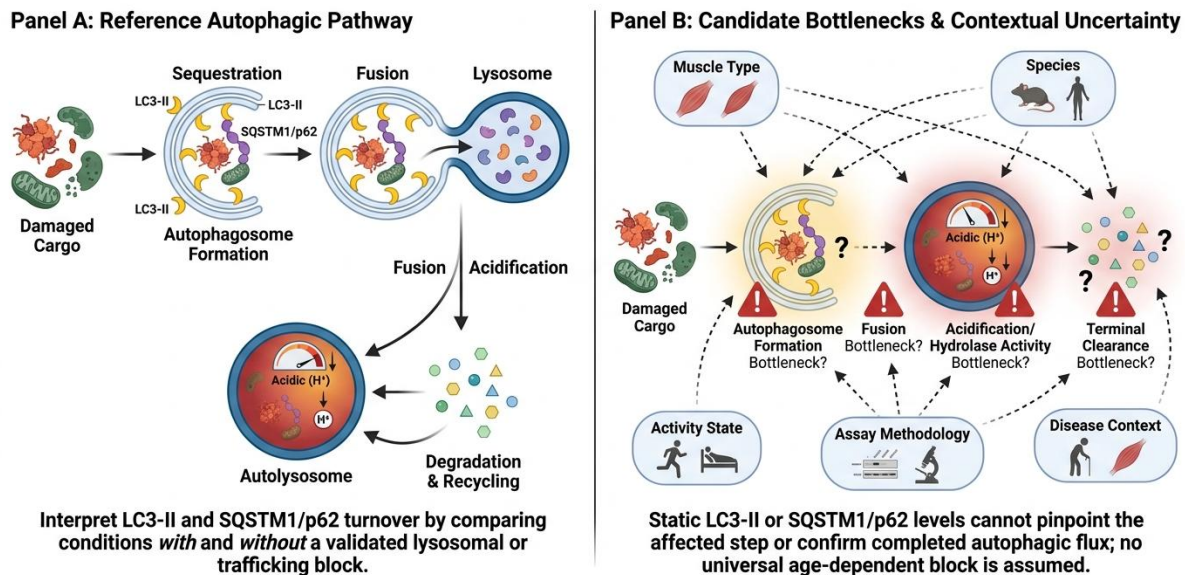
Figure 2 links each visual element to the interpretive claim. Panel A shows completed cargo processing and identifies LC3-II and SQSTM1/p62 turnover as quantities that require comparison with and without a validated block. Panel B maps candidate bottlenecks at autophagosome formation, fusion, acidification or hydrolase activity, and terminal clearance. Question marks and dashed arrows denote uncertainty, not a demonstrated universal block, and static LC3-II or SQSTM1/p62 abundance cannot identify the affected step.

4.2. Mitochondria–lysosome crosstalk

Mitophagy and inflammation are related in specific experimental contexts but are not synonymous or uniformly coupled. A mechanistic link requires evidence that defective mitochondrial clearance retains damaged organelles or mitochondrial damage-associated molecular patterns, that these signals engage an innate-immune sensor, and that the inflammatory response contributes to muscle injury. The BNIP3 study below supports one such sequence in experimental muscle; it should not be generalized to every form of aging-related mitophagy change.

Mitochondria are central to muscle energetics, calcium handling, and redox balance. Aging alters mitochondrial content, morphology, respiratory function, and network dynamics, but the direction and magnitude vary across muscles and activity levels [35]. Mitophagy must therefore be interpreted as one component of a broader quality-control system that includes fission, fusion, biogenesis, proteases, and intercellular transfer.

Loss of mitofusin 2 in aging mouse muscle impaired autophagy and mitochondrial quality while inducing a compensatory HIF1 α –BNIP3 pathway [36], cautioning against interpreting BNIP3 elevation as uniformly pathogenic or as proof of failed mitophagy. In C2C12 myotubes and adenovirus-mediated BNIP3 repression in mouse gastrocnemius, BNIP3 deficiency reduced bafilomycin-sensitive mitophagic flux, altered lysosomal activity, increased mitochondrial stress, and activated TLR9- and NLRP3-linked inflammatory signaling; repression in old mouse muscle worsened inflammation and atrophy [37]. Human muscle findings were associations between BNIP3 abundance and inflammatory or denervation markers, not causal tests. Thus, the study provides Tier 1 evidence for a defined experimental circuit and Tier 3 evidence for human association, not a universal mitochondria–inflammation sequence (Table 1, Section 4.2).



Abbreviations: LC3: microtubule-associated protein 1 light chain 3; SQSTM1/p62: sequestosome 1

Figure 2. Evidence-calibrated framework for interpreting autophagic flux in aging skeletal muscle. Panel A shows a reference pathway in which damaged cargo is sequestered, the autophagosome fuses with a lysosome, cargo is degraded in an acidic autolysosome, and metabolites are recycled. The annotation beneath Panel A specifies that LC3-II and SQSTM1/p62 turnover must be compared with and without a validated lysosomal or trafficking block. Panel B shows candidate bottlenecks at autophagosome formation, fusion, acidification or hydrolase activity, and terminal clearance. Question marks and dashed arrows denote uncertainty and heterogeneity across muscle type, species, activity state, assay, and disease context. Static LC3-II or SQSTM1/p62 abundance alone cannot identify the affected step or establish completed flux; no universal age-dependent fusion or degradation block is assumed. Abbreviations: LC3, microtubule-associated protein 1 light chain 3; SQSTM1/p62, sequestosome 1.

Lysosomal TRPML1 can sense mitochondrial reactive oxygen species and release calcium to promote autophagic adaptation in cell systems [38]. Such signaling offers a plausible mechanism by which transient stress coordinates organelle quality control. Chronic activation, failure of repair, or insufficient degradation could instead amplify damage. Testing these alternatives in intact muscle will require temporally resolved assays rather than endpoint marker panels.

4.3. Crosstalk with the ubiquitin–proteasome system

The ubiquitin–proteasome system (UPS) preferentially removes many short-lived, soluble, or misfolded proteins through proteasomal catalytic activity, whereas the autophagy–lysosome system is better suited to aggregates, membrane structures, and organelles. The systems are nevertheless coupled: SQSTM1/p62 and NBR1 can route ubiquitinated structures toward autophagy, proteasome stress can induce autophagy, and impaired autophagy can increase proteasomal demand [39, 40]. Consequently, ubiquitinated-protein accumulation, atroline expression, or SQSTM1/p62 abundance cannot identify the failed pathway. Attribution

requires parallel measurements such as proteasome catalytic activity, dynamic cargo turnover or autophagic flux, substrate synthesis, and pathway-specific perturbation or rescue.

Therapeutically, simultaneous inhibition or indiscriminate activation of degradative pathways could be harmful. Preserving muscle requires removal of damaged structures while avoiding excessive loss of functional proteins and organelles. Studies should therefore report pathway-selective pharmacodynamic effects, net protein balance, muscle mass, strength, and safety rather than relying on a single autophagy marker.

Satellite-cell and neuromuscular observations are considered next as downstream or contextual domains. They are not used as primary evidence that a myofiber lysosomal lesion causes human sarcopenia.

4.4. Contextual domain: satellite cells and regenerative capacity

Satellite cells are essential for adult muscle regeneration after injury [41, 42]. Basal autophagy helps maintain satellite-cell stemness by limiting proteotoxic and mitochondrial stress. In aged cells, reduced autophagy has

been associated with senescence and loss of regenerative capacity; restoring autophagy or manipulating *Atg7* in experimental models altered activation and regeneration [43, 44]. These studies support a role for autophagy in stem-cell maintenance, but most evidence comes from mice or isolated cells and injury paradigms rather than community-dwelling older adults with sarcopenia.

The contribution of satellite-cell depletion to non-injury-related myofiber atrophy also remains debated and may differ between maintenance, hypertrophy, and regeneration [45]. The most defensible conclusion is that autophagy supports regenerative competence and that age-related dysregulation could compound recovery from disuse or injury. It is premature to attribute progressive sarcopenia primarily to satellite-cell autophagy failure.

4.5. Contextual domain: neuromuscular and systemic influences

Denervation and motor-unit remodeling are major determinants of muscle weakness with age. Autophagy defects in motor neurons can contribute to neuronal injury, and age-related changes in cervical motor-neuron autophagy have been reported [46, 47]. These are neuronal findings and should not be presented as evidence that a myofiber acidification or cargo-clearance defect directly destabilizes the neuromuscular junction. Muscle, nerve terminal, Schwann cell, and activity-dependent mechanisms need to be separated in experimental design.

Inflammaging, adiposity, insulin resistance, medications, and chronic disease can alter nutrient sensing and degradative pathways in muscle. Conversely, damaged mitochondria and incompletely cleared macromolecules may amplify innate immune signaling. The BNIP3 study provides muscle-specific experimental support for one mitochondria–lysosome–inflammation circuit [37]. In humans, however, circulating cytokines and muscle markers are usually measured concurrently, limiting directionality. The autophagy–lysosome system should therefore be placed within a multilevel disease network rather than designated a universal upstream driver.

5. Human measurement: what can and cannot be concluded

Human biopsy studies can measure LC3 isoforms, SQSTM1/p62, LAMP proteins, cathepsins, TFEB/TFE3 localization, transcripts, ultrastructure, enzyme activity, and mitochondrial features. Each readout answers a different question. LC3-II abundance reflects the balance

between formation and clearance; SQSTM1/p62 is influenced by both degradation and transcription; cathepsin abundance must be paired with maturation and luminal-activity assays; and LAMP staining reports organelle abundance rather than competence. Assay timing relative to meals, exercise, circadian phase, and medication exposure can materially change interpretation [12].

Preanalytical and biological heterogeneity can be as important as the assay itself. Fresh-tissue viability and lysosomal pH can change during ischemia, transport, dissociation, freezing, or ex vivo incubation; neighboring biopsy cores may differ in fiber-type and non-myofiber-cell composition; and repeated sampling can introduce local injury or spatial mismatch. Muscle group, habitual activity, sex, recent exercise, feeding or fasting, circadian time, medications, and acute illness should therefore be standardized or recorded. Reproducibility should be tested within and between laboratories, and ex vivo or isolated-lysosome results should be validated against an in vivo or physiologically anchored reference rather than treated as direct equivalents.

Acute human exercise studies show changes in ULK1 phosphorylation, LC3 lipidation, SQSTM1/p62, and related transcripts. Some reports describe intensity-dependent responses or increased capacity after training [48, 49, 50]. These observations indicate pathway responsiveness but generally do not measure completed cargo degradation with a validated in vivo block. Statements should therefore use “autophagy-related signaling” or “marker abundance” unless a dynamic design supports flux.

Muscle MRI quantifies volume, fat infiltration, edema, and other compositional features; ³¹P magnetic resonance spectroscopy assesses aspects of muscle energetics; and PET reports tracer-specific biology. None is, by default, a direct measure of autophagic flux. Circulating autophagy-related proteins, microRNAs, extracellular vesicle cargo, and myokines are similarly non-specific and may originate from multiple tissues. Multi-omics profiles can identify associations and generate classifiers, but they require external validation and linkage to a clearly defined intended use [51].

Accordingly, a candidate biomarker should be described by sample, analyte, analytical validity, tissue and pathway specificity, relation to a muscle-relevant dynamic assay, and relation to clinical outcomes. Relabeled Table 2 presents this readiness framework. A composite ‘lysosomal/autophagy health index’ remains a research concept, not a validated clinical tool.

These validation domains are distinct. Analytical validity concerns measurement accuracy, precision,

range, and reproducibility; biological validity concerns whether the analyte reflects the proposed process; pharmacodynamic utility concerns whether it changes with target engagement; prognostic value concerns prediction of future clinical outcomes; and surrogate-

endpoint qualification requires evidence that treatment-induced biomarker change reliably predicts treatment effect on a clinical outcome. No candidate discussed here is qualified as a surrogate endpoint for sarcopenia, and evidence for one domain does not establish another.

Table 2. Validation and intended-use framework for candidate biomarkers of muscle autophagy–lysosome function.

Readout	Sample	Measured domain	Potential intended use	Validation status	Principal limitation
LC3-II, SQSTM1/p62, LAMP proteins, cathepsin abundance	Muscle biopsy	Static marker abundance	Exploratory pharmacodynamic or mechanistic context	Analytical feasibility is common; biological validity is assay- and context-specific	Cannot distinguish formation from clearance or abundance from activity without a dynamic comparator
Lysosomal pH, hydrolase activity, membrane integrity, cargo degradation	Fresh biopsy, isolated lysosomes, or validated ex vivo tissue	Direct organelle function	Mechanistic lesion and target engagement	Promising mouse-muscle method (27); human analytical and biological validity incomplete	Viability, heterogeneity, reproducibility, and relation to in vivo biology and outcomes require validation
Autophagy/mitophagy transcripts and omics signatures	Biopsy or blood	Regulatory association; not completed flux	Discovery, prognostic hypothesis, or stratification	Large human datasets exist, including SOMMA (32); external biological validation incomplete	Cellular origin, confounding, overfitting, and transportability
Circulating proteins, microRNAs, extracellular-vesicle cargo, myokines	Blood	Indirect, usually non-muscle-specific association	Minimally invasive exploratory monitoring	Candidate stage; no qualified diagnostic, prognostic, or treatment-selection use	Multiple tissue sources, preanalytics, and no established link to muscle dynamic flux
MRI and 31P-MRS	In vivo imaging/spectroscopy	Muscle composition and energetics	Quantity/quality phenotype or energetic pharmacodynamic measure	Analytically established for selected imaging outcomes; not lysosomal biological validation	Not a direct autophagic-flux measure; protocol and endpoint specificity
PET	In vivo tracer imaging	Tracer-specific biology	Research pharmacology or metabolism	Depends on tracer, analytical validation, and biological anchoring	No generally validated PET measure of skeletal-muscle autophagic flux
Strength, power, gait speed, chair stand, 6-min walk	Clinical performance	Clinical outcome; not pathway activity	Efficacy and prognosis	Consensus-supported clinical outcomes (1, 2)	Influenced by neural, cardiopulmonary, motivational, and musculoskeletal factors

Validation domains: analytical validity = accuracy, precision, range, and reproducibility; biological validity = relation to the proposed process; pharmacodynamic utility = response to target engagement; prognostic value = prediction of future outcome; surrogate qualification = evidence that treatment-induced biomarker change predicts clinical benefit. No listed candidate is qualified as a surrogate endpoint. Diagnostic, prognostic, pharmacodynamic, safety, and treatment-selection uses require different evidence.

6. Interventions and clinical translation

The interventions below are compared to show differences in mechanistic specificity and clinical maturity, not to imply therapeutic equivalence. Exercise is a multimodal standard intervention with established functional benefit; metformin and sirolimus are systemic agents tested in specific older populations; urolithin A has a mitochondrial and mitophagy-related rationale; and

spermidine or TFEB/TFE3-directed compounds remain preclinical or early translational for sarcopenia. Most were not designed to correct a prospectively validated lysosomal lesion in clinically defined sarcopenia.

6.1. Exercise

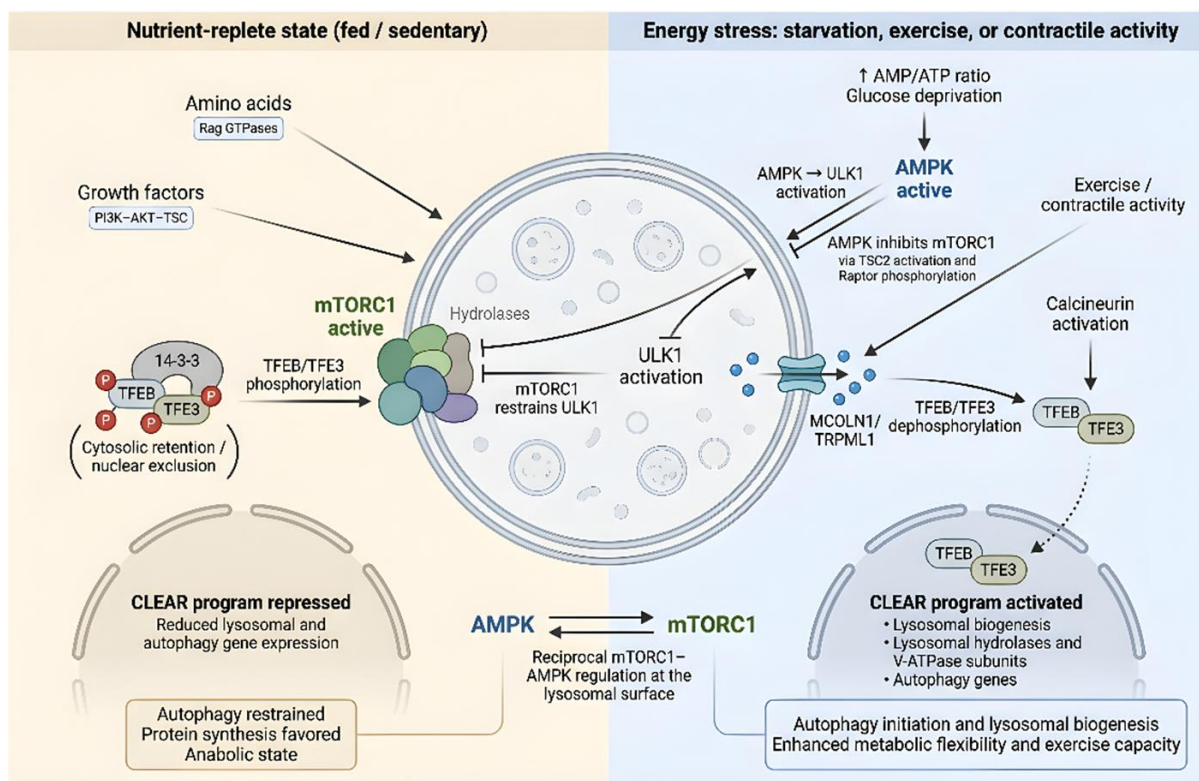
Resistance and multicomponent exercise improve clinically relevant outcomes in older adults with

sarcopenia. A network meta-analysis of 42 randomized trials found that resistance-based programs, alone or combined with nutritional or balance/aerobic components, were among the most effective approaches for strength, physical function, and quality-of-life outcomes [52]. These benefits justify exercise as standard management; evidence for autophagic-flux mediation is considered separately from the established clinical effect.

In experimental muscle, contractile activity can activate AMPK–ULK1 and TFEB/TFE3-linked programs, alter lysosomal capacity, and modify mitophagy. Human biopsy studies demonstrate acute and

training-associated changes in autophagy-related signaling and markers [20, 49, 50]. Marker changes vary with intensity, nutritional state, sampling time, and training status. Exercise can therefore be said to improve muscle outcomes and engage autophagy-related pathways, whereas mediation requires repeated, muscle-relevant dynamic assays.

Figure 3 summarizes the lysosome-centered mTORC1–AMPK–calcineurin–TFEB/TFE3 signaling framework and marks the unresolved links between signaling, completed autophagic flux, and clinical exercise benefits.



Schematic representation; upstream nutrient- and exercise-responsive signaling pathways are simplified.

Figure 3. Lysosomal nutrient and exercise sensing. The schematic contrasts nutrient-rich lysosomal mTORC1 signaling with energetic-stress or contractile-activity signaling through AMPK, MCOLN1/TRPML1-dependent Ca^{2+} release, calcineurin, and TFEB/TFE3. These signals may promote TFEB/TFE3 nuclear translocation and CLEAR-network transcription, with context-dependent contributions; the schematic maps signaling rather than completed flux or clinical mediation. Abbreviations: AKT, protein kinase B; AMPK, AMP-activated protein kinase; CLEAR, Coordinated Lysosomal Expression and Regulation; MCOLN1/TRPML1, mucolipin 1; mTORC1, mammalian target of rapamycin complex 1; PI3K, phosphoinositide 3-kinase; TFEB/TFE3, transcription factors EB/E3; TSC2, tuberous sclerosis complex 2; ULK1, unc-51-like autophagy activating kinase 1.

6.2. Nutrition and energy restriction

Adequate energy and protein intake support muscle protein synthesis and adaptation to resistance exercise. In frail or malnourished older adults, caloric restriction or fasting may worsen energy deficit and lean-mass loss.

These approaches should not be presented as general sarcopenia therapies. Any study of fasting, time-restricted eating, or caloric restriction should specify nutritional status, protein adequacy, supervision, weight-loss intent, and the population in which safety was established.

Spermidine, resveratrol, and other dietary compounds can influence autophagy-related pathways in

experimental systems, but many reports concern lifespan, non-muscle tissues, supraphysiological exposures, or surrogate outcomes [53, 54]. These agents should be

6.3. Pharmacological interventions: trial-level appraisal

Preclinical manipulation of mTORC1, AMPK, TFEB/TFE3, mitophagy, or lysosomal capacity can

described as preclinical or hypothesis-generating for sarcopenia until adequately powered trials demonstrate benefits in prespecified muscle and functional outcomes. improve selected aging phenotypes. For example, partial mTORC1 inhibition preserved muscle mass in aged rats, and muscle-specific TP53INP2 expression improved sarcopenia-related phenotypes in mice [33, 55]. These data provide target rationale but do not define an effective or safe human regimen.

Table 3. Human intervention trials relevant to autophagy, mitophagy, or mTOR modulation.

Study/population	Regimen	Duration	Prespecified primary endpoint	Main result	Safety	Autophagy/mitophagy readout	Funding/COI note
MET-PREVENT; probable sarcopenia with pre-frailty/frailty; n=72	Metformin 500 mg three times daily	4 months	4-m walk speed at 4 months	Adjusted effect 0.001 m/s (95% CI, -0.06 to 0.06); P = 0.96 (56)	110 vs 77 adverse events; 12 vs 3 hospital admissions; poor tolerability	None	NIHR-funded; one author reported consultancy fees and another disclosed funding and committee roles; all other authors declared no competing interests
RAPA-EX-01; sedentary adults 65–85 years; n=40	Sirolimus 6 mg once weekly plus home exercise	13 weeks	Change in 30-s chair-stand repetitions	ITT difference -2.13 (95% CI, -4.61 to 0.34); P = 0.089; sensitivity analyses favored placebo (57)	99 vs 63 total events; one possibly drug-related serious pneumonia	None	Crowdfunded via Lifespan.io/VitaDAO; author industry roles disclosed; Pfizer had no input
Older adults 65–90 years; n=66	Urolithin A 1000 mg daily	4 months	6-min walk distance and hand-muscle maximal ATP production	Neither primary outcome differed significantly from placebo; selected endurance and biomarker signals (58)	No significant between-group adverse-event difference	Human energetic readout (MRS)	Amazentis SA funded and participated in study/manuscript processes; company employment, board/shareholding, and patent interests disclosed
Overweight, low-endurance adults 40–64 years; n=88	Urolithin A 500 or 1000 mg daily	4 months	Peak power output	Primary peak-power outcome not significant; selected strength, VO ₂ , 6-min walk, and biomarker signals (59)	Reported as tolerated in studied population	Static abundance of mitophagy-related proteins	Amazentis SA funded/sponsored; multiple authors disclosed employee, board, or advisory roles

Abbreviations: CI, confidence interval; COI, conflict of interest; ITT, intention-to-treat; MRS, magnetic resonance spectroscopy; NIHR, National Institute for Health and Care Research; VO₂, oxygen consumption. Funding and COI summaries reproduce published disclosures for transparency and do not by themselves establish bias. None of the listed trials included a validated dynamic skeletal-muscle autophagic-flux measurement.

Clinical translation remains uncertain. In MET-PREVENT (n=72), metformin at 500 mg three times daily

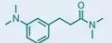
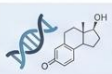
for four months did not improve the prespecified four-month gait-speed outcome in older adults with probable

sarcopenia and pre-frailty or frailty, and tolerability was poorer than with placebo [56]. In RAPA-EX-01 (n=40), once-weekly sirolimus at 6 mg for 13 weeks alongside home exercise did not significantly improve the primary intention-to-treat 30-second chair-stand outcome (between-group difference, -2.13 repetitions; 95% confidence interval, -4.61 to 0.34; $P = 0.089$). Sensitivity analyses favored placebo, and adverse events were more frequent with sirolimus [57]. These findings apply to the tested populations, regimens, durations, and endpoints; they neither prove class-wide failure nor support routine use.

Urolithin A trials have produced selected signals but require endpoint-specific interpretation. In older adults, supplementation improved muscle endurance and selected mitochondrial biomarkers, while the prespecified primary outcomes—change in six-minute walk distance and maximal hand-muscle ATP production—were not significantly different from placebo [58]. In 88

overweight, low-endurance adults aged 40–64 years, a separate trial of 500 or 1000 mg/day reported improvements in selected strength, performance, and mitochondrial-health measures, but the prespecified peak-power primary outcome was not significant [59]. Both trials were funded by the product developer and included investigators with disclosed company relationships. These disclosures are reported as contextual information and do not by themselves establish bias; population, formulation, endpoint hierarchy, multiplicity, duration, and independent replication determine evidentiary weight.

Table 3 therefore reports population, regimen, prespecified primary endpoint, effect estimate, safety, and whether muscle autophagy or mitophagy was directly measured. None of these trials validates an autophagy biomarker for treatment selection, and clinical benefit cannot be inferred solely from a mitochondrial or molecular signal.

	Mechanistic rationale (icon + text)	Human functional evidence (icon + short text)	Evidence-calibrated interpretation (icon + summary text)
Exercise	 Induces autophagy via AMPK activation	 Replicated functional benefit; autophagic flux mediation unproven	 Functional gain; flux mediation lacks direct proof
Metformin	 AMPK/mTORC1 modulation	 MET-PREVENT: benefits in tested regimen/population	 Regimen/population-specific; no class-wide inference
Once-weekly sirolimus	 mTORC1 inhibition	 RAPA-EX-01: evidence in tested regimen/population	 Specific to trial; no class-wide inference
Urolithin A	 Promotes mitophagy	 Selected signals; key outcomes not met	 Sarcopenia confirmation required
Spermidine	 Induces autophagy	 No dedicated human sarcopenia efficacy	 Lacking human efficacy data
TFEB/TFE3-directed compounds	 Transcriptionally targets autophagic/lysosomal genes	 No dedicated human sarcopenia efficacy	 Lacking human efficacy data

"No row includes a validated completed human skeletal-muscle autophagic-flux measurement."
 "Rows compare evidence maturity, not effect size." Most interventions were not designed to correct a validated lysosomal lesion.

AMPK: AMP-activated protein kinase ITT: intention-to-treat mTORC1: mammalian target of rapamycin complex 1 TFEB/TFE3: transcription factors EB/E3

Figure 4. Human evidence for autophagy- or mitophagy-related interventions. The simplified matrix compares mechanistic rationale, human functional evidence, and an evidence-calibrated interpretation for exercise, metformin, once-weekly sirolimus, urolithin A, spermidine, and TFEB/TFE3-directed compounds. Exercise has replicated functional benefit, but mediation by completed autophagic flux remains unproven. MET-PREVENT and RAPA-EX-01 conclusions are restricted to their tested regimens and populations and do not support class-wide inference. Urolithin A trials reported selected signals while not meeting key primary outcomes; sarcopenia-specific confirmation is required. Dedicated human sarcopenia efficacy evidence is lacking for spermidine and TFEB/TFE3-directed compounds. No row includes a validated completed human skeletal-muscle autophagic-flux measurement. Rows compare evidence maturity, not effect size, and most interventions were not designed to correct a validated lysosomal lesion. Abbreviations: AMPK, AMP-activated protein kinase; ITT, intention-to-treat; mTORC1, mammalian target of rapamycin complex 1; TFEB/TFE3, transcription factors EB/E3.

Figure 4 converts the trial appraisal into a simplified evidence-status matrix with larger labels. Each pharmacological statement is restricted to the tested population, dose, schedule, duration, and endpoint, and the matrix explicitly separates clinical outcomes from completed human muscle-flux measurement.

6.4. Safety and trial design

Autophagy is dependent on context: both insufficient degradation and excessive removal of cellular material can be harmful [60, 61]. Systemic agents may affect immune, hepatic, cardiac, neural, and metabolic function, and older adults often have polypharmacy and reduced physiological reserve. A favorable molecular signal is therefore insufficient without functional benefit and careful adverse-event reporting.

Future trials should specify the causal chain being tested: target engagement, change in a muscle-relevant dynamic process, preservation or gain of muscle function, and acceptable safety. Primary outcomes and analysis of populations should be prespecified; effect estimates and confidence intervals should be reported alongside P values. Duration must be long enough to detect clinically meaningful adaptation, while interim stopping rules should address infection, metabolic toxicity, weight loss, and functional deterioration. Sponsor role, author conflicts, and access to the data and analysis code should be transparent.

7. A measurement-led research agenda

The agenda is prioritized sequentially: first validate the lesion in humans; second standardize measurement and resolve biological heterogeneity; third qualify biomarkers for a stated intended use; and only then test lesion-directed interventions through a complete mechanistic and clinical chain.

7.1. Priority 1: validate the lesion before targeting it

The first priority is to determine whether terminal lysosomal degradation is impaired in naturally aging human muscle and in clinically defined sarcopenia. Studies should pair direct or near-direct measurements—lysosomal pH, hydrolase activity, membrane integrity, cargo degradation, organelle isolation, or validated *ex vivo* flux—with muscle strength, power, physical performance, and longitudinal change. Sampling should document sex, age, race or ethnicity where relevant, fiber composition, muscle group, nutritional state, recent

exercise, circadian time, comorbidity, and medication exposure.

Repeated biopsy designs could test whether a candidate defect predicts decline, responds to intervention, and mediates functional benefit. Cross-sectional contrasts between young and old groups are useful for discovery but cannot determine temporal sequence. Where a systemic flux block is not acceptable, investigators should develop and validate *ex vivo* or tracer-based methods against established animal reporters and include positive and negative controls.

7.2. Priority 2: standardize measurement and resolve heterogeneity

Whole-muscle homogenates combine myofibers, satellite cells, fibro-adipogenic progenitors, endothelial cells, immune cells, and neural elements. Single-nucleus, spatial, proteomic, metabolomic, and organelle-resolved approaches can identify cellular sources, but computational signatures must be anchored to functional assays. The SOMMA data demonstrate the value of deep human phenotyping while also showing that autophagy-related expression is multidirectional rather than a unitary score [32].

Fiber-type composition, muscle group, habitual loading, sex, hormonal context, age range, and disease context may modify lysosomal biology. Findings from predominantly glycolytic mouse muscles should not be generalized automatically to oxidative human postural muscles, and a biopsy from vastus lateralis should not be assumed to represent every locomotor or respiratory muscle. Studies should report biological sex, muscle and sampling site, fiber composition or an appropriate proxy, habitual activity, recent exercise, feeding state, comorbid disease, and medication exposure. Prespecified interaction analyses should be adequately powered and interpreted with multiplicity control.

7.3. Priority 3: qualify biomarkers for a defined intended use

Candidate biomarkers should progress through an explicit ladder: analytical validity and interlaboratory reproducibility; biological validity and tissue or pathway specificity; association with a dynamic muscle assay; pharmacodynamic responsiveness to target engagement; prognostic association with a meaningful clinical outcome; and, only if intended, formal surrogate-endpoint qualification. Until those steps are completed, circulating

proteins, extracellular-vesicle cargo, imaging, and omics signatures should be labeled indirect or exploratory.

The intended use must also be defined before performance is evaluated. Diagnostic biomarkers must discriminate a defined clinical state; prognostic markers must predict future outcomes; pharmacodynamic markers must track target engagement or biological response; safety markers must detect harm; and treatment-selection markers must identify differential benefits or risk. A marker that changes after exercise may show biological responsiveness without predicting who benefits, and a cross-sectional correlate of strength may be neither a flux measure nor a treatment-response marker.

7.4. Priority 4: test interventions with a complete translational chain

Intervention studies should distinguish pathway activation from completed flux and target engagement from clinical efficacy. A rigorous design would include: (i) a prespecified, muscle-relevant molecular or organelle endpoint; (ii) a validated functional endpoint; (iii) adequate power for the primary comparison; (iv) blinded outcome assessment where feasible; (v) effect estimates with confidence intervals; (vi) systematic adverse-event and adherence reporting; and (vii) transparent sponsor and conflict-of-interest disclosures.

Exercise remains the clinical benchmark because it improves patient-important outcomes even though its autophagy-mediated mechanism is unresolved. Novel agents should be compared with, or added to, optimized exercise and nutrition when ethically and practically appropriate. “Personalized autophagy tuning” and a “muscle microenvironment map” are useful hypotheses for future stratified trials, not current clinical tools.

7.5. Limitations of the review process

This review has limitations inherent to a mechanism-focused narrative synthesis. Study selection was not exhaustive or protocol-registered, screening was not performed by two independent reviewers, and a formal study-level risk-of-bias tool was not applied. Publication bias may favor positive mechanistic perturbation or rescue studies, while null human flux or biomarker-validation studies may be underrepresented.

Cross-study comparison is further limited by heterogeneity in species, age, sex, muscle group, fiber composition, activity and feeding state, disease context, assay definitions, perturbations, and sampling schedules. Sparse direct human measurements and inconsistent

laboratory protocols prevent quantitative synthesis of a single age-related lysosomal lesion. The evidence tiers improve transparency of individual claims but do not convert the review into a systematic review or eliminate these limitations.

8. Conclusion

Experimental evidence supports an essential role for basal autophagy and lysosomal competence in skeletal muscle homeostasis. Severe or targeted disruption can impair mitochondrial quality control, proteostasis, regeneration, and contractile function. In naturally aging human muscle, however, the magnitude, timing, and causal importance of defects in acidification, fusion, hydrolase activity, membrane integrity, recycling, cargo degradation, or completed autophagic flux remain uncertain because most studies rely on cross-sectional or static readouts. The available evidence therefore supports altered autophagy-lysosome competence as a plausible contributor to sarcopenia rather than a clinically validated core lesion.

Translation will require greater measurement and evidentiary discipline. Exercise improves muscle strength and physical function, but autophagy-mediated causality is not established. Metformin and once weekly sirolimus did not improve their prespecified functional outcomes in the tested populations and regimens, whereas urolithin A produced selected signals without uniformly meeting key primary endpoints. Future studies should distinguish pathway activation from completed flux, predefine muscle-relevant pharmacodynamic and functional outcomes, report safety and effect estimates, and stratify evidence by species, sex, muscle group, activity, and disease context. Such work will determine whether a specific lysosomal lesion is a modifiable therapeutic axis in clinically defined human sarcopenia or primarily a marker of broader age-related cellular change.

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data, make authorship decisions, or replace authorial scientific judgment.

Author Contributions

OD and JGK contributed equally to conceptualization, literature investigation, data curation, and preparation of the original draft. YHL and HJ contributed to investigation, validation, and critical revision of the manuscript. SSL conceived and supervised the review and contributed to interpretation, funding acquisition, project administration, and critical revision. All authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no competing interests.

Data Availability

No new datasets were generated or analyzed for this narrative review.

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