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Opinion

A perspective on muscle phenotyping in musculoskeletal research

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Musculoskeletal research should synergistically investigate bone and muscle to inform approaches for maintaining mobility and to avoid bone fractures. The relationship between sarcopenia and osteoporosis, integrated in the term 'osteosarcopenia', is underscored by the close association shown between these two conditions in many studies, whereby one entity emerges as a predictor of the other. In a recent workshop of Working Group (WG) 2 of the EU Cooperation in Science and Technology (COST) Action 'Genomics of MusculoSkeletal traits Translational Network' (GEMSTONE) consortium (CA18139), muscle characterization was highlighted as being important, but currently underrecognized in the musculoskeletal field. Here, we summarize the opinions of the Consortium and research questions around translational and clinical musculoskeletal research, discussing muscle phenotyping in human experimental research and in two animal models: zebrafish and mouse.

Highlights

There is a close relationship between muscle loss (sarcopenia) and bone loss (osteoporosis), integrated in the term 'osteosarcopenia'; however, detailed muscle phenotyping is under-represented in musculoskeletal studies.

Muscle phenotyping in both humans and animal models, such as mice and zebrafish, requires validation and agreement.

Parameters of muscle strength and function should be measured in health and disease while considering age and sex differences.

Animal models, such as mice and zebrafish, add valuable information and detail on muscle development and function, but need careful assessment to provide translatable insights.

The global impact of musculoskeletal health

Musculoskeletal disorders affect physical function and metabolic homeostasis, thereby impacting several other organ systems [1]. Their ultimate importance is captured by the 'Global Burden of Disease' study [2], which ranked five musculoskeletal conditions (low back pain, neck pain, falls, osteoarthritis and 'other musculoskeletal' diseases) among the top 11 leading causes of disability.

Osteoporosis (see Glossary) is the systemic deterioration of bone tissue, characterized by low bone mass and microarchitectural impairment, causing an increased fracture risk. Corresponding with low bone mass and impaired bone architecture in osteoporosis, **sarcopenia** has been defined as an age-associated loss of muscle mass, strength, and/or function, and is a major contributor to falls, a key event portending worsening musculoskeletal health and the subsequent injuries [3,4]; sarcopenia is also a predictor of fracture risk in both men and women, and

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quadriceps weakness predicts mortality after a fragility fracture [5,6]. The close correlation between osteoporosis and sarcopenia is reflected in the term '**osteosarcopenia**'. While the age-related deterioration of the musculoskeletal system might not be completely avoidable, identification of at-risk groups, timely diagnoses, and initiation of appropriate prophylaxis and treatment can prevent rapid deterioration [7]. Therefore, musculoskeletal research should investigate bone and muscle characteristics synergistically to generate comprehensive directives for maintaining mobility and avoiding fractures.

The COST Action GEMSTONE (CA18139) is an EU initiative that aims to make knowledge, data, and technologies available to a wider range of researchers, to help generate additional discoveries in musculoskeletal science. Of the six WGs in GEMSTONE, members of WG2 (phenotyping) recently summarized and discussed developments and open questions in skeletal phenotyping [8], giving a comprehensive review of techniques available for clinical and basic research applications in bone tissue. In a recent workshop, detailed muscle characterization was highlighted as an important but often neglected part of phenotyping. The Consortium members presented techniques of muscle phenotyping and engaged in discussions regarding potential knowledge gaps, both topics that may be valuable to skeletal experts.

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Box 1. Methods of muscle phenotyping

Human phenotyping

Physical measurements include muscle volume and function using the surrogate parameters mass, strength and, performance [10].

Muscle mass assessment includes imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), and dual X-ray absorptiometry (DXA). The appendicular lean mass measured by DXA was part of the initial definition of sarcopenia. Bioelectrical impedance analysis based on electric current attenuation and calculations based on anthropometric measurements can approximate muscle mass.

Muscle strength in clinical settings is most commonly assessed by hand grip. Impaired results are better associated with poor mobility and clinical outcomes compared with muscle mass alone [107]. Knee flexion and extension can be assessed with variable techniques with and without the use of equipment. Peak expiratory flow in people without lung disorders can determine the strength of the respiratory muscles, but data on its use as a sarcopenia surrogate are limited.

Physical performance can be measured with a variety of tests. Most commonly used are variations of speed tests for gait, stair climb, and combinations such as the 'Short Physical Performance Battery' (SPPB).

Muscle biopsies are not common in clinical use, but can provide invaluable information, usually done and considered tolerable at the vastus lateralis (largest muscle in the thigh). Muscle biopsy analysis can provide details of morphological characteristics, gene transcription and expression, and metabolism, allowing identification of the mechanisms of skeletal muscle loss/sarcopenia [108–111].

Mouse phenotyping

Besides biological and histological analyses of muscles in the mouse, in analogy to humans, muscle mass can be assessed by imaging using micro-CT, MRI, and DXA. Muscle strength as the minimal force required to oppose the forelimb of a mouse and whole-limb grip can be measured with a grip strength meter. The four-limb hang test is designed to measure endurance as the time until grip strength exhausts. Exercise capacity and endurance can be tested by treadmill exhaustion tests [112,113].

Fish phenotyping

Among the strengths of the zebrafish model for microscopy techniques is the transparency of the larvae. Muscle function via locomotion assay analyzes larval swimming parameters, such as speed, distance traveled, and number of movements. Commonly performed in larvae 5 days post fertilization, tests include assessing evoked responses by metal touch or acoustic stimuli and tests assessing swimming within a time frame [114]. The force generated during muscle contraction can be measured from live larvae subjected to an electrical stimulus, but is technically challenging [115].

In this review, we present narrative expert statements on muscle phenotyping in human experimental muscular research. While many insights into human muscular function and its changes upon aging can be studied from biopsies and by functional assessment of the muscle (Box 1), model organisms are essential to study the function of genes and their products via genetic modifications. As a result, two animal models, zebrafish and mouse, are also discussed.

Aging muscle and sarcopenia in humans

Sarcopenia is a heritable trait influenced by lifestyle factors [9]. In the original definition, appendicular lean mass measured by **dual X-ray absorptiometry (DXA)** was used alongside grip strength and gait speed, and many studies rely on DXA to define sarcopenia. While these measures are predictors of adverse health events, such as falls, mobility limitation, and mortality, there is now more emphasis on muscle strength (dynapenia), as opposed to muscle mass, as the primary criterion for defining sarcopenia. However, general consensus on the definitions and assessment of sarcopenia is yet to be reached [4,10].

Human muscle changes in sarcopenia

Human muscle comprises various fibers (Table 1) and specialized cell types [4], and is structurally and functionally adaptive to neural stimulation, loading, availability of substrates, and hormonal signals, among others [11]. With age, declining blood flow to skeletal muscles limits the diffusion of substrates, oxygen, hormones, and nutrients in the tissue, thereby contributing to sarcopenia and reduced aerobic capacity [12,13].

Comparing older patients who are immobile with those who are mobile can provide valuable insights into physiological versus pathological muscle aging. Sedentary octogenarians display a very high abundance of hybrid fibers [14,15], which are transitory intermediates between different fiber types that are observed during skeletal muscle development, adaptation to exercise, and aging [16]. Muscle deterioration is accompanied by fiber deformation [17], variations in the number of myonuclei [18], and imbalance in protein synthesis by muscle cells [19]. For example, the synthesis of **myosin** heavy chain declines with age [20], as do **myofiber** number and size [21]. Type II fast myofibers responsible for strength are typically more affected compared with type I slow fibers. Gait speed is strongly influenced by muscle strength and, thus, by the age-

Table 1. Main types of human muscle fiber and their characteristics

Characteristic	Type I	Type IIA	Type IIB
	Slow	Fast	Fast
Function	Endurance	Speed	Speed
Endurance	High	Intermediate	Low
Power	Low	High	High
Speed	Low	High	High
Fatigue	Low	Intermediate	High
Myosin-ATPase	Low	High	High
Activity	Aerobic	Aerobic and anaerobic	Aerobic
Capillarization	High	Intermediate	low
Mitochondria	High numbers	Intermediate numbers	Low numbers
Myoglobin	High	High	Low
Color	Dark red	Red	White
Lipid content	High	Low	Low
Creatin phosphate	Low	High	High

Glossary

Altricial: in bird and mammal biology, describes the dependence of newly hatched or born young on adults for food and protection.
Axial musculature: muscles that originate on the axial skeleton (i.e., bones in the head, neck, and core of the body).
Bone mineral density (BMD): amount of mineral in bone tissue, defined as mass per volume.
Dual X-ray absorptiometry (DXA): X-ray based method to measure bone density, considered the gold standard for osteodensiometry. Capable of whole-body scans, DXA can also be used to measure body composition and fat/lean mass.
Fibro-adipogenic precursors (FAPs): of mesenchymal stromal cell with stem cell potential. They are resident between muscle cells and are important for muscle regeneration and homeostasis.
Inbred mouse strains: individuals with at least 20 generations of inbreeding (mating brother × sister or offspring × parent). Individuals of inbred strains can be considered genetically identical for practical purposes.
Myoblast: precursor cells with single nuclei that fuse to form muscle fibers.
Myofibers: fibrous muscle cells.
Myosin: group of motor proteins abundant in the sarcomere region of muscle. They ATP-dependently mediate actin-based motility.
Myostatin: member of the TGF-β superfamily and an important regulator of muscle mass and function. It acts on muscle cells and inhibits muscle growth. Deletion of the gene encoding myostatin, *MSTN*, results in extremely muscular animals.
Osteoporosis: systemic deterioration of bone tissue, characterized by low bone mass and microarchitectural impairment, causing an increased fracture risk.
Osteosarcopenia: co-occurrence of osteoporosis and sarcopenia in a patient.
Phenotype: set of observable characteristics or traits of an organism. The phenotype is defined as the result of the genetic code (genotype) of an organism and the influence of environmental factors.
Sarcomere: basic contractile unit of a myocyte, comprising actin and myosin filaments.

associated reduction of these type II fibers [10,22]. The decline of myofibers with age might be caused partially by mitochondrial dysfunction driven by decreased neuronal input [23]. Mitochondrial density is known to vary with physical training and activity. This, along with the decrease in mitochondrial protein synthesis, may explain increased fatigue and reduced endurance [24–26]. Indeed, decreased mitochondrial bioenergetic capacity is observed in the transition from physiological to pathological muscle aging [27].

Sarcopenia: degenerative loss of muscle mass, quality, and strength.
Satellite cells: small multipotent cells found in mature muscle that reside between the basement membrane of the muscle and the muscle fibers, where they are important for tissue repair.

Satellite cells beneath the basal lamina of skeletal muscle fibers are important for myofiber maintenance, and satellite cell numbers decline during aging [28]. However, this loss does not appear to be causative for sarcopenia in the uninjured muscle, since mice lacking satellite cells show severe defects in muscle regeneration after injury, but are little affected during normal maintenance [29,30]. Multipotent human satellite cells were suspected to be driven toward the adipogenic lineage by adverse stimuli *in vitro* [31], but this was later refuted since accurate labeling showed them committed to a myogenic lineage [32]. By contrast, **fibro-adipogenic precursors (FAPs)**, a type of mesenchymal stromal cell, are necessary for both muscle regeneration and maintaining muscle homeostasis [33,34]. They respond to proinflammatory signaling and can become senescent cells, which impair muscle regeneration and support aberrant fibrogenic and adipogenic commitment and differentiation [35–37]. Adipogenic differentiation of FAPs can be delayed by exercise, whereas muscle regeneration can be rescued by senolytic depletion of senescent FAP populations [38]. 'Fatty infiltration' is present in both bone and muscle in osteosarcopenia and is being explored as an indicator and propagator of disease development. Both intermuscular adipose tissue accumulation (i.e., the accumulation of adipocytes between the muscle fibers) and intracellular lipid infiltration within the muscle cells are increased in aging and sarcopenia [39]. Fatty and fibrotic infiltration can be measured by distinct computed tomography (CT) and magnetic resonance imaging (MRI) approaches, and there is intense ongoing research to determine the extent to which these quantitative measures may serve as surrogates for impaired muscle function [40]. In addition, resident macrophages are also important modulators of muscle regeneration currently under investigation [41,42].

Muscle–bone crosstalk

It was long assumed that the interaction between muscle and bone is mainly mechanical, but evidence for a secretory crosstalk between the two organs is increasing, as comprehensively reviewed elsewhere [43,44]. Examples of proteins involved in such crosstalk include **myostatin**, a transforming growth factor beta (TGF-β) family member, which negatively regulates **myoblast** proliferation, thus affecting muscle mass and strength (although with conflicting results on its age-related expression) [45–47]. Its downregulation increases muscle mass and osteogenic differentiation of mesenchymal stem cells (MSCs), but its signaling can also lead to inflammation, insulin resistance, and cancer cachexia, among others [48]. Interleukin (IL) 6 and 7 promote osteoclastogenesis [49]. Irisin is mainly secreted from the muscle in response to exercise and its administration prevented both bone loss and muscle atrophy in hind limb-suspended mice [50]. While the mechanism involved is not yet fully elucidated, irisin appears to act through integrin receptors *in vitro*, blocking osteocyte cell death and stimulating sclerostin expression [51].

Energy metabolism is well linked to muscle and bone. IGF-1 causes muscle hypertrophy and promotes the differentiation of osteoblasts into osteocytes and, thus, bone formation [52]. Osteocalcin is secreted by bones during endurance exercise and its signaling in myofibers promotes glucose uptake and the breakdown of glucose into pyruvate [53].

Modeling sarcopenia in mice

As mammals, mice are anatomically similar to humans and their tissues are identical in cellularity, organization, and function. They have, or can be induced to have, many of the same disorders as

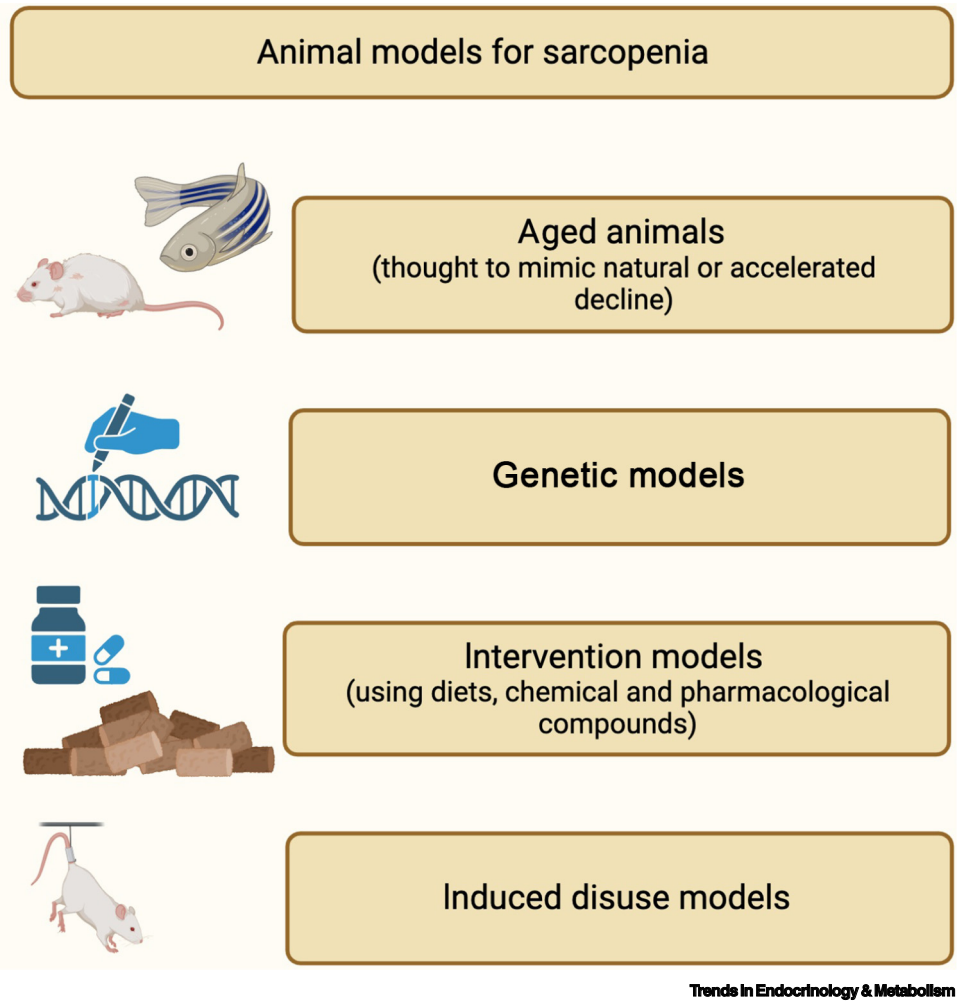

Figure 1. Animal models of sarcopenia. Figure created with BioRender (www.biorender.com).

aging humans and, therefore, serve as a model to study age-related processes [54,55]. Only ~1% of human protein-coding genes lack a homolog in mice [56]. Despite the advent of alternative models, mice remain a preferred choice for molecular studies of muscle aging, pharmacological tests, and therapeutic interventions designed to slow down or prevent muscle wasting [57,58]. **Inbred mouse strains** and transgenic mice are valuable to model human aging, including studies on the musculoskeletal system and sarcopenia in particular (Figure 1) [54,59–61].

What age in human years is my mouse?

Mice progress through the same developmental milestones as humans. They are **altricial** when they are born, depending on their mothers for food and protection. A period of rapid postnatal growth precedes an ordered sexual maturation phase, and a long adulthood with high reproductive fecundity, followed by reproductive and systemic decline [55]. However, the lengths of each life phase versus chronological age do not follow a linear relationship between mice and human. For most inbred strains, median lifespan is just under 3 years [62]. By comparison, the global life expectancy at birth for humans is currently 71 years for men and 76 years for women (being slightly higher in industrialized nations) [63]. Data collected over the past 3 months of the natural

life of a mouse reflect the accumulated pathological burden; thus, it is best to study aging processes, such as age-related musculoskeletal decline, from the advent of adulthood to 3 months before the median lifespan of the strain [64]. Similar to the concept of 'healthy lifespan' versus 'chronological lifespan' in humans [65], 3 months before the median total lifespan for a given mouse strain is achieved is considered the end of healthy aging in mice.

In female C57BL/6J (B6) mice, a common laboratory strain [66], sexual maturity occurs relatively late for inbred strains, at ~5 weeks of age [62]. They can become pregnant as early as 6.8 weeks of age [67]. Male mice generally produce mature sperm at 5.7 weeks of age, but are not considered to have completed the pubertal/adolescent phase until ~8.6 weeks of age [68]. The median lifespan for B6 mice is 901 days for males and 866 days for females [62], diverging from humans, where women usually outlive men [69]. Assuming puberty at 10 years for girls and 12 years for boys in humans, extrapolation would suggest that 1 month of healthy adulthood in B6 mice is operationally equivalent to ~2.6 years of adulthood in humans (averaging for males and females). Other models use more generalized median lifespans and ages of sexual maturity [70], and may be used when actual data for a specific mouse strain are missing.

Placing benchmarks around musculoskeletal development and aging highlights why considering these differences matters when studying musculoskeletal **phenotypes** in mouse models. Peak bone mass is reached at ~4 months of age in both males and females of most inbred mouse strains [71]. In humans, peak **bone mineral density (BMD)** is reached during the early 20s for both sexes [72]. Thus, peak BMD in mice largely reflects corresponding human timelines. Human bone growth is completed between 17 and 22 years of age on average, with epiphyseal fusion triggered by puberty. By contrast, the long bone epiphyses of mice do not fuse at sexual maturation: continued femur lengthening in B6 females has been observed up 12 months of age [71], when the growth plates finally bridge [73]. This would be the equivalent of a human growing up until their late 30s. In C57BL/6jRj mice, a strain closely related to B6 mice, the mass of major hind limb muscles is maintained until 18 months of age and then appears to steadily decline, beginning at 22 months of age in males [74], equivalent to ~64 years of age in humans. By contrast, humans show a slow decline of muscle mass beginning at ~30 years of age until age 60, when decline accelerates [75]. Therefore, C57BL/6jRj mice might mirror this accelerated decline phase.

Mouse and human physical parameters

For practical purposes, all mice within an inbred strain are genetically identical, allowing for repeated measures within strains. By using panels of inbred strains, 'surveys' across genetic backgrounds can be conducted allowing for the identification of trends and exploration of relationships between aging and age-related decline in musculoskeletal health, and highlighting strain-specific differences that need consideration when interpreting murine musculoskeletal aging studies. For example, considering measures of muscle mass, in a cross-sectional study of body composition in 30 inbred strains, lean mass was measured with DXA at 6, 12, and 18 months of age in male and female mice (data set Ackert1, data set number 25046 in the Mouse Phenome Database[i]). The analysis showed no consistent pattern for either gain or loss of whole-body lean mass. Females of strains such as MRL/MpJ, A/J and DBA/2J consistently lose lean body mass over time, while other females of 129S1/SvImJ and BALB/cByJ strains consistently increase lean mass, with similar patterns observed in males. These data suggest strong strain (genetic) differences for the 'lean mass' phenotype. Therefore, one needs to consider the genetic background of the mouse model for the interpretation of studies of age effects on lean mass. As a further example, considering functional assessments of muscle strength, grip strength decreases from 12 months of age onwards (chronologically 'equivalent' to ~38 years in humans) in male C57BL/6N mice and in an F1 C57BL/6×BALB/c cross [76]. Thus, these strains of mice lose

grip strength at a slightly older 'equivalent' age compared with the peak hand grip strength in humans, which is at ~30 years [77]. Analysis of data on grip strength in a wider study of 32 different inbred strains suggests that most male mice lose grip strength between 6 and 12 months of age. By contrast, female mice show a plateau effect, with grip strength loss not beginning until after 18 months of age (data set Seburn2, data set number 24801 in the Mouse Phenome Database[i]). The phenotypic diversity of grip strength between inbred strains signifies that the trait is highly genetically determined in mice.

Mice largely share the same major bone and muscle groups as humans. However, there are specializations of the **axial musculature** reflecting the need of wild mice to exert quick bursts of speed and to jump to avoid predators [78]. Muscle fiber excursion and biomechanical properties of individual muscles used in walking differ down to the molecular level when comparing four-legged mice with humans [79]. Mice widely express the gene encoding myosin heavy chain IIb isoform (found in high-force, fast-contracting fibers), whereas, in humans, expression of this gene was found in only a few muscles as mRNA and was not detected as a protein [80]. During muscle fiber growth in mice, the addition of myonuclei to fibers precedes myonuclear domain expansion, whereas, in growing humans, these two processes occur simultaneously [81].

Multiple studies across several tissues have shown that there are substantial differences in gene regulation between mice and humans, evidenced by a lack of conservation of gene regulation across cell types and divergences in gene expression patterns in biological pathways [82]. Therefore, it is not surprising that similar issues arise when comparing muscle gene expression profiles across ages in mice and humans. Although molecular changes affecting the mitochondria, and systemic contributors to aging such as inflammation, do occur in rodents as in humans, these processes are conserved at the pathway level rather than for individual genes. Furthermore, age-related changes in muscle gene expression occur progressively in mice, but in two distinct stages in humans [74], which may contribute to the two phases of muscle loss seen in humans [75]. A key accelerator of human bone and muscle decline is menopause, which has to be modeled in mice [83]. Such differences may explain the difficulties translating therapeutic agents from mice to humans [81].

The above caveats notwithstanding, mice are overall attractive models to study age-related muscle decline and can be used to model aspects of sarcopenia. Careful consideration of the genetics is needed because not all inbred strains experience the same age-related changes in muscle function or mass at the same rate. Chronological age correlated between mice and humans should not be used to select time points for observations, but instead the model life stage needs to be appropriately calculated. Lastly, analysis at the pathway level rather than for individual genes may be key for the translatability of studies between mice and human.

Zebrafish: a model organism for muscle conditions

Zebrafish are small-sized freshwater fish requiring relatively simple and cost-effective maintenance [84,85] with several potential advantages over mammalian models, including their fast development, transparency, genetic amenability, and the availability of a plethora of reporter lines (Figure 2). Although the evolutionary divergence of mammals from zebrafish dates to ~450 million years ago, zebrafish have the same bone and muscle cells as mammals. Approximately 80% of human disease-causing genes have at least one copy in zebrafish [86]. Muscle contractile units corresponding to those in mammals, formed by actin and myosin filaments (**sarcomeres**), are found in zebrafish [87]. Specific cell types are also traceable *in vivo* and can enable the study of cell interaction dynamics. Zebrafish have extraordinary regenerative capacity in their exoskeleton and muscles (including in response to heart injuries), serving as models for regeneration; however,

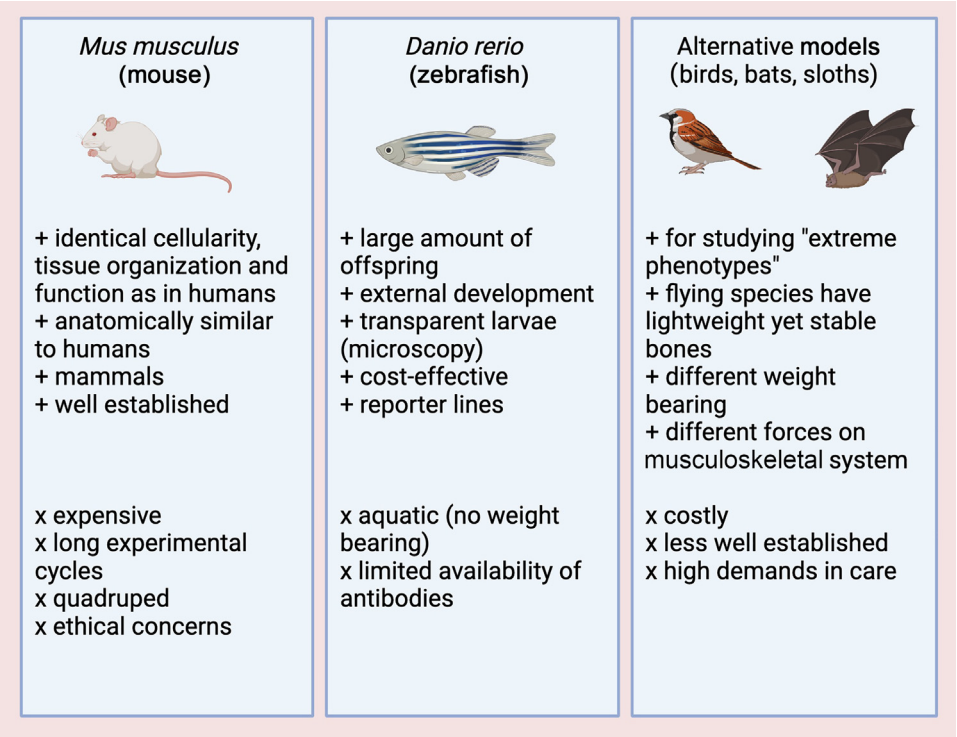

Figure 2. Pros and cons of different animal models for the study of musculoskeletal processes. Figure created with BioRender (www.biorender.com).

this capacity does not halt the development of sarcopenia and osteoporosis during aging [87]. Thus, although muscle research in zebrafish is lagging behind that in mice, they are an alternative and promising model system to advance our understanding of the genetics and mechanisms of muscle conditions and to identify potential therapeutics against age-related sarcopenia.

Embryos and larval states of zebrafish are versatile models in muscle research. They are decreasingly transparent and can be phenotypically assessed *in vivo* and *ex vivo*. Larval analysis has been used in large forward genetic screens aiming to identify genes associated with muscle diseases, reverse genetics with genetic targeting of specific genes using CRISPR/Cas9, drug screening, *in vivo* muscle phenotyping, and *in vivo* muscle function assessment [88,89]. Muscle fiber morphogenesis can be rapidly analyzed through the anterior–posterior axis of the zebrafish embryo. Within 24 h post fertilization, all 30 segments of muscles (myotomes) are formed and the first spontaneous muscle contractions are observed. More differentiated muscle fibers are located rostrally (toward the head), formed by fused myoblasts, resulting in multinucleated myofibers, while the posterior developing myotomes harbor developing muscle cells undergoing elongation and attachment to somite boundaries (myotendinous junctions). When analyzing zebrafish muscles, the superficial slow-twitch fibers of slower contractions and high endurance, and the fast-twitch fibers responsible for rapid high intensity contractions, located more internally, should be considered [90].

Assessments of zebrafish muscle physiology and function

Muscle birefringence, an optical property of muscle, is frequently used for a quick phenotypic assessment of muscle fibers of zebrafish larvae *in vivo* and has been intensively used in

phenotypic and drug screenings [88,89]. Besides reporter lines to label specific muscle types, Evans Blue Dye, a vital stain that enters cells with damaged membranes, reveals sarcolemmal damage *in vivo* [91]. For *ex vivo* phenotyping, immunostainings to distinguish slow- (e.g., anti-F59 antibody) and fast-twitch fibers (e.g., anti-Phalloidin, anti-F20, and anti-F310) are available [92]. Histological sections and electron microscopy can also be used for fine and ultra-fine muscle fiber phenotyping in zebrafish [93].

While most zebrafish muscle research is done in larvae, adult zebrafish phenotyping and disease modeling comprise an emergent research niche, highly relevant for advancing our understanding of muscle degeneration during aging. Zebrafish transparency is lost during their transition from the larval to juvenile stage (during approximately the first month of life). Therefore, the assessment of zebrafish muscles in 3D can be done *ex vivo* using enhanced contrast micro-CT (μCT) [94]. As in larvae, slow muscle fibers are located externally and fast fibers, representing most zebrafish muscles, are located more internally. Swimming behavior analysis is used as surrogate of muscle function [94].

Zebrafish show a natural age-related reduction in muscle mass, along with reduced swimming performance with increasing age, indicative of a decline in muscle function [95–97]. In older zebrafish, skeletal muscle degeneration is accompanied by increased senescence-associated β-galactosidase activity, mitochondrial dysfunction, oxidized protein accumulation, as well as spinal curvature (kyphosis) [98,99]. Similar to mammals, fish have slow and fast muscle fibers; however, adults are able to restore muscles via hypertrophy [100]. Aged zebrafish still show a reduced fiber cross-sectional area, although it is unknown whether this is specific to a fiber type. Given that the average lifespan of zebrafish is 2–3 years or more, waiting for natural aging for experimentation is not feasible. Modeling of muscle mass reduction can be achieved by genetic manipulations and environmental changes. Recently, a sarcopenia phenotype was induced through a high-fat diet [101]. In addition, mutant models that display premature aging phenotypes are commonly used to study the biology of sarcopenia.

Although fewer in zebrafish than in mice, various models of skeletal muscle aging have been reported in recent years with the development of CRISPR/Cas9 technology [102]. In addition, an age-related muscle phenotype has been identified in zebrafish with mutations in celsr1a (a non-classical cadherin involved in maintenance of progenitor cells) and tert (telomerase reverse transcriptase involved in maintenance of telomere length) [103,104]. An age-related sarcopenia phenotype in zebrafish might be mitigated by a prophylactic exercise regime, whereby an 8-week swimming exercise regime in zebrafish was sufficient to restore muscle mass in 21-month-old fish [87]. Exercise training improves swimming performance in young (8–12 months) and middle-aged (15–20 months) fish, but not old fish (25–30 months) [98]. This might suggest that, as in the humans, exercise alone is not sufficient to treat sarcopenia.

Concluding remarks and future perspectives

Clinical muscle phenotyping in human health and disease has significantly progressed over the past few years, although many questions remain (see Outstanding questions) [40]. There is a need for an agreement on specific and reproducible measurement parameters in health and disease, while considering age and sex differences. Such standardization is crucial not only for enhancing clinical assessments, but also for promoting data exchange and facilitating research endeavors. Drawing analogies from research on bone disease, the concept of a muscle-equivalent to the FRAX (a fracture risk assessment tool) score for osteoporosis has been proposed [105], emphasizing the importance of similar initiatives for muscle health. Additionally, there is a need for the development or refinement of questionnaires, laboratory biomarkers,

Outstanding questions

What could we learn from alternative model organisms in musculoskeletal research, such as birds (which have lightweight yet robust skeletons) or sloths (which show reduced skeletal muscle mass despite high strength and fatigue resistance)?

Can insights (clinical, preclinical, and 'omic studies) from the bone field be extrapolated to skeletal muscle research?

What surrogate parameters should be used to study musculoskeletal physiology and disease and how would these tools be standardized?

Which biomarkers (e.g., laboratory markers or imaging features) could be recommended in clinical assessment and studies?

What would be an appropriate model of sarcopenia scoring comparable to FRAX, and what parameters should it be based on?

and imaging tools, and consolidation of diagnostic criteria for various musculoskeletal diseases [106].

The interaction of preclinical and clinical scientists is a key goal for the GEMSTONE COST action. Many further aspects emerged following the writing of this review, including the complexity of measuring interactions between muscles, bones, joints, and tendons both during movement and at rest, and the need for better definitions of human disease phenotypes. Finally, we anticipate that, in addition to advances in clinical and preclinical muscle phenotyping, human aspects, such as social interactions, movement, and behavioral patterns and their feedback to the musculoskeletal function, will be a focus for future research.

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Declaration of interests

No interests are declared.

Resources

[i]https://phenome.jax.org

Supplemental information

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