Rodent Model of Muscular Atrophy for Sarcopenia Study

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The hallmark symptom of sarcopenia is the loss of muscle mass and strength without the loss of overall body weight. Sarcopenia patients are likely to have worse clinical outcomes and higher mortality than do healthy individuals. The sarcopenia population shows an annual increase of ~0.8% in the population after age 50, and the prevalence rate is rapidly increasing with the recent worldwide aging trend. Based on International Classification of Diseases, Tenth Revision, a global classification of disease published by the World Health Organization, issued the disease code (M62.84) given to sarcopenia in 2016. Therefore, it is expected that the study of sarcopenia will be further activated based on the classification of disease codes in the aging society. Several epidemiological studies and meta-analyses have looked at the correlation between the prevalence of sarcopenia and several environmental factors. In addition, studies using cell lines and rodents have been done to understand the biological mechanism of sarcopenia. Laboratory rodent models are widely applicable in sarcopenia studies because of the advantages of time savings, cost saving, and various analytical applications that could not be used for human subjects. The rodent models that can be applied to the sarcopenia research are diverse, but a simple and fast method that can cause atrophy or aging is preferred. Therefore, we will introduce various methods of inducing muscular atrophy in rodent models to be applied to the study of sarcopenia.

Key Words: Aging · Muscular atrophy · Muscle, skeletal · Rodentia · Sarcopenia

INTRODUCTION

Sarcopenia is a degenerative disease in which the mass, quality, and strength of skeletal muscle are lost by aging.[1] The sarcopenia population shows an annual increase rate of ~0.8% in the population after the age of 50, and the prevalence rate is rapidly increasing with the recent worldwide aging trend.[2] Based on International Classification of Diseases, Tenth Revision, a global classification of diseases published by the World Health Organization, issued the disease code (M62.84) given to sarcopenia in 2016.[3]

Although the study of sarcopenia has been active for a long time, it is expected that such study will be further activated based on the classification of disease codes in the aging society.
codes in the aging society. The study of sarcopenia can be largely divided into epidemiologic studies, meta-analyses, and experimental studies based on human and rodent interventions. Epidemiologic studies and meta-analyses focus on the study of the prevalence of sarcopenia and the interrelationship between environmental factors. These studies have demonstrated that muscle mass decreases with aging, which leads to physical disability,[4] and that in a highly active population, loss of muscle mass may not be as important as strength loss for predicting functional decline.[5] In experimental studies, research on the effects of exercise and dietary interventions and drug therapy on humans [6, 7] and rodents has been done.[8] However, many studies have sought to understand the biological mechanism of sarcopenia based on molecular biological methods rather than on human studies. Thus, in vitro studies using cell lines and rodent studies are also being conducted.[9-11]

Because of the advantages of saving time and cost and of using various analytical applications than cannot be used in human studies, laboratory rodent models can be widely used in sarcopenia studies. However, the causes of sarcopenia are very diverse, and it is very important to select an appropriate rodent model according to the research purpose. The rodent models that can be applied to the sarcopenia research are diverse, but a simple and fast method that can cause atrophy or aging is preferred. Therefore, we will introduce various methods of inducing muscle atrophy in rodent models to be applied to the study of sarcopenia (Fig. 1).

AGED-RODENT MODEL

Aged rodents have been widely used for studying sarcopenia (Table 1). Aged-rodent models are the most natural

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**Fig. 1.** Rodent model of muscular atrophy for sarcopenia study.
model and have several advantages compared to other sarcopenia models, including hindlimb unloading (HU) and disuse atrophy models. Aged rodents have morbidities and mechanisms fairly similar to those found in human sarcopenia patients, but the high cost and limited availability of aged rodents make the use of this model somewhat difficult.[12]

Female C57BL/6J mice developed sarcopenia with significant loss of quadriceps muscle mass by 24 months that was more pronounced by 27 to 29 months,[13] at a time when there is denervation and altered neuromuscular junctions (NMJ) morphology of myofibers.[14] Insulin-like growth factor-1 (IGF-1) signaling is a central regulator for protein metabolism and maintenance of normal muscle mass,[13,15] and key molecules of this signaling pathway are also important in aging skeletal muscles. Aging is closely related to a decrease in insulin sensitivity, which can impair IGF-1 activity. However, binding of muscle IGF-1 to the IGF-1 receptor through an intracellular signaling pathways involving tyrosine kinase activity may exerts an anti-apoptotic effect and reduce muscle atrophy via phosphatidylinositol 3-kinase (PI3K)-dependent Akt-dependent phosphorylation.[16] Gait characteristics were also changed in aged mice. Compared to young mice (3 months old), aged mice (24 months old) exhibited significantly decreased cadence, increased stride-time variability, and altered footfall patterns.[17]

The aged-rat model also showed patterns of muscle decrease similar to those of an aged-mouse model. Old male Wistar Han rats (19 months old) decreased body weight by 1.8 ± 0.9%, lean body mass by 0.3 ± 1.0%, and fat mass by 13.0 ± 3.0% for 4 weeks.[18] Because high calorie intake is known to accelerate the setup of sarcopenia, some studies gave a high-fat diet to animals.[19,20] When Sprague–Dawley rats were fed a high-fat diet at 6 months old, a loss of muscle cross-sectional area was observed in males at 16 months of age. But female rats were resistant to sarcopenia induced by a high-fat diet.[21] Hence males seem susceptible for lipotoxic properties, and gender difference should be considered in this condition. The loss of muscle mass in the rat fed a high-fat diet is not because of reduction of the Akt pathway or an upregulation of the ubiquitin proteasomal degradation of muscle protein, because of unchanged expression of the main ubiquitin ligases of

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**Table 1.** Aged-rodent models that applicable for study on sarcopenia, and key findings from individual studies

| References | Strain | Intervention/phenotype | Age at sacrifice (month) | Checked variable/key findings |
|------------|--------|------------------------|--------------------------|-----------------------------|
| Shavlakadze and Grounds [13], Chai et al. [14], Schiaffino and Mammucari [15], Tarantini et al. [17] | C57BL/6 mice | Natural aging | 24-29 | NMJ morphology of myofibers, lower IGF-1 in aged mice, poor gait characteristics in aged mice |
| Pötsch et al. [18] | Wistar Han rats | Natural aging | 19 | Decreased body weight, LBM and fat mass in aged rats |
| Kob et al. [21] | SD rats | Aged with HFD | 16 | Loss of muscle cross-sectional area in male rats (female was resistant to HFD) |

SD, Sprague-Dawley; HFD, high-fat diet; NMJ, neuromuscular junction; IGF-1, Insulin-like growth factor-1; LBM, lean body mass.

**Table 2.** Hindlimb unloading rodent models that applicable for study on sarcopenia, and key findings from individual studies

| References | Strain | Intervention/phenotype | Age at sacrifice | Checked variable/key findings |
|------------|--------|------------------------|-----------------|-----------------------------|
| Deavers et al. [27] | SD rats | Head-down suspension with single hindlimb support | 7 days | Lower muscle mass-to-body mass in soleus, plantaris and gastrocnemius |
| Halloran et al. [32] | SD rats | Tail traction with tape | 4 weeks | Bone formation and apposition rate were low in tibiofibular junctions of unloaded rats |
| Fell et al. [33] | SD rats | Whole-body suspension with hindlimb-load bearing | 1 week | Muscle atrophy significantly increases fatigability in gastrocnemius |
| Fitts et al. [34] | SD rats | HS, HI | 2 weeks | HS produced increases in muscle shortening. HI did not differ in muscle shortening |
| Jaspers and Tischler [35] | SD rats | Hindlimb tail-cast suspension | 6 days | Six days is the optimum duration for muscle unloading studies |

SD, Sprague-Dawley; HS, hindlimb suspension; HI, hindlimb immobilization.
MUSCLE ATROPHY INDUCTION MODEL

1. Hindlimb unloading

The rodent HU model was originally devised to investigate the astronauts’ musculoskeletal response to weightlessness or low-gravity conditions and has since been widely used as a muscle-wasting model mimicking the condition of muscle-wasting disease, inactivity, bed rest, and immobilization (Table 2).[23] Since weightlessness has been predicted to yield deficits in the principal tissues needed for structure and movement on Earth, primarily muscle and bone, the National Aeronautics and Space Administration (NASA) Ames Research Center (ARC) set up the HU model to study the mechanisms, responses, and treatments for the adverse consequences of spaceflight in the mid-1970s. After inception of the HU model at NASA, many laboratories have used the HU model to simulate weightlessness and subsequently used it as a muscle-wasting model. Since the standard operating procedure for applying the HU model to young and adult rodents was updated and approved by the NASA ARC Institutional Animal Care and Use Committee on August 8, 2001, more than 1,500 papers have published data that used this model system.[24]

The primitive HU model tested in 1975 was very simple, but led to suggestions for modifications ultimately incorporated into succeeding designs. The first HU model described in a full-length paper in 1979 used a hexcelite back harness and a cantilevered rotating beam that allowed the head-down animal to move in a 360° arc.[25] Data were compared with the weight and bone changes found in the Cosmos 782 and 936 biological satellites, and the author concluded that the changes of body weight, food consumption, and bone-formation rates in HU rats were very similar to spaceflight. So the HU model closely mimics results from rat and man exposed to near-weightlessness during orbital spaceflight and will allow preliminary answers to questions posed by spaceflight experiments.

Musacchia and colleagues used a modified first HU model.[26] In this model, they used a denim harness and a rotating beam that allowed the animal to move in a 140° arc. Body weight and food consumption in the HU rats were significantly less than those of the control group, and the animals exhibited adrenal hypertrophy at the end of the 7-day experiment. The authors concluded that the muscle changes were similar to those found during spaceflight and recovery from spaceflight. To find out whether the cephalad fluid shift contributed to changes in metabolism, Deavers and colleagues included a horizontal control and found that the head-down position was required for the diuresis and natriuresis that occurred during HU.[27] Stump and colleague[28] modified the model to measure muscle changes and blood flow. Deavers et al.[27] and Bouzeghrane et al.[29] advocated for a horizontal control, particularly for studies investigating fluid shifts. Hargens and colleagues[30] addressed the importance of the unloading angle. They found that the angle of unloading determined the amount of weight supported by the forelimbs as well as the tension applied to the tail, and showed that the HU rat applies 50% of its body weight to its forelimbs when the angle between the torso and the floor of the cage is 30°. As the angle increased, mechanical loading of the forelimbs declined and traction on the tail increased. If the angle was too steep, then the animals appeared stressed. A 30° angle of unloading was recommended, because it provided normal weight bearing on the forelimbs, unloaded the lumbar vertebrae but not the cervical vertebrae,[31] and induced a cephalad fluid shift.[30]

The HU model has not changed conceptually from the beginning. Data from all laboratories that used the model showed differential muscle atrophy, a cephalad fluid shift, animals having the freedom to move, eat, and groom with the forelimbs, and unloading of the hindlimbs without paralysis so that animals could recover from unloading. However, the harness system and degree of mobility differed significantly between laboratories. One problem possibly related to these differences was the reduced weight gain in growing rats or weight loss in adult animals that persisted throughout the experimental period. Harnesses tested at ARC included a combination of elastic and Velcro straps and hexcelite bonded with an epoxy resin to the back of the rat.[25] Each of these harnesses was only partially successful, and less stressful harness designs were sought. The concept of a tail harness originated with our Russian colleagues, who used a plaster of Paris mold for tail traction. In the early 1980s, orthopedic surgeons from the University of Southern California toured our laboratory and recommended that the tail cast be replaced with the tape that...
they used for placing human limbs in traction. Traction tape could be applied to an unanesthetized animal and would allow the tail to grow without restriction. In fact, the body weights of growing rats unloaded with the use of tail traction remained comparable to those of controls fed the same amount of food (i.e., group-mean-fed controls), in contrast to rats unloaded with the use of back harnesses. Tail traction appears to be less stressful to animals than are whole-body harnesses, as assessed by corticosterone levels and adrenal, thymus, and body weights.[32]

Unlike other rat models, the HU model did not require confinement of animals in small cages, limb casting, or flaccid paralysis by nerve section or surgical tenotomy. None of these techniques produced the differential muscle atrophy characteristic of spaceflight, i.e., a decreased in mass of the extensor muscles but not of other muscles associated with movement. In addition, recovery from disuse was difficult or impossible with the existing surgical models. But the many studies that used the HU model clearly showed that independent variables can influence results obtained and, ultimately, the analog’s validity in terms of understanding the mechanisms and physiological responses to spaceflight. Additional HU variables known to influence experimental results include age (growing vs. adult), sex, species (rat vs. mouse), and strain.

Chronic hindlimb suspension (HS) (unweighting) has been shown to limit growth and result in significant losses in hindlimb muscle mass, slow-twitch properties, and show myosin content.[33-37] This loss in muscle mass is more extensive in those muscles predominantly composed of slow-twitch (type I) fibers.[33,35,36,38] Although weight-bearing activity (mechanical stress) appears to be a primary factor in maintaining muscle weight in the context of HS,[37] evidence is lacking as to whether anabolic steroid treatment can serve as an independent stimulus to preserve muscle weight in the absence of weight-bearing activity.

2. Denervation model

It is thought that complex degeneration of the neuron-muscular system contributes to dynapenia.[2,39-42] Neuromuscular changes contributing to myofiber denervation occur within the central and peripheral nervous systems as well as within skeletal muscle tissue. Changes include diminished function or loss of neurons in the brain and spinal cord, demyelination of nerves, and progressive degeneration of NMJs.[43,44] Skeletal muscle denervation, caused by such problems as traumatic peripheral nerve injury, disease, pharmacologic intervention, and aging (Table 3), diminishes the function leads to immediate muscle atrophy.[14,45,46] Early muscle atrophy could be restored by a timely and appropriate reinnervation occurrence, but without one, myofiber atrophy progresses to irreversible changes in the muscle with muscle fibrosis and myofiber death.[47,48] Denervation is a common phenomenon in an aged NMJ. The tibial- or sciatic-nerve transection model to induce the denervation is commonly employed and a well-validated model in rodents. Only a single dose of analgesic is necessary in the immediate postoperative period. With the use of proper sterile technique, soft-tissue infection is rare.[49] This model allows the investigator to use genetically engineered mice to study the process of muscle atrophy in vivo in the absence of proteins crucial to the regulation of muscle mass.[50,51]

The tibial-nerve transection model is a validated, reproducible, and well-tolerated model of denervation-induced skeletal muscle atrophy in rodents, and is used to study the physiologic, cellular, and molecular biologic mechanisms that underlie muscle atrophy in vivo in the gastrocnemius and soleus muscle. The tibial nerve is a mixed motor-sensory peripheral nerve in the rodent hindlimb and is 1 of the 3-terminal branches of the sciatic nerve. Transection of the tibial nerve denervates the gastrocnemius, soleus, and plantaris muscles (and the 3 small deep flexor muscles of the foot, including the tibialis posterior, flexor digitorum longus, and flexor hallucis longus), and is a well-standardized and validated model in rats.[52,53] Also, various knockout (KO) and transgenic (Tg) mice allow us to assess the specific functions of proteins in the induction, development, and maintenance, or alternatively the resolution of muscle atrophy and fibrosis in vivo in this model. The tibial nerve supplies the gastrocnemius, soleus, and plantaris muscles, so its transection permits the study of denervated skeletal muscle composed of fast twitch (type II) fibers and/or slow twitch (type I) fibers. The gastrocnemius muscle is a mixed-fiber muscle (type I and type II, although predominantly type II), and the soleus muscle is composed of a large proportion of type I fibers, thereby providing both fast- and slow-twitch muscles for assessment.[54,55] The tibial-nerve transection model is suitable
| References | Strain | Intervention/phenotype | Age at sacrifice | Checked variable/key findings |
|------------|--------|-----------------------|------------------|------------------------------|
| Batt et al. [52] | Lewis rats | Tibial nerve transection | 1 and 3 months | Temporality of recruitment of signaling networks involved in protein degradation and cell death |
| Bain et al. [53] | 129J/C57BL/6 chimeric mice, C57BL/6 mice | Tibial nerve transection | 2 weeks | Absence of caspase-3 protects against denervation-induced skeletal muscle atrophy |
| Varejão et al. [59] | C57BL/6 (Sod−/−) mice | Oxidative stress | 3-4 weeks | Sod1−/− mice are 17 to 20% smaller and have a significantly lower muscle mass than WT mice as early as 3 to 4 months of age |
| Willand et al. [60] | C57BL/6 (Sod−/−) mice | Oxidative stress | 18-22 months | Muscle atrophy in Sod1−/− mice is accompanied by a progressive decline in mitochondrial bioenergetic function and an elevation of mitochondrial generation of ROS |
| Richner et al. [61] | C57BL/6 (Sod−/−) mice | Oxidative stress | 1-4 months | Preferential denervation of fast-twitch muscles beginning between 1 and 4 months of age, with relative sparing of slow-twitch muscle |
| Salmon et al. [63] | G1H mice (G93A) | ALS | Clinical disease started at 91±14 days of age, paralysis and death by 136±7 days of age | The age-dependent penetrance of motor neuron disease in this Tg model is due to the gradual accumulation of pathological damage in select populations of cholinergic neurons |
| Fulle et al. [64] | G1H mice (G93A) | ALS | 5 months | Motor neuron degeneration |
| Sastre et al. [65] | G1H mice (G93A) | ALS | 45-144 days | Neuronal cytoskeletal abnormalities may be implicated in the pathogenesis of human ALS |
| Park [66] | G1H mice (G93A) | ALS | 28, 47, 100, and 120 days | In ALS, denervation and reinnervation changes in muscle but normal appearing motor neurons |
| Muller et al. [67] | G1H mice (G93A), progressive neuropathy mice, Thy1-GAP43 Tg mice | Purified botulinum toxin A applied at 0.01 U/gm | 7, 30 days | Gradual and selective loss of synaptic connections that begun long before the onset of clinical deficits and correlated with the timing of disease progression |
| Jang et al. [68] | SOD1 (G93A), Bax heterozygote | ALS | 120-140 days | Clinical symptoms in the SOD1 G93A model of ALS result specifically from damage to the distal motor axon and not from activation of the death pathway, and cast doubt on the utility of anti-apoptotic therapies to combat ALS |
| Fischer et al. [69] | G1H mice (G93A) | FALS | 130-140 days | Impairment of motor neuron function precedes by 6 weeks the onset of apparent clinical signs (shaking, tremor) and the beginning of motor neuron loss. Neuromuscular deficits in FALS mice do not result from motoneuronal cell death but rather from loss of axonal integrity |
| Fischer et al. [70] | G1H mice (G93A) | FALS | 16 weeks | Longitudinal MRI hindlimb muscle volume measurements may provide a straightforward, rapid, non-invasive and sensitive, way of monitoring outcome of experimental ALS treatments |
| Chiu et al. [71] | G93A-SOD1 mice | ALS | 8, 12, 15, and 18 weeks | Muscle degeneration occurs before any evidence of neurodegeneration and clinical signs, supporting the postulate that motor neuron disease can initiate from muscle damage and result from retrograde dying-back of the motor neurons |
| Gurney et al. [72] | IL-10tm/tm mice | ALS | Young (4-5 months), old (22-28 months) | NMJ in other muscles, such as ocular muscles, is resistance to changes in aging. Moreover, damage to NMJs in aged muscles correlated with altered expression and distribution of CRMP4a and TDP-43, which are both altered in motor neurons affected by ALS |
| Tu et al. [73] | C57BL/6 mice | Tibial nerve transection | 2-3 month old mice denervated for 1, 2, or 4 weeks | Gastrocnemius muscle atrophy and decrease in myofiber CSA |

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for studying the process of denervation-induced muscle atrophy in both the short term (days) [50] and long term (weeks to months) [51,52]. The gastrocnemius and soleus muscles, both deprived of denervation model, can be easily and rapidly dissected with minimal handling, thus providing excellent-quality mRNA and protein for subsequent molecular analyses. Similarly, because of the size of the muscles, they can be split, providing tissue from the same animal for concurrent histologic and morphometric analyses. If hindlimb functional assessment is required, walking-track analysis can be serially done. The feet are dipped in ink, and the mouse is walked through an enclosure with paper on the bottom. Characteristics of the prints can be reliably measured and scored to indicate the extent of neuromuscular disability and gait compromise, since footprint characteristics reflect the functional muscle groups [56,57]. Although originally developed and validated in rats [56], walking-track analysis can also be used with mice [58].

| References | Strain | Intervention/phenotype | Age at sacrifice | Checked variable/key findings |
|------------|--------|------------------------|------------------|-----------------------------|
| Fischer et al. [74] | Outbred male White Wistar rats | The sciatic nerve with its 3 major branches was exposed through a gluteal muscle-splitting incision | Only functional tests were performed | The sciatic functional index, tibial functional index, and peroneal functional index offer the peripheral nerve investigator a noninvasive quantitative assessment of hindlimb motor function in the rat with selective hindlimb nerve injury |
| Frey et al. [75] | Male Lewis rats | Sciatic, tibial, and peroneal transection | Only functional tests were performed | Individual walking print length measurements alone can be used to characterize functional recovery after tibial and peroneal nerve injury, whereas toe spread reflected recovery after SNI |
| Gould et al. [76] | PTPα+/−, PTPα+/+ and PTPα−/− mice | SNC, SNT, and microsurgical repair, sciatic nerve allografting | 6 weeks | The loss of PTPα results in faster peripheral nerve regeneration and significant abnormalities of axon guidance |
| Brooks et al. [78] | Lewis rats | Tibial nerve transection and immediately repair with the fibular nerve, 1 month of electrical stimulation | 2 months | Short-term electrical muscle stimulation after nerve repair significantly reduces muscle atrophy and does not affect motor reinnervation |
| Marcuzzo et al. [79] | C57BL/6 mice | Sprayed nerve injury | Only Von Frey tests was performed | C57BL/6 mice experience profound allodynia as early as the day following surgery and maintain this for several weeks |
| Valdez et al. [80] | Vglut2[loxP::Psimte] mice | Partial sciatic nerve ligation surgery | 8 weeks | VGLUT2 is a major mediator of nociception in primary afferents, implying that glutamate is the key somatosensory neurotransmitter |

Tg, transgenic; SOD, superoxide dismutase; ALS, amyotrophic lateral sclerosis; FALS, familial amyotrophic lateral sclerosis; SNC, sciatic-nerve crush; SNT; sciatic-nerve transection; WT, wild-type; ROS, reactive oxygen species; MRI, magnetic resonance imaging; CSA, cross-sectional area; SNI, sciatic-nerve injury.
ligated, but the sural is left intact) serve as models of neuropathic pain.\cite{61,62} Thus, allodynia and thermal hyperalgesia could occur in the foot in our model as well, but we have not seen overt pain behavior in the mice with normal daily activity on soft bedding.

One of the leading theories on mechanisms underlying age-related muscle denervation points to oxidative stress.\cite{63-65} Reactive oxygen species (ROS) are natural byproducts of mitochondrial activity involved in respiration and energy production. ROS-mediated oxidative damages to DNAs, proteins, and lipids are normally kept in check by antioxidants. However, excessive ROS production can overwhelm the antioxidant defense, leading to increased oxidative damage of cellular machinery. Two mouse models, one lacking the Cu/Zn superoxide dismutase (\textit{Sod1}) gene and another harboring the Tg mutant human SOD1 gene, display progressive changes at the NMJ, including muscle-endplate fragmentation, nerve-terminal sprouting, and denervation. These changes at the NMJ share many of the common features observed in the NMJs of aged mice.\cite{66} SOD1 is a cytoplasmic antioxidant enzyme involved in the scavenging of superoxide free radicals. Mice lacking the SOD1 enzyme (\textit{Sod1} \textsuperscript{-/-} mice) show increased oxidative damages to proteins, lipids, and DNAs.\cite{67} In addition, these mice display progressive muscle denervation, weakness, and loss, changes seen despite the absence of a spinal cord motor neuron and ventral root axon loss.\cite{68-70} NMJ denervation and sprouting are observed in these mice at between one and 4 months of age and precede muscle loss,\cite{69,70} which is observed at between 3 and 4 months of age.\cite{67} Furthermore, muscle denervation and loss are greater in the gastrocnemius and tibialis anterior compared to the soleus.\cite{67,69,70} The G93A SOD1 mouse line Tg for SOD1 containing a point mutation at amino acid position 93 (G -> A) present in patients with familial amyotrophic lateral sclerosis. G93A SOD1 mice recapitulate many of the pathological hallmarks of amyotrophic lateral sclerosis, such as progressive muscle weakness and denervation, motor neuron loss, and paralysis.\cite{71-73} It has been demonstrated that muscle denervation is observed as early as at 47 days of age in these mice\cite{74-77} and precedes both motor neuron loss,\cite{62,63} and muscle atrophy.\cite{78,79} These characteristics are similar to those observed in the rodent models of aging\cite{14,80} as well as in \textit{Sod1} \textsuperscript{-/-} mice.\cite{69,70}

3. Immobilization

Immobilization-induced skeletal muscle atrophy is characterized by a decrease in muscle mass and an increase in the risk of debilitating diseases and orthopedic problems (Table 4). The cast immobilization is the most frequently used model for studying muscle atrophy because it simulates conditions after fractures that require casts and wrap the leg with a plaster bandage or spiral wire; so this model can mimic prolonged immobilization.\cite{12} The cast immobilization model may prove useful in studies on therapeutic interventions of muscle atrophy using Tg and mutant mouse strains.\cite{81} This model could also evaluate the muscle loss, because muscles that are fixed in a contracted state show greater atrophy than do stretched muscle.\cite{82} However, this model is time consuming, needs some skill, and may cause adverse events, including skin injury, local edema or necrosis, probably because of the retention of urine by the cast, and problems of escaping from the cast; so it requires experience, frequent observation, and replacement.\cite{83-85} There are several methods for construction of a cast immobilization model. The traditional method is using the plaster cast on a unilateral or bilateral hindlimb.\cite{86-91} After anesthesia, one or both hindlimbs was immobilized with a plaster cast and monitored on a daily basis for chewed plaster cast, abrasions, and problems with ambulation. Some modified methods have also been developed. Onda and colleagues used steel bonsai wire, which enables repeated direct access to the immobilized muscle and allows concurrent application and assessment of various therapeutic interventions.\cite{92} In this study, the weight of the soleus and planters muscles were decreased significantly in both bilateral and unilateral immobilization and the mRNA expression of \textit{Fbxo32} (MAFbx [also known as atropin-1] protein coding gene) and \textit{Trim63} (MuRF1 protein coding gene) were also decreased in both muscles.\cite{92} Another group developed a Velcro immobilization method using the commercially available hook-and-loop fastener that is faster and has less adverse events than does cast immobilization. They insist that Velcro immobilization could substitute for cast immobilization and allow the immobilization-intervention process to be repeated easily.\cite{85} Speecht and colleagues explored disuse-induced muscle atrophy by using a unilateral casting model in conjunction with HS. They showed in the study that a 2-week HS resulted in a significant decrease in gastrocnemius and quadriceps...
### Table 4. Immobilization models that applicable for study on sarcopenia, and key findings from individual studies

| References | Strain | Intervention/phenotype | Age at sacrifice | Checked variable/key findings |
|------------|--------|------------------------|------------------|-------------------------------|
| Madaro et al. [81] | Male C57BL/6 mice | Unilateral hindlimb immobilization | 7 days | Significant reduction in muscle fiber size in both the EDL and TA. Expression of the muscle-specific ubiquitin ligases MAFbx/Atrogin-1 and MuRF1 genes |
| Herbert and Balnave [82] | Female New Zealand White rabbits | Ankle was immobilized in a plaster cast | 10 days | Immobilization in a shortened position was associated with a significantly greater decrease in length and weight. Immobilization produced significant increase in the resting stiffness of MTU |
| Ohmichi et al. [83] | Male SD rats | Hindlimb CI | 10 weeks of electrical testing and observational study without sacrifice | CI induces ischemia/reperfusion injury in the hindlimb and consequent production of oxygen free radicals, which may be involved in the mechanism of widespread hyperplasia in chronic post-cast pain rats |
| Guo et al. [84] | Male SD rats | Tibia fracture CI | 4 weeks | Early limb mobilization after fracture may prevent the development of complex regional pain syndrome |
| Aihara et al. [85] | Male C57BL/6 | CI, VI | 2 weeks | VI induced muscle atrophy to the same extent as CI, but in a shorter time and with less complications |
| Booth and Kelso [86] | Male SD rats | CI | 4 weeks | Methods for production of muscular atrophy, casting is the least destructive to the animal. Moreover, removal of a cast most closely approximates the physiologic situation in studies upon the recovery from muscle atrophy |
| Williams and Goldspink [87] | 129 Re strain (mice) | One hindlimb immobilized by means of plaster of Paris bandage (held in shortened position) | 4 weeks | Muscles which have been immobilized in the shortened position there are quantitative and possibly qualitative alterations in the connective tissue which are likely to result in the reduced elasticity observed in immobilized muscles |
| Williams et al. [88] | Male rabbits of strain NZW | Immobilized in a shortened position | 7 days | Connective tissue accumulation that occurs in inactive muscle can be prevented either by passive stretch or by active stimulation |
| Williams [89] | S/Hy mice | One hindlimb of each animal was immobilized by means of plaster of Paris bandage with the ankle extended (soleus muscle shortened) | 10 days | Intermittent stretch prevented the connective tissue changes but did not prevent the reduction in muscle fiber length, which itself resulted in considerable loss of range of motion |
| Zemková et al. [90] | Male Wistars rats | Right HI using a cast of plastic-like material (shortened position) | 7 days | In the rat soleus immobilized for 7 days in the shortened position, the muscle mass loss was greater than the stretched soleus and shortened EDL |
| Karpakka et al. [91] | Male SD rats | Right HI with plaster of Paris (full plantarflexion of ankle) | 0, 1, or 3 weeks | Immobilization causes opposite changes in the enzyme activities, but due to the higher initial level after exercise protocol, training may slow down the deadaptive changes caused by disuse during the first week of immobilization |
| Onda et al. [92] | C57BL/6 mice | Spiral wire immobilization | 3, 5, or 10 days | The spiral wire immobilization model is more useful than the conventional HI model |
| Speacht et al. [93] | C57BL/6J mice | HS and casting | 2 weeks | Combination of HS and immobilization by casting exaggerates sarcopenia by stimulating autophagy but does not worsen osteopenia |

SD, Sprague-Dawley; CI, cast immobilization; VI, Vekro immobilization; EDL, extensor digitorum longus; TA, tibialis anterior; MTU, muscle-tendon units; HI, hindlimb immobilization; HS, hindlimb suspension.
weight of about 9% to 10%, but over a 2-fold greater decrease in HS with casted limb.[93]

CONSIDERATION FOR USING MOUSE MODEL IN THE STUDIES OF SARCOPENIA

The mouse is a good animal model for studying the sarcopenia because of low cost, short life span, and relative ease of genetic manipulation.[94,95] Moreover, there are many previous studies shown similarities in aging processes between human and mouse.[96-98] But, of course, there are also significant differences between humans and mice, and this could be a limitation for specific applications. Mice exhibit higher regenerative capacities, their muscle mass only minimally declines with age, mice have high telomerase activity in many organs, and they can synthesize vitamin C.[99] Moreover, mouse breeding technology allows researchers to reduce biological variation as a source of experimental noise and also allows the exploitation of strain and cohort differences as a tool in aging research.[100]

The composition of the skeletal muscle fiber also differs between humans and mice. There are 2 types of skeletal muscle fiber (myofiber): type I is slow myofiber that have a slow contraction time and rely on oxidative phosphorylation pathways and resist fatigue. In contrast, type II is quick myofiber that have a fast contraction time and rely on glycolytic pathways and fatigue more easily. Human muscles are composed predominantly of type I myofibers, while mice are mainly type II: such differences between species need to be considered when extending observation from animal models to humans. Furthermore, there is more time for more secondary consequences to become pronounced in humans where sarcopenia becomes progressively manifest over 20 to 30 years, whereas the duration is far shorter in mice; >1 year (18-30 months), with the normal lifespan of mice being a mere 3 years or less. Innervation of myofiber is clearly required for skeletal muscle contraction in mice and humans, but different conclusions may be reached from initial studies.[101] Examination of aged mice (up to 29 months old) revealed marked denervation of NMJs of hind limb muscles without any change in number or size of motor neuron cell bodies in the lumbar spinal cord, suggesting a primary problem at the level of the muscles per se.[102] In contrast, many changes in motor neuron function are noted from electrophysiological studies in aging humans supporting changes in the central nervous system,[103] although it is difficult to determine whether these changes are secondary to earlier NMJ changes, since invasive examination of the NMJ status is rare in human studies. Further experiments in animal models can help to define the precise timing of these key events.

Although there are sarcopenia or senescence-accelerated rodent models with aging-related metabolic diseases which were generated by genetic modification, the models of muscular atrophy introduced in this study are relatively easy to apply for the experiments due to its time efficiency to obtain. Genetic modification models take long time and high cost to breed. Nevertheless, aging model still would be the most recommendable choice for the sarcopenia study among all the muscular atrophy models because the ‘sarcopenia’ means the ‘aging-related muscular atrophy’. Therefore, when judged comprehensively, selecting an animal with an extreme-value after evaluating skeletal muscle mass and grip strength, etc. in aging rodents is probably the most appropriate method in sarcopenia research.

As mentioned above, using the aging rodent in the dictionary sense of the word “sarcopenia” may be the most desirable, but there is always a different perspective. In a variety of studies dealing with sarcopenia, muscular atrophy is also an important part of studying biological and molecular mechanisms, so choosing an aging rodent may not be the best option. Therefore, what is ultimately important is to rationally choose the animal model appropriate for research purpose.

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Conflict of interest
No potential conflict of interest relevant to this article was reported.

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