REVIEW

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Mouse models of sarcopenia: classification and evaluation

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Abstract

Sarcopenia is a progressive and widespread skeletal muscle disease that is related to an increased possibility of adverse consequences such as falls, fractures, physical disabilities and death, and its risk increases with age. With the deepening of the understanding of sarcopenia, the disease has become a major clinical disease of the elderly and a key challenge of healthy ageing. However, the exact molecular mechanism of this disease is still unclear, and the selection of treatment strategies and the evaluation of its effect are not the same. Most importantly, the early symptoms of this disease are not obvious and are easy to ignore. In addition, the clinical manifestations of each patient are not exactly the same, which makes it difficult to effectively study the progression of sarcopenia. Therefore, it is necessary to develop and use animal models to understand the pathophysiology of sarcopenia and develop therapeutic strategies. This paper reviews the mouse models that can be used in the study of sarcopenia, including ageing models, genetically engineered models, hindlimb suspension models, chemical induction models, denervation models, and immobilization models; analyses their advantages and disadvantages and application scope; and finally summarizes the evaluation of sarcopenia in mouse models.

Keywords Sarcopenia; Mouse model; Ageing; Genetic engineering; Hindlimb unloading; Chemical induction

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Introduction

The term 'sarcopenia' was first proposed by the Rosenberg team to describe the decrease in skeletal muscle mass with age.[1] In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) first proposed the widely used comprehensive diagnostic criteria for sarcopenia and recommended that muscle quality, muscle strength, and muscle function should be evaluated simultaneously in the diagnosis of sarcopenia.[2] In 2018, the EWGSOP2 revised the definition of sarcopenia. In the revised version, low muscle strength (the most reliable indicator of muscle function) replaced low muscle mass as the primary parameter of sarcopenia.[3] Previous studies and reviews have also shown that muscle strength is an appropriate tool for screening sarcopenia and dynapenia.[4] For low physical activity or disability, the number and intensity of association of low muscle strength is greater than that of low muscle mass.[5] As grip strength cannot be used as a representative of overall muscle strength, the measurement of muscle strength cannot be limited to grip strength.[6] Then, the Asian Working Group for Sarcopenia (AWGS) proposed the concept of 'possible sarcopenia', which refers to the decrease in muscle strength and/or body function.[7] It is also recommended that lifestyle interventions be carried out and related health education offered for patients with sarcopenia in community primary medical

institutions and that patients be encouraged to transfer to hospitals for diagnosis. At present, the prevalence data of sarcopenia vary greatly among different regions and races. According to the data published by the AWGS, the prevalence of sarcopenia in the elderly in Asia is approximately 5.5–25.7%.[7] According to the data of the EWGSOP, the prevalence rate of sarcopenia in European communities is 1–29%, and that in nursing homes is 14–33%[8]; some studies suggest that the prevalence of sarcopenia is as high as 15% in elderly people over 65 years old and 50% in elderly people over 80 years old.[9] In the USA, data are obtained by using different diagnostic criteria for sarcopenia: the prevalence of sarcopenia in women ranges from 2.5% to 27.2%, and that in men ranges from 3.1% to 20.4%.[10] Bouchard *et al*.[11] conducted an epidemiological survey on people aged 68–82 in Canada, which showed that the prevalence of sarcopenia was 38.9% in men and 17.8% in women. According to a survey in the USA in 2019, the total hospitalization cost of patients with sarcopenia was approximately 40.4 billion US dollars, which represents a huge economic burden on the health care system of the USA.[12] In addition, data from the UK[13] and the Czech Republic[14] also show that muscle weakness (including sarcopenia) can lead to an increase in direct medical costs. In a systematic review of 14 studies, patients with sarcopenia were found to have higher medical costs, despite some confounding factors.[15] Sarcopenia increases the risk of disability and death in the elderly. With the expansion of the ageing population and the substantial increase in medical cost burden, a great economic burden is shouldered by patients' families and society.

It is well known that sarcopenia is a chronic disease of the elderly that takes years to develop in humans, which makes research time-consuming and expensive.[16] Moreover, there are serious ethical problems in the use of patients with sarcopenia as test subjects, so treatment-related research must be carried out on animal experimental subjects, and only after it is confirmed to be safe and effective can it be carried out in humans. Experimental animals, such as mice, rats, rabbits, pigs, cats, dogs, and monkeys, are not only highly homologous with humans in genetic composition but also have organs and systems similar to those of human beings that perform their physiological functions in roughly the same way. At the same time, they also suffer from various diseases similar to human diseases.[17] Therefore, animal models can be used to simulate human physiological activities and the occurrence and development of diseases. To better understand the physiological and biochemical pathways of sarcopenia, some animal models have been established worldwide. Although these models can help us understand the occurrence of sarcopenia, they are still not perfect.[18]

Therefore, to better explore the molecular mechanism of sarcopenia and test the effectiveness of candidate interventions, the selection of reasonable animal models is particularly important. In this paper, we will review the different mouse models of sarcopenia, summarize the progress in the pathophysiology of sarcopenia, and evaluate the different therapeutic strategies. In addition, we will evaluate the advantages and disadvantages of each model and determine their effectiveness in studying the pathophysiology of sarcopenia and evaluating treatment. We will also summarize the evaluation methods adopted with respect to whether the mouse model truly simulates sarcopenia.

The importance of mouse models of sarcopenia

Mice are the most commonly used model organism in human disease research and are also commonly used as the preferred mammalian model for genetic intervention and drug intervention against muscle degeneration.[19] Mouse models have been widely used to gain insight into the underlying mechanisms of many diseases, to explore the efficacy of candidate drugs, and to predict the response of patients. Many different kinds of animals have been used to design animal models of sarcopenia, such as rodents (rats, mice, and guinea pigs), *Drosophila*, *Caenorhabditis elegans*, and zebrafish.[18,20] Most studies of sarcopenia use mice as animal models for several reasons. First, and most importantly, the ageing process of humans is similar to that of mice, which enables the mouse model to simulate the ageing process of humans in a relatively short time.[21] Although mouse ageing occurs in a condensed time frame, many developmental milestones are conserved.[22] There are obvious similarities in cell biology and tissue and system biology between aged mice and aged humans.[23] At the biochemical and histological levels, biopsies of the lens, liver, thymus, skin, and muscle of adult mice can hardly be distinguished from those of adult humans.[24] Humans and mice are also highly similar at the gene level. Ninety-nine per cent of human genes exist in mice, and the gene homology is as high as 78.5%; 93% of the regional gene sequence of the mouse genome is the same as that of humans.[25] Moreover, compared with other animals, the technology of mouse genome transformation is very mature.[26] Second, mice have a rapidly developing musculoskeletal system, and mice are relatively inexpensive, which makes them an economic choice for large sample size studies.[27] Various groups have used mouse models of muscle stem cell depletion to examine the contribution to sarcopenia-related phenotypes.[28–31] Among them, Liu *et al*.[31] showed that deficiencies in muscle stem cell fate and postsynaptic myogenesis provide a cellular basis for age-related neurological junction degradation and associated skeletal muscle degradation. Rapid and economical cultivation of a large number of available samples makes mice an ideal choice for studying the pathophysiology and progression of sarcopenia. Two of the most significant characteristics of animal models of

human diseases are the accuracy of their aetiology (it uses a physiologically relevant method of disease induction) and its performance (it can summarize the characteristics of human diseases).[32] In this regard, the mouse model of sarcopenia has corresponding excellent performance. Therefore, the mouse model can provide a preliminary research basis for drug intervention and various exercise therapy interventions for sarcopenia.

However, the mouse model of sarcopenia also has some shortcomings, including differences in immune system activity, metabolic function, stress response, anatomy, and physiology.[33] There is inherent variability in the composition of fibre types in human muscle in terms of fibre type proportion and fibre size.[34] Senescence, including sarcopenia, is associated with a decrease in muscle fibre number along with a reduction in the size of Type II but not Type I fibres.[35] However, mouse muscle is mainly composed of Type II (fast muscle) fibres.[36] Moreover, the composition of human skeletal muscle fibres is different from that of mice. The heterogeneity in fibre types needs to be taken into account when extending the results of mouse models to humans.

Classification of mouse models of sarcopenia

Aged model

Because ageing is the main risk factor for most musculoskeletal diseases (including osteoarthritis, osteoporosis, and sarcopenia), ageing mouse models have been widely used in the study of sarcopenia.[37] For the forms of ageing, natural ageing, high-fat diet-induced ageing, and accelerated ageing mouse models are commonly used. The life expectancy of mice is approximately 24 months; 3-month-old mice are equivalent to 20-year-old humans, and 18- to 24-month-old mice are equivalent to 56- to 69-year-old humans.[38] Therefore, most natural ageing mice are 18 months old or older. Moreover, compared with 18-month-old mice, more studies have used mice after 18 months of age. In addition, according to previous studies, biomarkers associated with ageing are mainly detected after at least 18 months.[25] Kim *et al*.[39] found that grip strength, exercise endurance, muscle volume, and muscle mass of 18-month-old mice were significantly lower than those of 10-week-old C57BL/6J mice, indicating the presence of sarcopenia in this model. Subsequently, they used 18-month-old C57BL/6J mice to determine the inhibitory effect of 5,7-dimethoxyflavone (DMF) on sarcopenia, suggesting that DMF may have a potential role in the development of sarcopenia.[39] More studies have been conducted on mice with sarcopenia after 18 months of age. In a study of 10-, 16-, 21-, and 25-month-old C57BL6/6J male mice, it was found that compared with 10-month-old mice, the weight of hindlimb muscle of 25-month-old mice was lower and the daily activities, muscle grip strength, and maximum muscle strength *in vitro* were significantly reduced.[40] The results showed that natural ageing of 25-month-old C57BL/6J mice was a reasonable model for the study of sarcopenia (defined as low muscle mass, strength, and function). Some studies have demonstrated that 5-aminolevulinic acid (ALA) can be used to treat sarcopenia and glucose intolerance by activating muscle mitochondria.[41] Of course, there are also natural ageing mouse models of other ages, such as 27 months old,[42] 88–96 weeks old,[43] 22 months old,[44,45] and 24 months old.[46] For sex selection, most studies tend to choose male mice because male mice live longer than female mice and high hormone variation can be avoided.[47] Compared with male mice, female mice secrete more oestrogen (even old female mice).[48] However, oestrogen can protect female mice from adipocyte hypertrophy, oxidative stress, and inflammation in adipose tissue, the latter of which is the pathogenic factor of sarcopenia.[49] Although there is controversy, oestrogen is a potential treatment for sarcopenia.[3,16] However, the use of female mice may increase the modelling time of a sarcopenia mouse model, and male mice are more vulnerable to diet-induced obesity than female mice.[50] This gives male mice a natural advantage as a high-fat diet-induced sarcopenia mouse model. Because the natural ageing mouse model can reproduce the ageing process to the greatest extent, it may be the most suitable for the study of ageing-related sarcopenia.

As the natural ageing mouse model requires a lot of time to build, an increasing number of researchers choose the compound model method to shorten the sarcopenia modelling time. A high-fat diet can be considered an ageing accelerator. An increasing number of studies have found that a high-fat diet is an important risk factor for sarcopenia. Elderly patients with long-term high-fat diet-related obesity and type 2 diabetes are more likely to have muscle loss and muscle strength decline than their peers.[51] The reason why older animals fed a high-fat diet can be used to study sarcopenia is that fat consumption has been proven to be a constant risk factor for the development of obesity and age-related muscle consumption.[52] Lee *et al*.[53] also found that in the obese mouse model induced by a high-fat diet, fat accumulation between muscles of obese mice decreased and muscle mass was lost. Hu *et al*.[54] found that in a high-fat feeding model, not only did the muscle regeneration ability of stem cells decrease but also the process of muscle cell differentiation into myotubes was damaged. Although some studies have shown that a high-fat diet for 13 weeks does not aggravate age-related bone or muscle decline in C57BL/6J mice,[55] the results are not applicable to ageing mouse models considering that they are selected from 20-week-old mice. Mice fed a high-fat/high-sucrose diet will have rapid metabolic changes, including obesity,

hyperleptinaemia, lack of physical activity, and dysglycaemia. Long-term feeding may lead to sarcopenia.[56] Studies on high-fat diets accelerating ageing in mice are rarely used because the observation indexes of experimental results may be affected by changes in food intake and diet composition. However, because a high-fat diet can accelerate sarcopenia and cause obesity at the same time, mouse models of sarcopenia accelerated by a high-fat diet can be an ideal choice for animal models of sarcopenic obesity.

Another accelerated ageing model is the senescence-accelerated prone (SAMP) mouse model, which is characterized by accelerated ageing, a shortened life span, and a healthy life span.[57] The senescence accelerated mouse (SAM) is an accelerated ageing model that was established by selective inbreeding of AKR/J strain mice donated by Jackson Laboratory in 1968.[58,59] SAMs can be divided into SAMPs and senescence-resistant inbred strains (SAMRs), which are often used as animal models of ageing acceleration and age-related diseases.[60,61] Compared with the SAMR strain, the SAMP strain showed a faster ageing process and shorter life span and showed earlier onset and faster progression of age-related pathological phenotypes, similar to human senile diseases.[59] The age-related pathological phenotypes of SAMPs in the ageing process are similar to those observed in the elderly, such as senile/secondary amyloidosis, contracted kidney, and senile osteoporosis.[62] SAMP8 is the most commonly used accelerated ageing mouse model in the study of sarcopenia. Compared with SAMP6 and SAMR1 (senescence-accelerated resistant mice 1), the typical characteristics of muscle ageing of SAMP8 mice appear at a relatively young age, which is almost twice as fast as other models.[57] Moreover, compared with SAMR1, SAMP8 can be used as an animal model to study
osteoporotic fracture healing in the presence of sarcopenia.[63] SAMP8 mice at 8 months old are considered to be in the early stage of sarcopenia, while SAMP8 mice at 10 months old may be in the sarcopenia stage.[64] Therefore, when SAMP8 mice are used to study sarcopenia, they are mostly used at 8 months of age and later. Many studies have used SAMP8 mice to validate the effect of exercise intervention on sarcopenia and to assess its intervention effect.[65,66] For example, Takigawa *et al*.[65] used the SAMP8 model to test the effect of long-term exercise on the prevention of sarcopenia, and the results showed that long-term spontaneous physical exercise may help to recover from ageing-related sarcopenia. In addition to exercise intervention, SAMP8 mice were also used to study and evaluate the efficacy of new drugs for sarcopenia.[67–69] Moreover, in SAMP8 mice induced by a high-fat diet, green tea extract has an inhibitory effect on muscle mass reduction.[70] Of course, other strains of SAMP mice can also be used in the study of sarcopenia, such as SAMP6,[71] SAMP10,[72] and SAMP1.[73] SAMPs may not represent sarcopenia caused by ageing, so they can only be used to study the effects of various interventions on sarcopenia and not to study the pathophysiological changes of sarcopenia. The ageing mouse model of sarcopenia and its research findings are shown in *Table* 1.

Genetically engineered models

Many genetically engineered mouse models are also designed to accelerate ageing or muscle ageing, so they can be used in the study of sarcopenia. Most studies have used knockout mice. For example, in the IL-10 knockout [IL-10 (−/−)] ageing mouse model, microarray analysis of gene expression in skeletal muscle showed that many of the 125 differentially expressed genes between 50-week-old IL-10 knockout and wild-type C57BL/6 mice were related to mitochondrial metabolism and apoptosis.[74] IL-10(−/−) mice also showed mitochondrial dysfunction, with a low adenosine triphosphate synthesis rate in skeletal muscle and a high level of damaged mitochondria.[75,76] Homozygous deletion of the gene for the anti-inflammatory cytokine IL-10 can promote the expression of NF-κB inflammatory mediators, so it can simulate the typical characteristics of human weakness, muscle weakness, inflammation, physical function decline, and overall activity reduction. Chronic inflammation is one of the important mechanisms of sarcopenia. The IL-10(−/−) mouse model can be used to study the pathogenesis of sarcopenia in the presence of inflammation. Another commonly used gene knockout mouse model is the copper zinc superoxide dismutase [CuZnSOD (SOD1)] knockout Sod1 (−/−) mouse, which shows many phenotypes of sarcopenia at the age of 5 months.[77,78] Sod1(−/−) mice show high levels of oxidative damage and the characteristics of normal ageing muscles in an accelerated manner and show weakness and destruction of neuromuscular junctions.[79] Ahn *et al*.[80] showed that neuron-specific expression of CuZnSOD could reverse the production of free radicals in the skeletal muscle of the Sod1(−/−) mouse model and prevent muscle atrophy. These results further support the feasibility of using *in vivo* redox status assessment in the development of pathological processes such as sarcopenia. Bhaskaran *et al*.[81] found that neuron-specific deletion of CuZnSOD was enough to cause the loss of motor neurons in young mice, but damage to neuromuscular junctions, muscle atrophy, and weakness were not obvious until middle age. It is suggested that we should choose middle-aged and older mice when selecting Sod1(−/−) mice. In addition, Asnsd1(−/−),[82] MIP(−/−),[83] HtrA2$^{mnd2(-/-)}$,[84] and NLRP3(−/−)[85] knockout mice have also been used to study the pathogenesis of and therapeutic interventions for sarcopenia.

Gene overexpression mouse models, such as transgenic mice with TNF-alpha overexpression (TNF-Tg mice), develop sarcopenia from adolescence to adulthood as they age. Pharmacological inhibition of TRAF6 signal transduction in skeletal muscle during ageing can treat/prevent sarcopenia

Table 1 The ageing mouse model of sarcopenia and its research findings

Aged model	Strain	Mouse age[a]	Key findings	References
Natural ageing	C57BL/6J	18 m	Studies suggest that DMF may have a potential inhibitory role in the development of sarcopenia.	39
	C57BL/6J	25 m	The results showed that natural ageing of 25-month-old C57BL/6J mice were reasonable models for the study of sarcopenia; Studies have demonstrated that ALA can be used to treat sarcopenia and glucose intolerance by activating muscle mitochondria.	40,41
	C57BL/6	27 m	The coffee treatment had a beneficial effect on age-related sarcopenia.	42
	—	22–24 m	Sarcopenia is associated with complex changes in mitochondrial morphology that could interfere with mitochondrial function and mitophagy.	43
	C57BL/6J	22 m	Myostatin inhibition can be treated as a potential therapeutic strategy for ageing-associated sarcopenia and insulin resistance. Testosterone reverses sarcopenia through stimulation of cellular metabolism and survival pathway, together with inhibition of death pathway.	44,45
	C57BL/6J	24 m	The uphill treadmill HIIT exercise sessions were well tolerated by aged mice and they led to performance gains.	46
HFD-induced ageing	C57BL/6	9 + 5 m HFD	High-fat diet appeared sufficient to generate myofibre atrophy in mice that are at an age preceding prevalent sarcopenia.	53
	C57BL/6	6 w + 8 m HFD	Insulin resistance impairs muscle regeneration by preventing myofibre maturation.	54
	C57BL/6J	8 w + 15 m HFHSD	Long-term HFHSD feeding may lead to sarcopenia	56
Accelerated ageing	SAMP8	6–10 m	SAMP8 can be used as an animal model to study osteoporotic fracture healing in the presence of sarcopenia.	63
	SAMP8	6–10 m	SAMP8 animals at Month 8 should be at pre-sarcopenia stage while Month 10 at sarcopenia stage.	64
	SAMP8	25 w	The results showed that long-term and spontaneous physical exercise may help to recover from ageing-related sarcopenia.	65
	SAMP8	27 w	Long-term habitual exercise attenuates muscle mass and strength decline, possibly through maintenance of muscle protein synthesis and mitochondrial maintenance.	66
	SAMP8	28 w	LPPS23 extenuates sarcopenia progression during ageing.	67
	SAMP8	32 + 8 w oligonol	Oligonol alleviated sarcopenia by regulating pathways involved in protein turnover and mitochondrial quality.	68
	SAMP8	7 + 31 w a normal diet supplemented with GJG	GJG has a therapeutic effect against sarcopenia.	69
	SAMP6	20–60 w	The mean muscle fibre SDH activities and CSAs in SAMP6 decreased at 60 weeks.	71
	SAMP10	25 w + 4 m Exercise train	Long-term exercise train modulates muscle-regenerative actions in a SAMP10 model.	72
	SAMP1	10 + 10 w SM-lipo	The SM-lipo are well absorbed into the body and improve muscle weakness caused by senescence.	73

ALA, 5-aminolevulinic acid; DMF, 5,7-dimethoxyflavone; GJG, Go-sha-jinki-Gan; HFD, high-fat diet; HFHSD, high-fat/high-sucrose diet; HIIT, high intensity interval training; LMHFV, Low-magnitude high-frequency vibration; LPPS23, *Lactobacillus paracasei* PS23; SAMP, senescence accelerated mouse prone; SM-lipo, sphingomyelin-based liposomes.
[a]m, months; w, weeks.

associated with age and rheumatoid arthritis by preventing TNF-alpha-induced proteolysis and inhibiting muscle fibre regeneration.[86] Yoshida *et al.*[87] established a functional acquisition model of age-related sarcopenia by transgene expression of (pro)renin receptor [(P)RR] under the control of the CAG promoter. It was observed that (P)RR-Tg mice died early, showing the histological characteristics of muscular atrophy with sarcopenia. All of these findings provide us with

a new type of sarcopenia mouse model, which allows researchers to choose the most reasonable mouse model according to their research purposes.

The accumulation of mitochondrial DNA (mtDNA) mutations is believed to contribute to mitochondrial dysfunction and may shorten life span. mtDNA mutant mice are a mouse model with proofreading-defective mtDNA polymerase γ (POLG) that has been proven to have a premature ageing phenotype, including sarcopenia.[88] Studies have shown that mtDNA mutation may directly affect the transcription and translation of the electron transport chain (ETC) complex and may prevent the assembly of the functional ETC complex in the mitochondrial inner membrane.[89] However, deficiency of the ETC complex can lead to a decrease in oxidative phosphorylation without increasing oxidative stress and can eventually lead to skeletal muscle cell apoptosis and sarcopenia.[89] mtDNA mutation in these pathways may be the cause of sarcopenia. Moreover, POLG mice also showed an increased mtDNA mutation rate, mitochondrial dysfunction, and premature ageing phenotypes (including sarcopenia), such as weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spin), osteoporosis, and sarcopenia.[90,91] This evidence suggests that the POLG mouse model may be a useful model for sarcopenia when the mtDNA mutation load increases. Most of the studies on sarcopenia and mtDNA mutations are carried out in animal models (rats, mice, etc.). In human sarcopenia patients, there is no study on mtDNA mutations, and only a few studies have examined the relationship between mtDNA deletion and sarcopenia.[92,93] Some disadvantages of this model include its high cost, large sample size, and considerable time and work.

In general, compared with SAMP8 mice, genetically engineered mice can be used to establish a sarcopenia mouse model faster, but the disadvantage is that genetically engineered mice usually do not have the appearance of typical characteristics observed under normal ageing conditions. Moreover, in mice, only one or two genes can be knocked out at a time, so it is difficult to understand gene interactions. There are also problems with accuracy in genetic engineering. The classification and research findings of genetically engineered mouse models of sarcopenia are shown in *Table* 2.

Chemical or dietary induction models

Another common model to induce sarcopenia in mice is the chemically induced model. In these models, substances that induce muscle ageing are injected directly into or perfused into the mouse models to be induced. These compounds undergo a series of complex processes in mice and eventually induce sarcopenia. Dexamethasone is a synthetic glucocorticoid with anti-inflammatory, anti-allergic, and anti-shock effects. However, long-term injection of dexamethasone can cause side effects such as weight gain, muscle atrophy, and cardiac accumulation of fat.[94] Interestingly, dexamethasone-induced muscle atrophy results mainly from a decrease in Type II muscle fibres, which is consistent with the muscle atrophy caused by ageing.[95] Chiu *et al*.[96] showed that dexamethasone can successfully establish a rat model of sarcopenia. At present, dexamethasone is widely used to establish muscle atrophy mouse models, and all of them are in young rats. Although dexamethasone can cause muscle degeneration in young rats, it also causes weight loss.[97] This may not be consistent with the phenomenon wherein a large proportion of elderly people with muscle wasting syndrome have a higher weight than healthy elderly people (i.e. oligomyxosis).[98] In view of the fact that dexamethasone can cause muscle atrophy, we consider that dexamethasone can be injected subcutaneously into mice to induce sarcopenia. Of course, the age of the mice, the dosage, and the days of administration still need further study.

The ageing mouse model established by subcutaneous injection of D-galactose may also be used for the study of sarcopenia. The principle of establishing an ageing model by subcutaneous injection of D-galactose is the metabolic theory of ageing, that is, it affects the functional metabolism of body cells (oxidative stress, inflammation, etc.) to reduce the function of some important enzymes related to it and induce experimental animals to show biochemical changes similar to natural ageing.[99] At present, in the field of ageing and anti-ageing at home and abroad, the D-galactose subacute ageing model, as an ideal mouse model, has been widely used in research on senile diseases, anti-ageing measures, and anti-ageing drug screening.[100] Chen *et al*.[101] used D-galactose to induce senescence of C2C12 myoblasts in mice and further concluded that targeted sarcolipin may be a new therapeutic strategy to alleviate muscle fibrosis associated with sarcopenia. Although there is no report on a D-galactose-induced sarcopenia mouse model, D-galactose is also a potential inducer of sarcopenia, similar to dexamethasone. It can be injected subcutaneously. This method can not only reduce stimulation to animals and avoid unnecessary death but can also increase the degree of ageing gradually, which is more suitable for studying the natural ageing process of elderly individuals.

The mouse model of sarcopenia induced by diet can also help us to study the coexistence of sarcopenia and other diseases. To determine the effect and molecular basis of colitis-associated sarcopenia, Saul *et al*.[102] treated 10-week-old male C57BL/6N mice with drinking water containing 0.75% dextran sodium sulfate (DSS) for 14 days to establish a mouse model of acute colitis coexisting with sarcopenia. It was found that after DSS treatment, quadriceps femoris and gastrocnemius muscle were reduced, creatine kinase, a marker of muscle injury, was slightly increased, and the size of muscle fibres (Type I and Type II fibres) was

Table 2 The classification and research findings of genetically engineered mouse models of sarcopenia

Genetically engineered models	Strain	Mouse age[a]	Key findings	References
Gene knockout	IL-10(−/−)	23 m	Energetic abnormalities may contribute metabolism to skeletal muscle weakness in this geriatric syndrome.	75
	IL-10(−/−)	22–24 m	Ageing in IL-10(−/−) is associated with reduced skeletal muscle mitochondrial death signalling and altered formation of autophagosomes.	76
	Sod1(−/−)	4–10 m	ROS-associated motor nerve terminal dysfunction is a contributor to the observed muscle changes in Sod1(−/−) mice.	77
	Sod1(−/−)	1–9 m	Mitochondrial hydroperoxide generation is elevated prior to muscle atrophy.	78
	Sod1(−/−)	18–22 m	Sod1(−/−) mice display characteristics of normal ageing muscle in an accelerated manner.	79
	Sod1(−/−)	8–11 m	Studies have confirmed the feasibility of using *in vivo* redox status assessment in the progression of pathological processes such as sarcopenia.	80
	Sod1(−/−)	18–24 m	Reduced expression of the superoxide scavenger CuZnSOD in neuronal tissue can lead to later disruption of the neuromuscular junction and muscle atrophy and weakness in aged mice.	81
	Asnsd1(−/−)	8–11 m	Asnsd1(−/−) mice demonstrated severe muscle weakness.	82
	MIP(−/−)	4–20 m	Studies suggest a putative role for MIP in the decline of muscle function during ageing.	83
	$HtrA2^{mnd2(-/-)}$	5–8 m	Loss of HtrA2/Omi protease activity induces mitonuclear imbalance via differential regulation of mitochondrial biogenesis in sarcopenia.	84
	NLRP3(−/−)	3, 12, 24 m	Studies have confirmed the NLRP3 inflammasome implication in muscular ageing and sarcopenia onset and progression.	85
Gene overexpression	TNF-Tg	10 m	Studies suggest that pharmacologic inhibition of TRAF6 signalling in skeletal muscles during ageing could treat/prevent age-related and RA-related sarcopenia by preventing TNFα-induced proteolysis and inhibiting of muscle fibre regeneration.	86
	(P)RR-Tg	25 m	The present study demonstrates the use of (P)RR-Tg mice as novel sarcopenia models.	87
mtDNA mutations	MCKPGC-1αMut	10 m	The results suggest that increased muscle PGC-1α expression is able to improve some premature ageing phenotypes in mutant mice without reversing the accumulation of mtDNA mutations.	88
	D257A	11,13 m	These findings demonstrate that mutations in mtDNA can be causal in sarcopenia by affecting the assembly of functional ETC complexes.	89
	D257A	8–15 m	Muscles from mtDNA mutant mice have been found to exhibit higher levels of mitochondrial fission and autophagy, which may contribute to the sarcopenic phenotype observed in premature ageing.	90

ETC, electron transport chain; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; ROS, reactive oxygen species; TRAF6, TNF receptor-associated factor 6.
[a]m, months.

reduced, which all supported the successful induction of the sarcopenia model.[102] It was finally concluded that DSS-induced colitis can lead to severe muscle loss in mice, so it is an appropriate model for inducing inflammation-related sarcopenia. Because sarcopenia is the loss of muscle mass and strength due to ageing or secondary to chronic diseases such as chronic liver disease, some studies have used 4-month-old male C57BL/6 mice supplemented with the hepatotoxin 5-diethoxycarbonyl-1,4-dihydrocolidine (DDC) diet and fed for 6 weeks to induce a chronic liver disease -related sarcopenia model.[103,104] Sato *et al.*[105] fed 7-week-old male C57BL/6 mice 0.2% adenine (Wako) for 6 weeks to induce a mouse model of uraemia and sarcopenia. The model induced by diet is non-invasive, economic, and convenient. However, the mechanism of sarcopenia is complex, so it is not relevant for studying sarcopenia alone. The authors believe that in addition to the abovementioned diseases, there must be many other diseases worthy of study when they

coexist with sarcopenia. It is hoped that different mouse models of sarcopenia coexisting with other diseases can be designed according to the research needs in the future.

Hindlimb suspension

The animal model of hindlimb unloading was developed by the Ames Research Center of National Aeronautics and Space Administration (NASA) in the mid-1970s and has been proven to be able to simulate weightlessness.[106] Currently, it has been accepted as a method for adaptation to reduced skeletal muscle use.[107] In this model, the hindlimbs of rodents are raised to produce a 30° head down tilt, resulting in head fluid displacement and avoiding hindlimb weight-bearing.[108] Hindlimb suspension (HLS) is a commonly used method for modelling sarcopenia in elderly mice, and it has been proven that it can simulate patients with clinical sarcopenia.[106,109] The principle of HLS is that it can promote muscle atrophy by destroying a variety of cellular processes, such as inducing oxidative imbalance, mitochondrial dysfunction, intercellular interaction, and abnormal protein synthesis/degradation.[110] In addition, ageing and/or disuse (such as HLS) can increase the oxidative stress response in the muscle tissue of rodents.[111] It is well known that increased oxidative stress is one of the important pathogeneses of sarcopenia. Most sarcopenia mouse models can be induced by HLS in 2 weeks.[108] When Anderson *et al*.[112] studied the effect of isosorbide dinitrate on muscular atrophy and sarcopenia, they unloaded the hindlimbs of mice for 18 days to simulate the sarcopenia model. Finally, they found that isosorbide dinitrate treatment may reduce atrophy and metabolic changes, prevent disuse, and counteract/prevent age-related sarcopenia. HLS mouse models have also been used in combinations with hindlimb exercise force tests, cage wheel running, and *in vitro* muscle electrophysiology to evaluate sarcopenia and muscle performance. The results show that the combination of these three methods seems to provide an effective and complementary method to measure muscle performance and functional differences.[113] With the aggravation of the ageing population, disuse sarcopenia has received increasing attention. Various clinical complications caused by disuse sarcopenia caused by long-term bed rest after surgery have become an important clinical problem. The present evidence suggests that the mouse HLS sarcopenia model can be used as a model of sarcopenia unrelated to exercise rather than that related to skeletal muscle ageing. The use of elderly animals and short-term HLS may be an effective model for sarcopenia with ageing-related inactivity.

Denervation model

The growth, development and normal function of skeletal muscle depend on the innervation and regulation of motor nerves. Denervation of skeletal muscle caused by traumatic peripheral nerve injury, disease, drug intervention, and ageing can reduce muscle function and lead to muscle atrophy.[114] Denervated atrophy of skeletal muscle is due to the loss of innervation of the muscle, which leads to the disuse and atrophy of skeletal muscle. Therefore, denervated atrophy of skeletal muscle is also called dystrophic atrophy or disuse atrophy. Denervated models have been widely used in the study of muscle atrophy and muscle loss.[115,116] Methods of denervation include tibial nerve transection and sciatic nerve stage truncation.[20] In the process of studying the expression and function of the inflammatory mediator angiopoietin-like protein 2 (ANGPTL2) in the skeletal muscle of ageing mice, a model of sarcopenia was established by resecting 0.5–1 cm of the sciatic nerve in the left hindlimb of 12-week-old mice without resection of the contralateral sciatic nerve.[117] It has been found that the upregulation of ANGPTL2 in skeletal muscle cells accelerates muscle atrophy and the decreased expression of Angptl2 in these tissues induced by exercise may partly explain how exercise training can prevent sarcopenia.[117] Because the establishment of the denervated mouse model requires more precise surgical procedures, the denervated rat model has also been used for the study of sarcopenia. Kinoshita *et al*.[118] induced a sarcopenia animal model by sciatic nerve axotomy to study the therapeutic effect of the antioxidant *N*-acetyl-L-cysteine on sarcopenia and verified that *N*-acetyl-L-cysteine can be used as a potential candidate drug for sarcopenia. However, the application scope of the denervated mouse model of sarcopenia is limited, and it is only suitable for research on neurogenic sarcopenia (including nerve injury, diseases, and drugs).

Immobilization

Skeletal muscle atrophy and sarcopenia are very serious problems in patients with limb limitation due to surgery (e.g. arthrodesis), joint pathology (e.g. arthralgia) or simple plaster fixation. Disuse related to long-term bed rest or disease can also induce sarcopenia.[9] Plaster fixation is the most commonly used model for the study of muscle atrophy because it simulates plaster fixation after fracture and wrapping of the leg with a plaster bandage or spiral thread, and it can be fixed for a long time.[109] In addition to plaster fixation, cast immobilization can also be used, and the model can be used to evaluate muscle loss.[119] Many immobilization methods are used in the mouse model of sarcopenia, such as the use of surgical suture nails to fix the ventral part of the hindlimb to the distal part of the leg,[120] the use of a 1.5 mL microfuge tube, metal paper clip, and Velcro loops for posterior limb immobilization,[121,122] or the use of AUTOCLIP 9 mm wound clips and the CLIP applier to fix the position of the hip, knee, and ankle joints in maximum flexion to the back skin and to fix the midfoot of the left hindlimb to the back skin.[123] Some

studies even used fixation combined with hindlimb unloading to exaggerate the muscle loss induced by no load to build a mouse model of sarcopenia.[124] The immobilization time ranged from 1 to 3 weeks, depending on the researchers' experimental design.[120,122–124] Compared with traditional plaster fixation, surgical staplers and other immobilization models are simple and can be carried out in a few seconds, which makes it possible to carry out high-throughput analysis with high reproducibility. In addition, the time for mice to adapt to immobilization is short. The immobilization model can be used to simulate the occurrence and development of sarcopenia in long-term bedridden populations. Dietary induction, HLS, denervation, and immobilization models of sarcopenia and their research findings are shown in *Table* 3.

All the models discussed earlier have been proven to be effective in the study of sarcopenia, and some progress has been made in the treatment of sarcopenia using these

Table 3 Dietary induction, HLS, denervation and immobilization models of sarcopenia and their research findings

Other models	Strain	Mouse age[a]	Key findings	References
Dietary induction models	C57BL/6N	10w + 14 d 0.75% DSS	It has been shown that DSS-induced colitis leads to severe muscle loss in mice and therefore is a suitable model to induce inflammation-associated sarcopenia.	102
	C57BL/6	4 m + 6 w (DDC)	The results demonstrate mice with CLD develop sarcopenia involving decreased levels of myofibrillar proteins, increased UPS, and oxidative stress.	103
	C57BL/6J	16 + 6w (DDC)	The data supports the protective role of Ang-(1–7) on the sarcopenia by CLD in mice.	104
	C57BL/6	7 + 6w (0.2% adenine)	The results indicate that indoxyl sulfate is a pathogenic factor for sarcopenia in CKD.	105
Hindlimb suspension	C57BL/6	4 w and 8 w + 18 d HLS	NO-donor treatment has potential to attenuate atrophy and metabolic changes and prevent regulatory changes during disuse and offset/prevent wasting in age-related sarcopenia or space travel.	112
	C57BL/6J	12 + 2 w HLS	The combination of HEFT, cage wheel running and *in vitro* muscle electrophysiology seems to provide effective and complementary methods to measure muscle performance and functional differences.	113
Denervation	C57BL/6	12 w, 52 w, and 78 w (sciatic denervation)	Studies have confirmed that strategies targeting CaVβ1E or GDF5 may be effective in reducing muscle mass loss in ageing.	115
	ICR	5–6 w (sciatic denervation)	Animal studies showed that low-dose alendronate by oral administration ameliorated the muscular malfunction in mouse models of denervation-induced muscle atrophy.	116
	C57BL/ N	12 w (sciatic denervation)	The study is the first to report that ANGPTL2 signalling may accelerate sarcopenia pathologies.	117
Immobilization	C57BL/6	21 w	It was confirmed that losartan may have clinical benefits to combat injury-related muscle remodelling and provide protection against disuse atrophy in humans with sarcopenia.	120
	FVB/N	8–10 w	Activation of mTOR signalling is a viable therapeutic target aims at preventing muscle atrophy during periods of mechanical unloading.	121
	C57BL/6	5 w	The soluble whey protein hydrolysate is a necessary and probable candidate for developing functional food to prevent sarcopenia.	122
	C57BL/6	9 w	They conclude that vitamin D antagonizes immobilization-induced muscle atrophy via VDR expressed in neural crest-derived cells.	123
	C57BL/6J	5 m	The findings suggest that casting exacerbates unloading-induced muscle loss via activation of autophagy.	124

Ang-(1–7), angiotensin 1–7; ANGPTL2, angiopoietin-like protein 2; CaVβ1, CaV1.1 β subunit; CKD, chronic kidney disease; CLD, chronic liver disease; DDC, 5-diethoxycarbonyl-1,4-dihydrocollidine; DSS, dextran sodium sulfate; GDF5, growth differentiation factor 5; HEFT, Hindlimb Exertion Force Test; HLS, hindlimb suspension; NO, nitric oxide; UPS, ubiquitin-proteasome system; VDR, vitamin D receptor.
[a]d, days; m, months; w, weeks.

models, as shown in *Table* 4. However, each model has its own advantages and disadvantages. When deciding which model is most suitable for particular research, we must first consider these advantages and disadvantages and then choose the model that is most suitable for the ongoing research. To choose the most effective and applicable method, we must consider the purpose of the study (focusing on the pathophysiology of the disease and its progress or evaluating the treatment effect), budget, availability of equipment and surgeons, and time requirements. We summarize the different types of models and their uses, advantages, and disadvantages in *Table* 5.

Table 4 Therapeutic studies and results of sarcopenia mouse model

Models used	Treatment	Key findings	References
Natural ageing	DMF	DMF can be used to inhibit sarcopenia via improving protein turnover and mitochondria function.	39
	ALA	ALA can be used to treat sarcopenia and glucose intolerance by activating muscle mitochondria.	41
	Coffee	The coffee treatment had a beneficial effect on age-related sarcopenia.	42
	Anti-myostatin antibody	Anti-myostatin antibody can be treated as a potential therapeutic strategy for ageing-associated sarcopenia.	44
	Testosterone	Testosterone reverses sarcopenia through stimulation of cellular metabolism and survival pathway, together with inhibition of death pathway.	45
	Uphill treadmill HIIT exercise	The uphill treadmill HIIT exercise sessions were well tolerated by aged mice and they led to performance gains.	46
Accelerated ageing	Long-term physical exercise	The results showed that long-term and spontaneous physical exercise may help to recover from ageing-related sarcopenia.	65
	Long-term habitual exercise	Long-term habitual exercise attenuates muscle mass and strength decline, possibly through maintenance of muscle protein synthesis and mitochondrial maintenance.	66
	LPPS23	LPPS23 extenuates sarcopenia progression during ageing.	67
	Oligonol	Oligonol alleviated sarcopenia by regulating pathways involved in protein turnover and mitochondrial quality.	68
	GJG	GJG has a therapeutic effect against sarcopenia.	69
	Green tea extracts	Green tea extracts ameliorate high-fat diet-induced muscle atrophy in senescence-accelerated mouse prone-8 mice.	70
	Long-term ET	Exercise restores muscle stem cell mobilization, regenerative capacity and muscle metabolic alterations via adiponectin/AdipoR1 activation.	72
	SM-lipo	The SM-lipo are well absorbed into the body and improve muscle weakness caused by senescence.	73
Dietary induction	Ang-(1–7)	Ang-(1–7) has a protective role on the sarcopenia by CLD in mice.	104
Hindlimb suspension	Isosorbide dinitrate	NO-donor treatment has potential to attenuate atrophy and metabolic changes, and prevent regulatory changes during disuse and offset/prevent wasting in age-related sarcopenia or space travel.	112
Denervation	Alendronate	Alendronate can be a promising therapeutic strategy for management of muscle wasting-related diseases and sarcopenia	116
Immobilization	Losartan	It was confirmed that losartan may have clinical benefits to combat injury-related muscle remodelling and provide protection against disuse atrophy in humans with sarcopenia.	120
	Soluble whey protein hydrolysate	The soluble whey protein hydrolysate is a necessary and probable candidate for developing functional food to prevent sarcopenia.	122
	Vitamin D	The low vitamin D status will worsen immobilization-induced muscle atrophy in mice	123

ALA, 5-aminolevulinic acid; Ang-(1–7), angiotensin 1–7; CLD, chronic liver disease; DMF, 5,7-dimethoxyflavone; ET, Exercise train; GJG, Gosha-jinki-Gan; HIIT, high intensity interval training; LPPS23, *Lactobacillus paracasei* PS23; NO, nitric oxide; SM-lipo, sphingomyelin-based liposomes.

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Table 5 Uses, advantages, and disadvantages of different types of sarcopenia mouse models

Model	Usefulness and advantages	Disadvantages
Natural ageing	Suitable for sarcopenia related to ageing Most natural progression No specially trained personnel required No specialized equipment needed	Long progression time Low incidence Variability in severity and onset
HFD-induced ageing	Suitable for obesity related sarcopenia research Relatively short progress time	High cost Greatly influenced by various factors such as diet composition With more complications
Accelerated ageing	Suitable for studying the effect of various interventions on sarcopenia Rapid progression High incidence No specially trained personnel required No specialized equipment needed	High cost Progression not similar to natural progression in humans
Gene knockout	Most rapid progression High incidence With many genes available for editing	High cost Progression not similar to natural progression in humans Possibility of confounding symptoms as a result of genetic alterations
Gene overexpression	Most rapid progression High incidence	High cost Progression not similar to natural progression in humans Specially trained personnel required Specialized equipment needed Possibility of confounding symptoms as a result of genetic alterations
mtDNA mutations	Suitable for studying sarcopenia related to mitochondrial dysfunction High incidence	High cost Progression not similar to natural progression in humans Specially trained personnel required Specialized equipment needed
Dietary induction	Suitable for the study of sarcopenia coexisting with other diseases Relatively short progress time	High cost Progression not similar to natural progression in humans With more complicated mechanism
Hindlimb suspension	Suitable for studying sarcopenia unassociated with exercise Relatively short progress time	Specially trained personnel required Specialized equipment needed Progression not similar to natural progression in humans
Denervation	Suitable for the related research of neurogenic sarcopenia High incidence	Need specially trained surgeon Risk of infection
Immobilization	Suitable for simulating the occurrence and development of sarcopenia in long-term bedridden people High incidence Relatively short progress time	Need specially trained surgeon Risk of infection

HFD, high-fat diet.

Evaluation of mouse models of sarcopenia

As discussed earlier, we can see that there are many types of mouse models of sarcopenia, but many researchers have not evaluated whether the mouse models after modelling meet the criteria of sarcopenia. In the author's opinion, this is very important because the results of studies conducted with unsuccessfully induced sarcopenia models are likely to be wrong and even misleading for subsequent studies. Moreover, these methods are also needed to assess muscle quality, strength, and function when examining the effects of various interventions and treatments on sarcopenia. At present, the diagnosis and evaluation of sarcopenia are mainly based on muscle quality, muscle strength, and function.[49] Therefore, we will review the commonly used evaluation methods of sarcopenia mouse models to provide suggestions for the

evaluation of sarcopenia mouse models. The commonly used evaluation methods of the mouse model of sarcopenia are shown in *Figure* 1.

Muscle strength

Previous guidelines suggested that muscle quality should be evaluated first in the diagnosis of sarcopenia, but the EWGSOP2 in the 2018 consensus regarded weak muscle strength as an important feature of sarcopenia and considered that muscle strength is currently the most reliable measurement index.[3] In the mouse model, various instruments were used to measure the strength of mice.[125] For the sarcopenia mouse model, the first choice is the non-invasive measurement of forelimb gripping force, which can measure the maximum contraction force of the forelimb in the state of autonomous activity. The study by Lauretani *et al*.[126] shows that hand grip strength (forelimb gripping force), lower limb muscle strength, and leg muscle cross-sectional area are closely related. Comprehensive evaluation is a method for diagnosing muscle strength and sarcopenia, and grip strength can be used as the preferred method for diagnosis because of its simplicity. Before the test, the mice were allowed to adapt to the gripping force instrument for 10 min. The forelimbs of the mice were placed on the sensing cross bar of the gripping force instrument. After the forelimbs grasped the cross bar, the tail of the mouse was dragged backward horizontally until the forelimb was released. The maximum gripping force of mice in the process of grasping the rod was automatically recorded by the gripping instrument.[40,68,103] The measurement was repeated several times, and the maximum or average value was recorded as the forelimb grasp force of the mice. In addition to the strength of the forelimbs, we can also measure the grip strength of all four limbs of mice. For example, Huang *et al*.[127] placed a mouse on a

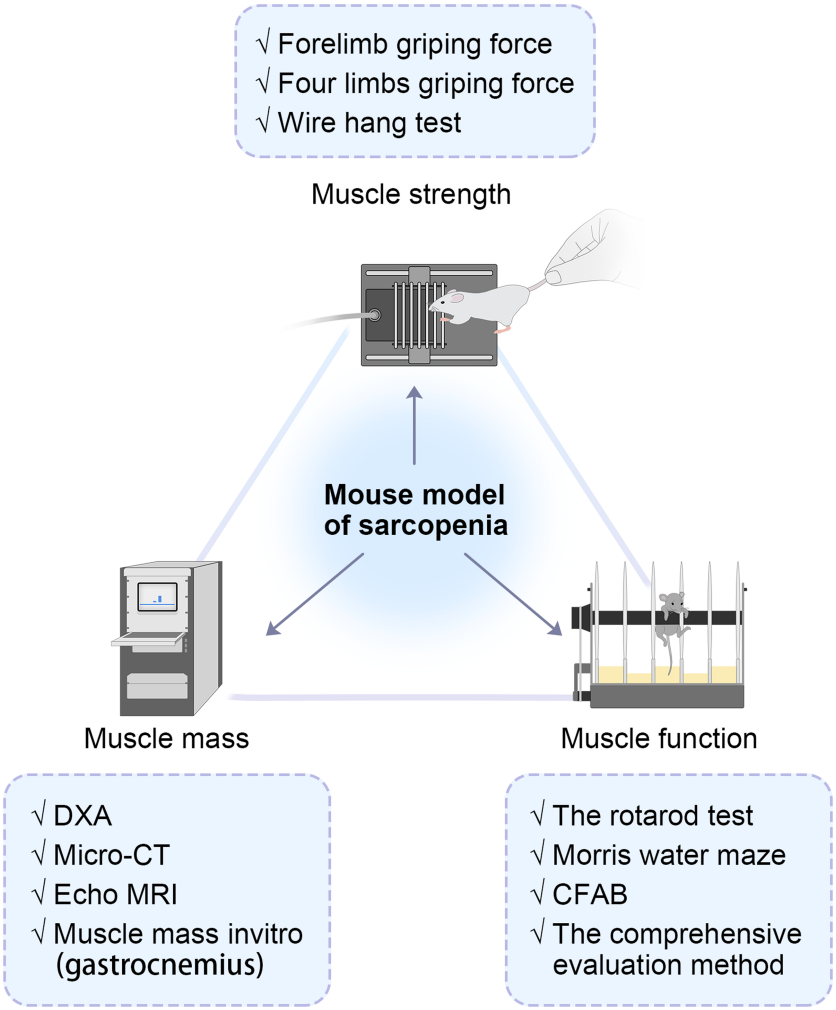

Figure 1 The commonly used evaluation methods of the mouse model of sarcopenia. CT, computed tomography; CFAB, comprehensive functional assessment battery; DXA, dual X-ray absorptiometry; MRI, magnetic resonance imaging.

dynamometer to make all four claws grasp the grid and then slowly dragged the mouse backward until it lost grip strength. During the experiment, the mice were kept in a horizontal position. Of course, the grip strength of mice can be reflected by the wire hang test.[128] It should be noted that the body weight of mice tends to increase with age and the absolute muscle strength is positively correlated with body weight. To exclude the influence of body weight on skeletal muscle strength, it is suggested that grip strength after weight correction should be used as an index to evaluate the skeletal muscle strength of mice.

Muscle mass

Computed tomography (CT), magnetic resonance imaging (MRI), dual X-ray absorptiometry (DXA), or bioelectrical impedance analysis are commonly used in the clinical evaluation of muscle quality in patients with sarcopenia. Among them, CT and MRI can measure skeletal muscle mass most directly and accurately.[129] These methods are also applicable in mouse models, but the cost has to be considered in the study. Non-invasive micro X-ray CT (micro-CT) imaging can be used in combination with special algorithms to detect muscle mass changes in mice.[130] At the same time, accurate and non-invasive longitudinal assessment of mouse skeletal muscle quality can also be achieved by micro-CT based on a 3D u-net deep learning network.[131] Pasetto *et al*. also developed a non-invasive program based on micro-CT without a contrast agent, which can be widely used in disease models characterized by muscle wasting.[132] Mice can also be placed on the DXA scanning platform after anaesthesia, and DXA can be used to measure their fat and muscle mass.[133] It is even possible to directly measure total fat content, muscle mass, free water content, and total body water content through an echo MRI body composition analyser based on MRI.[80] Compared with DXA, echo MRI has the advantages of fast detection, accurate positioning, and no use of radiation in mice. However, unlike humans, a sarcopenia mouse model can be used to directly detect muscle mass *in vitro*. Some studies directly measured the skeletal muscle mass of isolated hindlimbs (tibialis anterior muscle, soleus muscle, plantar muscle, and gastrocnemius muscle) of mice to represent the mass of all muscles.[40] However, most studies mainly choose the mass of the gastrocnemius[64,103,134] or soleus[57] to represent the overall muscle mass. Gastrocnemius muscle is one of the most easily acquired skeletal muscles in mouse limbs, and it is also the most important muscle in lower limb activities. At the same time, the gastrocnemius is also the muscle most affected by ageing in wild-type mice, which is a good representative of skeletal muscle mass and strength.[135] Most of the previous mouse models of sarcopenia used the gastrocnemius as the target muscle to weigh, and it can also be used for the subsequent determination of various biochemical indicators.[64,85,134] In addition, the ratio of gastrocnemius weight to body weight can be considered a diagnostic basis for sarcopenia in mouse models, but the specific diagnosis cut-off point remains to be further studied. More importantly, instruments that can accurately measure the muscle mass of living mice are not common, which limits the application of this method in research.

Muscle function

Compared with the earlier two assessments, muscle function assessment is the most easily ignored. The evaluation of muscle function in patients with sarcopenia is often reflected by the 6 or 4 m walking speed,[136] and for mice, the gait speed can also be evaluated, but the methods used are different. The rotarod test is usually used to indirectly reflect the walking speed of mice.[137] The rod cylinder rotates at a speed of 4 rpm and accelerates to 40 rpm over 5 min. The mouse is placed on the cylinder, and the rotating speed (rpm) is recorded when the mouse falls.[128] Of course, the authors believe that this method is not limited to the measurement of the final rotational speed but can also be used to measure the number of mice falling in the appropriate range of rotational speed to reflect the muscle function of mice. The Morris water maze is commonly used to evaluate the learning and memory ability of mice regarding the spatial sense of position and orientation (spatial location).[138] It has the potential to evaluate muscle function in mice with sarcopenia because swimming can also reflect muscle function to a large extent. In addition to the earlier simple assessments of muscle function, we can also use different combinations to comprehensively evaluate muscle function. For example, Liu *et al*.[128] developed a comprehensive evaluation of the weakness index through grip strength, walking speed, physical activity, and endurance. Graber *et al*.[139] developed a fully validated and tested comprehensive functional assessment battery and proposed that the comprehensive functional assessment battery can be used to determine the relationship between different parameters related to sarcopenia. In addition, some studies have comprehensively evaluated the body function of mouse models of sarcopenia.[127] However, it is worth noting that some mouse models may have cognitive impairment in the process of modelling, so we should exclude a decline in motor ability that is affected by the decline in cognitive ability.

Conclusion

To better understand the pathophysiology of sarcopenia and formulate the corresponding therapeutic intervention strategies, the development of a mouse model of sarcopenia has unique prospects. The modelling methods of sarcopenia mice

include aged models, genetically engineered models, HLS models, chemical-induced or diet-induced models, denervation models, and immobilization models. Each type of modelling method has its own advantages and disadvantages. In the research selection, we must select the appropriate mouse model of sarcopenia according to the research design. Although some progress has been made in the study of sarcopenia mouse models, many potential mechanisms of its action are still unclear. At present, there are no diagnostic criteria for sarcopenia in animal models, which makes the evaluation results of sarcopenia and other intervention methods different from each other. In the future, more research should focus on the development of more sarcopenia mouse models suitable for all kinds of research, and a unified cut-off point standard in the evaluation of sarcopenia mouse models should also be generated.

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Conflict of interest

The authors declare that they have no competing interests.

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