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Muscle mass, structural and functional investigations of senescence-accelerated mouse P8 (SAMP8)

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Abstract: Sarcopenia is an age-related systemic syndrome with progressive deterioration in skeletal muscle functions and loss in mass. Although the senescence-accelerated mouse P8 (SAMP8) was reported valid for muscular ageing research, there was no report on the details such as sarcopenia onset time. Therefore, this study was to investigate the change of muscle mass, structure and functions during the development of sarcopenia. Besides the average life span, muscle mass, structural and functional measurements were also studied. Male SAMP8 animals were examined at month 6, 7, 8, 9, and 10, in which the right gastrocnemius was isolated and tested for ex vivo contractile properties and fatigability while the contralateral one was harvested for muscle fiber cross-sectional area (FCSA) and typing assessments. Results showed that the peak of muscle mass appeared at month 7 and the onset of contractility decline was observed from month 8. Compared with month 8, most of the functional parameters at month 10 decreased significantly. Structurally, muscle fiber type IIA made up the largest proportion of the gastrocnemius, and the fiber size was found to peak at month 8. Based on the altered muscle mass, structural and functional outcomes, it was concluded that the onset of sarcopenia in SAMP8 animals was at month 8. SAMP8 animals at month 8 should be at pre-sarcopenia stage while month 10 at sarcopenia stage. It is confirmed that SAMP8 mouse can be used in sarcopenia research with established time line in this study.

Key words: contractile properties, muscle fiber typing, sarcopenia, senescence-accelerated mouse P8 (SAMP8)

Introduction

Sarcopenia is used to describe the age-induced progressive loss of skeletal muscle mass and muscle strength, as well as poor physical performance [21, 25, 26]. In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) defined sarcopenia as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death [8, 14, 21]. Sarcopenia is divided into 3 stages: pre-sarcopenia, sarcopenia and severe sarcopenia. The

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"pre-sarcopenia" stage is characterized by decrease of skeletal muscle mass with no impact on muscle function or physical performance. At the "sarcopenia" stage, low skeletal muscle mass and strength appear, with or without poor physical performance. The "severe sarcopenia" is defined when low muscle mass, low muscle strength and poor physical performance appear [8].

For pre-clinical investigation on sarcopenia, a good animal model is needed. Generally, rats and mice are mostly used as animal models in skeletal muscle ageing research [18]. Senescence-Accelerated Mouse (SAM), which consists of 18 lines: 11 senescence-prone inbred strains (SAMP) and 7 senescence-resistant inbred strains (SAMR), are regarded as a good choice for sarcopenia study [28, 29]. According to a previous study on SAMP8 mice at 10 (young), 25 (adult) and 60 (old) weeks of age [9], SAMP8 exhibited typical features of accelerated muscle ageing with a short life span and fast ageing progress due to high oxidative stress status [6, 10], greater decrease in muscle mass and contractility, larger reduction of type II muscle fibers in size [20, 23]. All findings indicate that SAMP8 mouse is a reasonable model for sacopenia research. However, there is no report to validate when sarcopenia occurs in SAMP8. The onset of the alteration of muscle mass and function, as well as muscle structural alteration, should be investigated for future interventional or preventive research.

Therefore, we aim to investigate the onset time of sarcopenia in SAMP8 mouse model at different timepoints in this study. The sarcopenia onset time was speculated between month 6 and 10, as Takeda's study reported that 25-week-old SAMP mouse was at adult stage and the median survival time was 9.7 month [5]. According to the definition proposed by EWGSOP, sarcopenia is characterized by the decrease of muscle mass and muscle force [8, 14, 16]. Hence, muscle mass, structural and functional parameters were assessed between month 6 and 10 in SAMP8 mice.

Materials and Methods

This is a descriptive study to validate the onset time of sarcopenia in SAMP8 mouse. Based on the diagnostic criteria of sarcopenia proposed by EWGSOP, skeletal muscle mass and muscle function were regarded as the primary outcome in this study. It has been reported that the SAM strain mouse met most criteria for the use of mammalian models for sarcopenia research [6]. The SAMP strain mouse showed high oxidative status [28] and the SAMP8 mouse was recommended as the animal model for sarcopoenia research [9].

Animals and study design

Male senescence accelerated mouse P8 (SAMP8) were obtained from the Laboratory Animal Service Center (LASEC), the Chinese University of Hong Kong, where male was used to avoid high hormonal variation. All animals were kept under conventional conditions, on a 12:12 h light:dark cycle with food and water *ad libitum*. The research protocol was approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong (Ref: 12/012/MIS). A total of 52 animals were included in this study, with 22 for survival rate investigation from month 8 to month 15. The other 30 mice were examined at 6–10-month old with 6 mice in each time-point. Body weight of each mouse was measured once a month before euthanasia at the designated time-point. All animals that survived before euthanasia were included in the final analysis. Along the progression of ageing, skeletal muscle fiber type II is much more easily affected [16]. As a fast-twitch muscle, gastrocnemius was selected as the target muscle by considering its important roles in the posture holding and body movement.

Survival rate (SR) calculation

A total number of 22 SAMP8 mice were used for SR calculation. From month 8 to month 15, the SR were calculated every couple weeks [9]. The SR at different timepoints were obtained as the following equations [2]:

$$SR(t) = \frac{R - Dt}{R} \times 100\%$$

SR (t): The survival rate of the animal at least to timepoint t

R: The number of alive mice at the beginning of timepoint

Dt: The total deaths of mice within the time-point t

Muscle mass measurement

Under general anaesthesia, the gastrocnemius of the left hindlimb was isolated. After weighing the muscle mass, the isolated muscle was put into the frozen 2-methylbutane under optimal length for 20seconds and then stored at −80°C for the following biochemical assay.

Muscle functional assessment

Under general anaesthesia, the mice were incised and gastrocnemius of the right hindlimb was isolated carefully along with the Achilles's tendon and femur condylar. The muscle was then mounted on a holder vertically between the platinum electrodes with the Achilles's tendon attached to the dual-mode muscle lever arm system (300C-LR, Aurora Scientific Inc.). Muscle functional test was performed according to the established protocol [9, 20]. The whole muscle was incubated in the organ bath of the *ex vivo* muscle functional test system (800A, Aurora Scientific Inc.) containing mammalian Ringer solution (121 mmol/l NaCl, 5.4 mmol/l KCl, 1.2 mmol/l $MgSO_4.7H_2O$, 25 mmol/l $NaHCO_3$, 5 mmol/l HEPEs, 11.5 mmol/l Glucose, 2.5 mmol/L $CaCl_2$), which was maintained at room temperature and continuously pumped gas with a mixture of 95% O_2 and 5% CO_2.A 15 min stabilization period was needed after mounting. The optimal length (L_o) of the muscle was measured after two tetanic contractions (1A, 300 ms duration, 150 Hz stimulation frequency) with 5 min intervals. Under the L_o, the muscle was electronically stimulated two more times by a single stimulus with 1 min interval to evaluate the twitch characteristic (twitch force, F_0). A continuous stimulus was given three times for 300 ms at 80 Hz with 5 min rest to evaluate the tetanic contraction ability (tetanic force, F_t). The contraction strength, contraction time, half-relaxation time were acquired directly. Descending tetanic stimuli (300 ms) of 80, 70, 60, 50, 40, 20 Hz with a 5 min interval were given to assess the force-frequency relationship. To evaluate the fatigability, pulses of 300 ms tetanic stimuli were provided at 80 Hz. The total time of 30 consecutive pulses at 5 s intervals was 150 s. After the functional test, the muscle mass was weighed. The muscle cross-sectional area (MCSA) was calculated by dividing the muscle mass by the muscle optimal length (L_0) and the density of mammalian skeletal muscle (1.06 mg/mm^3). Normalized by MCSA, the specific twitch force (SF_0) and specific tetanic force (SF_t) were obtained as the following equations [20].

$$\mathrm{MCSA}\left(\mathrm{mm}^2\right)=\frac{1,000\times \mathrm{MM}(\mathrm{g})}{\mathrm{D}\times 10\times L_0\ (\mathrm{cm})}$$

$$\mathrm{SF}_0(\mathrm{g}/\mathrm{mm}^2)=\frac{\mathrm{F}_0(\mathrm{g})}{\mathrm{MCSA}\ (\mathrm{mm}^2)}$$

$$\mathrm{SF}_t(\mathrm{g}/\mathrm{mm}^2)=\frac{\mathrm{F}_t(\mathrm{g})}{\mathrm{MCSA}\ (\mathrm{mm}^2)}$$

where, D=Muscle density=1.06 mg/mm^3
MM=Muscle mass (g): ×1,000 makes it mg
L_0=Optimal muscle length (cm): ×10 makes it mm

Muscle structural assessment

Adenosine triphosphatase (ATPase) staining was performed to identify different kinds of muscle fibers with ATP disodium salt ($C_{10}H_{14}N_5Na_2O_{12}P_3$, abcam, UK) according to the protocol established in our previous study [5]. Under −20°C, the muscle transverse sections were cut at 8 μm with a cryostat (Cryocut 1800, Leica, Germany). All sections were brought to room temperature and incubated in pre-incubating solution (barbital acetate solution 5.0 ml, 0.5N HCl 2.0 ml, mQH_2O 12.0 ml) at pH 4.45 for exactly 5 min and then incubated in ATP solution at pH 9.4 for 25 min. Washed with 1% (w/v) $CaCl_2$ for 3 × 3 min, the sections were incubated in the 2% $CoCl_2$ for 10 min. Then the sections were washed with 1:20 0.1 M sodium barbital and mQH_2O for 5 times. Incubationin2% (v/v) $(NH_4)_2S$ solution for 20 s was performed for color development. After rinsing the slides with mQH_2O for 5 times, the sections were dehydrated and were mounted with Canada balsam. Two digital images were taken from each section with a microscope (Leica Microsystems Ltd.) at 50 × and 200 × magnifications. Morphometric analysis was performed with Image-Pro Plus Software (Version 6.0, Media Cybernetics, Inc. USA). The muscle fiber could be identified as type I (darkest), type IIA (lightest) and type IIB (moderate). The muscle fiber distribution as percentage number and the average fiber size were quantified for each fiber type.

Statistical analysis

Except the survival rate, all other quantitative data were expressed asmean ± standard deviation (SD), and analyzed by one-way analysis of variance (ANOVA) with post-hoc Tukey tests using SPSS (Version 19.0, SPSS Inc., IBM, USA) among different time-points. The linear slope was calculated for the fatigue rate (FR) analysis and one-way ANOVA was performed for the slope values. Two-way ANOVA with post-hoc Tukey tests was performed for the force-frequency relation and fatigability comparison among different time-points. The significance level was set at $P \leq 0.05$.

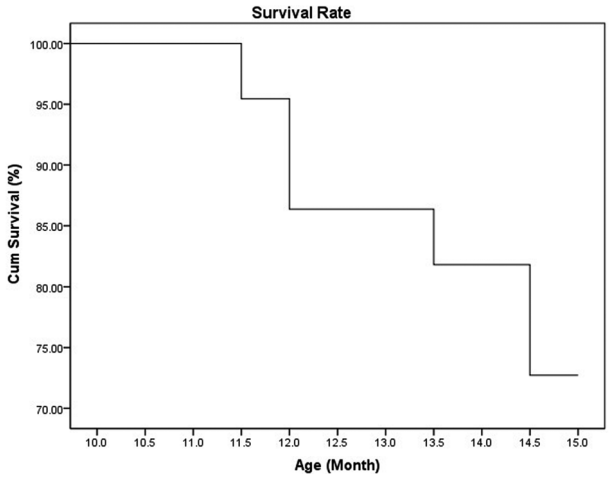

Fig. 1. Cumulative survival rate of SAMP8. Survival rate decreased from month 11 (100%) to month 15 (72%).

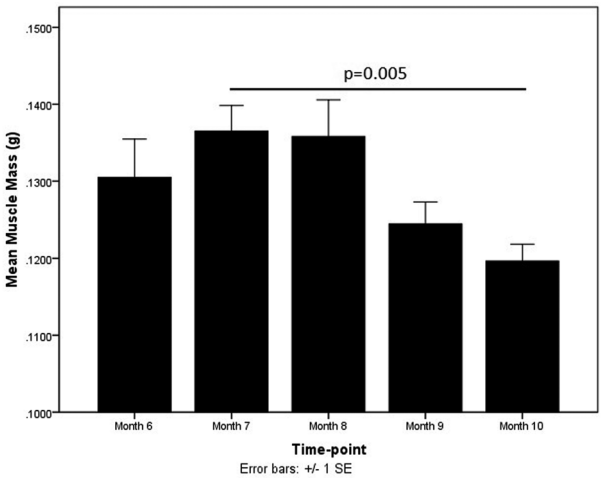

Fig. 2. Muscle mass (MM) of gastrocnemius. Data are presented as means ± SD. Differences among time-points were analyzed by one-way ANOVA followed by post-hoc Tukey's test. Peak of MM appeared at month 7 and there was significant difference between month 7 and month 10.

Table 1. *Ex vivo* contractile properties at 5 time-points

	F_0 (g)	F_t (g)	SF_0 (g/mm^3)	SF_t (g/mm^3)	SF_0/SF_t ratio
Month 6	32.14 ± 8.36*	62.56 ± 11.30*	4.10 ± 1.14*	8.21 ± 1.79*	0.50 ± 0.11^a
Month 7	42.62 ± 12.56	93.83 ± 12.67^a	5.39 ± 1.59	11.88 ± 1.59^a	0.45 ± 0.09
Month 8	45.14 ± 7.90^a	91.35 ± 8.29	5.65 ± 0.97	11.43 ± 0.68	0.49 ± 0.06
Month 9	44.06 ± 6.44	92.21 ± 11.69	5.67 ± 0.86^a	11.73 ± 1.28	0.48 ± 0.22
Month 10	37.52 ± 4.66*	76.99 ± 4.69*	5.01 ± 0.59*	10.31 ± 0.89*	0.49 ± 0.07

Data are presented as means ± SD of 6 mice. The "a" stands for the peak of the same parameter among time-points. Differences among time-points were analyzed by one-way ANOVA followed by post-hoc Tukey's test. A *P* value less than 0.05 was considered to be statistically significant. *P<0.05 compared with the time-point with peak value.

Results

Survival rate and body mass

The cumulative survival rate from month 8 to month 15 of SAMP8 was shownin Fig. 1. Atmonth 8, the survival rate was 100%; at month 15, the survival rate was 72%. There was no death reported before 10 months. There was no significantchange in body mass among different time-points.

Muscle mass

Muscle mass (MM) increased from month 6 to 7.The peak MM at month 7 was 0.137 ± 0.007g (Fig. 2). Afterwards, a decreasing trend was observed until month 10, when a significant lower MM was found to be 12.41% lower than the peak MM at month 7 with statistically significant (P=0.005).

Muscle contractile properties

Table1 summarized the contractile properties of the right gastrocnemius in SAMP8.

From month 6 to 8, twitch force (F_0) increased and the peak appeared at month 8, followed with a decreasing trend. Compared with the peak, the F_0 at month 10 reduced significantly (P=0.049). Although F_0 at month 6 was lower than the peak (P=0.036), there was no significant change from month 7 to 9. The same trend was observed in the SF_0 except that the peak appeared at month 9.

Both of F_t and SF_t increased from month 6 to 7 and then decreased from month 7 to 10, with the peak appeared at month 7. F_t at month 6 and month 10 were significantly lower than that of month 7 (P=0.000 and P=0.001, respectively), and the SF_t showed a similar result (P=0.000 and P=0.001, respectively).

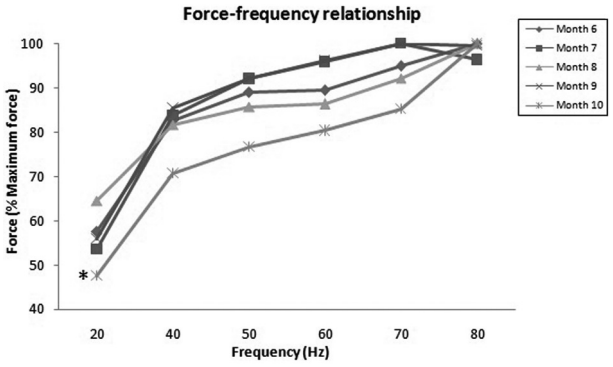

Fig. 3. Force-frequency relationship. Differences among time-points were analyzed by two-way ANOVA followed by post-hoc Tukey's test. SDs were less than 8.91%. The force-frequency relationship curve at month 10 was lower than other time-points (month 6: P=0.000; month 7: P=0.001; month 8: P=0.001; month 9: P=0.000). No significant difference of the curves among the other time-points was found.

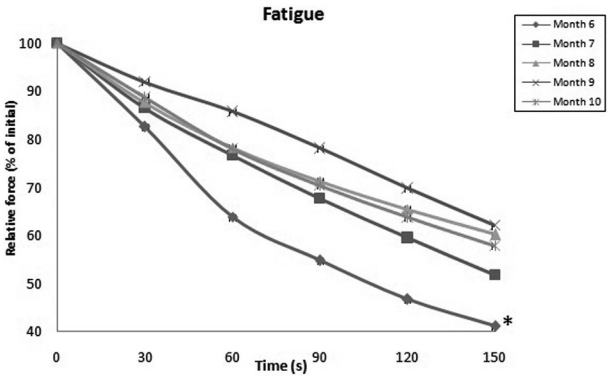

Fig. 4. Tetanic force (relative to initial force) during 150 s of repeated fatiguing stimulation. SDs were less than 8.34%. Differences among time-points were analyzed by two-way ANOVA followed by post-hoc Tukey's test. The tetanic force curve (under repeated stimuli, normalized by the initial force) at month 6 was significantly lower than other time-points (month 7: P=0.037; month 8: P=0.000; month 9: P=0.000; month 10: P=0.020).No significant difference of the curves among the other time-points was found.

Table 2. Contraction time (CT) and half relaxation time (RT50) during tetanic contraction, fatigue rate (FR) and percentage loss of tetanic force (FL%) during 150 s fatigue contraction

	CT(ms)	RT50 (ms)	dF/dt (g/s)	FR	FL%
Month 6	52.77 ± 5.48	34.71 ± 8.76	2,090 ± 480	20.31 ± 1.47	59.73 ± 20.73[a]
Month 7	43.18 ± 7.23	27.20 ± 5.45	2,096 ± 432	26.46 ± 1.88[a]	53.61 ± 5.84
Month 8	56.11 ± 4.78[a]	34.85 ± 4.68[a]	2,280 ± 434[a]	21.20 ± 5.83	45.63 ± 10.77
Month 9	43.74 ± 5.48	22.36 ± 4.86	1,652 ± 218*	17.33 ± 5.34	43.77 ± 8.41
Month 10	46.72 ± 3.30	23.25 ± 4.02	1,688 ± 257*	21.05 ± 6.69	55.07 ± 12.59

Data are presented as means ± SD of 6 mice. The "a" stands for the peak of the same parameter among time-points. Differences among time-points were analyzed by one-way ANOVA followed by post-hoc Tukey's test. A P value less than 0.05 was considered to be statistically significant. *P<0.05 compared with the time-point with peak value.

Although the peaks of SF_0 and SF_t appeared at different time-points (SF_0 at month 9, SF_t at month 7), the SF_0/SF_t ratio remained stable. The peak of SF_0/SF_t ratio was at month 6, with no significant difference among different time-points.

Force-frequency relation and fatigability

As shown in Fig. 3, compared with other strains, the force-frequency curve at month 10 was lower than other time-points (month 6: P=0.000; month 7: P=0.001; month 8: P=0.001; month 9: P=0.000). The maximum tetanic force at month 7 was induced by 70 Hz stimuli and those at other time-points were induced by 80 Hz stimuli.

Table 2 showed the largest contraction time (CT) and half-relaxation time (RT50) at month 8. The rate of force development (dF/dt) increased slightly from month 6 to 8, with the peak at month 8. Thereafter, a sharp decrease of dF/dt was observed. The dF/dt at both month 9 and 10 were significantly lower than the peak (P=0.008 and 0.017 respectively) at month 8. Fatigue rate (FR) at month 7 was higher than all other time-points, while month 6 showed the peak of percentage loss of tetanic force (FL%). The differences in all the fatigue related parameters among different time-points were not significant. However, Fig. 4 indicated that the tetanic force curve (under repeated stimuli, normalized by the initial force) at month 6 was much lower than other time-points (month 7: P=0.037; month 8: P=0.000; month 9: P=0.000; month 10: P=0.020).

Fiber cross-sectional area (FCSA) and proportion

Results of muscle FCSA were shown in Table 3. Muscle fibre type I showed a decreasing trend from

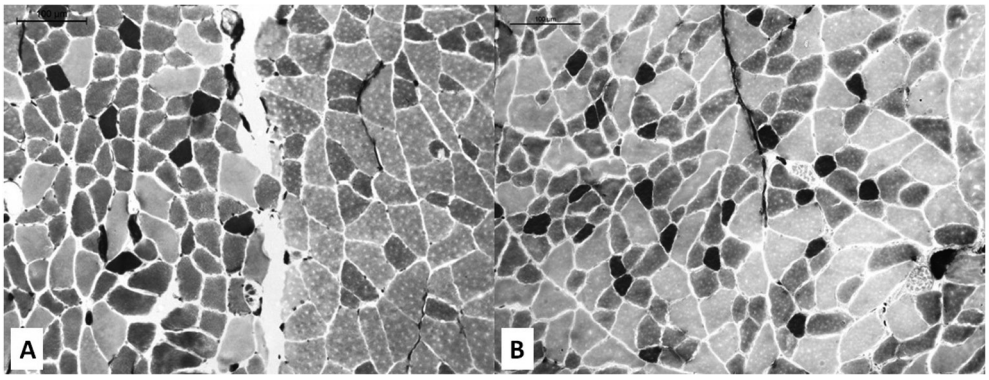

Fig. 5. ATPase staining of SAMP8 gastrocnemius showed the distributed myofibers (Stain, ATPase; magnification, ×20). The darkest, lightest, and intermediate signals represent type I, type IIA and type IIB, respectively.**A:** Month 8; **B:** Month 10. Reduced type IIB muscle fibers were generally observed.

Table 3. Muscle FCSA of gastrocnemius from SAMP8 at different time-points (μm^2)

	Type I	Type IIA	Type IIB
Month 6	570 ± 152[a]	1,150 ± 284*	851 ± 162[a]
Month 7	529 ± 138	1,200 ± 362	839 ± 170
Month 8	499 ± 114*	1,320 ± 337[a]	806 ± 164
Month 9	502 ± 132*	1,200 ± 285*	803 ± 159
Month 10	479 ± 125*	1,160 ± 315*	802 ± 158

Data are presented as means ± SD of 6 mice. The "a" stands for the peak of the same parameter among time-points. Differences among time-points were analyzed by one-way ANOVA followed by post-hoc Tukey's test. A *P* value less than 0.05 was considered to be statistically significant. **P*<0.05 compared with the time-point with peak value.

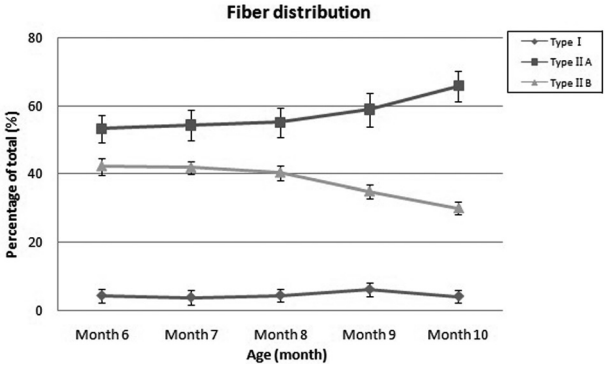

Fig. 6. Distribution of fiber types in gastrocnemius. Data are presented as means ± SD. From month 6 to month 10, the percentage of muscle fiber type IIA increased from 53% to 66%; type IIB decreased from 42% to 30%, with no significant change in type I.

month 6 to 10. The FCSA at month 8 to 10 were lower than that of month 6 with significant difference (*P*=0.05, *P*=0.027 and *P*=0.002, respectively). Fiber type IIA increased from month 6 to 8 (*P*=0.000), followed with a sharp reduction until month 10. FCSA of type IIA at month 9 and 10 were much smaller than the peak (*P*=0.028 and *P*=0.001,respectively). The largest type IIB FCSA appeared at month 6. Type IIB FCSA showed a continuous decrease from month 6 to 10, with no significant difference among different time-points.

As shown in Fig. 5 and Fig. 6, gastrocnemius was mainly composed of fiber type IIA, and the percentage became larger during the process of ageing from 53% at month 6 to 66% at month 10. The percentage of muscle fiber type IIB in decreased from 42% at month 6 to 30% at month 10. The percentage of fiber type I in gastrocnemius was about 4 to 5%, with no significant change observed over time.

Discussion

The SAMP8 mouse was reported by Takeda *et al.* to meet most criteria for the use as a mammalian model on sarcopenia research [28]. Based on the diagnostic criteria of sarcopenia proposed by EWGSOP in 2010, skeletal muscle mass, muscle strength as well as physical performance were regarded as the primary outcome domains. In this study, we investigated muscle mass, structural and functional properties of gastrocnemius in order to validate the onset time of sarcopenia in SAMP8 mice. The reduction of gastrocnemius mass from month 7 indicated that sarcopenia should start between month 7 and month 8. Muscle functional test of contractile properties and fatigability suggested that the decrease of muscle strength and function started at month 8. ATPase

staining showed that the largest FCSA of muscle type IIA appeared at month 8; fiber type IIB decreased both in FCSA and number along with ageing process. All the above findings indicated that the onset time of sarcopenia in SAMP8 should be month 8.

The muscular system accounts for over 40% of the total body mass and the loss of skeletal muscle mass leads to diminished strength and exercise capacity [13, 30]. Since body weight loss caused by the muscle mass loss may be obscured by an increase of fat mass, not all sarcopenic individuals would demonstrate low body mass. However, the loss of strength is directly related with the reduction of muscle mass, though the relationship is not linear [13]. The progressive decline of skeletal muscle mass with ageing is one of the most well-recognized features of sarcopenia [24]. The absolute age-dependent muscle mass reduction should be a reflection of the onset of sarcopenia [8]. In this study, age-dependent muscle loss of gastrocnemius was analyzed to validate the onset of sarcopenia in SAMP8. The largest muscle mass of gastrocnemius in SAMP8 appeared atmonth 7, then followed with a decreasing trend. This indicated that sarcopenia in SAMP8 started between month 7 and month 8.

Body movements and maintenance of posture require skeletal muscle contraction. Skeletal muscle strength and postural stability are closely related to the fall risk and clinical outcomes, such as gait speed and response time [3].The absolute muscle contraction strength is mainly determined by muscle cross-sectional diameter [9]. In this study, the peak of F_0 and F_t appeared at month 8 and month 7 respectively. Compared with absolute strength, SF_0 and SF_t (normalized by MCSA) showed the same trend along with ageing from month 6 to 10. Although the contraction strength (F_0 and F_t) is determined by muscle mass, some evidences suggest that skeletal muscle mass and muscle contraction strength do not vary in a linear fashion. The decrease of muscle strength is not linearly proportional to the muscle mass [19]. It was also reported that the age-related reduction of SF_0 and SF_t was due to the change of myosin structure and cross-bridge states [11]. The F_0/F_t ratio is regarded as an indirect measure of muscle stiffness and a high ratio indicates a high degree of stiffness [9]. Vuokko Kovanen *et al.* found that very old animals showed higher muscle stiffness than the young ones [15]. In this study, there was no significant change of F_0/F_t ratio along the progression of ageing from month 6 to month 10.The reason of this observation might be that 10-month old SAMP8 animals were not old enough to show the significant increase in muscle stiffness. Additional time-point might be needed to verify the above speculation.

One typical feature of skeletal muscle senescence is the extension of contraction time (CT), but larger contraction strength may also result in a longer contraction time. The dF/dt, which is independent of contraction strength and the duration of stimulation, is regarded as a distinct and functionally relevant variable in muscle function [9, 17]. As a reflection of reaction capability, dF/dt is regarded as an important factor on the investigation of physical performance [9]. In fast-twitch skeletal muscle, the reduction of dF/dt is probably related to the age-induced decrease of sarcoplasmic reticulum function [9]. In this study, both of CT and dF/dt peaked at month 8 followed by a sharp decrease of dF/dt, which indicated that the muscle senescence accelerated from month 8. As mentioned above, MM reduced between month 7 and month 8 while SF_t declined at month 7. Though there were no significant change in RT50, FR and FL%, the decreasing trend of FR and FL% from month 7 to 10 reflected an increased resistance to fatigue. Combining the reduction in dF/dt with the decline of MM and SF_t, we derive that sarcopenia started at month 8 in SAMP8.

Our findings showed that gastrocnemius of SAMP8 is fast-twitch dominant muscle mainly composed of type II fibers with approximately 5% type I fibers in composition. Ageing process resulted in skeletal muscle atrophy, primarily affecting type II fibers [4, 16]. Age-related type II skeletal muscle fibers atrophy is associated with the reduction of muscle fiber in number and size [4, 5]. Our results showed that type IIB skeletal muscle fibers decreased in both mean CSA and proportion with increasing age. Type IIB fibers are responsible for fast movements, so this phenomenon indicates a greater muscle power reduction during the process of ageing. Larger motor neurons with larger axons innervate type II fibers, especially type IIB. The reduction of type II is accompanied with a preferential loss of largest motor neurons with a lowest oxidative capacity [1, 16]. One of the distinctive features of ageing is the decrease of skeletal muscle fiber in size. In a previous clinical study, we observed a greater decrease of fiber CSA in type II fibers than in type I fibers in community elderly [16]. It proved that type II fibers was affected more by ageing, indicating that type II fibers played a more important role than type I in physical performance. In this study, the mean

peak CSA of type IIA fibers appeared at month 8 followed with a sharp decrease, showing a similar trend with that of the skeletal muscle mass and muscle force. The age-related changes in type I skeletal muscle fibers are controversial. The continuous decline of type I fibers in CSA from month 6 to 10 is mainly attributed to the whole muscle atrophy. Our data indicates that there is no age-related changes of type I in proportion, which confirms the previous studies [7, 17, 27, 31]. The discrepancy between type I fiber in size and proportion could be explained by a type IIA to type I fiber type switching during ageing [7, 12].

Several animal models were recommended for sarcopenia research. The Fisher 344×Brown Norway Rat (FBN rat) is a typical model with significant age-related decrease of muscle mass and muscle strength at month 33 [22]. C57BL/6 was also used for sarcopenia research. The decline of muscle mass and muscle strength occurred at month 18 with high correlation (R^2=0.94) [32]. Compared with the above two models, the advantage of SAMP8 mouse are the short lifespan accelerated senescence process. The onset time of sarcopenia in SAMP8 was month 8 with significant reduction of muscle mass (12.41%), muscle strength (11.64%) and contractibility (25.96%). Furthermore, mitochondrial dysfunction is the primary cause of high oxidative stress status in SAMP8, hence inducing senescence acceleration [6]. And it has been proved that the age-related pathological phenotypes during the progress of ageing in SAMP8 is similar to human geriatric disorders [28]. Therefore, SAMP8 is recommended as a high cost-effective animal model for sarcopenia research.

The present study had some limitations. The last timepoint in this study was month 10 because it was reported that the average life span was 9.7 months [9, 29]. However, our results showed that the significant decline of muscle outcomes appeared at month 10 compared with the peak, which suggested that month 10 was at sarcopenia stage. Therefore, recruiting one more timepoint at month 11 might present a clearer sarcopenia process. Also, the recovery of muscle force under optimal length after repeated tetanic stimulation was not measured in this study, which might help us distinguish the reversible and irreversible loss of force during skeletal muscle fatigue. Additionally, the molecular mechanism and the alteration of neuromuscular junctions during the ageing progression in SAMP8 should be investigated in the future.

In conclusion, the onset time of sarcopenia in SAMP8 was month 8. Month 8 SAMP8 mice should beat presarcopenia stage, while month 10 SAMP8 mice were at sarcopenia stage. These findings indicated that SAMP8 mouse could be used in sarcopenia research with established time line in this study. This animal model is useful to be utilized to further study the pathogenesis, treatment, or prevention of sarcopenia.

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