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Article

Habitual Exercise Attenuates the Aging Associated Muscle Atrophy and Muscle Strength Decline in SAMP8

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Abstract: Loss of muscle mass and strength are progressing with aging. Exercise is a beneficial method to prevent physical disfunction and habitual exercise improve the muscle quality. Therefore, we evaluated the effects of a long-term habitual exercise on the senescence-accelerated mice prone8 (SAMP8). 27wk SAMP8 were used in this study. Mice were classified into 28 (28w) and 44 weeks old. The 44-week group was divided into the sedentary group (44w) and a group exercising for 16 weeks (44w+Ex). The 44w+Ex performed habitual exercise from 28 to 44 weeks. Additionally, grip strength tests were performed with mice aged 28 and 44 weeks. Mice were dissected and collected muscle samples and measured muscle weight at 44w. Gastrocnemius was decreased in 44w but were unchanged in 44w+Ex. Grip strength in 44w was lower trend, but there was no change in 44w+Ex. The phosphorylation levels of Akt and p70S6K as a protein synthesis marker were decreased in 44w. Cytochrome c oxidase subunit IV(COXIV) mRNA and protein levels decreased in 44w. These results suggested that long-term habitual exercise attenuated muscle mass and strength decline through improving muscle protein synthesis and mitochondrial function. In conclusion, long-term habitual exercise attenuated muscle mass and strength decline.

Keywords: Aging, Habitual exercise, SAMP8, Sarcopenia

1. Introduction

Aging is an inevitable process in animals, including humans, and has various effects on the body. For example, aging affects the functioning of the brain, nerves, and intestines. Skeletal muscle aging includes skeletal muscle loss and muscular weakness, known as sarcopenia [1], Sarcopenia was initially described by Rosenberg [2]. The European Working Group on Sarcopenia in Older People (EWGSOP) provides a definition of age-related sarcopenia and a statement on diagnostic criteria. The EWGSOP recommend using both the loss of skeletal muscle mass and the loss of muscle function (muscle and physical ability) for diagnosis of sarcopenia. This definition is more complex because

muscle strength is not only dependent on muscle mass, but also function [3, 4]. In recent years, studies have reported that various forms of exercise have positive effects in preventing sarcopenia. For example, resistance training can stimulate muscle protein synthesis [5, 6]. Kryger reported that resistance training by women at an advanced age could increase skeletal muscle mass and muscle strength [7]. More recently, the effects of habitual exercise have also been studied [8, 9, 10].

Aging affects insulin sensitivity and reduces muscle protein synthesis [11]. Akt is one of the muscle protein synthesis related protein, and is upstream protein of muscle protein synthesis. On the other hands, downstream of Akt is 70kDa S6 kinase (p70S6K), which activates muscle protein synthesis. Phosphorylation levels of Akt is decreasing in the old mice has been reported. As a result, decrease of Akt phosphorylation suppress the activation in downstream of Akt [12]. However, habitual exercise improves the phosphorylation levels of Akt and activate the muscle protein synthesis [11]. Furthermore, mitochondria contents and function decline with aging [13, 14]. Reductions in mitochondrial function causes muscle ATP synthesis reduction and muscle weakness [15, 16]. However, habitual exercise regulates the mitochondrial contents and function during aging [17]. Short et al. reported that long-term habitual exercise can increase cytochrome c oxidase subunit IV (CoxIV) mRNA levels regardless of age [18]. Moreover, habitual exercise increases the Pgc-1a mRNA levels, promoting mitochondria production [19, 20]. Positive correlations between muscle ATP synthesis and muscle function have been reported [16], therefore, if mitochondrial functions improve, muscle function may also increase.

In the study of sarcopenia, aging model animals are required, however, natural aging models require a lot of time to evaluate. We used senescence-accelerated mice (SAM). SAM represent a multi-factor approach and are an accepted model for accelerated senescence and study of age-related pathologies [21]. In particular, SAMP8 (prone 8 of SAM) were reported as useful in the sarcopenia model [22, 23]. To our knowledge, there are no studies on the effects of long-term habitual exercise programs on the sarcopenia model. Hence, the aim of this study was to evaluate the effects of long-term habitual exercise on the sarcopenia model.

2. Materials and Methods

2.1. Animals and Experimental Grouping

The animal experiments in this study were approved by the Animal Care Committee, University of Tsukuba. An overview of the experiments is shown in Figure 1. Male SAMP8 aged 28 weeks (Japan SLC, Hamamatsu, Japan) were kept under conventional in individually at 20–26℃ with a humidity level of 40–60% and a 12-h light/dark cycle. Female mice were excluded from this study due to the effects of menstruation and menopause. Mice were freely fed a standard feed (MF, Oriental Yeast, Tokyo, Japan). Mice were divided into the 28 weeks old group (28w) and the 44 weeks old group, split into the sedentary group (44w) and group undergoing exercise for 16 weeks (44w+Ex). At the age of 28 weeks, the number of mice were 28 w (n = 10), 44 w (n = 10) and 44 w + Ex (n = 10), but there are individuals which died after the initial grip strength measurement and during study. Therefore, the data used in this study were 28w (n = 8), 44w (n = 7), and 44w + Ex (n = 9).

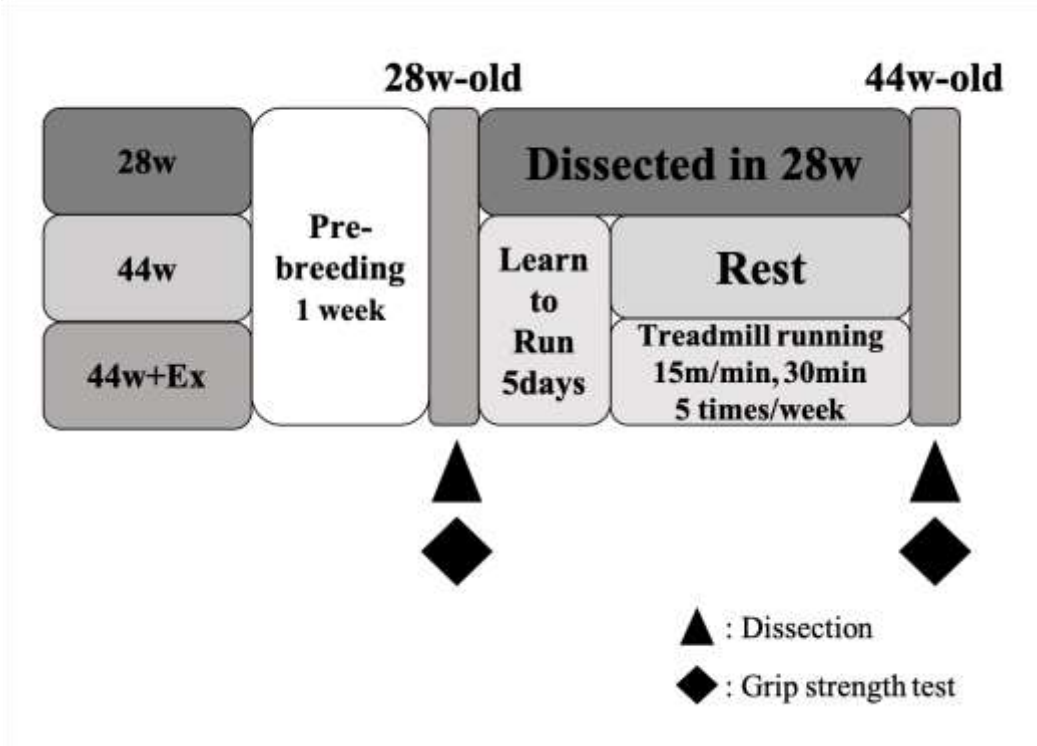

Figure1. Over view in this study

2.2. Exercise Protocol

The mice ran on a treadmill for small animals (FVRO, 4E9S-6, Fuji Medical Science, Chiba, Japan). The mice learned to run for 5 days followed by training performed at 15 m/min for 30 min a day in light cycle, 5 days per week from 28 to 44 weeks of age. Training protocol were modified from Murase et al [18]. The back of each treadmill lane contained an electrified grid, which delivered a shock stimulus to mice if mice are resting on grid. The mice were monitored during running training to prevent escape. If mice are caught in a treadmill, immediately rescued and excluded from the experiment.

2.2. Grip Strength Test

The grip strength test reflected a muscle strength index of all four limbs and was conducted using a small animal grip measurement device (GMP-100B, MELQUEST, Toyama, Japan). Mice were held in the limb-grip, the tail was pulled backward, and the maximum value of the force applied to release the grip was taken as the measurement value. The measurement was performed five times, and the average value, excluding the maximum value and the minimum value, was taken as the index for each mouse. The same researcher conducted the measurements each time. Measurements were conducted so that the researcher was blind to the individual mouse and measured value to remove bias.

2.3. Sampling

At the time of physiological investigation (28 and 44 weeks old; Figure 1), mice were anaesthetized by an intraperitoneal injection of pentobarbitone sodium (Kyoritsu Seiyaku Corporation, Tokyo, Japan). Soleus muscles, plantaris muscles, and gastrocnemius muscles were harvested, following

which mice were euthanized by cervical dislocation. The muscle wet weights were measured at the time of euthanasia and then immediately frozen in liquid nitrogen and stored at -80℃ for later biochemical assays.

2.4. Western Blot Analysis

Total proteins were extracted from gastrocnemius muscle with radioimmunoprecipitation assay (RIPA) buffer (1% NP-40, 0.1% SDS, 20 mM Tris-HCl (pH 8.0), 5 mM EDTA, 150 mM NaCl including proteinase inhibitor tablets [cOmplete™ mini, Roche, Basel, Switzerland] phosphatase inhibitor [PhosSTOP™, Roche, Basel, Switzerland]). Lysate were centrifuged at 12,000 g for 15 min at 4 °C. Total protein concentrations for each sample were measured by BCA protein assay kit (Thermo Fisher Scientific, MA, US) and 20 μg/lane of total protein were used for 10% SDS-polyacrylamide gel electrophoresis. In western blot analysis, the primary antibodies used were rabbit monoclonal Akt antibody (Cell Signaling Technology, #4691T, MA, US, 1:2000), p-Akt antibody (Ser473) (Cell Signaling Technology, #4060T, US, 1:2000), p70S6K antibody (Cell Signaling Technology, #2708T, US, 1:2000), p-p70S6K(Thr389) (Cell Signaling Technology, #9205S, US, 1:2000), COXIV antibody (Cell Signaling Technology, #4850, US, 1:2000) and OXPHOS antibody (abcam, ab110413, Cambridge, UK, 1:4000). Anti-rabbtit IgG, HRP-linked antibody (Cell Signaling Technology, #7074, US, 1:2000) and Anti-mouse IgG, HRP-linked antibody (Cell Signaling Technology, #7076, US, 1:10000) was used as the secondary antibody. The list of antibodies used in this study are shown in Table 1. Signals were detected by chemiluminescence reagent (SignalFireTM Elite ECL Reagent, Cell Signaling Technology, US). Detected images were scanned using a Light-Capture Cooled CCD Camera System (Image Quant LAS-4000, GE Healthcare, UK).

Table 1. List of antibodies.

Product code	Protein name	Spiecies	Dilution
CST #4691T	Akt	Rabbit	1:2000
CST #4060T	p-Akt(Ser473)	Rabbit	1:2000
CST #2708T	p70S6K	Rabbit	1:2000
CST #9205S	p-p70S6K(Thr389)	Rabbit	1:2000
CST #4850	COXIV	Rabbit	1:2000
CST #7074	Anti-rabbit IgG, HRP-linked Antibody	Rabbit	1:2000

2.5. Quantitative RT-PCR Analysis

Total RNA was extracted from the gastrocnemius muscle using Sepasol®-RNA I Super G (Nacalai Tesque, Kyoto, Japan). The total RNA concentration was measured with a spectrophotometer (NANO DROP 2000, Thermo Fisher Scientific, MA, USA). Based on the values obtained, the samples were adjusted to 400 ng/μL, with sterile water. After adding 5x PrimeScript RT Master Mix (RR 036 A, Takara Bio, Shiga, Japan) and RNase-free water to the diluted RNA, reverse transcription was carried out in a thermal cycler (TP 350, Takara Bio) (37 °C, 15 min; 85 °C, 5 s; 4 °C, ∞). After reverse transcription, SYBR Premix Ex Taq II [Tli RNaseH Plus] (Takara Bio) was used for a quantitative polymerase chain reaction (qPCR) technique. Amplification of a reverse transcription polymerase chain reaction (RT-PCR) involved an initial decomposition step at 95 °C for 20 s, decomposition at 95 °C for 30 s, and annealing and extension at 60 °C for 30 s for 40 cycles using the real-time PCR system 7500 Fast (Applied Biosystems, CA, USA). The mRNA expression of TATA-box binding protein (TBP) was measured as a control. The cycle threshold (Ct) value of the target gene was standardized by the Ct value of the housekeeping gene (ΔΔCt method). The relative expression level of the target gene was calculated as the relative value compared to the 28 weeks old value. The sequences of primers used in this study are shown in Table 2.

Table 2. List of primers.

Accession No.	Gene name	Primer Sequence	bp
NM_008904.2	PPAR gamma coactivator 1-alpha (PGC-1α)	5'-GCGAACCTTAAGTGTGGAACTC-3' 5'-GCCTTGAAAGGGTTATCTTGG-3'	93
NM_001293559.1	Cytochrome c oxidase subunit 4 (CoxIV)	5'-TGATGTGGCCCATGTCAC-3' 5'-GTTAAACTGGATGCGGTACAACT-3'	121
NM_007505.2	mitochondrial H+-ATP synthase alpha subunit (Atp5a1)	5'-TCCATGCCTCTAACACTCGAC-3' 5'-GCTTAACACACGCCCAGTCT-3'	122
NM_013684.3	TATA box binding protein (Tbp)	5'-CCAATGACTCCTATGACCCCTA-3' 5'-CAGCCAAGATTCACGGTAGAT-3'	104

2.6. Statistical Analysis

Data are shown as the mean ± SEM. Data were subjected to a one-way analysis of variance was used to evaluate significance. Post hoc test was used Tukey's method. Statistical analyses were performed using SPSS statistics ver. 26 for Mac (SPSS Japan, Tokyo, Japan).

3. Results

3.1. Body Weight

There were no changes in body weights when the groups were compared. The body weight graphs are displayed in Figure 2.

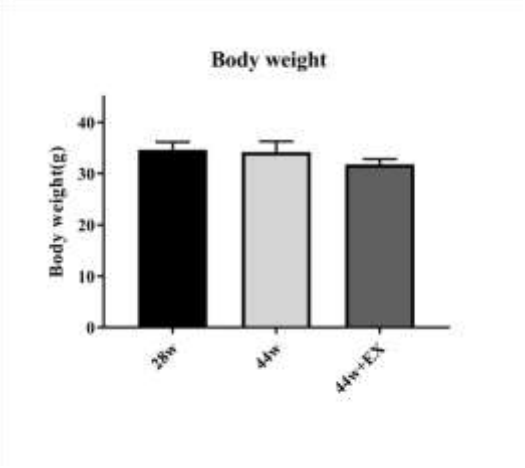

Figure 2. Body weight in each group. All mice were dissected in 28w and 44w. Body weights were measured before dissection in each time points. Values are means ± SE (n=7-9).

3.2. Skeletal Muscle Mass and Muscle Strength Reduction

To evaluate the effect of aging and habitual exercise on muscle, skeletal muscle mass (Figure3-A), relative skeletal muscle mass and muscle strength were measured (Figure 3-B). In the 44w group, gastrocnemius muscle mass declined with aging and grip strength tended to decrease. In the 44w+Ex group, there were no significant changes in skeletal muscle and muscle strength. Thus, habitual exercise prevented reductions in skeletal muscle mass and muscle strength associated with aging.

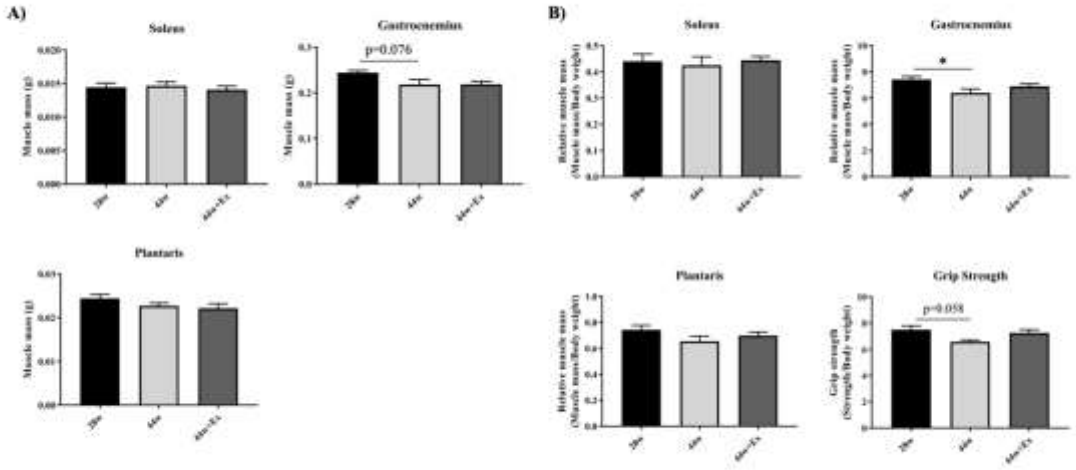

Figure3. Muscle weight and Grip strength. A) Muscle mass in each group. B) Relative muscle mass and Gripstrength. Muscle weights were measured in each time points. The grip strength reflected a muscle strength index of all four limbs and was conducted using a small animal grip measurement device. 28week-old(28w), 44week-old(44w) and Exercise(44w+Ex). Values are means ± SE (n=7-9). Single asterisk indicates significant differences ($P<0.05$)

3.3. Protein Synthesis Related Protein Expression

To evaluate the effect of aging and habitual exercise on the phosphorylation level of Akt and p70S6K were measured (Figure 4). In Akt and p70S6K, 44w was significant lower compared to both 28w and 44w+Ex group.

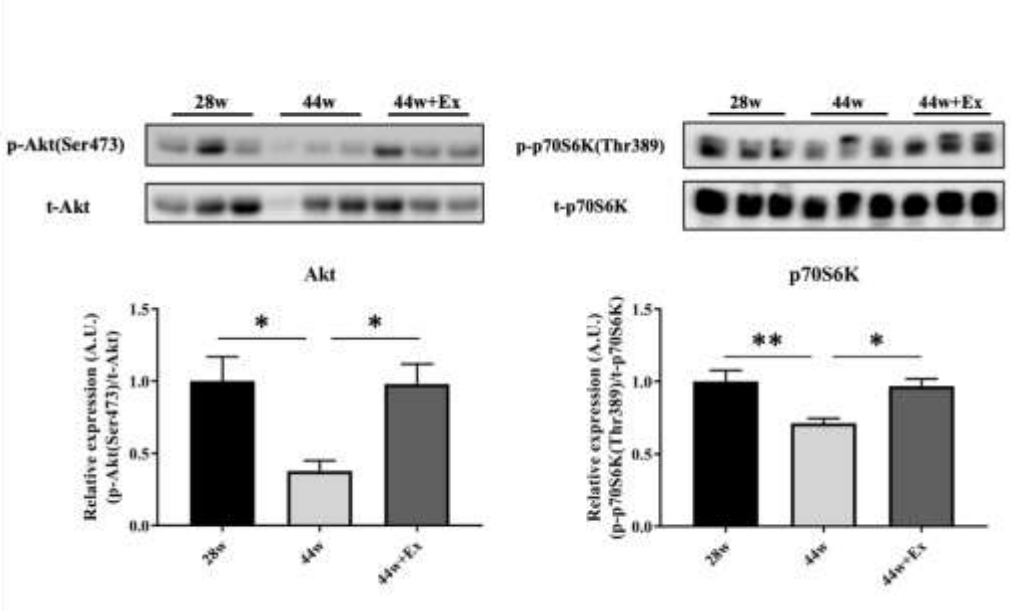

Figure 4. The phosphorylation level of Akt and p70S6K in gastrocnemius muscle. Total protein was extracted from gastrocnemius muscle and both proteins were analyzed by Western Blot analysis. Akt: Protein kinase B, p70S6K: 70-kDa ribosomal protein S6 kinase. 28week old (28w), 44week old (44w) and exercise (44w+Ex). Values are means ± SE (n=6). Double asterisks indicate significant differences ($P<0.01$). Single asterisk indicates significant differences ($P<0.05$).

3.4. Mitochondrial Functioning Gene and Protein Expression

Habitual exercise increased the expression level of genes related to mitochondrial functioning. To evaluate the effect of aging and habitual exercise on the expression levels of genes related to mitochondrial function, the expression levels of Pgc-1a, CoxIV and Atp5a1 were measured (Figure 5-A). In the 44w group, CoxIV mRNA levels declined with aging and there were no changes in Pgc-1a and Atp5a1. In the 44w+Ex group, there were no changes in CoxIV and Atp5a1. CoxIV genes expression was changed 44w so we examined the protein expression in COXIV. In COXIV, 44w was significant lower compared to both 28w and 44w+Ex group.

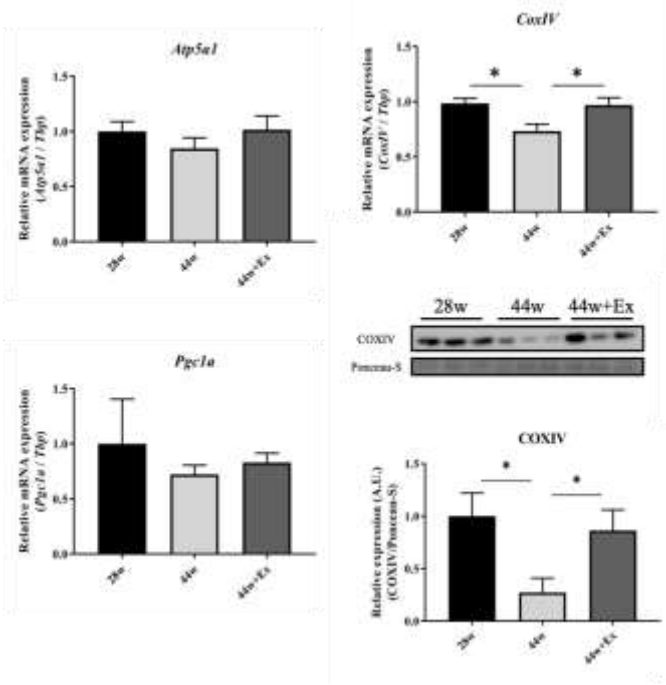

Figure 5. Genes and protein expressions related to mitochondrial function in gastrocnemius muscle. Genes expression level related to mitochondrial function. RNA was extracted from gastrocnemius muscle and converted to cDNA. cDNA was used as template for real time RT-qPCR. Pgc-1α: PPAR gamma coactivator 1-alph, CoxIV: Cytochrome c oxidase subunit IV, ATP5a1: ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1. Values are means ± SE (n=7-9). Proteins expression level related to mitochondrial function. Total protein was extracted from gastrocnemius muscle and both proteins were analyzed by Western Blot analysis. COXIV: Cytochrome c oxidase subunit IV, 28week old (28w), 44week old (44w) and exercise (44w+Ex). Values are means ± SE (n=6). 28week old (28w), 44week old (44w) and exercise (44w+Ex). Single asterisk indicates significant differences ($P<0.05$).

4. Discussion

Muscle aging, known as sarcopenia, is significant problem in terms of quality of life, health, and decreased life expectancy. Muscle aging studies and muscle aging animal models are required since human life is of considerable length, making examinations very difficult. In this study, we used the SAMP8 for a sarcopenia model animal. First, we examined muscle mass and muscle strength changes following a habitual exercise program. Guo et al. reported that SAMP8 muscle mass peaked at 7 months old and muscle strength decline began at 8 months old; therefore, we used 28 weeks old (7 months old) SAMP8 [22]. Relative gastrocnemius muscle mass decreased with aging (Figure 3-B). Additionally, muscle strength decreased in the 44w group. These results were consistent with previous studies [22], and both aspects are important since EWGSOP refers to sarcopenia as not only a decrease in muscle mass, but also a decline in muscle strength. Although the absolute weights tended to be significant, there was no significant difference. This point was different from previous studies. Liu et al. reported that fat accumulates in SAMP8's muscles with aging [24]. Hence, ectopic fat may have accumulated in this study. Additionally, differences in breeding environment affected the amount of physical activity, etc., and there may have been no clear difference in changes in muscle weight. Nevertheless, it is useful to be able to evaluate the effects of aging on muscles in a short period of time, so SAMP8 is a useful model animal for sarcopenia.

To evaluate why sarcopenia was prevented by long-term, habitual exercise, muscle protein synthesis related proteins and genes related to mitochondrial function were investigated.

Muscle atrophy is caused by a decline in muscle protein synthesis [14], therefore, phosphorylation levels of Akt and p70S6K, which are related to muscle protein synthesis, were

measured. In this study, the phosphorylation level of proteins showed decling with aging and exercise suppressed its decline. Therefore, muscle atrophy at this age of SAMP8 may be primarily due to declined muscle protein synthesis, and contrary to the aging, exercise maintained the muscle protein synthesis. Previous study referred that 18~19 m/min is lactate threshold (LT) in mice [19]. Exercise below LT was low to moderate intensity, and it is considered that this study was low to moderate intensity of 15 m / min. Hence, this study revealed that habitual exercise of low intensity exercise can maintain muscle mass by maintaining muscle synthesis.

Long-term, habitual exercise can improve mitochondrial function and increase mitochondria content, therefore, we examined the genes and proteins related to mitochondrial function. Mitochondria is one of the important organellar to produce ATP, and mitochondrial function and muscle function are related [16]. During aging, mitochondrial function decline and this causes muscle strength to also decline. In this study, the mRNA and protein expression level of CoxIV, which is a mitochondrial marker, were decreased in 44w (Figure 5). In this sarcopenia model, aging may have caused a decrease in mitochondrial function and / or a decrease in mitochondrial content, but exercise had inhibitory effect. Generally, it is difficult for elderly people to exercise with high intensity. However, our results suggested that even though low intensity exercise that is safety and executable for elderly could attenuate the aging associated muscle mass and strength decline through the maintenance of mitochondrial function and maintaining muscle protein synthesis.

Despite these findings, the mitochondrial genome level and mitochondria content, were not measured in this study. In addition, muscle fiber type and fiber cross sectional area could not be examined using muscle sections, creating a limitation which must be addressed in future study.

5. Conclusions

The effects of long-term, habitual exercise in SAMP8 mice as a sarcopenia model were evaluated in this study. Long-term, habitual exercise preserved muscle mass and muscle strength. The mechanisms underlying this phenomenon could be preservation of the muscle protein synthesis and mitochondrial function.

Author Contributions: K.A. and H.O. conceived and designed the experiments; K.A., M.K. and K.H. performed the experiments: K.A. drafted the manuscript; K.A., M.K. and K.H. analyzed the data; T.S. provided the experiment technique. K.A., T.A., T.N., M.T., T.S., K.T. and H.O. provided critical comments and contributed to the discussion of the results; K.A. and H.O. edited and revised the manuscript. All authors approved the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Morley, J.E., Baumgartner, R.N., Roubenoff, R., Mayer, J. and Nair, K.S. 2001. Sarcopenia. *J. Lab. Clin. Med*. 137: 231
2. Rosenberg, I. 1989. Summary comments: epidemiological and methodological problems in determining nutritional status of older persons. *Am. J. Clin. Nutr*. 50: 1231–1233.
3. Goodpaster, B.H., Park, S.W., Harris, T.B., Kritchevsky, S.B., Nevitt, M. Schwartz, A.V., Simonsick, E.M., Tylavsky, F.A, Visser, M. and Newman, A.B. 2006. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J. Gerontol. A. Biol.* 61: 1059–64.
4. Janssen, I., Shepard, G.S., Katzmarzyk, P.T. and Roubenoff, R. 2004. The healthcare costs of sarcopenia in the United States. *J. Am. Geriatr. Soc.* 52: 80–85.
5. Dreyer, H.C., Fujita, S., Cadenas, J.G., Chinkes, D.L., Volpi, E. and Rasmussen, B.B. 2006. Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J. Physiol.* 576: 613–624.

6. MacDougall, J.D., Gibala, M.J., Tarnopolsky, M.A., MacDonald, J.R., Interisano, S.A. and Yarasheski, K.E. 1995. The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can. J. Appl.* 20: 480–486.
7. Kryger, A.I. and Andersen, J.L. 2007. Resistance training in the oldest old: Consequences for muscle strength, fiber types, fiber size, and MHC isoforms. *Scand. J. Med. Sci. Sports*. 17: 422–430.
8. Capelli, C., Rittveger, J., Bruseghini, P., Calabria, E. and Tam, E. 2016. Maximal aerobic power and anaerobic capacity in cycling across the age spectrum in male master athletes. *Eur. J. Appl. Physiol.* 116: 1395–410.
9. Tromm, C.B., Pozzi, B.G., Paganini, C.S., Marques, S.O., Pedroso, G.S. and Souza, P.S. 2016. The role of continuous versus fractionated physical training on muscle oxidative stress parameters and calcium-handling proteins in aged rats. *Aging Clin. Exp. Res.* 28: 833–841.
10. Ziaaldini, M.M., Koltai, E., Csende, Z., Goto, S., Boldogh, I., Taylor, A.W. and Radak, Z. 2015. Exercise training increases anabolic and attenuates catabolic and apoptotic processes in aged skeletal muscle of male rats. *Exp. Gerontol*. 67: 9–14.
11. Fujita, S., Rasmussen, B.B., Cadenas, J.G., Drummond, M.J., Glynn, E.L., Sattler, F.R. and Volpi, E. 2007. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. *Diabetes*. 56: 1615–162.
12. Li, M., Li, C., & Parkhouse, W. S. (2003). Age-related differences in the des IGF-I-mediated activation of Akt-1 and p70S6K in mouse skeletal muscle. *Mech Ageing Dev.* 124(7), 771–778.
13. Bua, E., Johnson, J. and Herbst, A. 2006. Mitochondrial DNA- deletion mutations accumulate intracellularly to detrimental levels in aged human skeletal muscle fibers. *Am. J. Hum. Genet*. 70: 469–480.
14. Zahn, J., Sonu, R. and Vogeletal, H. 2006. Transcriptional profiling of aging in human muscle reveals a common aging signature. *PLoS Genetics.*
15. Coen, M., Jubrias, S.A., Distefano, G., Amati, F., Mackey, D.C., Glynn, N.W., Manini, T.M., Wohlgemuth, S.E., Leeuwenburgh, C., Cummings, S.R., Newman, A.B., Ferrucci, L., Toledo, F.G., Shankland, E., Conley, K.E. and Goodpaster, B.H. 2013. Skeletal muscle mitochondrial energetics are associated with maximal aerobic capacity and walking speed in older adults. *J. Gerontol. A Biol. Sci. Med. Sci*. 68: 447–455.
16. Zane, C., Reiter, D.A., Shardell, M., Cameron, D., Simonsick, E.M., Fishbein, K.W., Studenski, S.A., Spencer, R.G. and Ferrucci, L. 2017. Muscle strength mediates the relationship between mitochondrial energetics and walking performance. *Aging Cell*. 16: 461–468.
17. Ghosh, S., Lertwattanarak, R., Lefort, N., Molina-Carrion, M., Joya- Galeana, J., Bowen, B. and Musi, N. 2011. Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance. *Diabetes.* 60: 2051– 2060.
18. Murase, T., Haramizu, S., Ota, N., and Hase, T. (2009). Suppression of the aging-associated decline in physical performance by a combination of resveratrol intake and habitual exercise in senescence-accelerated mice. *Biogerontology,* 10(4), 423–434.
19. Naidoo, A., Naidoo, K., Yende-zuma, N., and Gengiah, T. N. (2015). Effect of high-intensity exercise on aged mouse brain mitochondria, neurogenesis, and inflammation. *Neurobiol Aging,* 19(2), 161–169.
20. Safdar, A., Little, J.P., Stokl, A.J., Hettinga, B.P., Akhtar, M. and Tarnopolsky, M.A. 2011. Exercise increases mitochondrial PGC-1alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J. Biol. Chem.* 286: 10605–10617.
21. Takeda, T., Matsushita, M., Kurozumi, M., Takemura, K., Higuchi, K. and Hosokawa, M. 1997. Pathobiology of the senescence-accelerated mouse (SAM). *Exp. Gerontol.* 32: 117–127.
22. Short, K.R., Bigelow, M.L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S. and Nair, K.S. 2005. Decline in skeletal muscle mitochondrial function with aging in humans. *Proc. Natl. Acad. Sci. USA.* 102: 5618–5623.
23. Wim, D., Bert, E., Monique, R. and Peter, H. 2005. Soleus muscles of SAMP8 mice provide an accelerated model of skeletal muscle senescence. *Exp. Gerontol.* 40: 562–572.
24. Liu, H. W., Chan, Y. C., Wei, C. C., Chen, Y. A., Wang, M. F., & Chang, S. J. (2017). An alternative model for studying age-associated metabolic complications: Senescence-accelerated mouse prone 8. *Exp. Gerontol*, 99(1), 61–68.