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Study of Therapeutic Effects of Losartan for Sarcopenia Based on the F344xBN Rat Aging Model

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Abstract. *Background/Aim: Sarcopenia is an age-related disease in which muscle mass and strength are markedly reduced. There are few effective treatments, but the angiotensin II receptor antagonist losartan has been reported to be effective. Our aim was to evaluate the therapeutic effectiveness of losartan for sarcopenia and explore the underlying mechanisms. Materials and Methods: We investigated body weight, muscle mass (gastrocnemius, soleus, peroneus longus, and tibialis anterior muscles), and serum markers in an aged rat model population divided into four treatment groups: Control, exercise, losartan, and exercise plus losartan. The rats were sacrificed at 6, 12, or 18 months after the start of the experiment and autopsies were performed. Results: Compared with the control group, average muscle mass and weight increased in the two groups treated with losartan. AKT serine/threonine kinase (AKT) and extracellular signal-regulated kinase (ERK) muscle growth factors increased in the peroneus longus. mechanistic target of rapamycin kinase (mTOR) increased in tibialis anterior, peroneus longus, and soleus. Conclusion: Losartan treatment slowed muscle degeneration and activated the PI3K–AKT–mTOR and ERK/mitogen-activated protein kinase signalling pathways required for production of muscle growth factors when combined with exercise.*

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Key Words: Sarcopenia, skeletal muscle, aging, exercise, PI3K/AKT/mTOR signalling pathway, angiotensin II receptor blockade.

Sarcopenia is an age-related disease. Individuals begin to experience a linear decrease in skeletal muscle mass and strength from their early 40s; up to 50% of skeletal muscle mass is lost by the late 80s (1-3). Given that muscle accounts for up to 60% of body mass, pathological changes to this tissue can cause significant problems for older people. Sarcopenia develops together with frailty, cachexia, osteoporosis, and metabolic alterations that lead to death (4, 5). In modern society, sarcopenia develops due to various causes, including altered living environments and eating habits, decreased activity, excessive muscle use (for example, high-intensity repetitive labour), inflammation, and aging (6, 7). Despite the increasing incidence of age-related sarcopenia, the molecular and cellular mechanisms responsible for its occurrence are not fully understood. However, recent studies have shown that increased transforming growth factor-β (TGF-β) signalling affects satellite cell function and skeletal muscle damage. Antagonism of TGF-β signalling by losartan, an angiotensin II receptor antagonist commonly used in treating hypertension, improved muscle fibre and *in vivo* muscle function in sarcopenic mice (8). Levels of hormones, such as cortisol, oestrogen, testosterone, and insulin-like growth factor 1 (IGF-1), which affect skeletal muscle mass, were reduced, and muscle protein metabolism was affected. Decreases in growth hormone and IGF-1, mainly found in sarcopenia, are associated with decreases in muscle mass and bone density (9-12). The phosphoinositide 3 kinase (PI3K)–AKT serine/threonine kinase (AKT)–mechanistic target of rapamycin kinase (mTOR) pathway is activated when growth factor binds to the tyrosine kinase receptor, inducing an increased rate of cell growth and proliferation. Reduced growth factor expression leads to a decrease in downstream signalling factors. This process induces sarcopenia as protein synthesis is reduced and proteolysis is increased.

The extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) pathways contribute to the proliferation of muscle cells. The development of sarcopenia reduces the level of expression

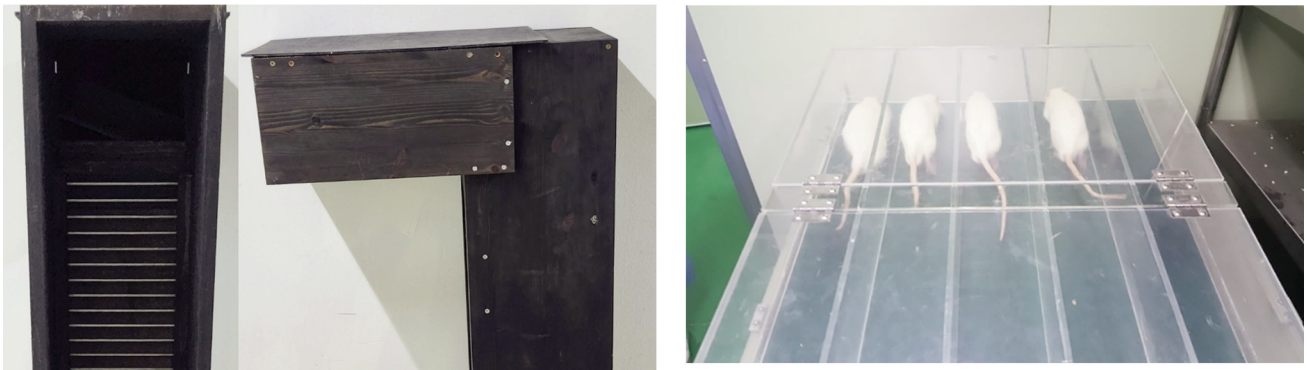

Figure 1. *Animal exercise experiment. Left: The Climb is an exercise device. The rats climbed ladders with weights hung from their tails. This was performed nine times per session, with three sessions per week. The weight was gradually increased from 150 g to 250 g. Right: A DJ-344 treadmill manufactured by Daejong Instrument Industry was used. Through training, the amount of exercise was gradually increased to 20 min at a 10° inclination and a speed of 30 m/min. Before the experiment, animals were adapted to the treadmill and the climb.*

of ERK and MAPK pathways and inhibits muscle cell proliferation (13, 14).

Several methods of sarcopenia treatment are currently under study. The most effective treatment is to increase the synthesis of AKT, ERK1/2, and mTOR, which are directly involved in muscle protein synthesis. The PI3K–AKT–mTOR pathway is activated when a growth factor binds to the tyrosine kinase receptor, increasing cell growth and proliferation. Activation of this pathway increases the synthesis of growth factors, which has been shown to have benefits in sarcopenia (15, 16). Losartan is a drug used to treat hypertension and is a renin-angiotensin II receptor antagonist. It reduces blood pressure by blocking activation of the angiotensin II receptor and reducing the production and secretion of aldosterone (17, 18). Losartan was selected because previous studies found that losartan, administered to treat patients with heart failure, improved muscle performance (19). In this study, losartan was used to increase growth factors involved in muscle protein synthesis. Losartan stimulates growth factors to increase PI3K–AKT and mTOR activity. It promotes muscle regeneration by inhibiting muscle fibrosis and promoting muscle fibre differentiation. TGF-β signalling activates myofibroblasts in the myofibers, resulting in muscle fibrosis; this reduces muscle mass. Furthermore, as angiotensin II is inhibited by losartan, the AKT, mTOR and ERK pathways are up-regulated through two crucial but different pathways for skeletal muscle homeostasis, the TGF-β, and IGF-1/AKT/mTOR signalling cascades. Up-regulation of AKT/mTOR and ERK pathways contributes to myofiber formation, which improves muscle remodelling and provides protection from sarcopenia (17, 20). A study on the C57BL/6 aged mouse model found improved skeletal muscle activity (8). However, as far as we are aware, there have been no studies in aged rat models and thus, there are no Food and Drug Administration-approved drugs for sarcopenia (15, 19, 21). Therefore, this study aimed to evaluate the therapeutic

Table I. *Treatment regimens.*

Group	Exercise	Agent
Group A	–	–
Group B	Treadmill, climb (3 times a week)	–
Group C	–	Losartan (0.6 g/l in drinking water)
Group D	Treadmill, climb (3 times a week)	Losartan (0.6 g/l in drinking water)

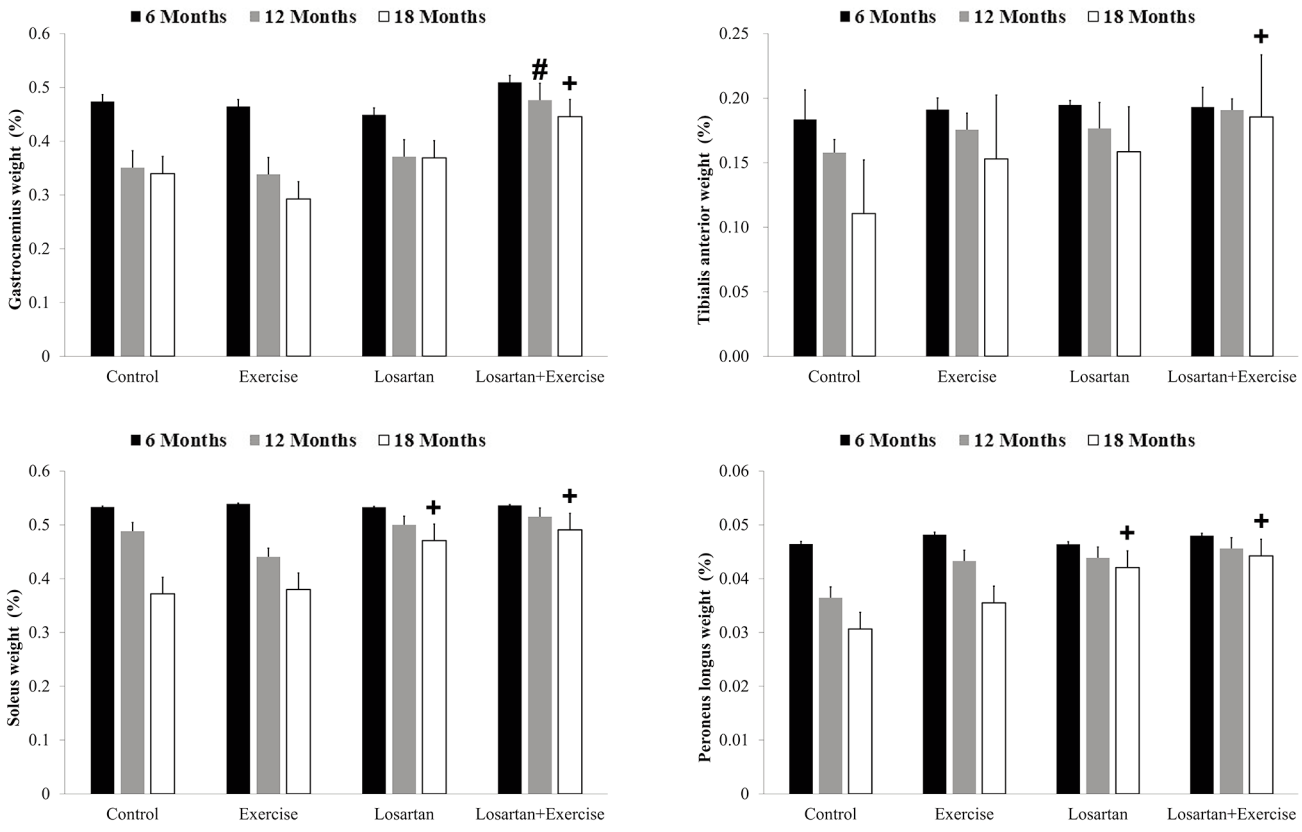

Figure 2. *Muscle mass at 6, 12, and 18 months. The effects of losartan and resistance exercise on muscle regeneration were evaluated. Relative muscle mass was quantified as muscle weight/total body weight×100. The relative weights of gastrocnemius, tibialis anterior, soleus and peroneus longus muscles, respectively, are indicated for each group. Values represent the mean±standard error of each treatment group (Tukey-Kramer method, n=5). Significantly different at p<0.05 vs. #12-month control group, +18-month control group.*

effect of the angiotensin II receptor antagonist losartan on sarcopenia through the activation of AKT, ERK, and mTOR cytokines in F344xBN F2 rats, which are suitable for the study of aging phenomena (22-24).

Materials and Methods

Animals and ethics. All procedures were performed according to the protocol, which was approved by the Institutional Animal Care and Use Committee (approval number: GIACUC-R2018028) of the Animal Experimental Ethics Committee of Gachon University Gil Hospital. Four-week-old F344 and BN rats were purchased from Japan SLC Inc (Japan SLC Inc, Hamamatsu, Japan). They were raised in the animal room of the Gachon University Bio-Nano Research Institute. F2 rats produced from mating F1 (F344xBN) offspring of F344 and BN rats were used for our experiments. This study was conducted in male F344xBN rats (aged 4 weeks). The animals were bred at a controlled temperature (24˚C±2˚C) and humidity (50±10%), with a 12-h light/dark cycle. The animals were self-fed with Purina laboratory animal diet (Nestlé Purina Pet Care Company, St Louis, MO, USA) and water. After the experiment, the animals were fasted for 12 h and were then euthanized after anaesthesia using isoflurane in an enclosed chamber (25, 26). After incision of the abdominal cavity, a 10 ml 23G needle was inserted into the inferior vena cava and 10 ml of blood was collected. When collecting blood, about 2 ml was put into a complete blood count tube (SEWON MEDICAL, Busan, Republic of Korea) containing a K2EDTA anticoagulant. The remaining blood was placed in a BD Vacuum® blood collection tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and centrifuged for 15 min at 1,814×*g*. Subsequently, the serum was separated and stored at −85˚C.

Experimental design. The total number of rats was 60. There were four experimental groups: A was the control group, B was the exercise group, C was the losartan-treated group, and in group D, losartan and exercise were combined. Losartan (Zhejiang Tianyu, Taizhou, PR China) was administered autonomously in the drinking water, diluted to a concentration of 0.6 g/l. The experimental design for each group is summarized in Table I. The number of rats was divided by the number of study time points (three, thus 20 rats per time point), and each was divided into four experimental groups (five per group). The rats were sacrificed for evaluation at 6 (n=20), 12 (n=20), or 18 (n=20) months (8, 27, 28).

Exercise. Physical exercise, which is known to prevent sarcopenia, was designated as a positive control in this experiment (29, 30). Treadmill and climbing exercises were performed in groups B and D (Figure 1).

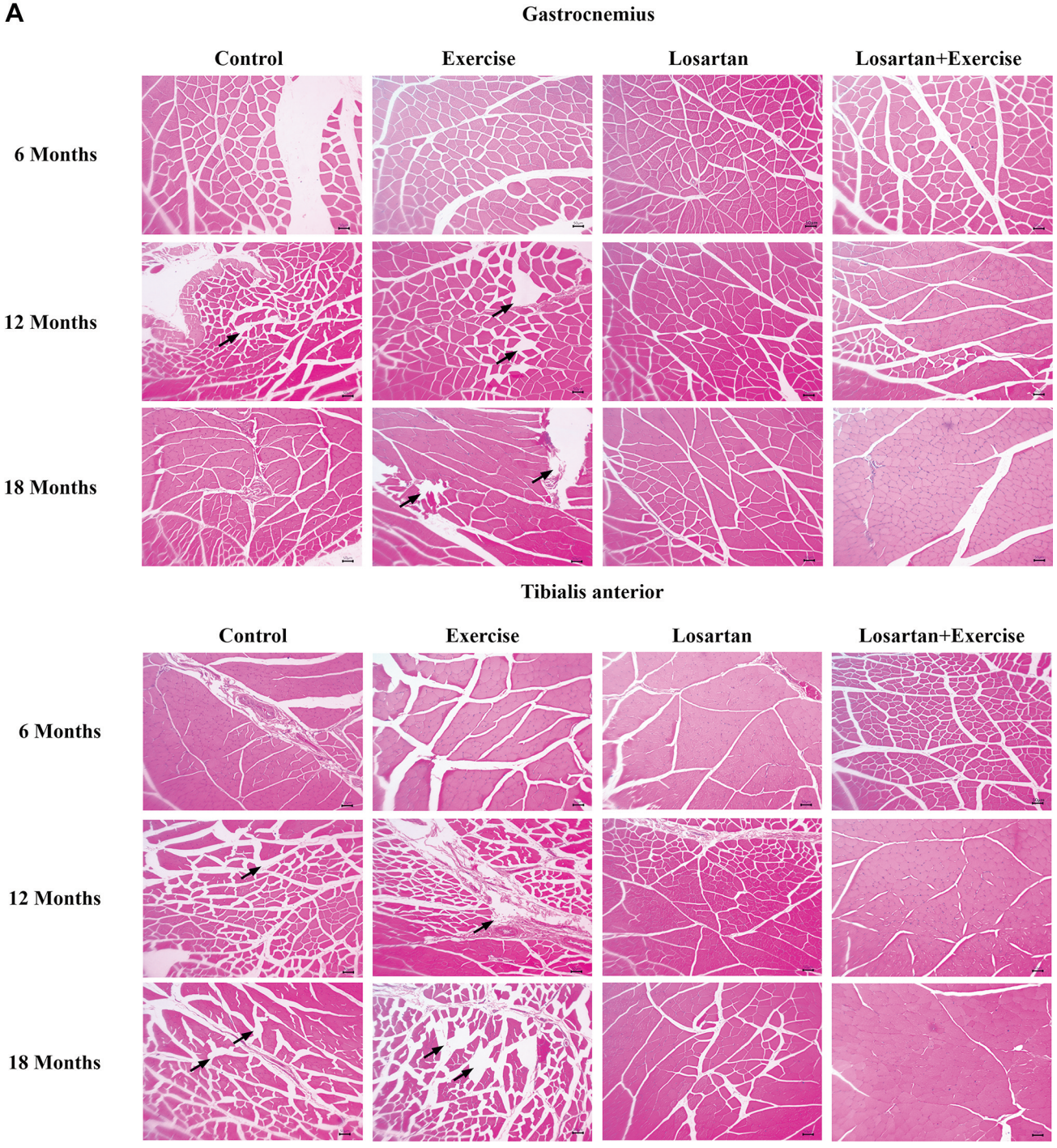

Figure 3. *Continued*

Rats performed treadmill exercises three days a week, each training session lasting for 20 min. Before the experiment, they started running at a speed of 5 m/min at a 10˚ inclination and the speed was gradually increased at 30-second intervals. This treadmill exercise was performed at 30 m/min for a week, to allow the rats to adapt to the device (31). Resistance exercise was carried out through climbing exercises. A load was applied to the tail of the rat. The rats were initially given a load of 150 g and the load was increased by 25 g weekly until 250 g was reached. The training was conducted nine times per session, with three sessions per week. There was a 2-min break between each session.

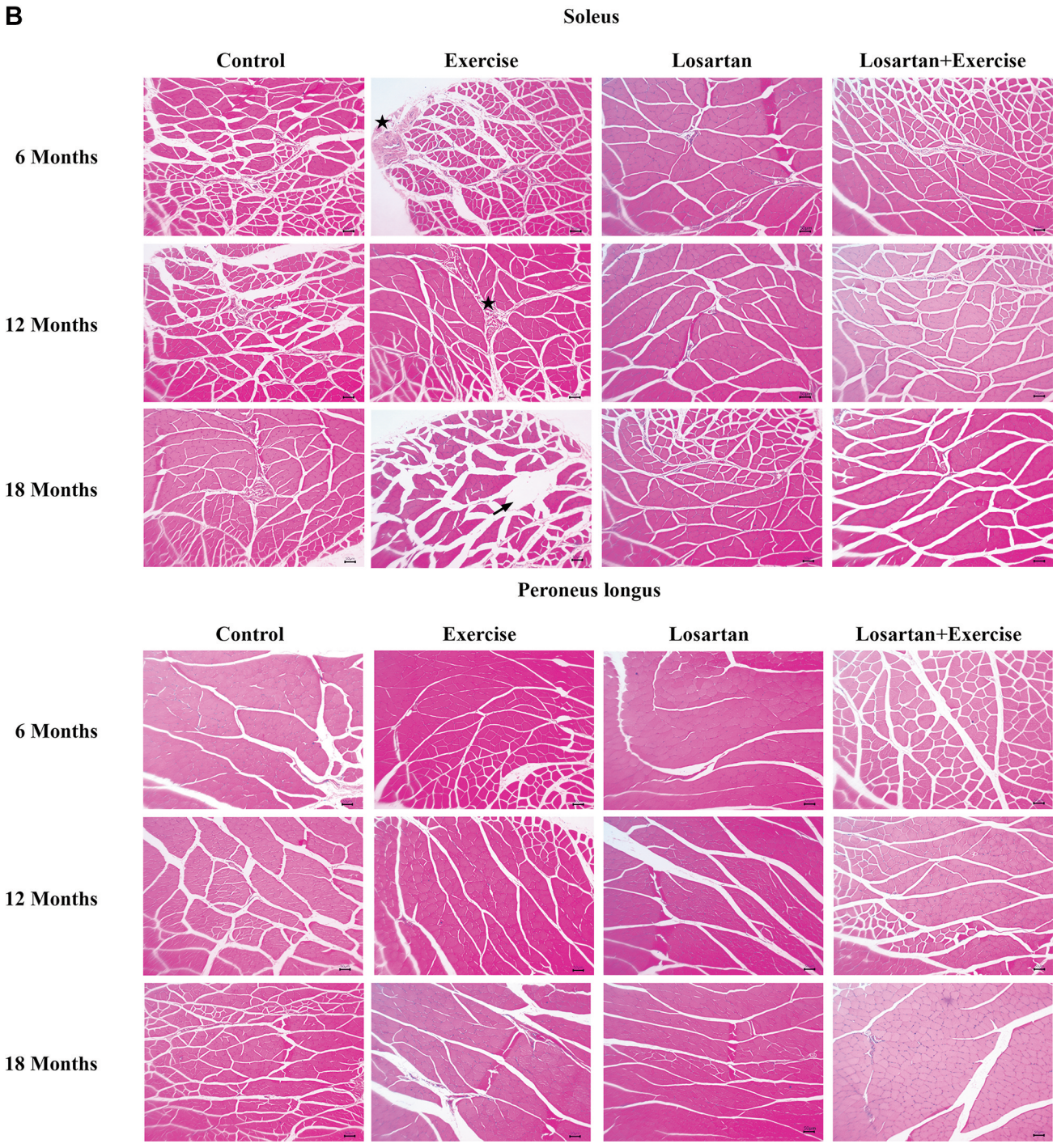

Figure 3. *Continued*

Weight measurement. The weight of each rat was measured at autopsy and compared by endpoint (6, 12 and 18 months). All rats had a 12-h food restriction before weighing and autopsy, but the water supply was unrestricted. During the autopsy after the rats were euthanized, the skin was incised to expose the femoral head, and the connective tissue was cut to separate the femur from the torso. The tibialis anterior (TL), peroneus longus (PL), gastrocnemius (GC), and soleus (SOL) muscles were separated and weighed individually.

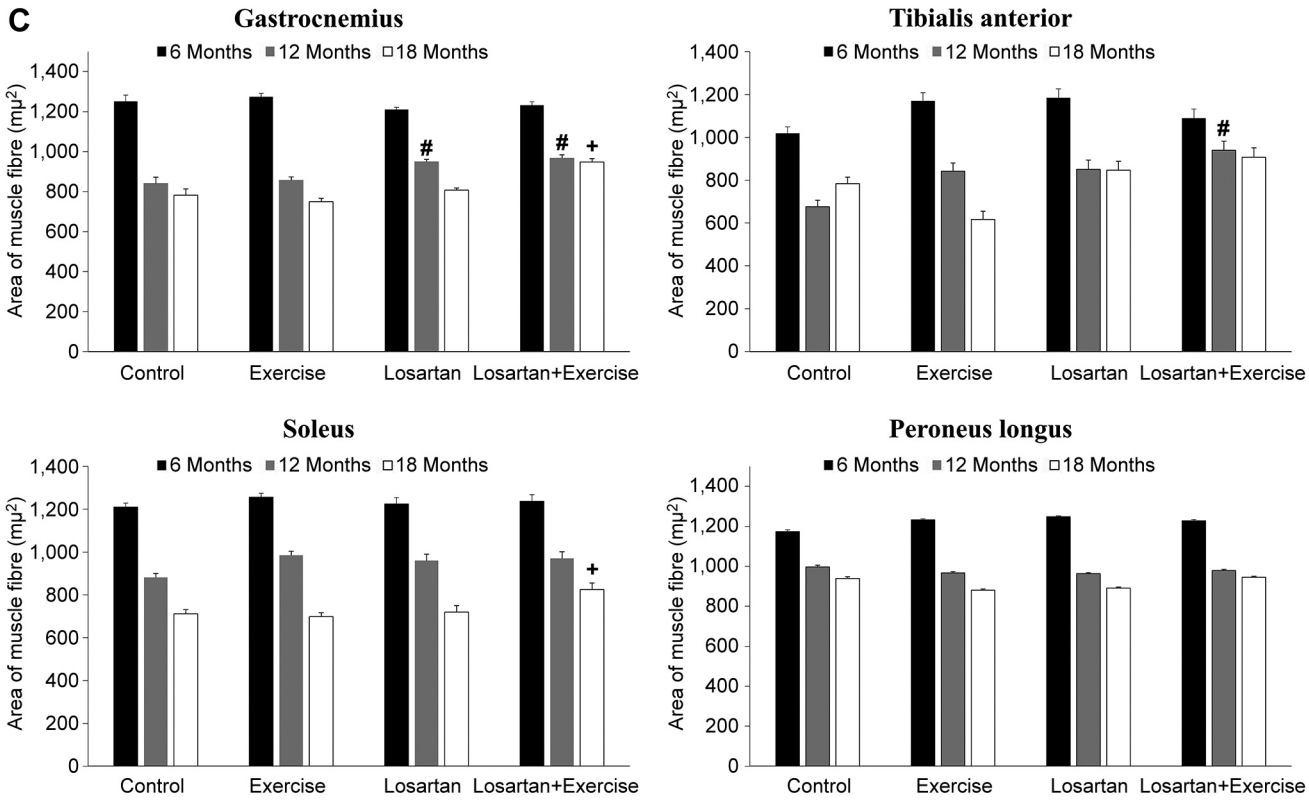

Figure 3. *Histological examination and muscle fibre area of tissue samples from gastrocnemius, tibialis anterior, soleus, and peroneus longus muscles at 6-, 12-, and 18-months in rats of the different treatment groups. A: Haematoxylin and eosin staining of tissue from gastrocnemius and tibialis anterior muscle group. Black arrows show myofascial detachment and ruptured muscle fibre. Asterisks indicate inflammation and connective tissue infiltration. Scale bar=50 μm, original magnification, ×100. B: Haematoxylin and eosin staining of tissue from soleus and peroneus longus muscle group. Black arrows show myofascial detachment and ruptured muscle fibre. Asterisks indicate inflammation and connective tissue infiltration. Scale bar=50 μm, original magnification, ×100. C: Muscle fibre areas in each group. Values represent the mean±standard error of the four treatment groups (Tukey–Kramer method, n=5). Significantly different at $p<0.05$ vs. #12-month control group, +18-month control group.*

Cytokine assay. Samples of TL, PL, GC, and SOL muscle tissue and blood serum collected during the autopsies at 6, 12, and 18 months underwent multiplex analysis using Luminex® (Koma Biotech, Seoul, Republic of Korea). The Luminex System uses bead-based technology to test cytokine signalling for AKT, ERK, and mTOR.

Histological analysis. Transverse incisions were made along the GC, PL, SOL, and TL muscle samples then these were fixed with 4% formaldehyde solution (Sigma-Aldrich, St. Louis, MO, USA) and dehydrated with progressively increasing concentrations of ethanol. The samples were cleared in xylene (BBC Biochemical, McKinney, TX, USA) and embedded in paraffin. These paraffin blocks were cut to 4–μm thickness, attached to slides, and stained with haematoxylin and eosin (H&E; (BBC Biochemical) (32-34). The muscle fibre area was measured using ImageJ 1.53K/Java 1.8.0_172 software (National Institute for Health, Bethesda, MD, USA) under H&E staining (35, 36).

Statistical analysis. All values are expressed as the mean±standard error. Comparison of means between groups were performed using SPSS software (version 26.0.0.0, IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Statistical analyses including one-way analysis of variance and Tukey test were performed with SPSS. In all statistical analyses, $p<0.05$ was considered to indicate a statistically significant difference between groups.

Results

Weight measure. The relative masses of the GC, SOL, PL, and TL muscles at 6, 12, and 18 months according to group were recorded (Figure 2). The aging of skeletal muscle is characterized by reduced muscle mass. The 12-month muscle masses of the TL and PL in the exercise group increased compared to the control group. However, the muscle mass of the exercise group at 18 months was lower in comparison to the control group, but there was no significant difference. Losartan treatment alone significantly increased SOL and PL muscle mass at 18 months compared to the control group (Figure 2). Losartan increases muscle mass by inducing the proliferation of

Table II. *Total body weight (g) at 6, 12, and 18 months.*

Group	6 Months	12 Months	18 Months
Group A	408.68±13.03	360.62±10.05*	313.31±4.95#
Group B	451.43±6.13	413.12±17.88	318.20±14.66#
Group C	412.06±12.45	384.84±18.51	352.83±22.01
Group D	436.40±25.38	419.36±20.81	392.83±22.01

Values represent the mean±standard error of the four treatment groups (Tukey-Kramer method, n=5). Significantly different at $p<0.05$ *vs*. *6-month control group, #12-month control group.

muscle cells to prevent muscle loss due to sarcopenia. The muscle mass at 18 months in the losartan plus exercise group was significantly increased compared to the control group (Figure 2). These results suggest that losartan and resistance exercise in combination can ameliorate skeletal muscle aging.

Total body weights were recorded at 6, 12, and 18 months depending on the group (Table II). In all groups, weight was continuously lost due to aging. The bodyweight of animals of the 18-month exercise group significantly decreased compared to the 12-month control group. However, body weight in the losartan and losartan plus exercise groups significantly increased compared to the control group during the experimental period. Therefore, combining losartan with resistance exercise helps maintain weight.

Histological analysis. The effects of resistance exercise and losartan on muscle fibre area at 6, 12 and 18 months were investigated by H&E staining. Muscles and the area of muscle fibres between groups were compared. All groups showed a tendency for muscle fibre decrease (Figure 3). Fascia separation and ruptured muscle fibres were observed in the control group and exercise group, and inflammation and connective tissue infiltration were observed in the exercise group (Figure 3A and B). Fascia separation was not observed in the losartan-treated group and losartan plus exercise group. Myofiber area in GC in the losartan-treated group was significantly increased compared to the control group at 12-months (Figure 3C). Similarly, in the losartan plus exercise group, the muscle fibre area was significantly increased in GC, TL, and SOL compared to the control group, suggesting that losartan and resistance exercise ameliorated skeletal muscle atrophy.

Cytokine assay. The AKT, ERK1/2, and mTOR cytokine assays of GC, SOL, PL, and TL muscles in 6, 12, and 18-month-old F344xBN rats showed that losartan modulated the PI3K-AKT–mTOR pathway during treatment. Cytokine analysis using antibodies for the pathway proteins showed the AKT level constantly decreased in all muscles of the control group (Figure 4A). The exercise group and losartan-treated group showed an increase in AKT level at 12 and 18 months compared to the control group, but there was no significant difference. In comparison, the AKT level in all muscles was significantly increased in the losartan plus exercise group compared to the control group. Losartan with resistance exercise also improved the ERK1/2 levels compared to the control (Figure 4B). The ERK 1/2 level in the GC of the exercise group was significantly increased at 12 and 18 months compared to the control group, while in the SOL and TL, only the 12-month result was significantly increased. ERK1/2 levels were significantly increased in the losartan group at 12 and 18 months compared to the control group in the GC, SOL, and TL. In the SOL of the losartan, and exercise plus losartan groups at 12 and 18 months, the ERK 1/2 levels were significantly increased compared to the control group (Figure 4B). In the exercise group, the mTOR level was increased compared to the control group, but there was no significant difference. At 12 and 18 months, the mTOR expression levels were significantly increased in the GC of both losartan-treated groups compared to the control group. The mTOR level in all muscle types in the losartan plus exercise group were significantly increased at 12 and 18 months compared to the control group (Figure 4C). Therefore, the losartan treatment modulated the PI3K–mTOR pathway. Compared with the control group, activation of the PI3K–mTOR pathway was significantly increased in the losartan plus exercise group.

Discussion

Skeletal muscle mass is preserved by maintaining a homeostatic balance between muscle regeneration, protein synthesis, and proteolysis. This balance breaks down during aging, leading to loss of muscle mass and function over time. In addition, the reduced regenerative capacity and muscle mass after injury induce an atrophic response to muscle use; this contributes to morbidity and mortality in the aging population (37, 38). This study found that the ability to repair skeletal muscle in F344xBN rats with induced sarcopenia rebounds when they were treated with the angiotensin II receptor blocker losartan. In addition, we observed that the combination of losartan and exercise prevented loss of muscle mass due to leg muscle sarcopenia. Previous studies have provided evidence that angiotensin II receptor blockade affects several important pathways related to skeletal muscle homeostasis, including the AKT/mTOR pathway and the ERK signalling pathway [reviewed in (39)]. In our cytokine assay, the levels of mTOR and ERK1/2 expression in the losartan plus exercise group were significantly increased in all muscles. This implies that the protein expression level increased as the muscles were strengthened due to exercise on the inclined treadmill (40). Moreover, the muscle fibre area significantly increased in the losartan plus exercise group compared to the control group, suggesting that losartan and resistance exercise protected skeletal muscle atrophy from sarcopenia (8, 41, 42).

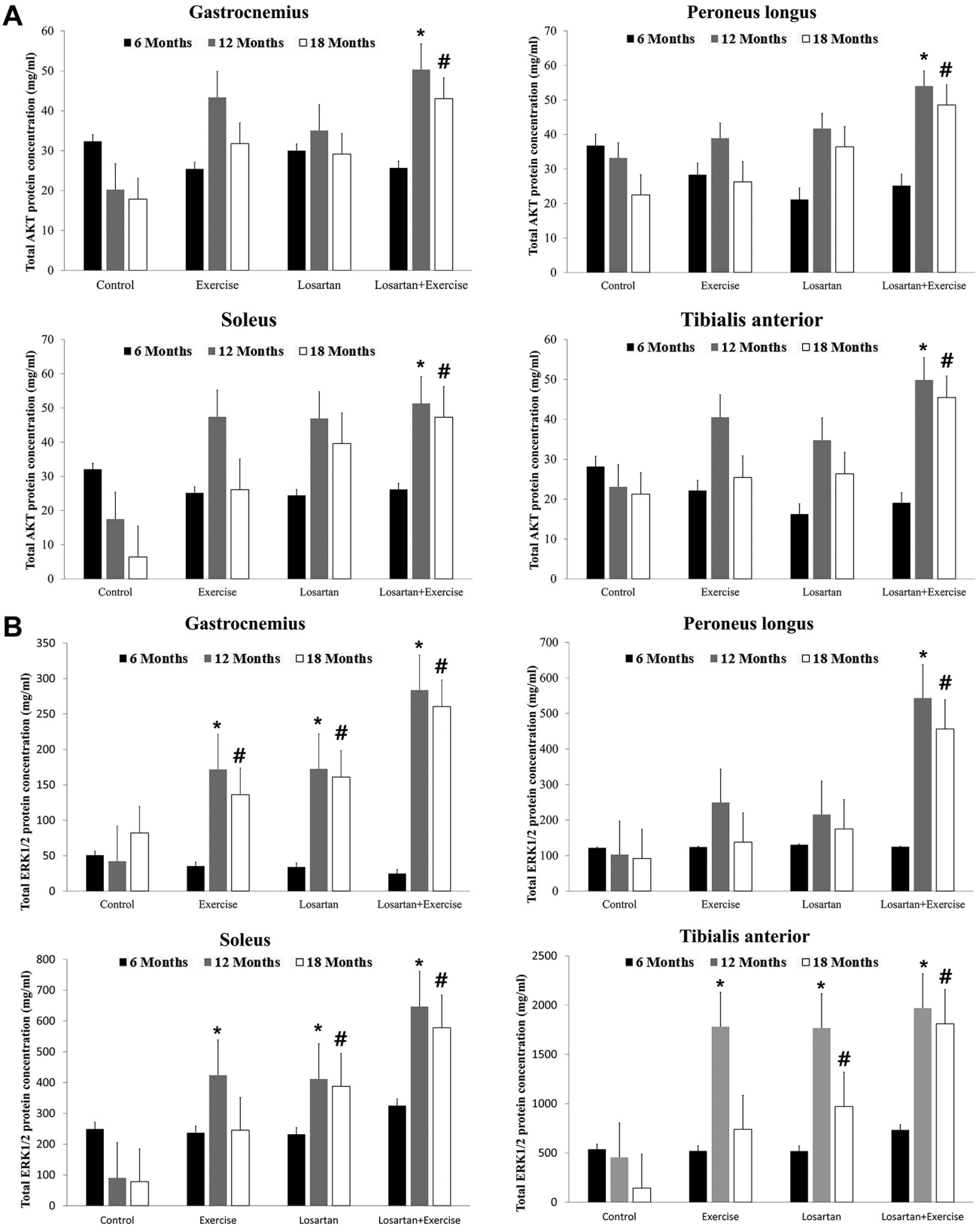

Figure 4. *Continued*

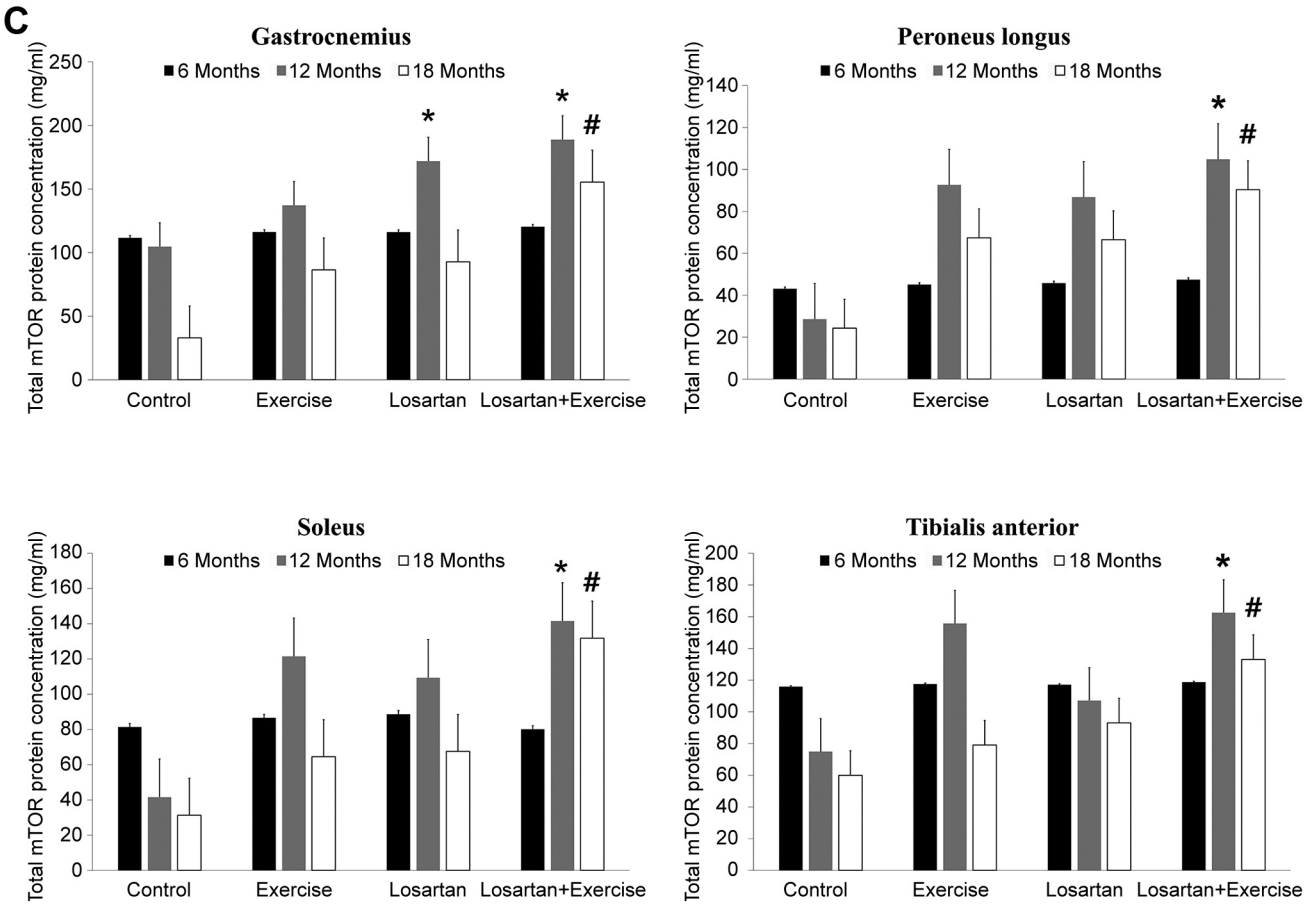

Figure 4. *Effects of losartan and exercise on expression of AKT serine/threonine kinase (AKT) (A), mitogen-activated protein kinase (ERK1/2) (B), and mechanistic target of rapamycin kinase (mTOR) (C) in muscle tissues and cells of sarcopenia-induced F344xBN rats. Values represent the mean±standard error of the four treatment groups (Tukey-Kramer method, n=5). Significantly different at p<0.05 vs. *6-month control group, #12-month control group.*

In conclusion, the results of our study showed that the losartan and losartan plus exercise groups had increased muscle mass and body weight compared to the control group. Cytokine activity and expression were also higher in the losartan plus exercise group. This suggests that losartan with resistance exercise ameliorates sarcopenia. We found that blocking the angiotensin II receptor led to AKT–mTOR–ERK signalling regulation that protected against sarcopenia-induced muscle decrease. Skeletal muscle remodelling was also positively affected by the increased skeletal muscle mass. These results demonstrate that losartan was effective in treating sarcopenia of skeletal muscle in rats. However, it would have been helpful in this study had the cytokine assay also measured levels of other molecules involved in aging, such as IGF-1, cortisol, oestrogen, and testosterone (9). Furthermore, follow-up studies should identify the appropriate concentration and possible side-effects of losartan according to age and explore the appropriate amount of exercise it should be combined with.

Conflicts of Interest

There are no conflicts to declare.

Authors' Contributions

Conceptualization, D.H.K., D.Y.K. and K.D.P.; methodology, D.H.K., D.Y.K. and K.D.P.; investigation, D.H.K., D.Y.K. and K.D.P.; writing—original draft preparation, D.H.K. and D.Y.K.; writing—review and editing, D.Y.K.; visualization, D.H.K. and D.Y.K.; supervision, D.Y.K. All Authors read and agreed to the published version of the article.

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References

1 Walston JD: Sarcopenia in older adults. Curr Opin Rheumatol *24(6)*: 623-627, 2012. PMID: 22955023. DOI: 10.1097/BOR.0b013e328358d59b

2 Kim EH and Kim SM: Sarcopenia of the old age. Korean J Clin Geriatr *16(1)*: 1-7, 2015. DOI: 10.15656/kjcg.2015.16.1.1

3 Marcell TJ: Sarcopenia: causes, consequences, and preventions. J Gerontol A Biol Sci Med Sci *58(10)*: M911-M916, 2003. PMID: 14570858. DOI: 10.1093/gerona/58.10.m911

4 Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinková E, Vandewoude M, Zamboni M and European Working Group on Sarcopenia in Older People: Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing *39(4)*: 412-423, 2010. PMID: 20392703. DOI: 10.1093/ageing/afq034

5 Greco EA, Pietschmann P and Migliaccio S: Osteoporosis and sarcopenia increase frailty syndrome in the elderly. Front Endocrinol (Lausanne) *10*: 255, 2019. PMID: 31068903. DOI: 10.3389/fendo.2019.00255

6 Wilson D, Jackson T, Sapey E and Lord JM: Frailty and sarcopenia: The potential role of an aged immune system. Ageing Res Rev *36*: 1-10, 2017. PMID: 28223244. DOI: 10.1016/j.arr.2017.01.006

7 Rom O, Kaisari S, Aizenbud D and Reznick AZ: Lifestyle and sarcopenia-etiology, prevention, and treatment. Rambam Maimonides Med J *3(4)*: e0024, 2012. PMID: 23908848. DOI: 10.5041/RMMJ.10091

8 Burks TN, Andres-Mateos E, Marx R, Mejias R, Van Erp C, Simmers JL, Walston JD, Ward CW and Cohn RD: Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. Sci Transl Med *3(82)*: 82ra37, 2011. PMID: 21562229. DOI: 10.1126/scitranslmed.3002227

9 Morley JE: Hormones and sarcopenia. Curr Pharm Des *23(30)*: 4484-4492, 2017. PMID: 27881060. DOI: 10.2174/1381612823666161123150032

10 Moustogiannis A, Philippou A, Zevolis E, Taso O, Chatzigeorgiou A and Koutsilieris M: Characterization of optimal strain, frequency and duration of mechanical loading on skeletal myotubes' biological responses. In Vivo *34(4)*: 1779-1788, 2020. PMID: 32606147. DOI: 10.21873/invivo.11972

11 Karalaki M, Fili S, Philippou A and Koutsilieris M: Muscle regeneration: cellular and molecular events. In Vivo *23(5)*: 779-796, 2009. PMID: 19779115.

12 Bian A, Ma Y, Zhou X, Guo Y, Wang W, Zhang Y and Wang X: Association between sarcopenia and levels of growth hormone and insulin-like growth factor-1 in the elderly. BMC Musculoskelet Disord *21(1)*: 214, 2020. PMID: 32264885. DOI: 10.1186/s12891-020-03236-y

13 Kalinkovich A and Livshits G: Sarcopenia—The search for emerging biomarkers. Ageing Res Rev *22*: 58-71, 2015. PMID: 25962896. DOI: 10.1016/j.arr.2015.05.001

14 Ziaaldini MM, Marzetti E, Picca A and Murlasits Z: Biochemical pathways of sarcopenia and their modulation by physical exercise: a narrative review. Front Med (Lausanne) *4*: 167, 2017. PMID: 29046874. DOI: 10.3389/fmed.2017.00167

15 Cohen S, Nathan JA and Goldberg AL: Muscle wasting in disease: molecular mechanisms and promising therapies. Nat Rev Drug Discov *14(1)*: 58-74, 2015. PMID: 25549588. DOI: 10.1038/nrd4467

16 Fannin J, Rice KM, Thulluri S, Dornon L, Arvapalli RK, Wehner P and Blough ER: Age-associated alterations of cardiac structure and function in the female F344xBN rat heart. Age (Dordr) *36(4)*: 9684, 2014. PMID: 25062714. DOI: 10.1007/s11357-014-9684-6

17 Abadir PM, Foster DB, Crow M, Cooke CA, Rucker JJ, Jain A, Smith BJ, Burks TN, Cohn RD, Fedarko NS, Carey RM, O'Rourke B and Walston JD: Identification and characterization of a functional mitochondrial angiotensin system. Proc Natl Acad Sci U S A *108(36)*: 14849-14854, 2011. PMID: 21852574. DOI: 10.1073/pnas.1101507108

18 Siegl PK, Kivlighn SD and Broten TP: Pharmacology of losartan, an angiotensin II receptor antagonist, in animal models of hypertension. J Hypertens Suppl *13(1)*: S15-S21, 1995. PMID: 18800451. DOI: 10.1097/00004872-199507001-00002

19 Vescovo G, Dalla Libera L, Serafini F, Leprotti C, Facchin L, Volterrani M, Ceconi C and Ambrosio GB: Improved exercise tolerance after losartan and enalapril in heart failure: correlation with changes in skeletal muscle myosin heavy chain composition. Circulation *98(17)*: 1742-1749, 1998. PMID: 9788828. DOI: 10.1161/01.cir.98.17.1742

20 Murphy AM, Wong AL and Bezuhly M: Modulation of angiotensin II signaling in the prevention of fibrosis. Fibrogenesis Tissue Repair *8*: 7, 2015. PMID: 25949522. DOI: 10.1186/s13069-015-0023-z

21 Lin CH, Yang H, Xue QL, Chuang YF, Roy CN, Abadir P and Walston JD: Losartan improves measures of activity, inflammation, and oxidative stress in older mice. Exp Gerontol *58*: 174-178, 2014. PMID: 25077714. DOI: 10.1016/j.exger.2014.07.017

22 Paturi S, Gutta AK, Katta A, Kakarla SK, Arvapalli RK, Gadde MK, Nalabotu SK, Rice KM, Wu M and Blough E: Effects of aging and gender on muscle mass and regulation of Akt-mTOR-p70s6k related signaling in the F344BN rat model. Mech Ageing Dev *131(3)*: 202-209, 2010. PMID: 20153766. DOI: 10.1016/j.mad.2010.01.008

23 Hepple RT, Hagen JL, Krause DJ and Baker DJ: Skeletal muscle aging in F344BN F1-hybrid rats: II. Improved contractile economy in senescence helps compensate for reduced ATP-generating capacity. J Gerontol A Biol Sci Med Sci *59(11)*: 1111-1119, 2004. PMID: 15602056. DOI: 10.1093/gerona/59.11.1111

24 Hagen JL, Krause DJ, Baker DJ, Fu MH, Tarnopolsky MA and Hepple RT: Skeletal muscle aging in F344BN F1-hybrid rats: I. Mitochondrial dysfunction contributes to the age-associated reduction in VO2max. J Gerontol A Biol Sci Med Sci *59(11)*: 1099-1110, 2004. PMID: 15602055. DOI: 10.1093/gerona/59.11.1099

25 Marquardt N, Feja M, Hünigen H, Plendl J, Menken L, Fink H and Bert B: Euthanasia of laboratory mice: Are isoflurane and sevoflurane real alternatives to carbon dioxide? PLoS One *13(9)*: e0203793, 2018. PMID: 30199551. DOI: 10.1371/journal.pone.0203793

26 Nagate T, Chino T, Nishiyama C, Okuhara D, Tahara T, Maruyama Y, Kasahara H, Takashima K, Kobayashi S, Motokawa Y, Muto S and Kuroda J: Diluted isoflurane as a suitable alternative for diethyl ether for rat anaesthesia in regular toxicology studies. J Vet Med Sci *69(11)*: 1137-1143, 2007. PMID: 18057828. DOI: 10.1292/jvms.69.1137

27 Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW and Dietz HC: Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med *13(2)*: 204-210, 2007. PMID: 17237794. DOI: 10.1038/nm1536

28 Sellers SL, Milad N, Chan R, Mielnik M, Jermilova U, Huang PL, de Crom R, Hirota JA, Hogg JC, Sandor GG, Van Breemen C, Esfandiarei M, Seidman MA and Bernatchez P: Inhibition of Marfan syndrome aortic root dilation by losartan: Role of angiotensin II receptor type 1-independent activation of endothelial function. Am J Pathol *188(3)*: 574-585, 2018. PMID: 29433732. DOI: 10.1016/j.ajpath.2017.11.006

29 Cho J, Choi Y, Sajgalik P, No MH, Lee SH, Kim S, Heo JW, Cho EJ, Chang E, Kang JH, Kwak HB and Park DH: Exercise as a therapeutic strategy for sarcopenia in heart failure: insights into underlying mechanisms. Cells *9(10)*: 2284, 2020. PMID: 33066240. DOI: 10.3390/cells9102284

30 Aoyama T, Nakazono M, Nagasawa S and Segami K: Clinical impact of a perioperative exercise program for sarcopenia and overweight/obesity gastric cancer. In Vivo *35(2)*: 707-712, 2021. PMID: 33622863. DOI: 10.21873/invivo.12311

31 Aamann L, Ochoa-Sanchez R, Oliveira M, Tremblay M, Bémeur C, Dam G, Vilstrup H, Aagaard NK and Rose CF: Progressive resistance training prevents loss of muscle mass and strength in bile duct-ligated rats. Liver Int *39(4)*: 676-683, 2019. PMID: 30394651. DOI: 10.1111/liv.13997

32 Olson LC, Nguyen TM, Heise RL, Boyan BD, Schwartz Z and McClure MJ: Advanced glycation end products are retained in decellularized muscle matrix derived from aged skeletal muscle. Int J Mol Sci *22(16)*: 8832, 2021. PMID: 34445538. DOI: 10.3390/ijms22168832

33 Sayed RK, de Leonardis EC, Guerrero-Martínez JA, Rahim I, Mokhtar DM, Saleh AM, Abdalla KE, Pozo MJ, Escames G, López LC and Acuña-Castroviejo D: Identification of morphological markers of sarcopenia at early stage of aging in skeletal muscle of mice. Exp Gerontol *83*: 22-30, 2016. PMID: 27435496. DOI: 10.1016/j.exger.2016.07.007

34 Wang C, Yue F and Kuang S: Muscle histology characterization using H&E staining and muscle fiber type classification using immunofluorescence staining. Bio Protoc *7(10)*: e2279, 2017. PMID: 28752107. DOI: 10.21769/BioProtoc.2279

35 Lin J, Lopez EF, Jin Y, Van Remmen H, Bauch T, Han HC and Lindsey ML: Age-related cardiac muscle sarcopenia: Combining experimental and mathematical modeling to identify mechanisms. Exp Gerontol *43(4)*: 296-306, 2008. PMID: 18221848. DOI: 10.1016/j.exger.2007.12.005

36 Mizunoe Y, Kobayashi M, Saito H, Goto A, Migitaka R, Miura K, Okita N, Sudo Y, Tagawa R, Yoshida M, Umemori A, Nakagawa Y, Shimano H and Higami Y: Prolonged caloric restriction ameliorates age-related atrophy in slow and fast muscle fibers of rat soleus muscle. Exp Gerontol *154*: 111519, 2021. PMID: 34416335. DOI: 10.1016/j.exger.2021.111519

37 Morris RT, Spangenburg EE and Booth FW: Responsiveness of cell signaling pathways during the failed 15-day regrowth of aged skeletal muscle. J Appl Physiol (1985) *96(1)*: 398-404, 2004. PMID: 14514701. DOI: 10.1152/japplphysiol.00454.2003

38 Chakravarthy MV, Davis BS and Booth FW: IGF-I restores satellite cell proliferative potential in immobilized old skeletal muscle. J Appl Physiol (1985) *89(4)*: 1365-1379, 2000. PMID: 11007571. DOI: 10.1152/jappl.2000.89.4.1365

39 Yoon JH and Kwon KS: Receptor-mediated muscle homeostasis as a target for sarcopenia therapeutics. Endocrinol Metab (Seoul) *36(3)*: 478-490, 2021. PMID: 34218646. DOI: 10.3803/EnM.2021.1081

40 Bavdek R, Zdolšek A, Strojnik V and Dolenec A: Peroneal muscle activity during different types of walking. J Foot Ankle Res *11*: 50, 2018. PMID: 30202446. DOI: 10.1186/s13047-018-0291-0

41 Lin CH, Chang PC, Chu PH, Chuang YF, Huang RC and Chen CN: Effects of losartan and exercise on muscle mass and exercise endurance of old mice. Exp Gerontol *165*: 111869, 2022. PMID: 35710057. DOI: 10.1016/j.exger.2022.111869

42 Yoshihara T, Takaragawa M, Dobashi S and Naito H: Losartan treatment attenuates hindlimb unloading-induced atrophy in the soleus muscle of female rats via canonical TGF-β signaling. J Physiol Sci *72(1)*: 6, 2022. PMID: 35264097. DOI: 10.1186/s12576-022-00830-8

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