Scientific Research Publishing

Chinese Medicine, 2023, 14, 276-285
https://www.scirp.org/journal/cm
ISSN Online: 2151-1926
ISSN Print: 2151-1918

Effect of Sijunzi Decoction on the Myonuclear Domain of Rat Soleus in Spleen Qi Deficiency

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How to cite this paper: Zhang, L.L., Ma, D., Wang, S.N., Liu, X.D., Wang, L.Z. and Shan, D.H. (2023) Effect of Sijunzi Decoction on the Myonuclear Domain of Rat Soleus in Spleen Qi Deficiency. *Chinese Medicine*, **14**, 276-285.
https://doi.org/10.4236/cm.2023.144015

Received: November 16, 2023
Accepted: December 19, 2023
Published: December 22, 2023

Abstract

Objective: To study the mechanism of Sijunzi decoction treating limb weakness in spleen Qi deficiency (SQD) based on the myonuclear domain (MND) theory. **Methods**: 40 male Sprague-Dawley rats were randomly divided into the normal group, SQD model group (model group), SQD+ still water group (SW group) and SQD+ Sijunzi decoction group (CM group), 10 rats each group; Grip-Strength Meter was used to measure limb grip strength; transmission electron microscope was employed to observe the ultrastructural changes of the myofibers, Image Pro 6.0 was used to measure the myonuclear numbers, cross-section area (CSA) and then their ratios (the MND sizes) were calculated, immunofluorescence assay was chosen to test the expressions of paired box gene 7 (Pax7) and myogenic differentiation antigen (MyoD). **Results**: Compared with those in the normal group, limb grip strength was decreased, sarcomeres were abnormal, and all the myonuclear numbers, CSA and MND sizes were reduced, but the Pax7+ cell numbers were increased, significantly, in the model and SW groups; Compared with those in the model and SW groups, limb grip strength was increased, sarcomeres were basically normal, the myonuclear number and CSA were both greater, and the Pax7+ and MyoD+ cell numbers were both increased, significantly, in the CM group. **Conclusion**: Sijunzi decoction might increase the myonuclear number by activating the MSCs to treat limb weakness in SQD.

Keywords

Sijunzi Decoction, Spleen Qi Deficiency, Limb Weakness, Myonuclear Domain, Muscle Stem Cell

1. Introduction

Sijunzi decoction is well known for treating limb weakness in spleen Qi defi-

ciency (SQD), and its mechanism is not clear yet. Muscle fibers are the engines of locomotion, and the muscle fiber is multinuclear, therefore every myonucleus has its own controlling domain, which is called the myonuclear domain (MND) [1]. In general, increasing the MND size means muscle growth or hypertrophy, and decreasing it leads to atrophy. However, it is thought that the MND size only expands to a certain extent, and new nuclei from the muscle stem cells (MSCs) must be added for further muscle growth [2]. The MSCs, also called the satellite cells, can be activated to proliferation, differentiation and fuse into the nearby myofibers [3] [4]. Based on the above findings, the MND size and MSCs were targeted in the present study, attempting to explore the possible mechanism of Sijunzi decoction treating limb weakness in SQD.

2. Materials and Methods

2.1. Animals

Forty male Sprague-Dawley rats, body mass (BM) 200 ± 10 g, Liaoning Chang Sheng Biotechnology Co. Ltd., SCXK (Liao) 2020-0001), were habituated at (22 ± 2)˚C, in 55% ± 5% humidity and with a 12 h light/dark cycle (lights on at 8:00 and off at 20:00). After one week of accommodation, rats were randomly divided into the normal group, SQD model group (model group), SQD model + stilled water group (SW group) and SQD model + Sijunzi decoction (CM group), 10 rats each group. Animal care procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and every effort was made to minimize the animal suffering.

2.2. Establishment and Evaluation of the SQD Model

The SQD model was established and assessed according to our previous study [5]. Briefly, in 2 weeks, rats in the model, SW and CM groups were provided with feed and water *ad libitum* on odd days and fed only with cabbage on even days, meanwhile they were forced to swim to fatigue every day. The evaluation standards included emaciation (BM decrease) checked by the electronic scales, poor appetite (decreases of food and water intakes) detected in the metabolic cages, mental fatigue (motion distances and vertical times) recorded with the OFT-100 opening activity experiment system (Chengdu TME Technology Co, Ltd., Chengdu, China), and limb weakness checked with YLS-13A Grip Strength Meter for Rats and Mice (Jinan Yi Yan Technology Development Co., Ltd., Shandong, China). Evaluation of the SQD model was finished by an investigator who was blinded for groups.

2.3. Treatment

Sijunzi decoction (Radix Ginseng 9 g, Rhizoma Atractylodis Macrocephalae 9 g, Poria 9 g and Radix Glycyrrhizae 6 g) was purchased from the First Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, and after being soaked and boiled in stilled water, the concentration of Sijunzi decoction was

adjusted to 1.40 g/mL. The equivalent dose for a rat was converted from the clinical dose of an adult, which was given to rats in the CM group by gavage twice a day with an interval of 8 h for 2 weeks. Rats in the normal group were not treated, those in the model and SW groups were separately applied actions of intrgastric administration and given 2 mL SW in the same way as the CM group.

2.4. Ultrastructures of the Myofibers

After rats were euthanized by inhaling carbon dioxide, soleus muscle was collected and cut into pieces of 1 mm^3 at 4˚C and fixed with 2.5% glutaraldehyde in phosphate buffer. After dehydration in ethanol with graded concentrations, specimens were treated with propyene oxide and embedded in Epon. Ultrathin sections stained with uranyl acetate and lead citrate were examined under a transmission electron microscope (JEM-1 200EX; Jeol, Tokyo, Japan).

2.5. The MND Size

Soleus muscle was stored at 4% paraformaldehyde solution, and then embedded in paraffin and sectioned at 6 μm. The sections were stained with hematoxylin and eosin and examined by a light microscopy. The myonuclei were dyed blue, and 100 myofibers each group (10 fibers each rat) were chosen by the investigator described above to measure their myonuclear numbers, cross-section area (CSA) by Image Pro 6.0 (Bethesda, MD, USA), and then the MND size was calculated by CSA/the myonuclear number [6] [7].

2.6. Proliferation and Differentiation of the MSCs

Simply, soleus muscle was quickly frozen in liquid nitrogen, embedded in optimal cutting temperature compound and then sectioned at 8 μm using a freezing CM1950 microtome (Leica, Wetzlar, Germany). The sections were fixed with 4% paraformaldehyde for 10 min, washed in PBS, and then blocked for 30 min in blocking buffer containing 5% bovine serum albumin at 37˚C. Sections were incubated with the the mouse anti-paired box gene 7 (Pax7) and rabbit anti-myogenic differentiation antigen (MyoD) (both were 1:200; Santa Cruz Biotechnlogy, sc-514352, sc-377460) overnight at 4˚C. Sections were washed 3 times in PBS and incubated for 5 min at room temperature (RT) in the blocking solution, and were incubated with the goat anti-mouse IgG and goat anti-rabbit IgG diluted in blocking solution for 90 min at RT, and the nuclei were stained with DAPI (0.3 μM). After incubation, sections were washed 3 times with PBS, and a drop of Alexa Fluor 488/594 secondary antibody mixture was added and incubated at RT in darkness for 1 h. Finally, sections were mounted with DAPI and fluorescence was visualized using an Olympus FV10i confocal microscope (Tokyo, Japan). The Pax7+ and MyoD+ nuclei in 100 myofibers each group (10 myofibers each rat) were counted in the blind way as described above.

2.7. Statistical Analysis

Data were processed using SPSS 25.0 and expressed as mean ± standard devia-

tion (SD). Analysis of variance was conducted to test differences between groups and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Treatment of Sijunzi decoction

Table 1 and Figure 1 showed that all items in the model and SW groups were significantly less than those in the normal group, indicating that the SQD mode was successfully established and SW could not treat SQD. Compared with those in the model and SW groups, data in the CM group increased obviously, which suggested that Sijunzi decoction treated SQD.

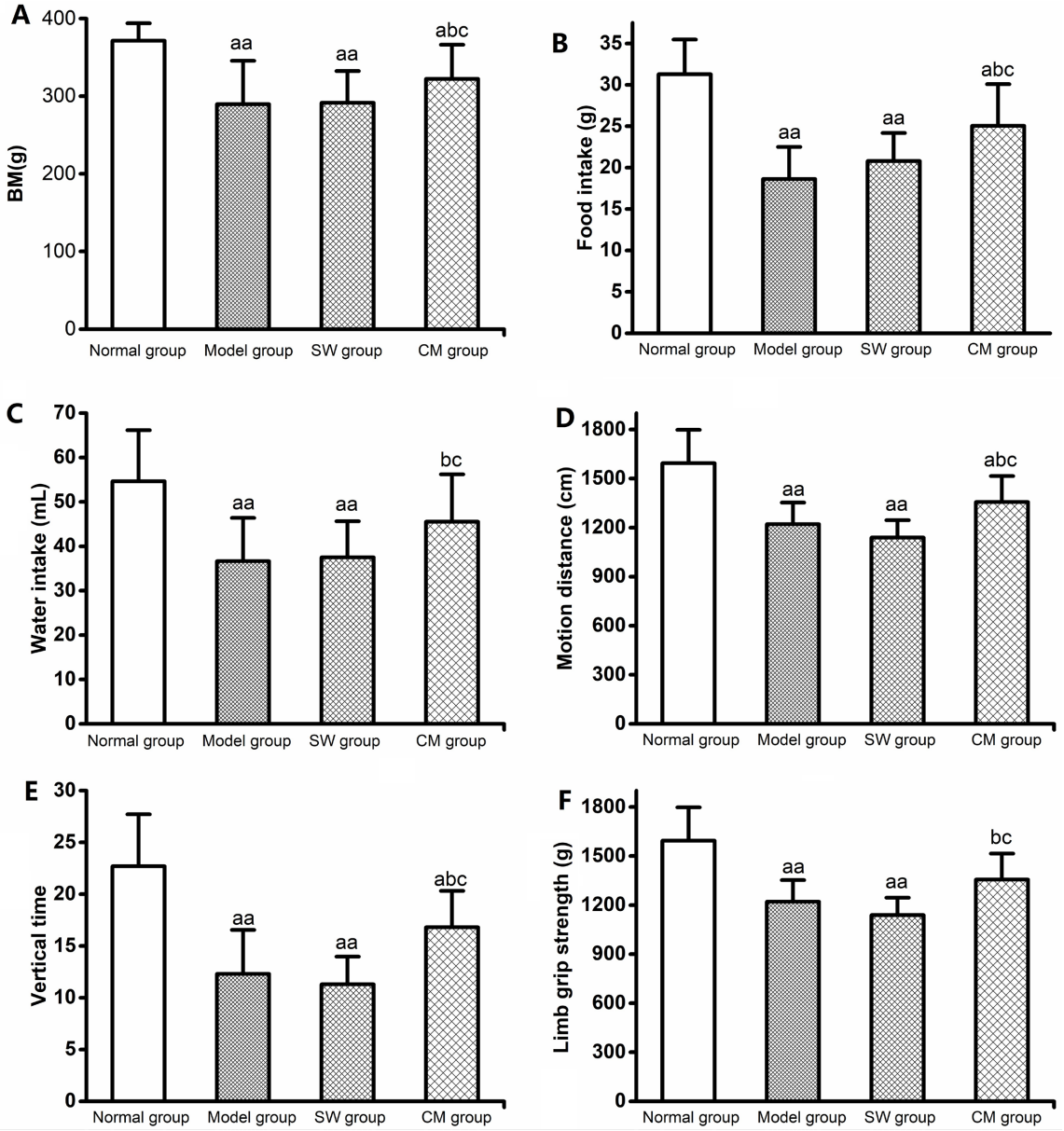

Figure 1. Effect of Sijunzi decoction on the symptoms of the SQD model in rats. Note: mean ± SD, n = 10; verse the normal group, $^{a}p < 0.05$, $^{aa}p < 0.01$; verse the model group, $^{b}p < 0.05$; verse the SW group, $^{c}p < 0.05$, $^{cc}p < 0.01$.

3.2. Ultrastructures of the Myofibers

Figure 2(A) was the typical structures of the sarcomeres in the normal group, where the Z and M lines were clear, and mitochondria were between the myofilaments. Figure 2(B) and Figure 2(C) were morphological changes in the model and SW group, where some Z and M lines were disorderly, and mitochondria were smaller; Figure 2(D) showed the sarcomeres of the CM group were basically normal, and the mitochondria were bigger than those in the model and SW groups.

Table 1. Effect of Sijunzi decoction on the symptoms of the SQD model in rats (mean ± SD, n = 10).

Group	Emaciation (BM) (g)	Poor appetite		Mental fatigue		Limb grip strength (g)
		Food intake (g)	Water intake (mL)	Motion distance (cm)	Vertical time	
Normal group	371.47 ± 22.46	31.27 ± 4.21	54.6 ± 12.0	8933.20 ± 1751.35	22.7 ± 5.2	1593.75 ± 204.66
Model group	289.67 ± 56.03^{aa}	18.63 ± 3.88^{aa}	36.7 ± 9.7^{aa}	6145.55 ± 1933.78^{aa}	12.3 ± 4.4^{aa}	1221.65 ± 198.11^{aa}
SW group	291.45 ± 40.89^{aa}	20.79 ± 4.33^{aa}	38.0 ± 8.1^{aa}	6605.10 ± 1653.23^{aa}	11.3 ± 2.7^{aa}	1138.93 ± 106.75^{aa}
CM group	322.13 ± 44.21^{abc}	25.05 ± 5.04^{abc}	45.6 ± 10.7^{bc}	7783.44 ± 1409.25^{abc}	16.8 ± 4.0^{abc}	1355.90 ± 171.58^{bc}

Note: verse the normal group, $^{a}p < 0.05$, $^{aa}p < 0.01$; verse the model group, $^{b}p < 0.05$; verse the SW group, $^{c}p < 0.05$, $^{cc}p < 0.01$.

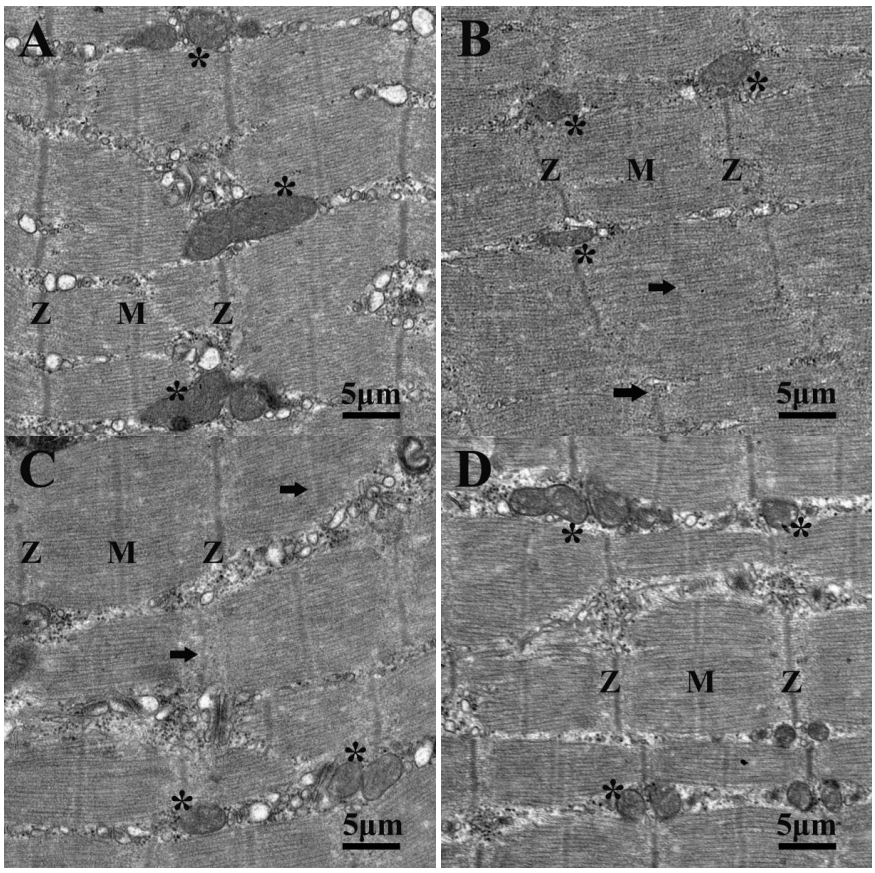

Figure 2. Ultrastructures of the myofibers (7000×). Note: A, the normal group; B, the model group; C, the SW group; D, the CM group; M, the M line; Z, the Z line; black arrow: the M or Z line was not clear; *, mitochondrion.

3.3. The Myonuclear Numbers, CSA and MND

Table 2 and Figure 3 showed that, compared with those in the normal group, three items were all decreased significantly in the model and SW groups, and the myonuclear number was increased, CSA and the MND were reduced obviously in the CM group; Compared with those in the model and SW groups, the myonuclear number and CSA were increased markedly in the CM group.

3.4. Assessment of Proliferation and Differentiation of MSCs

Pax7 promotes proliferation of the MSCs, so the Pax7+ cell number is used to detect MSCs' proliferation [8]. Figure 4 left and Table 3 showed that the Pax7+

Table 2. The myonuclear numbers, CSA and MND (mean ± SD, n = 100 myofibers).

Group	Myonuclear number	CSA (μm²)	MND (μm²)
Normal group	3.27 ± 1.19	1487.08 ± 610.33	473.59 ± 151.04
Model group	2.76 ± 0.98^{aa}	1064.75 ± 409.13^{aa}	409.51 ± 145.70^{aa}
SW group	3.01 ± 1.18^{aa}	1144.45 ± 482.23^{aa}	395.61 ± 131.09^{aa}
CM group	3.94 ± 0.80^{abbcc}	1380.98 ± 258.59^{abbcc}	354.21 ± 58.02^{aa}

Note: verse the normal group, $^{a}p < 0.05$, $^{aa}p < 0.01$; verse the model group, $^{bb}p < 0.01$; verse the SW group, $^{cc}p < 0.01$.

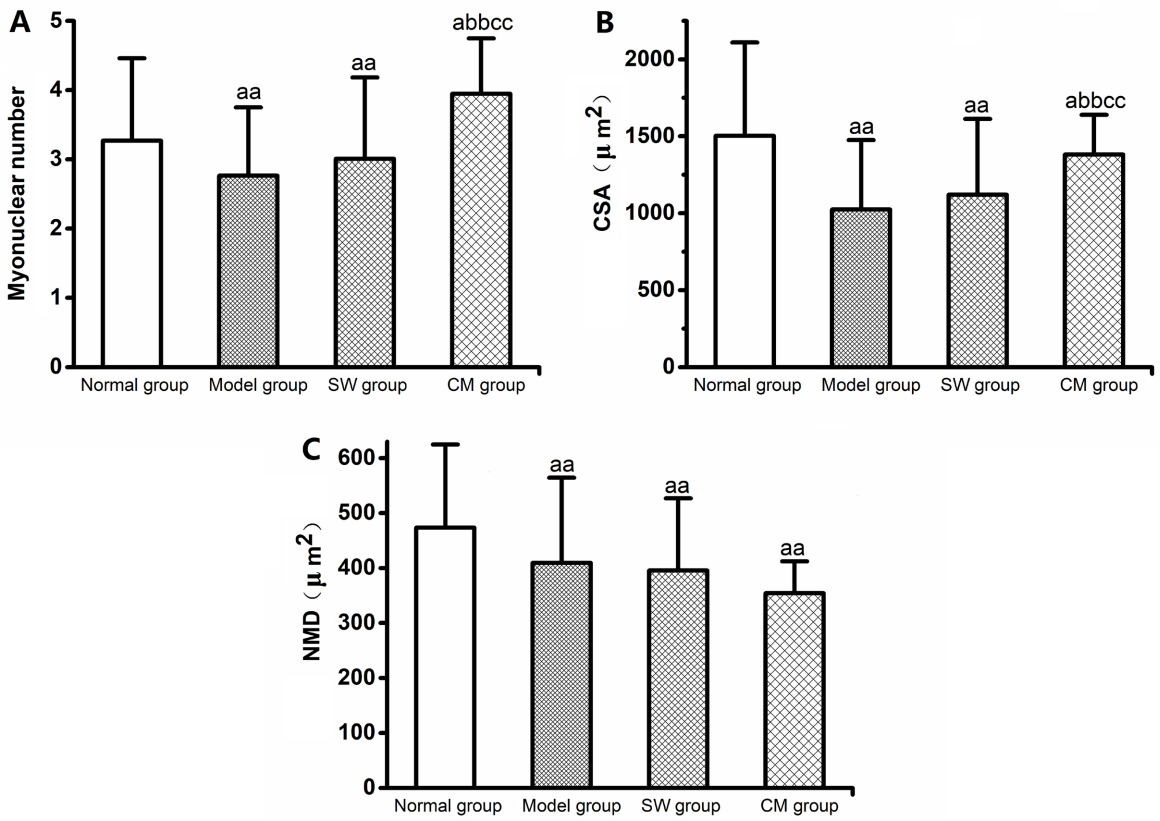

Figure 3. The myonuclear numbers, CSA and MND. Note: mean ± SD, n = 100 myofibers; verse the normal group, $^{a}p < 0.05$, $^{aa}p < 0.01$; verse the model group, $^{bb}p < 0.01$; verse the SW group, $^{cc}p < 0.01$.

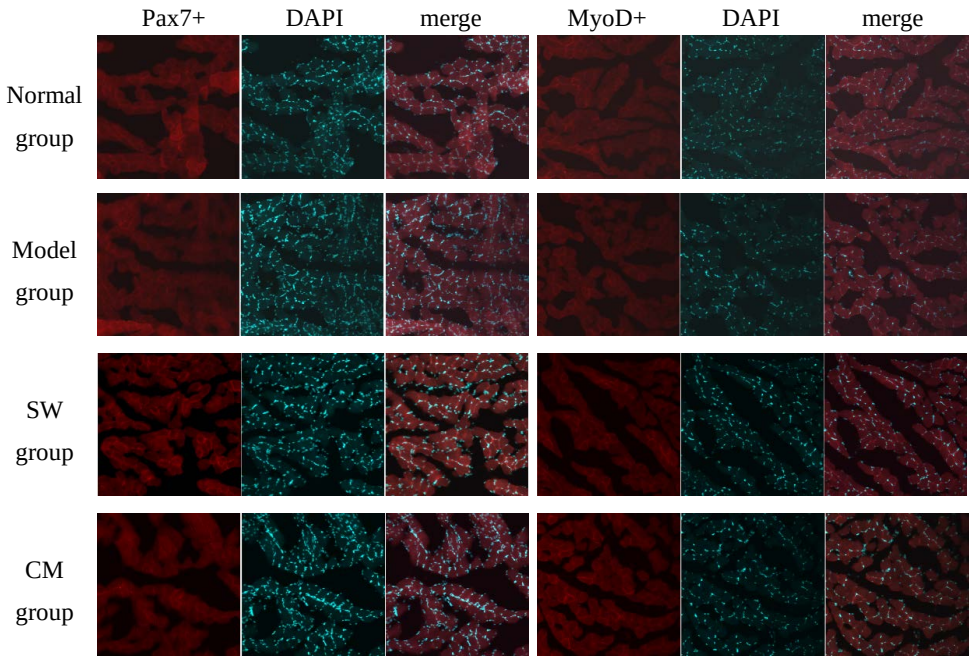

Figure 4. Pax7 and MyoD expressions.

Table 3. The Pax7+ and MyoD+ cell numbers (mean ± SD, n = 100 myofibers).

Group	Pax7+ cell number	MyoD+ cell number
Normal group	5.57 ± 1.27	2.85 ± 0.58
Model group	7.71 ± 1.38[a]	3.00 ± 0.48
SW group	6.86 ± 0.90[a]	2.90 ± 0.46
CM group	9.29 ± 1.11[aabcc]	3.65 ± 0.72[abc]

Note: verse the normal group, [a]$p < 0.05$, [aa]$p < 0.01$; verse the model group, [b]$p < 0.05$; verse the SW group, [c]$p < 0.05$, [cc]$p < 0.01$.

cell numbers in the later 3 groups were all greater than that in the normal group, and that in the CM group was higher than those in the model and SW groups. MyoD plays an important role in differentiation of the MSCs, therefore its positive cell number is used to assess MSCs' differentiation [9]. Based on **Figure 4** and **Figure 5** right and **Table 3**, the MyoD+ cell number in the CM group was higher than those in the former 3 groups significantly.

4. Discussion

In the theory of the traditional Chinese Medicine, muscle is governed by spleen, and muscle will become atrophy and weakness when SQD develops, which can be treated by Sijunzi decoction, a representative prescription for invigorating spleen Qi. The mechanism of Sijunzi decoction treating limb weakness in SQD is still not clear. As myonuclei are the control centers of the skeletal muscle fiber, the effect of Sijunzi decontion on the MND was studied herein, which has not been reported.

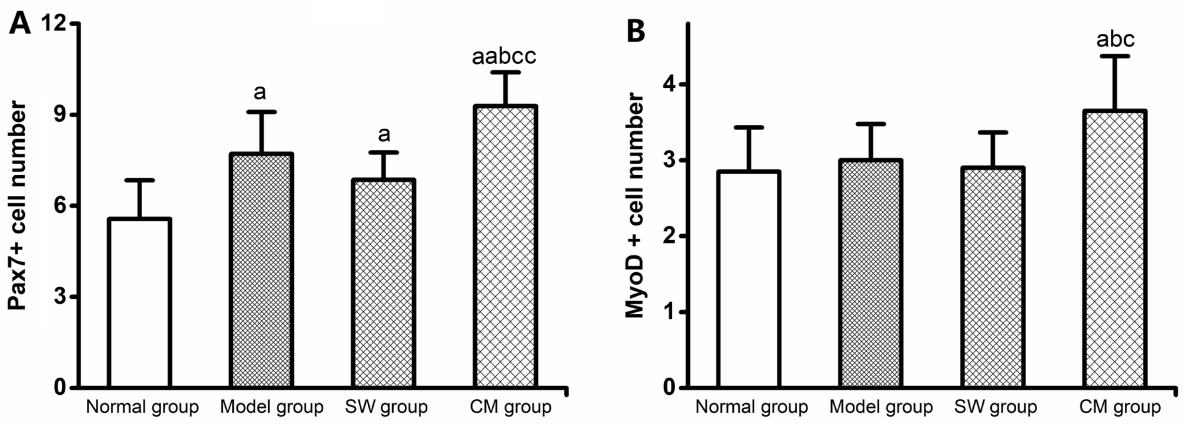

Figure 5. The Pax7+ and MyoD+ cell numbers. Note: mean ± SD, n = 100 myofibers; verse the normal group, $^{a}p < 0.05$, $^{aa}p < 0.01$; verse the model group, $^{b}p < 0.05$; verse the SW group, $^{c}p < 0.05$, $^{cc}p < 0.01$.

In the present study, the action of Sijunzi decoction treating limb weakness in SQD was first confirmed, and then its effect on the myofibrils was detected. Each myofibril is composed of myosin filaments and actin filaments, which are responsible for the actual muscle contraction. In the sarcomere, the myosin filaments and actin filaments partially interdigitate, and the centers of the former are the M line whereas the ends of the latter are attached to the Z lines. It was observed that both the M and Z lines were disorder when SQD developed, and Sijunzi decoction ameliorated such abnormality. These positive results made it possible for the next experiments focusing on the MND.

Different from other types of cells, the skeletal muscle fiber is multinuclear, and the myonuclei are pushed to the periphery of the cell by motor proteins which are densely packed in the core. The peripheral myonuclear placement means each nucleus owns its territory, that is the MND, and its enlargement in size and/or new nuclei addition can induce hypertrophy [1] [2].

The MND size differs between the muscle types [10] [11], and soleus targeted here is one of the typical slow muscle. It was observed that, first, when SQD occurred, the MND size became small due to the reduction of both the nuclei numbers and CSA; Secondly, the Pax7+ cell number was raised, but the MyoD+ cell number was unaltered. The activation of the MSCs needs many factors [12] [13] and among them, Pax7 promotes proliferation and MyoD stimulates differentiation, therefore their positive cell numbers are widely used to imply the corresponding phases of the MSCs' activation [14] [15] [16]. Accordingly, it was speculated that when SQD developed, the MSCs might split, but their daughters might not undergo differentiation, resulting in no new nuclei entering into the muscle fibers.

Sijunzi decoction can treat limb weakness in SQD, and we have reported that one of its mechanisms is related to mitochondrial protection [17], but its effect on the MND is still unknown. Our results showed that, first, Sijunzi decoction increased both the myonuclear number and CSA, indicating the positively effect on the muscle fibers. Although the MND size in the CM group was not signifi-

cantly bigger than that in the model group, it might become larger if the treatment of Sijunzi decoction was continued due to the increase of the myonuclear number. Secondly, both the Pax7+ and MyoD+ cell numbers were increased markedly in the CM group, meaning the increases of MSCs' proliferation and differentiation. Therefore, Sijunzi decoction was thought to activate the MSCs to add new nuclei into the nearby myofibers.

Based on the theory of the MND and our results, limb weakness in SQD might be caused by the reduction of the MND size and the inhibition of MSCs' activation, and it is concluded that Sijunzi decoction might increase the myonuclear number by activating the MSCs to treat limb weakness in SQD. It should be noted that the activation of the MSCs is complicated, and the in vitro experiments are needed to confirm the effect of Sijunzi decoction on the MSCs.

Funding

This study was supported by National Natural Science Foundation of China (81673851) and Department of Education of Liaoning Province (LJKMZ20221322).

Conflicts of Interest

The authors report no conflict of interest.

References

[1] Bagley, J.R., Denes, L.T., McCarthy, J.J., *et al.* (2023) The Myonuclear Domain in Adult Skeletal Muscle Fibres: Past, Present and Future. *The Journal of Physiology*, **601**, 723-741. https://doi.org/10.1113/JP283658

[2] Hansson, K.A. and Eftestøl, E. (2023) Scaling of Nuclear Numbers and Their Spatial Arrangement in Skeletal Muscle Cell Size Regulation. *Molecular Biology of the Cell*, **34**, pe3. https://doi.org/10.1091/mbc.E22-09-0424

[3] Koopmans, P.J., Ismaeel, A., Goljanek-Whysall, K., *et al.* (2023) The Roles of miRNAs in Adult Skeletal Muscle Satellite Cells. *Free Radical Biology and Medicine*, **209**, 228-238. https://doi.org/10.1016/j.freeradbiomed.2023.10.403

[4] Memczak, S. and Belmonte, J.C. (2023) Overcoming Muscle Stem Cell Aging. *Current Opinion in Genetics & Development*, **83**, Article ID: 102127. https://doi.org/10.1016/j.gde.2023.102127

[5] Ma, D., Liu, W.J., Wang, L., *et al.* (2019) Mitophagy in the Skeletal Muscle Is Suppressed in Spleen Qi Deficiency. *Chinese Medicine*, **10**, 11-18. https://doi.org/10.4236/cm.2019.101002

[6] Sara, B., Tatiane, G., Van Roie, E., *et al.* (2020) The Effect of Resistance Training, Detraining and Retraining on Muscle Strength and Power, Myofibre Size, Satellite Cells and Myonuclei in Older Men. *Experimental Gerontology*, **133**, 531-565. https://doi.org/10.1016/j.exger.2020.110860

[7] Karlsen, A., Bechshøft, R.L., Malmgaard-Clausen, *et al.* (2019) Lack of Muscle Fibre Hypertrophy, Myonuclear Addition, and Satellite Cell Pool Expansion with Resistance Training in 83-94-Year-Old Men and Women. *Acta Physiol*, **227**, e13271.

[8] Zhao, S., Chen, J., Wu, L., Tao, X., *et al.* (2023) Induced Pluripotent Stem Cells for Tissue-Engineered Skeletal Muscles. *International Journal of Molecular Sciences*,

24, 11520-11539. https://doi.org/10.3390/ijms241411520

[9] Yagi, M., Ji, F., Charlton, J., Cristea, S., *et al.* (2021) Dissecting Dual Roles of MyoD during Lineage Conversion to Mature Myocytes and Myogenic Stem Cells. *Genes & Development*, **35**, 1209-1228. https://doi.org/10.1101/gad.348678.121

[10] Hromowyk, K.J., Talbot, J.C., Martin, B.L., *et al.* (2020) Cell Fusion is Differentially Regulated in Zebrafish Post-Embryonic Slow and Fast Muscle. Developmental Biology, **462**, 85-100. https://doi.org/10.1016/j.ydbio.2020.03.005

[11] Brooks, N.E. and Myburgh, K.H. (2014) Skeletal Muscle Wasting with Disuse Atrophy Is Multi-Dimensional: The Response and Interaction of Myonuclei, Satellite Cells and Signaling Pathways. Frontiers in Physiology, **5**, 99. https://doi.org/10.3389/fphys.2014.00099

[12] Southerland, K.W., Xu, Y., Peters, D.T., *et al.* (2023) Skeletal Muscle Regeneration Failure in Ischemic-Damaged Limbs Is Associated with Pro-Inflammatory Macrophages and Premature Differentiation of Satellite Cells. Genome Medicine, **5**, 95. https://doi.org/10.1186/s13073-023-01250-y

[13] Keefe, A.C., Lawson, J.A., Flygare, S.D., *et al.* (2015) Muscle Stem Cells Contribute to Myofibres in Sedentary Adult Mice. Nature Communications, **6**, 7087-7097. https://doi.org/10.1038/ncomms8087

[14] Lee, J., Park, J., Choe, H. and Shim, K. (2022) Insect Peptide CopA3 Promotes Proliferation and PAX7 and MYOD Expression in Porcine Muscle Satellite Cells. *Journal of Animal Science and Technology*, **64**, 1132-1143. https://doi.org/10.5187/jast.2022.e81

[15] Loperfido, M., Steele-Stallard, H.B., Tedesco, F.S., *et al.* (2015) Pluripotent Stem Cells for Gene Therapy of Degenerative Muscle Diseases. *Current Gene Therapy*, **15**, 364-380. https://doi.org/10.2174/1566523215666150630121207

[16] Tian, Z.L., Wang, R.L., Yang, Q.F., *et al.* (2023) Detection of Multiple Biomarkers Associated with Satellite Cell Fate in the Contused Skeletal Muscle of Rats for Wound Age Estimation. *International Journal of Legal Medicine*, **137**, 875-886. https://doi.org/10.1007/s00414-023-02971-w

[17] Liu, W.J., Xu, X.Z., Duan, Z.Y., *et al.* (2021) Efficacy of Sijunzi Decoction on Limb Weakness in Spleen Qi Deficiency Model Rats through Adenosine Monophosphate-Activated Protein Kinase/Unc-51 like Autophagy Activating Kinase 1 Signaling. *Journal of Traditional Chinese Medicine*, **41**, 617-623.