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ORIGINAL

SHENLING BAIZHU POWDER ENHANCES SKELETAL MUSCLE RECOVERY VIA MIR-23A/PI3K/AKT PATHWAY: IMPLICATIONS FOR MANAGING SARCOPENIA AND OPTIMIZING PHYSICAL PERFORMANCE

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ABSTRACT

Objective: To investigate the effects of Shenling Baizhu Powder (SLBZP) on skeletal muscle atrophy in diabetic mice, focusing on its regulatory role in the miR-23a/PI3K/AKT pathway, and to explore its clinical implications for the treatment of sarcopenia and physical performance enhancement. **Methods:** A diabetic mouse model with skeletal muscle atrophy was established to evaluate the therapeutic effects of SLBZP. Mice were divided into control, diabetic model, and SLBZP-treated groups, with the latter receiving different doses of SLBZP. Muscle mass, grip strength, and fiber cross-sectional area were assessed as indicators of muscle function and morphology. miR-23a, PI3K, and AKT expression levels were analyzed using qRT-PCR and Western blot. Additionally, histological evaluations of muscle tissue were conducted to observe structural changes. Statistical analyses were performed to assess correlations between molecular changes and physical performance metrics. **Results:** SLBZP significantly improved muscle mass and grip strength in diabetic mice compared to the untreated model group ($P < 0.05$). Histological analysis revealed increased muscle fiber cross-sectional area and reduced atrophic changes in the SLBZP-treated group. Molecular analyses demonstrated upregulation of the miR-23a/PI3K/AKT pathway, which is associated with

muscle protein synthesis and reduced apoptosis. The therapeutic effects were dose-dependent, with higher doses of SLBZP showing greater improvements in muscle function and morphology. **Conclusion:** Shenling Baizhu Powder effectively mitigates skeletal muscle atrophy in diabetic mice by enhancing the miR-23a/PI3K/AKT pathway, promoting muscle regeneration and improving functional outcomes. These findings suggest its potential as a therapeutic agent for managing sarcopenia and optimizing physical performance, particularly in populations at risk for muscle loss, such as aging adults and athletes recovering from injury or metabolic disorders. Further clinical research is warranted to validate these results and explore its integration into sports and rehabilitation medicine.

KEYWORDS: SLBZP; MiR-23a/PI3K/AKT Pathway; Diabetes; Skeletal Muscle Atrophy; Sarcopenia

1. INTRODUCTION

Skeletal muscle atrophy, characterized by a reduction in muscle mass, strength, and function, is a common complication in diabetes and aging populations. This condition, often progressing to sarcopenia, significantly impacts physical performance (Beaudart et al., 2017; Chen et al., 2020), mobility, and quality of life. Sarcopenia not only increases the risk of falls and fractures but also limits participation in physical activities, impeding recovery and overall health (Cleasby et al., 2016; Cruz-Jentoft & Sayer, 2019; Dhillon & Hasni, 2017). In sports and rehabilitation medicine, managing muscle atrophy is critical for promoting optimal physical performance and ensuring long-term functionality in both athletes and aging populations. The miR-23a/PI3K/AKT pathway plays a pivotal role in regulating muscle protein synthesis and preventing protein degradation (Garhöfer et al., 2020; Laviano et al., 2014; Nomura et al., 2018), making it a key target for therapeutic interventions in skeletal muscle atrophy. Dysregulation of this pathway has been implicated in conditions such as diabetes-induced muscle wasting and age-related sarcopenia. While pharmacological treatments targeting this pathway have been explored, the potential of traditional herbal formulations in modulating these molecular mechanisms is gaining attention. Shenling Baizhu Powder (SLBZP) (Papadopoulou, 2020; Sanz-Cánovas et al., 2022), a classic formulation in traditional Chinese medicine, has been traditionally used to strengthen the spleen and enhance energy, making it a promising candidate for addressing muscle atrophy. Emerging evidence suggests that SLBZP may influence molecular pathways involved in muscle regeneration and repair, including the miR-23a/PI3K/AKT signaling pathway (Yan et al., 2020; Yin, Chen, et al., 2021; Zhang et al., 2022).

However, its specific effects on muscle atrophy and its potential

application in sarcopenia management remain underexplored. This study aims to investigate the therapeutic effects of SLBZP on skeletal muscle atrophy in diabetic mice, focusing on its role in upregulating the miR-23a/PI3K/AKT pathway (Zhang et al., 2018; Zhong et al., 2018). By evaluating functional and molecular changes, we seek to provide insights into its mechanism of action and explore its clinical relevance in managing sarcopenia.

These findings could have broad implications for enhancing physical performance and recovery, particularly in athletes, aging adults, and individuals with metabolic disorders, aligning with the multidisciplinary goals of sports and rehabilitation medicine.

2. Materials and Methods

2.1 Reagents and Instruments

SLBZP (approval number: national drug approval number Z14020346, specification: 12g/bags) was purchased from Shanxi Huakang Pharmaceutical Co., Ltd., which is composed of 10 kinds of traditional Chinese medicine, including *white lentils, atractylodes macrocephala, poria cocos, licorice, platycodon grandiflorum, lotus seed, ginseng, amomum villosum, yam* and *coix seed*. The drug was dissolved in distilled water within 24 hours before the animal experiment and stored in a refrigerator at -4 ° C for standby. Streptozotocin (STZ) was purchased from Wakack-IL Pfanstiehl Laboratory in the United States. Dissolve the drug in the corresponding volume of distilled water before use. The solvent of this experiment is distilled water. Urea nitrogen (BUN) kit was purchased from Shanghai Yubo Biotechnology Co., Ltd., China, and serum creatinine test kit was purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., China. Enzyme-linked immunosorbent assay (ELISA) kit is planned to be purchased from Shanghai Biyuntian Biotechnology Co., Ltd, China. Masson's modified IMEB training kit purchased from Abison (Shanghai, China) Biotechnology Co., Ltd.

The mice grip meter was purchased from Columbus Instruments, Columbus, Ohio, USA. Tissue Freezing Medium (TBS) was purchased from Solebo Technology Co., Ltd. in Beijing, China. Olympus 1X51 inverted fluorescent microscope was purchased from Puhe Optoelectronics (Shanghai, China) Technology Co., Ltd. and equipped with DP73-1-51-17MP color camera. Varioskan LUX multi-function enzyme marker (microplate detector) and Applied Biosystems PCR thermal cycler were purchased from ThermoFisher, USA.

2.2 Animal and Diabetes Mice Model

The experimental animals in this study are 100 male mice (because female mice will have diabetes resistance after STZ injection), purchased from

Shanghai Experimental Animal Center, Chinese Academy of Sciences (Shanghai, China). The study was carried out in strict accordance with the requirements of the Regulations on the Practical License for Experimental Animals of Hebei North University (Hebei, China), and the animals were fed in the standard temperature (24 ± 2 ° C), humidity (60 ± 10%) and light patients. After one week of adaptive feeding, 100 mice were randomly divided into study group and control group, with 50 mice each. Among them, the mice in the study group (C57BL/6J) were injected with STZ at a dose of 150mg/kg/d, and intraperitoneally for two consecutive days. In the control group, only the same volume of solvent was injected intraperitoneally, and intraperitoneally for 5 consecutive days. All groups in this study can get food free of charge. During the experiment, mice were regularly provided with drinking water. The experimental personnel participating in this study measured the food consumption and water consumption of mice at regular intervals every two days, and measured their body weight every week. Secondly, the mice muscle grip function is measured by using a mice grip meter with dual computer sensors. In addition, blood samples from the caudal pyramidal vein of the two groups of mice were collected at regular intervals every day, with a volume of 0.5 mL each time, and stored in a refrigerator at 4 ° C. Finally, separate the blood sample and extract the serum, and use the corresponding biochemical indicator detection kit to analyze the sample. Among them, the serum urea nitrogen level of mice was detected with the BUN kinetic program kit, and the serum creatinine level of mice was detected with the serum creatinine detection kit.

2.3 Grouping and Administration Method

Among the successfully constructed diabetes mice, 36 mice (C57BL/6J) with no significant difference in body weight ($P < 0.05$) were randomly divided into 4 groups: control group, low dose SLBZP group, medium dose SLBZP group and high dose SLBZP group. Among them, 9 cases in the control group were gavaged with 10ml of normal saline per animal; 9 cases of the low-dose group were given a dose of 5g SLBZP plus 10ml normal saline/kg/day by gavage; 9 cases of the medium-dose group were given a dose of 10g SLBZP plus 10ml normal saline//kg/day by gavage; and 9 cases in the high-dose group were given a dose of 20g SLBZP plus10ml normal saline/kg/day by gavage.

2.4 RT-PCR

The total RNA was extracted by the scheme of TRIZOL reagent (Invitrogen Corporation, Beijing, China). After grinding, the muscle tissue samples of mice were cracked by adding 3 times the volume of TRIZOL reagent. The sample was extracted according to the extraction method of TRIZOL reagent kit. PCR at 50 μ L volume. The primer sequence was designed according to the miRNA (miR-23a, miR-27a) and protein (IGF1/PI3K/Akt signal

pathway protein, myostatin-related protein) to be detected. For example, the forward primer of miR - 23a is 5 '- ACC ATG TGG TAC CCA GAA ATC-3', and the reverse primer is 5 '- GTG GGA CTT CCA CCA CTG CA-3'. The amplification was carried out under the following conditions: 15 minutes at 37 ℃, then 30 cycles at 95 ℃ for 5 seconds, 45 minutes at 60 ℃ and 1 minute at 72 ℃, and then extended for 10 minutes at 72 ℃. PCR products were analyzed by 1% agarose gel electrophoresis, and ethidium bromide (0.5 μ G/ml) and observed under ultraviolet light. The product was analyzed by molecular analysis software.

2.5 ELISA

The enzyme-linked immunosorbent assay (ELISA) of miR-23a, miR-27a and IGF1/PI3K/Akt signal path protein, myostatin-related protein was conducted in strict accordance with the experimental protocol provided by the supplier (Shanghai Biyuntian Biotechnology Co., Ltd, China.). All samples were measured three times.

2.6 Western Blotting

Western blot was used to analyze the expression of mice IGF1/PI3K/Akt signal pathway protein and myostatin-related protein in the muscle tissue samples of mice. First, the muscle tissues were washed with PBS for three times, and then the lysis was completed in the lysis buffer (Shanghai Biotech Co., Ltd., China), and the protein concentration was determined by Bradford method; Secondly, the protein was separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane (Millipore, MA, USA). Thirdly, the membrane sealed with 3% BSA at room temperature for 1.5 hours, the protein was incubated overnight with the corresponding first antibody of the protein to be tested at 4 ℃. Then, horseradish peroxidase (HPR) labeled sheep anti-rabbit secondary antibody and unmarked sheep anti-rabbit secondary antibody were used to complete the coupling. Finally, incubate the membrane with HRP substrate for 5 minutes, analyze the antigen-antibody interaction with chemiluminescence kit, and analyze the relative amount of protein with Image system.

2.7 Measurement of Muscle Tissue Morphology

Mice muscle tissue was fixed in 3.7% formaldehyde/PBS (pH = 7.4), dehydrated, paraffined, and sectioned. Masson trichromatic staining was performed with Masson's modified IMEB staining kit. Muscle tissue staining pictures are visualized with Olympus 1X51 inverted microscopes were used to observe the images, and DP73-1-51-17MP color camera was used to take photos. The area of mice kidney collagen was measured with CellSens 1.9 dimension and count and measure full software and calculated from 10

separate field areas.

2.8 Immunohistochemistry (IHC)

First, mice muscle was embedded in tissue freezing medium (TBS) and isopentane was cooled with dry ice. Secondly, the cross sections (10 mm) of different muscle midsection were fixed in 4% paraformaldehyde for 10 minutes. Then, the microstructure was infiltrated in 0.05% Triton X-100 (PBS) for 10 min and quenched in 50 mm NH4Cl for 10 min. Next, the sample was sealed with 5% bovine serum albumin for 1 hour and incubated with primary antibody overnight. Subsequently, the sections were washed with distilled water, and the anti-rabbit IgG labeled with fluorescein isothiocyanate (FITC) was used, and the nucleus was stained with 4',6-diamidino2-phenylindole (DAPI). Finally, the prepared tissue sections were observed with Olympus 1X51 inverted fluorescence microscope and photographed with DP73-1-51-17MP color camera. Note: The whole immunohistochemistry process uses anti-laminin antibodies and measures at least 500 individual muscle fibers per muscle.

2.9 Statistical Analysis

All data in this study were processed by SPSS 20.0 statistical analysis software (IBM, USA). The measurement data were expressed by "mean ± standard deviation" ($\bar{x} \pm s$), and independent sample t-test was used for comparison between groups. $P < 0.05$ indicates that the difference is statistically significant.

3. Results

3.1 The Effects of SLBZP Treatment on the Grip Endurance of Mice and Serum BUN and Creatinine Levels

We first analyzed the grip endurance of mice, and the results showed that SLBZP and STZ-treated mice displayed an increase in grip endurance (Table 1). Then, the study assessed the serum BUN and creatinine levels in the mice of each group, and the data presented that SLBZP treatment dose-dependently inhibited BUN and creatinine production when compared with the control group (Figure 1).

Table 1: The Grip Endurance of Mice After SLBZP and STZ Treatment

	CONTROL GROUP (N=9)	LOW SLBZP GROUP (N=9)	MEDIUM SLBZP GROUP(N=9)	HIGH SLBZP GROUP(N=9)	*F*	*P*
GRIP ENDURANCE	53.62±10.21S	67.23±11.20S*	82.56±10.26S*	100.36±12.23S*	34.36	<0.05

*Note: *P<0.05, compared with the control group.*

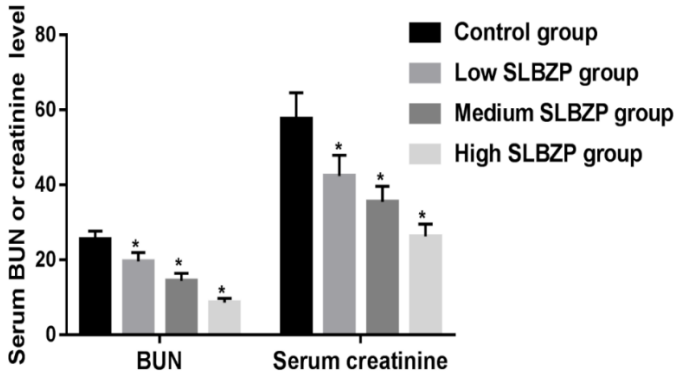

Figure 1: The Serum Levels of BUN and Creatinine in the Mice of Each Group. *P<0.05.

3.2 RNA Expression of Mir-23a, Mir-27a, IGF1/PI3K/Akt Signal Pathway Proteins, And Myostatin-Related Proteins in the Muscle Tissues of SLBZP And STZ-Treated Mice

As shown in Table 2, miR-23a, miR-27a, IGF-1, and AKT RNA expression were significantly upregulated, while Myostatin expression was downregulated in a dose-dependent manner in the muscle tissues of SLBZP and STZ-treated mice in comparison with the muscle tissues of control. In addition, the result showed that SMAD3 expression had no significant difference in the muscle tissues of SLBZP and STZ-treated mice in comparison with the control (Table 2).

Table 2: RNA Expression of miR-23a, miR-27a, IGF1/PI3K/Akt Signal Pathway Proteins in Mice of Each Group

GENE NAME	CONTROL GROUP (N=9)	LOW SLBZP GROUP (N=9)	MEDIUM SLBZP GROUP (N=9)	HIGH SLBZP GROUP (N=9)	*F*	*P*
MiR-23a	1.01±0.45	2.56±0.23*	3.64±0.45*	4.98±1.60*	33.7	<0.05
MiR-27a	0.98±0.12	1.87±0.25*	3.01±0.48*	4.12±0.62*	97.01	<0.05
IGF-1	1.02±0.23	2.42±0.41*	3.65±0.62*	5.01±0.78*	86.18	<0.05
AKT	0.99±0.13	2.56±0.45*	3.78±0.67*	5.26±0.81*	89.36	<0.05
Myostatin	3.98±0.67	2.52±0.43*	1.34±0.21*	0.97±0.16*	94.21	<0.05
SMAD3	1.02±0.14	0.95±0.15*	1.06±0.10*	1.12±0.17*	2.263	=0.10

*Note: *P<0.05, compared with the control group.*

3.3 GF1/PI3K/Akt Signaling Pathway Proteins and Myostatin-Related Protein Expression in the Muscle Tissues of SLBZP and STZ-Treated Mice

Subsequently, the study analyzed the protein expression of AKT, p-AKT(Ser473), p-AKT(Thr308), Myostatin, SMAD3 and p-SMAD3 by western blot to determine the effects of SLBZP on GF1/PI3K/Akt signaling pathway and

myostatin in STZ-treated mice. The results revealed that the relative protein expression of AKT and p-AKT(Ser473) was dramatically increased, while Myostatin and p-SMAD3 protein expression were decreased in the muscle tissues of SLBZP and STZ-treated mice in relative to the control (Table 3). Moreover, the results showed that p-AKT(Thr308) and SMAD3 production were not affected by SLBZP treatment in the STZ-treated mice (Table 3).

Table 3: AKT, p-AKT(Ser473), p-AKT(Thr308), Myostatin, SMAD3 and p-SMAD3 protein expression in mice of each group

GENE NAME	CONTROL GROUP (N=9)	LOW SLBZP GROUP (N=9)	MEDIUM SLBZP GROUP (N=9)	HIGH SLBZP GROUP (N=9)	*F*	*P*
AKT	1.01±0.12	2.03±0.34*	3.12±0.43*	3.96±0.63*	83.51	<0.05
P-AKT(SER473)	0.97±0.14	2.64±0.43*	3.95±0.62*	5.21±0.75*	103.1	<0.05
P-AKT(THR308)	0.98±0.13	1.03±0.14*	1.10±0.16*	1.09±0.15*	1.333	0.28
MYOSTATIN	3.82±0.63	2.34±0.38*	1.05±0.14*	0.75±0.11*	123.4	<0.05
SMAD3	1.03±0.14	0.98±0.13*	1.12±0.11*	1.09±0.14*	2.059	=0.13
P-SMAD3	4.65±0.72	3.32±0.43*	2.25±0.34*	1.13±0.06*	98.9	<0.05

*Note: *P<0.05, compared with the control group.*

3.4 GF1/PI3K/Akt Signaling Pathway Proteins and Myostatin-Related Protein Secretion in the Serum of SLBZP and STZ-Treated Mice

The study analyzed the serum levels of IGF-1, AKT, p-AKT and Myostatin by ELISA to determine the effects of SLBZP on GF1/PI3K/Akt signaling pathway and myostatin in STZ-treated mice. As presented in Table 3, IGF-1, AKT and p-AKT serum levels were dramatically increased, while Myostatin was decreased in the SLBZP and STZ-treated mice in relative to the serum of control (Table 4).

Table 4: The serum levels of IGF-1, AKT, p-AKT and Myostatin in the mice of each group

GENE NAME	CONTROL GROUP (N=9)	LOW SLBZP GROUP (N=9)	MEDIUM SLBZP GROUP (N=9)	HIGH SLBZP GROUP (N=9)	*F*	*P*
IGF-1	156.2±21.23(ng/mL)	221.3±34.45(ng/mL) *	314.5±36.12(ng/mL) *	397.5±35.43(ng/mL) *	95.88	<0.05
AKT	147.5±18.16(ng/mL)	209.6±28.21(ng/mL)*	298.5±32.02(ng/mL)*	375.3±34.18(ng/mL)*	108.3	<0.05
P-AKT	13.21±0.14(ng/mL)	34.52±4.59(ng/mL)*	53.14±7.45(ng/mL)*	71.89±9.23(ng/mL)*	140.7	<0.05
MYOSTATIN	495.2±62.41 (pg/mL)	378.5±54.36 (pg/mL) *	265.7±41.23(pg/mL) *	181.5±36.36(pg/mL) *	113.2	<0.05

*Note: *P<0.05, compared with the control group.*

3.5 The Effect of SLBZP Treatment on Muscle Fiber Cross-Sectional Area in STZ-Treated Mice

The study continued to analyze the effect of SLBZP on mouse skeletal muscle relative muscle fiber cross-sectional area using Masson's modified IMEB staining kit. The results showed that muscle fiber cross-sectional area had no significant difference in the low SLBZP group, but higher in the medium SLBZP group than in the control group, and the highest in the high SLBZP group (Figure 2).

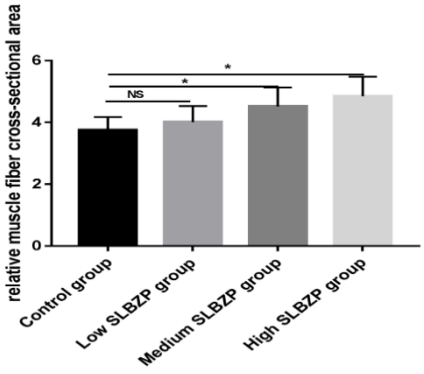

Figure 2: Mouse skeletal muscle relative muscle fiber cross-sectional area in each group. NS, no significant difference. *P<0.05.

3.6 The positive expression rates of IGF-1, AKT, Myostatin and SMAD3 in muscle tissues of SLBZP and STZ-treated mice

The study further investigated the effects of SLBZP on GF1/PI3K/Akt signaling pathway and myostatin pathway in STZ-treated mice by IHC assays. To this end, we detected the positive expression rates of IGF-1, AKT, Myostatin and SMAD3 in muscle tissues of each mouse. The data revealed that IGF-1 and AKT-positive expression rates were greatly increased, but Myostatin-positive expression rate was decreased in STZ-treated mice after SLBZP treatment when compared with the control groups (Figure 3). Additionally, the positive expression rate of SMAD3 had no significant difference in SLBZP and STZ-treated mice in relative to control (Figure 3).

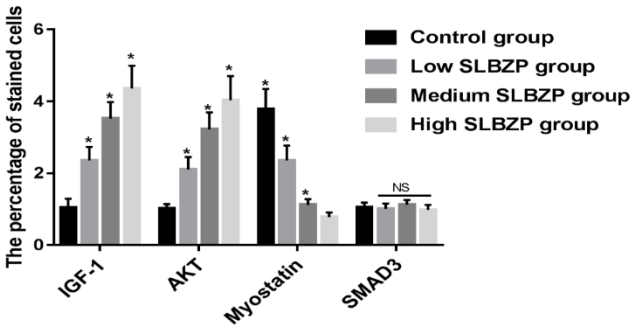

Figure 3: The positive expression rates of IGF-1, AKT, Myostatin and SMAD3 in muscle tissues of SLBZP and STZ-treated mice. NS, no significant difference. *P<0.05.

4. Discussion

Diabetes, as a basic metabolic disease with a relatively slow development process, is mainly due to the decline of human pancreatic function or the inability of the human body to effectively use insulin. At present, the disease and its complications have become an important factor threatening human health. In particular, hyperglycemia can not only damage multiple target organs of the human body, but also lead to a variety of complications, such as cardiovascular and cerebrovascular diseases, diabetes nephropathy, diabetes peripheral neuropathy, skeletal muscle atrophy, diabetes and cataract (Izzo et al., 2021; Liang et al., 2022). This not only reduces the life cycle of patients, but also brings huge economic and psychological burden to patients and their relatives. Among these complications, the mechanism and treatment of skeletal muscle atrophy and atrophy are hot issues in current clinical research (Purnamasari et al., 2022). As an important target organ of insulin action, skeletal muscle is not only the main part of insulin resistance, but also the main target organ of hyperglycemia damage. According to clinical research, various factors related to diabetes, such as high glucose toxicity, insulin resistance, lipid deposition, and neurovascular disease, can damage the structure or physiological function of human skeletal muscle to a certain extent, resulting in a decrease in the amount of human skeletal muscle, a decrease in the tension of muscle extension and contraction, and a continuous decline in motor function (Bonaldo & Sandri, 2013; Yin, Li, et al., 2021). (Kitada & Koya, 2021) concluded that insulin resistance not only accelerated the degenerative disease of human skeletal muscle, but also increased the risk of skeletal muscle mass loss in diabetes patients. In addition, according to the research of Tomoyo (Miyakuni et al., 2021), the decline of skeletal muscle volume and muscle stretch in diabetes patients is significantly related to the level of glycosylated hemoglobin (HbA1c). Therefore, enough research data show that diabetes is closely related to the degree of skeletal muscle damage, and even many scholars agree that sarcopenia is an important complication of diabetes. Sarcopenia is the result of gradual decline in muscle mass, which is generally considered as a gradual and generalized loss of skeletal muscle strength and function. sarcopenia can be divided into three stages: (1) The early stage of sarcopenia, the patients only have reduced muscle volume; (2) The sarcopenia stage, the patients have decreased muscle volume or muscle strength and muscle function; (3) Severe oligomyosis of sarcopenia, the patients is characterized by a significant reduction in muscle volume, muscle strength and muscle function. Relevant clinical studies have proved that sarcopenia significantly increases the risk of fracture, chronic heart and lung disease and swallowing dysfunction in the elderly (Nishikawa et al., 2021). Especially for elderly patients with diabetes, most of them are in skeletal muscle dystrophy, and elderly diabetes patients with sarcopenia often have poor prognosis. With the age of diabetes patients, insulin resistance gradually increases, and the function of pancreatic islet cells

gradually weakens, which will cause progressive insufficiency of insulin secretion. In common, insulin plays a crucial role in all aspects of protein synthesis, but when this hormone is severely reduced, it will cause imbalance in protein synthesis and degradation in muscle, increasing the risk of sarcopenia in diabetes patients. Secondly, with the increase of patients' age, the function of their motor neurons gradually degenerated and lost, the quality and strength of skeletal muscles gradually declined, and the incidence rate of sarcopenia also increased. In addition, if the elderly diabetes patients have poor blood glucose control, the incidence rate of sarcopenia is also significantly increased, which may be related to the inflammatory reaction caused by hyperglycemia (Bano et al., 2017). Sarcopenia usually has many adverse effects on the prognosis and treatment of elderly diabetes patients, such as increasing the incidence of pulmonary complications in elderly diabetes patients, increasing the incidence of cardiovascular adverse events, increasing the basic metabolic rate of elderly patients, aging and other clinical manifestations. In addition, the elderly patients with muscular dystrophy will also have hypoglycemic reactions caused by factors such as decreased appetite and body mass. In addition, once the glucose supply of elderly patients is insufficient for a long time and at a high frequency, it will further cause damage to liver, kidney, heart, brain and other organs, and even endanger life. According to the theory of traditional Chinese medicine, spleen deficiency can lead to blood stasis and phlegm turbidity, making hydration stop, and inducing spleen deficiency and dampness stagnation, which is a common symptom of diabetes patients. If the elderly patients with diabetes suffer from spleen deficiency, poor water movement, bladder bet and other conditions, it will lead to polydipsia, frequent urination, gastrointestinal metabolic disorder and other syndromes, and even with the development of the disease. There will be pathological wet turbidity, wet turbidity, trapped in the spleen, weak movement and other symptoms. For such clinical symptoms, the traditional Chinese medicine SLBZP can effectively improve (Meng et al., 2021). Among them, *Codonopsis pilosula*, *Chinese yam, atractylodes macrocephala and astragalus* can promote the growth of “yang qi” in the spleen and stomach, and can raise the clear and reduce the turbid, *Poria cocos* can pour out the fire of deficiency, and *Pinellia* ternate can dry and damp and dissipate phlegm. The compatibility of all drugs can play the role of drying and damp and reducing turbid, and invigorating the spleen. In addition, modern pharmacological research shows that *Atractylodes macrocephala* root can reduce the level of liver glycogen, while *Poria cocos* extract can inhibit blood sugar and effectively reduce blood pressure and regulate insulin resistance, reasonably regulate the level of adipose hormone, thereby promoting the proliferation of insulin cells and alleviating its failure (Li et al., 2010; Song et al., 2018). Therefore, in the treatment of elderly type 2 diabetes patients with spleen deficiency and dampness syndrome, it is of great clinical research value to further explore the

therapeutic mechanism and molecular pharmacology of SLBZP. According to relevant clinical studies, SLBZP can reduce inflammatory factors (MCP-1, TNF-α) by increasing the expression of miR-23a/27a. It can relieve the clinical symptoms of obese patients with T2DM (Zhang et al., 2022). MicroRNAs (miRs) are a class of non-coding small molecular RNAs with extensive biological activities, which carry out material transmission and signal pathway molecular regulation inside and outside the cell. Research shows that miR-23a/27a is involved in the body's inflammatory response, insulin resistance and insulin IKK/PKC signal pathway, and the expression level of miR-23a/27a in the serum of obese patients is negatively correlated (Lozano-Bartolome et al., 2018; Zhang et al., 2022). Our previous study of our research group found that the treatment effect of SLBZP combined with metformin in treating T2DM obese patients was better than that of metformin alone, which showed that HOMA-IR and GHb decreased after treatment. This may be due to the fact that SLBZP can increase the expression level of miR-23a/27a and reduce inflammatory factors (MCP-1 and TNF-α) Horizontal. However, due to the limited clinical sample data, this result needs further research to confirm. At the same time, we found that 22 miRs changed in the muscle atrophy model of CKD mice, especially the reduction of miR-23a was the most obvious. However, miR-23a is closely related to miR-27a and miR-24-2. When the current body miR is processed into a single miR-23a, miR-27a and miR-24-2, they will target different tissues. The expression of miR-23a/27a can play the role of insulin resistance, which can prevent CKD-induced muscle atrophy by changing the abundance of various proteins. In addition, clinical studies have shown that miR-23a in skeletal muscle of diabetes rats is reduced, which is consistent with muscle atrophy during diabetes (Yu et al., 2018). The present work showed that SLBZP and STZ-treated mice displayed an increase in grip endurance. SLBZP treatment inhibited BUN and creatinine production and increased miR-23a and miR-27a expression. Previous studies have indicated that IGF-1/PI3K/AKT pathway is involved in myoglobin synthesis (Hudson et al., 2014). The present work analyzed the effects of SLBZP on the regulation of IGF-1/PI3K/AKT pathway. The results showed that IGF-1, AKT and p-AKT (Ser473) expression were significantly upregulated, but p-AKT (Thr308) expression had no change in the muscle tissues of SLBZP and STZ-treated mice, indicating that SLBZP might activated the IGF-1/PI3K/AKT pathway by increasing p-AKT (Ser473) production. Given the regulatory functions of the Myostain/SMAD pathway in skeletal muscle regeneration (Latres et al., 2005; Zhao et al., 2012), we investigated the effects of SLBZP on the Myostain/SMAD pathway. As a results of analyses, Myostatin and p-SMAD3 expression were downregulated in the muscle tissues of SLBZP and STZ-treated mice, but SMAD3 production were not affected by SLBZP treatment in the STZ-treated mice. Further, muscle fiber cross-sectional area had no significant difference in the low SLBZP group, but higher in the medium SLBZP group and the highest in the high SLBZP group in

comparison with control groups (Sriram et al., 2014). Therefore, according to this clinical study and relevant literature evidence, we further believe that after the application of SLBZP, the levels of miR-23a and miR-27a in muscle can be increased, and then the skeletal muscle atrophy induced by diabetes can be improved by up regulating IGF1/PI3K/Akt signaling pathway and down regulating myostatin cascade reaction. In addition, the overexpression of miR-23a and miR-27a leads to an increase in the circulation of these miRs in the external body. These miRs seem to be absorbed by muscle fiber cells in other muscles, which is beneficial to the process of muscle atrophy.

5. Conclusions

This study demonstrates that Shenling Baizhu Powder (SLBZP) effectively alleviates skeletal muscle atrophy in diabetic mice by upregulating the miR-23a/PI3K/AKT pathway. The treatment not only improved muscle mass and strength but also enhanced muscle fiber morphology and reduced atrophic changes. These findings highlight the therapeutic potential of SLBZP in promoting muscle regeneration and restoring functional capacity, particularly in conditions characterized by muscle wasting, such as diabetes and sarcopenia. The activation of the miR-23a/PI3K/AKT pathway underlines the molecular basis of SLBZP's effects, emphasizing its role in enhancing muscle protein synthesis and reducing apoptosis. This mechanism is particularly relevant for sports and rehabilitation medicine, as it provides a targeted approach to managing muscle loss and improving physical performance. The dose-dependent effects observed in this study suggest that optimizing SLBZP dosage could further maximize its therapeutic benefits. The results have important implications for individuals at risk of muscle atrophy, including aging adults, athletes recovering from injury or metabolic disorders, and populations experiencing reduced physical activity. By addressing both functional and molecular aspects of muscle health, SLBZP offers a promising alternative or adjunct to conventional therapies for sarcopenia and muscle recovery. Future research should focus on validating these findings in clinical settings and exploring the long-term effects of SLBZP on muscle health and physical performance. Investigating its integration into rehabilitation programs and athletic recovery protocols could further enhance its applicability. By bridging traditional medicine and modern sports science, SLBZP presents a novel approach to optimizing physical health and performance across diverse populations.

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Mechanism of improving diabetes-induced sarcopenia by up-regulating miR-23a27a expression in Ginseng and Atractylodes macrocephala.

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REFERENCES

Bano, G., Trevisan, C., Carraro, S., Solmi, M., Luchini, C., Stubbs, B., Manzato, E., Sergi, G., & Veronese, N. (2017). Inflammation and sarcopenia: a systematic review and meta-analysis. *Maturitas*, *96*, 10-15.

Beaudart, C., Dawson, A., Shaw, S., Harvey, N. C., Kanis, J., Binkley, N., Reginster, J. Y., Chapurlat, R., Chan, D., & Bruyère, O. (2017). Nutrition and physical activity in the prevention and treatment of sarcopenia: systematic review. *Osteoporosis International*, *28*, 1817-1833.

Bonaldo, P., & Sandri, M. (2013). Cellular and molecular mechanisms of muscle atrophy. *Disease models & mechanisms*, *6*(1), 25-39.

Chen, L.-K., Woo, J., Assantachai, P., Auyeung, T.-W., Chou, M.-Y., Iijima, K., Jang, H. C., Kang, L., Kim, M., & Kim, S. (2020). Asian Working Group for Sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *Journal of the American Medical Directors Association*, *21*(3), 300-307. e302.

Cleasby, M. E., Jamieson, P. M., & Atherton, P. J. (2016). Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. *J Endocrinol*, *229*(2), R67-R81.

Cruz-Jentoft, A. J., & Sayer, A. A. (2019). Sarcopenia. *The lancet*, *393*(10191), 2636-2646.

Dhillon, R. J., & Hasni, S. (2017). Pathogenesis and management of sarcopenia. *Clinics in geriatric medicine*, *33*(1), 17-26.

Garhöfer, G., Chua, J., Tan, B., Wong, D., Schmidl, D., & Schmetterer, L. (2020). Retinal neurovascular coupling in diabetes. *Journal of Clinical Medicine*, *9*(9), 2829.

Hudson, M. B., Woodworth-Hobbs, M. E., Zheng, B., Rahnert, J. A., Blount, M. A., Gooch, J. L., Searles, C. D., & Price, S. R. (2014). miR-23a is decreased during muscle atrophy by a mechanism that includes calcineurin signaling and exosome-mediated export. *American Journal of Physiology-Cell Physiology*, *306*(6), C551-C558.

Izzo, A., Massimino, E., Riccardi, G., & Della Pepa, G. (2021). A narrative review on sarcopenia in type 2 diabetes mellitus: prevalence and associated factors. *Nutrients*, *13*(1), 183.

Kitada, M., & Koya, D. (2021). Autophagy in metabolic disease and ageing. *Nature Reviews Endocrinology*, *17*(11), 647-661.

Latres, E., Amini, A. R., Amini, A. A., Griffiths, J., Martin, F. J., Wei, Y., Lin, H. C., Yancopoulos, G. D., & Glass, D. J. (2005). Insulin-like growth factor-1 (IGF-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway. *Journal of Biological Chemistry*, *280*(4), 2737-2744.

Laviano, A., Gori, C., & Rianda, S. (2014). Sarcopenia and nutrition. *Advances*

in food and nutrition research, *71*, 101-136.

Li, T.-H., Hou, C.-C., Chang, C. L.-T., & Yang, W.-C. (2010). Anti-hyperglycemic properties of crude extract and triterpenes from Poria cocos. *Evidence-based Complementary and Alternative Medicine*, *2011*.

Liang, J., Bao, A.-l., Ma, H.-y., Dong, W., Li, W.-h., Wu, X., Li, H.-y., Hou, H.-y., Chen, Y.-q., & Fu, J.-l. (2022). Prevention of polycystic ovary syndrome and postmenopausal osteoporosis by inhibiting apoptosis with Shenling Baizhu powder compound. *PeerJ*, *10*, e13939.

Lozano-Bartolome, J., Llaurado, G., Portero-Otin, M., Altuna-Coy, A., Rojo-Martínez, G., Vendrell, J., Jorba, R., Rodríguez-Gallego, E., & Chacon, M. R. (2018). Altered expression of miR-181a-5p and miR-23a-3p is associated with obesity and TNF α-induced insulin resistance. *The Journal of Clinical Endocrinology & Metabolism*, *103*(4), 1447-1458.

Meng, M., Bai, C., Wan, B., Zhao, L., Li, Z., Li, D., & Zhang, S. (2021). A network pharmacology-based study on irritable bowel syndrome prevention and treatment utilizing shenling baizhu powder. *BioMed research international*, *2021*.

Miyakuni, T., Komiyama, H., Takano, M., Ikeda, T., Matsushita, M., Kobayashi, N., Otsuka, T., Miyauchi, Y., Asai, K., & Seino, Y. (2021). A preliminary pilot study investigating the impact of endovascular treatment on leg muscle volume in peripheral artery disease and its relation to baseline glycemic control. *Nutrition, Metabolism and Cardiovascular Diseases*, *31*(1), 269-276.

Nishikawa, H., Asai, A., Fukunishi, S., Nishiguchi, S., & Higuchi, K. (2021). Metabolic syndrome and sarcopenia. *Nutrients*, *13*(10), 3519.

Nomura, T., Kawae, T., Kataoka, H., & Ikeda, Y. (2018). Assessment of lower extremity muscle mass, muscle strength, and exercise therapy in elderly patients with diabetes mellitus. *Environmental health and preventive medicine*, *23*(1), 20.

Papadopoulou, S. K. (2020). Sarcopenia: A contemporary health problem among older adult populations. *Nutrients*, *12*(5), 1293.

Purnamasari, D., Tetrasiwi, E. N., Kartiko, G. J., Astrella, C., Husam, K., & Laksmi, P. W. (2022). Sarcopenia and chronic complications of type 2 diabetes mellitus. *Review of Diabetic Studies*, *18*(3), 157-165.

Sanz-Cánovas, J., López-Sampalo, A., Cobos-Palacios, L., Ricci, M., Hernández-Negrín, H., Mancebo-Sevilla, J. J., Álvarez-Recio, E., López-Carmona, M. D., Pérez-Belmonte, L. M., & Gómez-Huelgas, R. (2022). Management of type 2 diabetes mellitus in elderly patients with frailty and/or sarcopenia. *International Journal of Environmental Research and Public Health*, *19*(14), 8677.

Song, M. Y., Lim, S.-K., Wang, J.-H., & Kim, H. (2018). The root of Atractylodes macrocephala Koidzumi prevents obesity and glucose intolerance and increases energy metabolism in mice. *International Journal of Molecular Sciences*, *19*(1), 278.

Sriram, S., Subramanian, S., Juvvuna, P. K., Ge, X., Lokireddy, S., McFarlane, C. D., Wahli, W., Kambadur, R., & Sharma, M. (2014). Myostatin augments muscle-specific ring finger protein-1 expression through an NF-kB independent mechanism in SMAD3 null muscle. *Molecular endocrinology*, *28*(3), 317-330.

Yan, C., Zhou, Y., Chen, Q., Luo, Y., Zhang, J. H., Huang, H., & Shao, A. (2020). Dysfunction of the neurovascular unit in diabetes-related neurodegeneration. *Biomedicine & Pharmacotherapy*, *131*, 110656.

Yin, L., Chen, X., Li, N., Jia, W., Wang, N., Hou, B., Yang, H., Zhang, L., Qiang, G., & Yang, X. (2021). Puerarin ameliorates skeletal muscle wasting and fiber type transformation in STZ-induced type 1 diabetic rats. *Biomedicine & Pharmacotherapy*, *133*, 110977.

Yin, L., Li, N., Jia, W., Wang, N., Liang, M., Yang, X., & Du, G. (2021). Skeletal muscle atrophy: From mechanisms to treatments. *Pharmacological Research*, *172*, 105807.

Yu, Y., Du, H., Wei, S., Feng, L., Li, J., Yao, F., Zhang, M., Hatch, G. M., & Chen, L. (2018). Adipocyte-derived exosomal MiR-27a induces insulin resistance in skeletal muscle through repression of PPARγ. *Theranostics*, *8*(8), 2171.

Zhang, L.-J., Zhan, L.-B., Hang, T.-Y., Luo, J.-T., & Zhao, C.-Y. (2022). Shenling baizhu powder alleviates chronic inflammation to prevent type 2 diabetes of ZDF rats via intestinal flora. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, *47*(4), 988-1000.

Zhang, Y., Tang, K., Deng, Y., Chen, R., Liang, S., Xie, H., He, Y., Chen, Y., & Yang, Q. (2018). Effects of shenling baizhu powder herbal formula on intestinal microbiota in high-fat diet-induced NAFLD rats. *Biomedicine & Pharmacotherapy*, *102*, 1025-1036.

Zhao, T., Qi, Y., Li, Y., & Xu, K. (2012). PI3 Kinase regulation of neural regeneration and muscle hypertrophy after spinal cord injury. *Molecular biology reports*, *39*, 3541-3547.

Zhong, W., Xie, C., & Zhu, H. (2018). Experiment of Shenqi Compound Recipe Mediating IGF - 1 /PI3K /Akt Intervening Skeletal Muscle of Mice with Type 2 Diabetes Mellitus. *Liaoning Journal of Traditional Chinese Medicine*, *45*(5), 1072-1075.