# Investigation of Pharmacological Agent(s) and Molecular Mechanism thereof for the treatment of Sarcopenia associated Muscle Atrophy

SUMMARY

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**PRIYANKA PRAJAPATI**

**Enrollment No. - 409/19**

**Co- Supervisor**

**DR. SHUBHINI A. SARAF**

**Professor**

**Supervisor**

**DR. SAPANA KUSHWAHA**

**Assistant Professor**

**DEPARTMENT OF PHARMACEUTICAL SCIENCES**

**SCHOOL OF BIOMEDICAL & PHARMACEUTICAL SCIENCE**

**BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY**

**(A CENTRAL UNIVERSITY)**

**VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025 (U. P.), INDIA**


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## Summary

### 1. Introduction

According to the International Working Group on Sarcopenia (IWGS), sarcopenia is a syndrome that affects older people's skeletal muscle quality, muscle mass, and strength rendering them weak and physically inactive. The factors for sarcopenia include inactivity, hormonal imbalance, impaired neuronal signaling, cytokines production, and high amounts of macromolecule breakdown that lead to inadequate protein synthesis. The estimated expenditure for managing sarcopenia and related diseases in the United States of America is $18.5 billion, or 1.5% of all healthcare expenditures. Sarcopenia's demographic statistical growth rate has been noted in many nations, including China, Poland, Spain, Mexico, and India, with an incidence of 11 to 50% among people 65 and older. The European Working Group on Sarcopenia in Older People 2 (EWGSOP-2) defined sarcopenia as to decrease in skeletal muscle size, quality, and strength. Although the EWGSOP2 has underlined the significance of assessing muscle quality as a diagnostic hallmark of sarcopenia, there is not yet a broad consensus on the use of this assessment in routine clinical practice.

Various signaling pathways, including Insulin-like Growth Factor-1 (IGF-1), phosphokinase B (Akt), mammalian target of rapamycin (mTOR), Transforming Growth Factor-β (TGF-β), and Nuclear Factor-κB (NF-κB)/Mitogen-Activated Protein Kinase (MAPK), are being investigated as potential modulators of sarcopenia progression. Muscle lipid accumulation can impact muscle protein metabolism, insulin sensitivity, and mechanistic signaling pathways, providing potential pharmaceutical treatment options. Both catabolic and anabolic pathways play important roles in the degeneration of muscle associated with aging. Skeletal muscle fiber degradation in older individuals is regulated by several systems, including atrogene, Foxo, Akt, and myostatin. These signaling molecules influence oxidative stress, apoptosis, autophagy, hormone sensitivity, inflammation, and levels of inflammation-related proteins, leading to increased protein breakdown and reduced protein synthesis. Animal models such as hindlimb unloading, suspension, and immobilization-induced sarcopenia have been used to study the pathophysiology and mechanisms of sarcopenia. Findings also showed that the consumption of amino acids (AAs) is essential for boosting muscle protein synthesis or contributing to an anabolic response. Leucine regulates intracellular signaling pathways and is a precursor for muscle protein synthesis. Reduced leucine, isoleucine, and aromatic AA concentrations have

been observed in sarcopenic patients, which has been associated to muscle mass, physical performance, and muscle strength as diagnostic criteria. Plasma metabolomic findings indicated that L-alanine and proline may be pronounced sarcopenia biomarkers. Increased physical performance can be attributed, in part, to choline, an essential micronutrient that plays a critical role in a number of metabolic pathways. Further, studies found that older adults with high levels of non-EAA including proline had a greater risk of sarcopenia. Additionally, the AAs threonine and histidine are notable biomarkers for sarcopenia. The multi-analysis found that glutamine, serine, lysine, threonine, and proline could have been biomarkers for sarcopenia. The findings describe the elderly with suspected sarcopenia's significantly altered plasma AA metabolisms, allowing researchers to screen high-risk individuals to develop new preventive and therapeutic strategies. Therefore, above mentioned amino acid considerations may assist in preventing and treating sarcopenia and regulating muscle mass and activities.

Repurposed drugs, including selective androgen receptor modulators (SARMs), metformin, a select few angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin receptor blockers (ARBs), offer potential solutions. Positive results from clinical trials and animal studies suggest that losartan, an ARB, effectively enhances grip strength and muscle mass. Further research is needed to fully understand its capabilities. Notably, clinical trials with Perindopril, Losartan, and exercise have shown positive outcomes in older individuals, highlighting the potential of ARBs as a therapeutic option for sarcopenia and its consequences.

The pressing need to address sarcopenia-associated muscle atrophy highlights the importance of identifying and repurposing existing pharmacological drugs or agents. In light of this, the present study aims to investigate the repurposing of existing ARBs as a potential treatment for sarcopenia-induced muscle wasting.

## 2. Objectives

The present study aims to explore the potential of angiotensin receptor blockers (ARBs) in treating muscle wasting associated with sarcopenia. The specific objectives of this study are as follows:

1. *In-silico* study to analyze the interaction between existing ARBs and anabolic and catabolic proteins.
2. Investigate the effects of the identified lead compound on the C2C12 skeletal muscle cell line and uncover the underlying mechanisms.
3. Evaluate the effect of the identified lead compound on sarcopenic obesity induced by a

high-fat diet and natural aging sarcopenic rat models, while also elucidating the associated mechanisms.

4. Assess the effects of the identified lead compound on the metabolomic analysis of skeletal muscles in sarcopenic rats.

**3. Experimental Design**

***In silico* study design**

Molecular docking was conducted to predict the binding affinities of angiotensin receptor blockers (ARBs) such as Azilsartan, Telmisartan, Losartan, Eprosartan, Irbesartan, Olmesartan, Candesartan, and Valsartan with insulin-like growth factor-1 (IGF-1) as an anabolic protein, and MuRF-1 as a catabolic protein. The crystal structures of the proteins were obtained from the Protein Data Bank, and the active sites were determined using Biovia Discovery Studio Visualizer. PDBQT files for the proteins and ligands were prepared, and grid boxes were created using AutoDock Tools (ADT). AutoDock/Vina was used for the docking process, and the binding affinities (kcal/mol), probable hydrogens, and amino acids were calculated. The position with the highest binding energy or affinity was selected.

***In vitro* study**

C2C12 myoblast skeletal cell lines were cultured in DMEM high glucose (DMEM-HG) supplemented with 10% fetal bovine serum and antibiotics-antimycotics. To induce myotube differentiation, C2C12 cells were seeded in six-well plates and cultured until 80% confluency. Subsequently, they were washed with PBS and cultivated in DMEM supplemented with 2% horse serum until clear, multinucleated myotubes formed. To evaluate the effect of azilsartan on myogenic differentiation, 80% of confluent C2C12 myoblasts were serum-starved in DMEM-HG without serum for six days. Different concentrations of azilsartan (1, 10, 100, and 1000nM) were added to the media, with regular replenishment every 24 hours. After the treatment period, the C2C12 cells were terminated by trypsinization, followed by Western blotting analysis of cell lysates to assess the levels of MyoD and myogenin, early and late myogenic regulatory factors, respectively.

***In vivo* study**

In the present study, two sarcopenic rat models were utilized. Firstly, a high-fat diet intervention was employed to induce sarcopenia, resulting in the development of the sarcopenic obese rat model. Secondly, natural aging rats simulate sarcopenia associated with aging.

*(a) High-fat diet (HFD)-induced sarcopenic obesity rat model*

In the HFD diet-induced sarcopenic obese rat model, 14-17 months male *Sprague Dawley* rats

were fed a high-fat diet (HFD) for four months, resulting in old sarcopenic obese (SO) rats (18-21 months). At the 18$^{th}$ month, rats were switched to a standard chow diet *ad libitum*. Sarcopenic obesity in rats was confirmed by assessing fat mass, lipid profiles, and forelimb grip strength. The old SO rats were then divided into Old Control (OC) and Old Azilsartan Treatment (OT) groups. A vehicle (0.5% carboxymethyl cellulose, CMC) was administered to age-matched control groups (YC and OC). Treatment groups (YT and OT) received oral azilsartan (AZL) at a dose of 8 mg/kg for six weeks. The dose selection was based on a literature review and human equivalent dose (HED) calculation. After six weeks, forelimb grip strength and body composition tests were conducted. Following humane sacrifice, serum and gastrocnemius (GN) muscles were collected. Half of the GN muscles were fixed in 10% formalin, while the remainder was stored at -80°C.

*(b) Natural aged sarcopenic rat model*

This study used four-month-old and eighteen-month-old male *Sprague-Dawley* rats. Rats had *ad libitum* access to a conventional chow diet and water throughout the experiment. The eighteen-month-old rats, referred to as natural sarcopenic (NS) rats, were selected based on previous research. The young SD rats were divided into Young Control (YC) and Young Azilsartan Treatment (YT) groups. Similarly, the natural-aged rats were categorized into Aged Control (AC) and Aged Azilsartan Treatment (AT) groups. YC and AC/NS rats received 0.5% carboxymethyl cellulose (CMC) as vehicle control. Azilsartan (AZL) was orally administered to both young and AT rats at a dose of 8 mg/kg for six weeks. After the AZL treatment, the rats underwent various muscle coordination tests. Following sacrifice, serum samples and gastrocnemius muscles (GN) were collected for further analysis.

**4. Endpoints Parameters**

To explore the potential of angiotensin receptor blockers (ARBs) in treating muscle wasting associated with sarcopenia, the following methods/techniques were used:

a) **Muscle strength and muscle coordination test:** Hanging-wire (Horizontal-wire) test, Actophotometer analysis, Inclined plane test, Footprint analysis, Grip weighing test, Forelimb grip strength test, and Body composition analysis.
b) **Muscle markers:** Creatine kinase, myostatin, insulin, and testosterone levels
c) **Lipidemic markers:** Lipid profiles [triglycerides (TG), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL)]

d) **Oxidative stress and antioxidant levels:** Oxidative stress markers (malondialdehyde, protein carbonyl, nitric oxide, ROS levels) and antioxidants (glutathione, catalase, 2,2-diphenyl-1-picryl-hydrazyl-hydrate scavenging activity, and superoxide dismutase).

e) **Histology:** Histological examination was performed to observe structural changes and gastrocnemius (GN) muscle morphology of diabetic rats after salbutamol treatment.

f) **Fibrosis staining:** Sirius red and Masson's trichome staining.

g) **Muscle fiber typing using COX, SDH and combined SDH-COX staining:** Succinate dehydrogenase (SDH) and cytochrome c oxidase (COX-2) staining.

h) **Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM):** To visualize structure of GN muscle.

i) **Immunohistochemistry and Western blotting:** Akt-1, MuRF-1, TNF-α, myogenin and myoD

*j)* **1H NMR profiling:** Skeletal muscle (GN) profiling using 1H Nuclear Magnetic Resonance (NMR)

## 5. Results

### *Summary of Findings: An in-silico* study

In the present, we assessed the binding affinity of ARBs towards two key proteins involved in sarcopenia: insulin-like growth factor 1 (IGF-1) and muscle ring finger-1 (MuRF-1). Recent findings have highlighted the significance of these pathways in enhancing translational efficiency and controlling ribosomal biogenesis during skeletal muscle hypertrophy. The mammalian target of rapamycin (mTOR), an evolutionarily conserved serine/threonine kinase, plays a crucial role in protein synthesis and preventing muscle loss. The IGF-1, Akt/Protein Kinase B, and mTOR pathway serve as the primary driver linking mechanical contraction and protein synthesis, potentially experiencing dysregulation in older individuals. Additionally, MuRF-1, a catabolic protein, contributes to the degradation of contractile proteins and likely targets the same sarcomeric proteins, leading to skeletal muscle atrophy. MuRF1 has been demonstrated in multiple studies to restrict the activation of hypertrophy-related genes in response to hypertrophic stimuli.

Findings showed that among the selected ARBs, telmisartan and azilsartan showed a high binding affinity with IGF-1 and MuRF-1, displaying binding energies of -10.4 kcal/mol, -10.1 kcal/mol, -7.4 kcal/mol, and -7.2 kcal/mol, respectively. Based on the positive results from in-

silico studies, azilsartan was chosen for further evaluation of its efficacy in both *in vitro* and *in vivo* experiments. Additionally, previous findings indicating azilsartan's ability to modulate insulin sensitivity in skeletal muscle support its potential as an anti-sarcopenic agent and the rationale for its selection for this study.

***Summary of in vitro findings: Effect of Azilsartan on C2C12 Skeletal Muscle Cell Line***

Various skeletal muscle cell culture models have been employed for research on sarcopenia pathophysiology. Among these models, the rat skeletal muscle L6 cells and the murine C2C12 cells are commonly used in vitro systems to investigate molecular characteristics. The C2C12 murine myoblast cell line serves as an accessible and established model for studying muscle differentiation and growth regulation. This mouse-derived cell line has been extensively utilized in various research areas, including aging, diabetes, obesity, hyperlipidemia, muscle growth, hepatic steatosis, and growth impairment.

The results indicate higher concentrations of AZL (100 and 1000nM) resulted in a significant increase in MyoD expression compared to a lower concentration (1nM). AZL (azilsartan) significantly enhances the expression of MyoD, a regulator of early myogenesis stages, at a maximum dose of 1000nM AZL. Furthermore, myogenin, a regulator of late myogenesis stages, exhibited increased expression in a dose-dependent manner. Overall, these *in vitro* findings suggest that AZL promotes cell differentiation at certain doses and has the potential to alleviate muscle wasting associated with sarcopenia, as supported by significant expression of MyoD and Myogenin.

***Summary of Findings: Effects of azilsartan on high-fat diet-induced sarcopenic obese induced Skeletal Muscle Wasting***

In this study, we investigated the effects of AZL on skeletal muscle wasting in rats with sarcopenic obesity induced by a high-fat diet. The results demonstrated that AZL treatment improved grip strength and lean mass while reducing fat mass in sarcopenic obese rats.

AZL treatment in sarcopenic rats had several positive effects, including attenuating muscle mass and functional impairments, restoring oxidative stress balance, and increasing antioxidant levels. It also increased myofibrillar protein content, restored muscle anabolic markers, and improved muscle fiber quality. Skeletal muscle wasting, characterized by reduced grip strength, lean body mass, and increased fat mass, was evident in sarcopenic obese rats. However, AZL treatment increased lean muscle mass, suggesting its potential role in protein synthesis-related muscle metabolism.

Furthermore, AZL treatment reduced the production of reactive oxygen species (ROS) and improved antioxidant levels in sarcopenic obese rat muscles. It decreased cholesterol and triglyceride levels, improved HDL levels, and protected against lipid-related complications. The treatment also reduced the levels of muscle damage biomarkers, including myostatin and creatine kinase (CK), while increasing testosterone levels. The study also examined the effect of AZL on the cellular architecture, surface morphology, and ultrastructure of sarcopenic obese rat muscles. AZL treatment resulted in an increased cross-sectional area of skeletal muscle fibers and a reduction in collagen levels, indicating a decrease in fibrotic area. It also improved the organization of myofibrils, sarcomere structures, and muscle fiber structures, including increased mitochondrial number and size and enhanced Z line. Additionally, AZL treatment exhibits muscle fiber typing, shifting mitochondrial enzyme activity from slow oxidative muscle fibers to fast glycolytic muscle fibers. This shift was more prominent in sarcopenic obese rats compared to young rats. Findings from the immunohistochemical analysis revealed that AZL treatment increased the expression of phospho Akt-1 (p-Akt-1) and decreased the expression of MuRF-1 and TNF-α in sarcopenic obese rats. These findings suggest that AZL may prevent protein degradation and promote protein synthesis in sarcopenic obesity.

Overall, our findings demonstrate that AZL treatment prevents protein degradation and promotes protein synthesis in high-fat diet-induced sarcopenic obese rats. The increased expression of p-Akt-1, along with the decreased expression of MuRF-1 and TNF-α in AZL-treated rats, showed that AZL could be beneficial in preventing additional muscle wasting and promoting muscle protein synthesis in sarcopenic obesity.

***Summary of Findings: Effects of Azilsartan on Natural Aging Induced Skeletal Muscle Wasting***

In our study, we investigated the effect of AZL on skeletal muscle loss in naturally aged sarcopenic rats. The results showed that AZL treatment significantly increased body weight, GN muscle weight, and motor coordination functions in these rats. It also improved muscle grip strength, locomotion activity, and motor coordination compared to age-matched control rats. These findings suggest that AZL could be useful in preventing age-related sarcopenia. Furthermore, AZL treatment restored antioxidant levels and reduced oxidative stress in skeletal muscles, indicating its antioxidant properties in preventing age-related muscle loss. Levels of lipid peroxidation, protein carbonyl, and hydrogen peroxide were reduced by AZL treatment in the GN muscle of naturally aged sarcopenic rats. Imbalance between ROS production and antioxidant defenses can contribute to muscle wasting in the elderly, and AZL helped restore

this balance.

AZL treatment also decreased muscle biomarkers, such as myostatin and creatine kinase, in naturally aged sarcopenic rats. Additionally, it increased serum insulin levels, which has metabolic and anabolic effects on skeletal muscle, promoting protein synthesis and reducing protein breakdown. AZL treatment also increased testosterone levels, which is known to improve physical performance by promoting skeletal muscle growth.

Moreover, AZL improved the cellular architecture, fibrosis, surface morphology, and ultrastructure of GN muscle in naturally aged sarcopenic rats. Scanning electron microscopy and transmission electron microscopy showed that AZL treatment restored the surface morphology and ultrastructure of aged skeletal muscles to some extent. The stiffness of the epimysium, which contributes to tissue stiffness and muscle function in sarcopenic rats, was also alleviated by AZL treatment. Furthermore, AZL treatment influenced myofiber typing, causing a transition from type I (slow oxidative) to type II (fast glycolytic) muscle fibers in naturally aged sarcopenic rats. Type I fibers have a higher rate of protein synthesis and breakdown and are more resistant to fasting than type II fibers.

To conclude, immunohistochemical analysis showed that AZL treatment increased the expression of p-Akt-1 and decreased the expression of MuRF-1 and TNF-α in naturally aged sarcopenic rats. These findings suggest that AZL treatment reduces protein breakdown through MuRF-1 and TNF-α downregulation and increases protein synthesis through p-Akt-1 signaling. It also accelerated the transition of muscle fibers from type I to type II.

In summary, our study demonstrates that AZL treatment attenuates age-related muscle wasting in sarcopenia by restoring the muscle markers, antioxidant levels, cellular architecture, and myofiber typing.

***Summary of Findings: Effects of Azilsartan on muscle metabolomic analysis of sarcopenic rats***

Several lines of evidence have shown that metabolomics could be a valuable approach for studying metabolites and predicting alterations in the diagnosis of sarcopenia and related complications. In addition to supplying amino acids to organs and tissues, the process of muscle protein degradation plays a crucial role in human health. Excessive protein degradation can lead to muscle loss, motor dysfunction, and accelerated aging. Among the twenty amino acids, nine are essential amino acids (EAAs) that cannot be synthesized by the body at physiological levels and must be obtained through a balanced diet. Leucine, isoleucine, and valine, also known as branched-chain amino acids (BCAAs), are EAAs that contribute to muscle protein synthesis and

anabolism. The remaining eleven amino acids, including alanine, arginine, aspartate, cysteine, glutamate, glycine, proline, serine, taurine, and tyrosine, can be produced by the body and are necessary for sufficient amounts for the synthesis of new muscle protein.

The present study aims to investigate the effect of AZL on skeletal muscle metabolomes in sarcopenic rats and further explore the regulation of associated AA metabolism in protein synthesis in sarcopenic skeletal muscle. Additionally, this study aims to elucidate the role of metabolomics in the pathophysiology of sarcopenic muscle. The spectral analysis primarily revealed signals of various metabolites, including essential amino acids (methylhistidine, leucine, isoleucine, phenylalanine, methionine, threonine, and valine), non-essential amino acids (alanine, glycine, glutamate, glutamine, proline, serine, tyrosine, betaine, and sarcosine), energy metabolites (acetate, creatine, fumarate, formate, glycerol, lactate, pyruvate, and succinate), ketone bodies contents (acetone and 3-hydroxybutyrate), and the precursor of acetylcholine (choline). By analyzing the VIP score plot from PLS-DA simulated analysis, we identified the metabolic entities with discriminatory potentials. The VIP score plot revealed the dominance of resonances from creatine, alanine, isoleucine, valine, methylhistidine, proline, phenylalanine, creatinine, glutamate, and sarcosine in the PLS-DA models. Metabolites that appeared in the variables importance plot of the PLS-DA and had a Variable Importance in Projection (VIP) score > 1.0 were subjected to the ANOVA test for identification of significant variables and achieving satisfactory classification. Random forest analysis (RFA) demonstrated the importance of each variable in data classification for each metabolite. The random forest plot illustrated the loss of accuracy when excluding a variable from the model, compared to the control (AC & OC) and treated groups of old/aged (AT & OT) rats, with more crucial variables leading to a greater accuracy drop. Box cum whisker plots generated with metaboanalyst 5.02 software showed the quantitative variations of significant discriminatory metabolites such as alanine, choline, creatinine, formate, N, N-dimethylglycine, phenylalanine, proline, threonine, tyrosine, and uracil in the treatment groups compared to their respective sarcopenic control groups.

After comparing the metabolite levels between the sarcopenic and AZL-treated groups, we associated these differences with known pathways in muscle tissues. The altered metabolites showed statistical significance in both groups, specifically in energy production and muscle protein synthesis pathways. By utilizing the KEGG database, we identified several pathways associated with these metabolites in muscle tissues. Additionally, we observed interconnected metabolic pathways involved in sarcopenic muscle loss, including phenylalanine tyrosine

metabolism, methylamine metabolism, glycolysis, ketogenesis, and the TCA cycle. In this study, we analyzed significant metabolites such as alanine, choline, creatinine, formate, N, N-dimethylglycine, phenylalanine, proline, theronine, tyrosine, and uracil, which have been implicated in sarcopenic muscle loss. These metabolites are highlighted in green in Fig. 38. Furthermore, we found that other metabolites, such as serine, pyruvate, 3-hydroxybutyrate, glutamine, glutamate, histidine, succinate, betaine, isoleucine, valine, leucine, inosine, and PTR, play an essential role in sarcopenia. Although they exhibited insignificant increases after AZL treatment, they are of significant importance in the context of sarcopenic muscle loss.

Alterations in protein and amino acid metabolism can have a significant impact on the pathophysiology of sarcopenia. Essential amino acids (EAAs) and branched-chain amino acids (BCAAs) play crucial roles in activating satellite cells and regulating muscle protein synthesis and proteolysis. Our findings indicate a notable decrease in choline levels in aged skeletal muscle, which were increased with AZL treatment. Insufficient choline levels have been associated with various changes in myoblasts, including muscle tissue atrophy, as evidenced by elevated blood CK levels. Sarcosine, a naturally occurring compound found in skeletal muscles and various tissues, is synthesized in the body from dietary choline and the amino acid methionine. Sarcosine has been shown to induce dose-dependent activation of autophagy in mouse fibroblasts, highlighting its significance as a crucial factor in the pathogenesis of sarcopenia. In our study, we observed an increase in sarcosine and methionine levels in AZL-treated rats. The analysis of a generalized linear model revealed a positive correlation between gait speed and dietary intake of lysine, threonine, leucine, valine, BCAAs, and aromatic AAs. This suggests that the increased levels of these amino acids in the treated groups may be responsible for improving gait speed in sarcopenic rats. Additionally, our study showed increased creatinine levels with AZL treatment, indicating a potential reduction in muscle wasting. Furthermore, we observed a significant improvement in fumarate and N, N-dimethylglycine levels in aged skeletal muscle following AZL treatment. These findings are consistent with previous studies conducted on aged mice, which also reported lower levels of tricarboxylic acid (TCA) intermediate fumarate in aged soleus muscles compared to adult soleus muscles.

TNF-α modulates the expression of polyamine synthesis and proline production, and lower levels of proline metabolites were observed in older adults. As discussed in previous chapters, the immunoexpressions of TNF-α were found to be increased in the sarcopenic rat muscle. This

increase may be interconnected with the reduction of proline metabolite levels in sarcopenic muscle due to inflammation, while the levels were restored after AZL treatment. While most amino acids are broken down in the liver, branch-chain ketoacid dehydrogenase and mitochondrial dehydrogenase are primarily responsible for the metabolism of BCAAs in muscle tissue. Glutamate serves as a crucial intermediate in the metabolism of muscle energy under both physiological and pathological circumstances, as it can support respiration. Therefore, when HFD-treated sarcopenic obese rats were administered with the azilsartan, the aforementioned metabolic alterations reverted to normal, indicating that azilsartan had both protein-building and sarcopenic obesity-associated muscle loss treating activities.

## 6. Conclusions

In this study, we screened eight angiotensin receptor blockers (ARBs) and found that telmisartan and azilsartan exhibited good binding affinity for the anabolic protein IGF-1 and the catabolic protein MURF-1. Based on these results, we selected azilsartan for further investigation in treating muscle loss in sarcopenia. In vitro analysis showed that AZL increased the expressions of MyoD and Myogenin, indicating its potential to promote skeletal muscle differentiation. In a high-fat diet-induced sarcopenic obese rat model, AZL treatment increased lean muscle mass, grip strength, and muscle coordination, and restored the balance between oxidative stress and antioxidants. It also improved muscle structure, anabolic markers, and decreased fibrosis. Similar positive effects were observed in a natural aging sarcopenic rat model. The skeletal muscle metabolomics analysis revealed significant alterations in amino acids involved in muscle protein synthesis, which were restored by AZL treatment. Overall, our findings suggest that azilsartan has the potential to effectively mitigate muscle wasting in sarcopenia, making it a promising candidate for further exploration in age-associated muscle loss.