

Effects of Eight Weeks of Functional Training with Blood Flow Restriction and Betaine Supplementation on Follistatin, Myostatin, and Functional Outcomes in Older Women: A Randomized Controlled Trial Protocol

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Abstract

Background: Sarcopenia is characterized by age-related declines in muscle mass and function. Functional training with blood flow restriction (BFR) and betaine supplementation may improve anabolic adaptations; however, their combined effects in elderly women remain unclear. Therefore, this study aims to investigate their effects on serum follistatin and myostatin levels in elderly women.

Methods: This randomized, double-blind, placebo-controlled clinical trial protocol will enroll 56 community-dwelling older women (≥ 60 years) in Tehran, Iran. Participants will be randomized (1:1:1:1 allocation via a computer-generated sequence with allocation concealment) into four parallel groups ($n=14$ each) including functional training with blood flow restriction plus betaine (FBFR+B), functional training with blood flow restriction plus placebo (FBFR+P), betaine supplementation alone (B; 4 g/day), or non-exercising control (CON) for 8 weeks (exercise 3 sessions/week). Primary outcomes (plasma follistatin and myostatin measured by enzyme-linked immunosorbent assay) and secondary outcomes [muscle strength via 1RM bench/leg press, functional performance via Timed Up-and-Go (TUG) and 10-Meter Walk Test (10-mWT), health-related quality of life (WHOQOL-BREF), and pain characteristics (McGill Pain Questionnaire)] will be evaluated at baseline and 48 hours following the 8-week intervention. Between-group differences will be analyzed using ANCOVA adjusted for baseline values, with significance set at $P<0.05$.

Discussion: This study will evaluate the effects of functional training combined with blood flow restriction (BFR) and betaine supplementation on serum follistatin and myostatin levels in elderly women. Findings from this research may inform future guidelines for non-pharmacological interventions to preserve muscle health, preserve independence, and manage sarcopenia in aging populations.

Trial Registration: The protocol was registered in the Iranian Registry of Clinical Trials with the code of IRCT20250418065376N1.

Keywords: Sarcopenia, Betaine, Follistatin, Myostatin, Resistance Training

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1. Introduction

Aging is a complex, multidimensional process accompanied by progressive biological, physical, psychological, and social changes, with older adulthood commonly defined as beginning at 60 years of age (1). The global population of older adults is increasing rapidly, with the number of people aged 60 years and older projected to reach approximately 2.1 billion by 2050 (2). With advancing age, multiple biological processes contribute to the progressive decline in physiological function and cellular homeostasis, including genomic instability, telomere attrition, cellular senescence, and impaired tissue regeneration (3). These age-related changes

contribute to the development of conditions such as sarcopenia, which is characterized by impaired muscle function and, particularly, reduced muscle strength and muscle quantity or quality (4).

One of the important molecular pathways associated with sarcopenia is the follistatin–myostatin axis. Myostatin is an inhibitory cytokine belonging to the transforming growth factor-beta (TGF- β) superfamily, expressed in skeletal muscle. This cytokine acts as a negative regulator of muscle growth and limits muscle hypertrophy (5). Myostatin binds to activin IIB, activates muscle-reducing signaling pathways, and, with increasing atrogen expression, suppresses myogenesis-related genes,

leading to muscle atrophy. Myostatin and IGF-1/PI3K/Akt signaling pathways regulate protein synthesis and have interactions; however, the mechanism of these interactions remains unknown (6).

In contrast, follistatin is a glycoprotein that binds strongly to myostatin and inhibits its activity. By reducing myostatin-mediated inhibitory signaling, follistatin may support satellite cell activity and muscle hypertrophy (4). The myostatin-follistatin ratio (F:M) is a significant indicator for assessing body composition and muscle strength following resistance training (7). At the molecular level, physical activity regulates myostatin and follistatin expression. Physically active individuals have higher basal follistatin and lower myostatin levels than inactive individuals, which may contribute to better muscle growth and maintenance (8).

Resistance training is an effective non-pharmacological approach for preventing and managing sarcopenia, as it enhances muscle mass, strength, physical function, balance, and quality of life while reducing fall risk in older adults (9). However, guidelines from the American College of Sports Medicine indicate that conventional high-intensity resistance training (>70% 1RM) may pose joint discomfort, poor compliance, and an elevated risk of musculoskeletal injury in older populations with compromised functional capacity (10).

In this regard, functional training (FT) is intended to improve the capacity to perform basic activities of daily living, such as stair climbing, stepping over obstacles, and rising from a chair, based on the principle of training specificity. This method typically includes resistance exercises using body weight, elastic bands, or free weights, performed on stable or unstable surfaces to enhance functional movement performance (11).

Blood flow restriction training (BFR) is a novel method that generates high metabolic stress with low mechanical load and releases hormonal stimuli. BFR induces short-term ischemia, lactate accumulation, cellular swelling, activates mTOR signaling pathways, and increases IGF-1, which promotes similar adaptability to (or even greater than) high-intensity exercise and reinforces anabolic responses (12). For instance, Ramadhan and colleagues reported that six weeks of low-intensity resistance training with BFR improved balance performance in elderly women compared

with moderate-intensity resistance training (13). Similarly, Espinosa-Méndez and co-workers (14) concluded that low-intensity strength training (20–50% 1RM) with partial arterial occlusion in the lower limbs improved overall and specific muscle strength in older adults, but did not affect muscle mass or functional task performance. Pazokian and colleagues found that functional training with BFR in elderly men led to an increase in muscle strength, elevated follistatin levels, reduced myostatin levels, and an improvement in the follistatin-to-myostatin (F:M) ratio, demonstrating stronger anabolic effects compared to traditional resistance training (15).

Furthermore, betaine, a compound derived from choline and found in foods such as sugar beets and spinach, has recently gained scientific attention. This molecule, as a methyl donor, can increase IGF-1, MyoD, and mTOR expression, decrease myostatin expression, and enhance anabolic pathways (16, 17). Supplementation with 2.5–6 g/day has been suggested to increase circulating betaine levels and support its physiological functions (17, 18). A review by Machek and colleagues suggested that combining BFR with betaine supplementation may offer a synergistic strategy to improve strength, muscle hypertrophy, and functional performance, particularly in older individuals with sarcopenia (18).

Although low-intensity BFR training has gained considerable attention in recent years, the existing literature has predominantly evaluated traditional resistance training modalities, whereas investigations combining BFR with functional movement patterns remain scarce (8, 19). Furthermore, evidence on the synergistic effects of betaine supplementation combined with BFR functional training is limited, particularly among older women. Preliminary evidence suggests that betaine supplementation exerts positive effects on muscle anabolic pathways (20). However, few studies have examined the combined effects of these interventions on molecular biomarkers associated with muscle growth and preservation. Therefore, this study protocol was designed to investigate the effects of eight weeks of functional training combined with BFR and betaine supplementation on serum follistatin and myostatin levels in women aged 60 years and older. The protocol specifically focuses on follistatin and myostatin as key regulatory biomarkers of muscle mass homeostasis, providing novel insight into a safe, feasible, and scalable strategy for mitigating

sarcopenia risk in older women.

2. Method

2.1. Design

This study is designed as a randomized, placebo-controlled, double-blind clinical trial with a pretest–posttest parallel-group design recruiting 56 older women from Tehran, Iran. Participants will be randomly allocated to one of four intervention groups (1:1:1:1):

FBFR+B group: Functional training with blood flow restriction (FBFR) plus betaine supplementation (4 g/day)

FBFR+P group: Functional training with blood flow restriction (FBFR) plus placebo (4 g/day maltodextrin)

B group: Betaine supplementation alone (4 g/day) without exercise training

CON group: Control group maintaining habitual daily activities

The study protocol was developed in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013 guidelines, as outlined in Figure 1 and Supporting Information (21). The intervention details are structured following the TIDieR checklist (22).

Timepoint	Trial Period				
	Enrollment	Allocation	Baseline	After 8 Weeks	
	-t ₁	0	Pr ₁	Po ₁	Po ₂
Enrollment:					
Eligibility Screen	X				
Informed Consent	X				
Socio-demographic and Clinical Profile	X				
Randomization		X			
Allocation		X			
Intervention/ Comparator:					
FBFR+B		X			X
FBFR+P		X			X
B		X			X
CON		X			X
Assessments:					
Food Frequency Questionnaire (FFQ)	X				
Health questionnaire	X				
BMI (Body Mass Index)	X				X
Plasma Follistatin and Myostatin	X				X
Muscle Strength (Bench Press, Leg Press)	X				X
Functional Performance (Sit-To-Stand, TUG)	X				X
Quality of Life (WHOQOL-BREF)	X			X	
Pain Intensity (MPQ)	X			X	

Figure 1: The figure shows the SPIRIT schedule of enrollment, interventions, and assessments. FBFR + B: Functional exercises with blood flow restriction (FBFR) and betaine supplementation (B) group; FBFR + P: Functional exercises with blood flow restriction (FBFR) and placebo (P) group; B: Betaine supplementation (B) group; CON: Control group; -t₁: Pre pre-allocation time point; Pr₁: Pre-test; Po₁: Post-test; Po₂: Post-test (48 h After 8 Weeks); TUG: Timed up-and-go test; 10mWT: Ten-Meter Walk Test; WHOQOL-BREF: World Health Organization Quality of Life Brief; MPQ: McGill Pain Questionnaire

Recruitment began on July 23, 2025, and is anticipated to be completed by October 22, 2025, with overall study completion planned for January 22, 2026.

2.2. Selection and Description of Participants

Community-dwelling older women (≥ 60 years) from Tehran, Iran, will be recruited via advertisements at local senior and sports centers. A study physician will screen all volunteers to confirm eligibility. Inclusion criteria were (1) age ≥ 60 years; (2) no musculoskeletal, joint, or mobility impairments; (3) no fracture within the past 6 months; (4) medical clearance for exercise; (5) no diagnosed cardiovascular, hypertensive, diabetic, neuromuscular, or metabolic disorders; (6) no ongoing dietary supplement or hormone therapy use; (7) no restrictive diets; (8) completed health screening; (9) no other concurrent structured exercise; (10) ability to attend all sessions; and (k) written informed consent. Exclusion criteria included (1) failure to complete baseline testing; (2) ≥ 2 consecutive or ≥ 4 total unexcused absences; (3) withdrawal of consent; (4) non-adherence to the exercise or supplementation protocol; (5) occurrence of any acute medical or psychological condition; or (6) substantial change in health status. Baseline demographic, anthropometric (weight, height, BMI), medical history, and dietary data (via a validated Food Frequency Questionnaire) will be

collected prior to allocation.

2.3. Intervention Protocols

The four study groups will follow distinct protocols: the two exercise groups (FBFR+B and FBFR+P) will perform functional training with blood flow restriction (BFR) three times weekly for eight weeks. The FBFR+B group will consume 4 g/day of betaine, while the FBFR+P group will receive a maltodextrin placebo (4 g/day). The supplementation-only group (B) will ingest 4 g/day of betaine without exercise, and the control group (CON) will maintain their habitual daily activities throughout the study. All exercise sessions will be conducted at the Barman Sports Club, Tehran, Iran supervised by an experienced coach, with a duration of 40–55 minutes per session.

2.3.1. Functional Training Protocol

Participants in the FBFR+B and FBFR+P groups will complete an 11-station functional circuit (Figure 2). The session structure includes a 5-minute warm-up, the 11-station circuit, and a 5-minute cool-down. Exercises include Swiss ball wall squats, dumbbell flies, triceps extensions, BOSU lunges, crunches, squats, shoulder presses, band curls, hyperextensions, seated rows, and alternating bicep curls (14, 15).

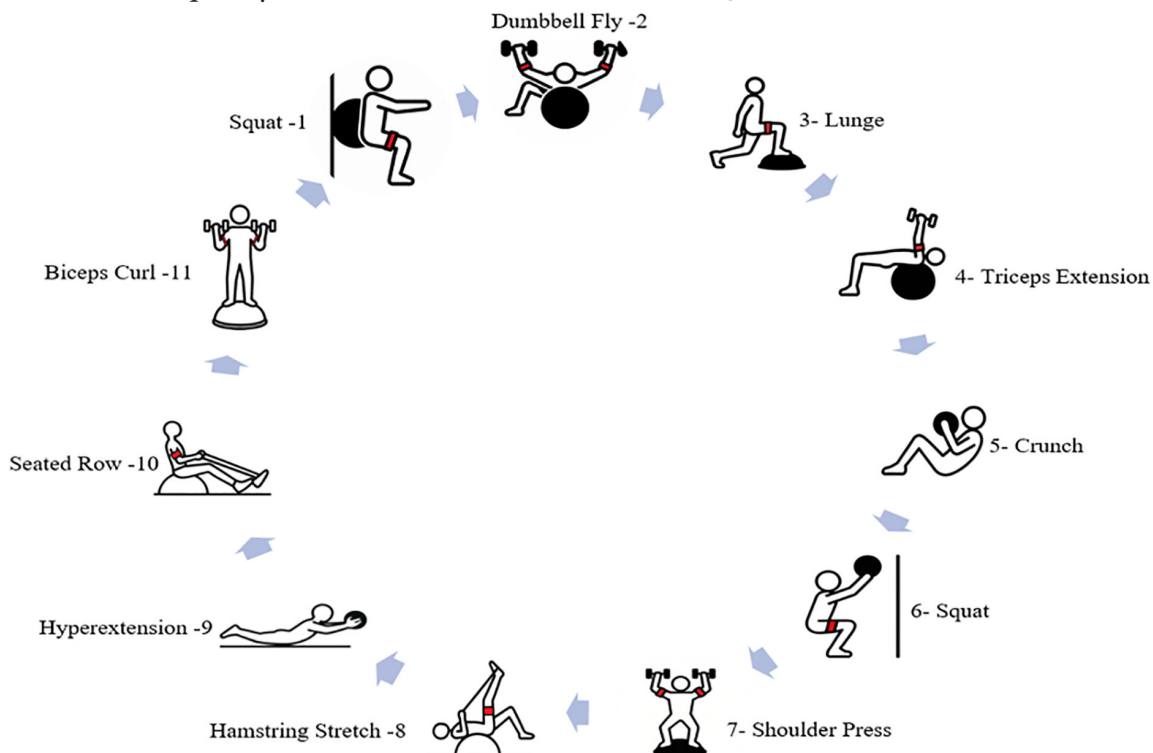


Figure 2: The figure shows the functional circuit training.

Table 1: Functional training with blood flow restriction (BFR) protocol

Timepoints	Intensity				Sets (N)	Repetitions (N)	Rest between Set (min)
	Cuff Pressure (%AOP)	Dumbbells (%1RM)	Medicine Ball (kg)	Power Band (Color)			
Week 1	50%	20%	2	Yellow	2	10	1 min
Week 2	55%	25%					
Week 3	60%	30%	3	Red	3	10	1 min
Week 4	65%	35%					
Week 5	70%	40%	4	Green	4	10	1 min
Week 6	75%	45%					
Week 7	80%	50%	5	Blue	4	10	1 min
Week 8	85%	55%					

Training load prescription is detailed in Table 1. Intensity for stations 1–5 and 7 will be prescribed as a percentage of one-repetition maximum (%1RM), while stations 8 and 9 (resistance bands) and stations 6, 10, and 11 (medicine ball) will be based on specific resistance increments. Training intensity will commence at approximately 20% of 1RM, with a 5% weekly progression. 1RM will be estimated using the Brzycki equation (23):

$$1RM = \frac{\text{Weight(kg)}}{(\text{Reps} \times 0.0278) - 1.0278}$$

2.3.2. Blood Flow Restriction (BFR) Protocol

Partial BFR will be applied using 5-cm-wide pneumatic cuffs (Ghamat Pouyan Co., Iran) placed on the proximal region of both upper arms and thighs. Cuffs will remain inflated during exercise sets and deflated during rest periods to facilitate tissue reperfusion. To determine the required arterial occlusion pressure (AOP), arm and thigh circumferences will be measured using a standard measuring tape, and both systolic blood pressure (SBP) and diastolic blood pressure (DBP) will be assessed using a digital sphygmomanometer. These variables will then be applied to validated prediction equations (15, 24) to calculate the target cuff pressure:

Upper body AOP (mmHg)=

$$(1.461 \times \text{arm circumference}) + (0.514 \times \text{systolic BP}) + (0.339 \times \text{diastolic BP}) + 17.236$$

Lower body AOP (mmHg)=

$$(5.893 \times \text{thigh circumference}) + (0.912 \times \text{systolic BP}) + (0.734 \times \text{diastolic BP}) - 220.046$$

Cuff pressure will be set at 50% of AOP during

the first two weeks (Table 1), increasing by 5% weekly to reach 85% by week eight (14, 15, 25).

2.3.3. Betaine Supplementation

Participants in the FBFR+B and B groups will consume 4 g of anhydrous betaine (Bulk Supplements Inc., USA) daily, divided into two 2 g doses taken 30 minutes after breakfast and dinner. The FBFR+P group will receive a matching daily dose of maltodextrin (4 g/day) (16, 17, 18). To ensure double-blinding, both the betaine and maltodextrin powders will be distributed in identical, coded sachets, and participants in all intervention groups will be instructed to dissolve each dose in 125 mL of warm water and consume it immediately. Compliance will be monitored via weekly collection of empty sachets and daily follow-up calls.

2.4. Outcomes

All outcomes will be assessed at baseline and 48 hours following the 8-week intervention.

2.4.1. Primary Outcomes

For the measurement of follistatin and myostatin, 5 mL of venous blood will be collected from the antecubital vein by a trained laboratory technician. Blood samples will be collected after an overnight fast of 8–10 hours. Samples will be drawn into 5-mL EDTA tubes and centrifuged at $2000 \times g$ for 10 minutes at 4°C to separate the plasma. The plasma will then be divided into aliquots and stored at –80°C until analysis. Plasma concentrations of follistatin (Catalog No. CSB-E13516h, Cusabio, USA) and myostatin (Catalog No. CSB-E11300h, Cusabio, USA) will be measured in duplicate using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions.

2.4.2. Secondary Outcomes

2.4.2.1. Muscle Strength

Upper- and lower-body dynamic strength will be estimated as a 1-repetition maximum (1-RM) based on submaximal performance, following the National Strength and Conditioning Association (NSCA) guidelines, for the bench press and leg press, respectively. To ensure consistent and maximal effort throughout the test, standardized verbal encouragement will be provided by the lead assessor.

2.4.2.2. Functional Performance

Functional mobility will be assessed using the Timed Up and Go (TUG) test. Participants will stand up from a standard chair, walk 3 meters at a comfortable pace, turn around, walk back, and sit down again. Time will be measured from the verbal command “go” until the participant’s back contacts the chair backrest. The test will be performed three times, and the mean of the three trials will be used for analysis, with a time of ≤ 12 seconds representing the cut-off value for independent mobility (26).

Gait performance will be assessed using the Ten-Meter Walk Test (10-mWT). Participants will be instructed to walk at their usual, self-selected comfortable pace along a 14-meter walkway. The additional distance at the beginning and end of the walkway will allow participants to reach and maintain a steady walking speed before entering the measured section, and only the time required to walk the central 10 meters will be recorded. Based on walking speed, participants will be classified as slow walkers (<0.7 m/s), moderate walkers (0.7 – 1.0 m/s), or fast walkers (>1.1 m/s) (27).

2.4.2.3. Quality of Life

Quality of life will be evaluated using the Persian version of the World Health Organization Quality of Life–Brief questionnaire (WHOQOL-BREF). This 26-item instrument assesses four domains, including physical health, psychological health, social relationships, and environment, as well as two items addressing overall quality of life and general health. Items are rated on a 5-point Likert scale. Domain scores will be transformed to a 0–100 scale, with higher scores

indicating better quality of life. The Persian version has demonstrated satisfactory validity and test–retest reliability in Iranian populations (ICC: 0.75–0.84) (28).

2.4.2.4. Pain

Pain will be evaluated using the validated Persian version of the McGill Pain Questionnaire (MPQ). The questionnaire includes the Pain Rating Index (PRI), comprising 78 descriptors categorized into sensory, affective, evaluative, and miscellaneous dimensions; the Present Pain Intensity (PPI), assessed using a 0–5 verbal rating scale; and a body diagram for pain localization. The PRI score and the Number of Words Chosen (NWC) will be analyzed. Higher PRI scores will indicate greater pain intensity, whereas higher NWC values will reflect a broader qualitative pain experience. The Persian version has demonstrated satisfactory psychometric properties (Cronbach’s $\alpha \approx 0.85$; test–retest $r \approx 0.97$) (29, 30).

2.5. Sample Size Calculation

Sample size calculation was performed a priori using G*Power software (version 3.1.9.6). A power analysis was conducted based on a one-way ANOVA (fixed effects, omnibus) with a significance level of 0.05 and a statistical power of 80%. To ensure a rigorous estimation, a conservative effect size ($f=0.25$) was adopted, rather than the larger effect sizes reported in previous literature, to avoid the overestimation of intervention efficacy (15). Based on these parameters, the analysis indicated a requirement of 12 participants per group. Considering an anticipated attrition rate of 15%, we will recruit 14 participants per group, resulting in a total sample size of 56.

2.6. Randomization

To ensure adequate randomization, participants will be assigned to one of the four study groups using a simple random allocation method with an equal ratio (1:1:1:1), generated through a web-based randomization tool. The random sequence will be prepared by an independent researcher who will not be involved in participant recruitment, intervention delivery, or outcome assessment. Allocation concealment will be maintained using sequentially numbered, opaque, sealed envelopes to prevent the prediction of group assignment.

After the completion of baseline assessments and verification of eligibility criteria, the next envelope in the sequence will be opened by the study coordinator to reveal the participant's allocated group.

2.7. Blinding

A double-blind protocol will be maintained for supplementation, using identical betaine and placebo units. An independent researcher will oversee supplement coding and distribution, with unblinding occurring only after the completion of statistical analysis. To mitigate detection bias, outcome assessors and laboratory personnel will remain masked to participant group allocation for the duration of the study.

2.8. Data Analysis

Statistical analyses will be conducted using SPSS version 25.0, and results will be reported as mean $\pm$ standard deviation. The Shapiro–Wilk test will be used to assess data normality, and Levene's test will be used to verify the homogeneity of variance. To ensure the analysis of covariance (ANCOVA) is appropriate, the assumption of homogeneity of regression slopes will also be examined. Pre-test values will be included as covariates in an ANCOVA to examine between-group differences adjusted for baseline values. Bonferroni-adjusted post hoc tests will be used for pairwise comparisons where there are significant main effects. Paired-sample t-tests will be used to evaluate within-group pre-to-post changes.

2.9. Safety Monitoring and Adherence

To ensure participant safety and protocol adherence, all exercise sessions will be directly supervised by trained exercise physiologists. Adherence will be promoted through individualized support and ongoing participant feedback regarding exercise volume and intensity. Any necessary program adjustments based on participant tolerance or feedback will be reviewed by the research team. Concomitant care will be monitored, and participants will be required to refrain from consuming prohibited medications or non-prescribed dietary supplements throughout the study. Safety protocols will include weekly check-ins to monitor clinical status. All participants retain the right to withdraw from the study at any

time without penalty or impact on their ongoing medical care.

2.10. Harms and Adverse Event Reporting

Participants will be monitored continuously during exercise sessions for any adverse reactions related to blood flow restriction (e.g., excessive petechiae, numbness, or localized pain beyond expected discomfort) or betaine supplementation (e.g., gastrointestinal symptoms such as nausea or diarrhea), as well as excessive exercise-induced muscle soreness. Participants will be instructed to immediately report any discomfort or adverse symptoms to the supervising exercise physiologist or the principal investigator. All adverse events, regardless of severity, will be documented in a dedicated adverse event logbook. In the event of an adverse occurrence or clinical deterioration, the study physician will conduct a medical evaluation and determine whether to modify, temporarily interrupt, or permanently discontinue the intervention. Any serious adverse events (SAEs) will be reported to the local Ethics Committee within 24–48 hours in accordance with institutional regulatory requirements.

2.11. Data Monitoring

Given the single-center, short-term nature of this 8-week intervention (low-risk study), a formal, independent Data Monitoring Committee (DMC) was deemed unnecessary. Data integrity and quality control will be managed directly by the principal investigator and the research team. This includes regular audits of raw data against case report forms (CRFs) to ensure accuracy. Interim analyses are not planned; however, the research team will continuously review adherence and data quality throughout the intervention period to ensure protocol compliance.

2.12. Post-Trial Care and Dissemination

Following the trial, participants will continue to receive necessary routine clinical care, and the study physician will remain available to address any post-trial health concerns related to the study procedures. Study findings will be disseminated through publication in peer-reviewed scientific journals and presentations at relevant academic conferences.

3. Discussion

This study protocol outlines a randomized controlled trial designed to investigate the effects of eight weeks of functional training combined with blood flow restriction (BFR) and betaine supplementation on serum follistatin and myostatin levels, as well as functional outcomes, in older women. Aging and the postmenopausal state are characterized by dynapenia, progressive neuromuscular decline, and blunted anabolic responsiveness. We hypothesize that this multi-modal intervention will favorably modulate follistatin and myostatin regulatory signaling, thereby promoting clinically meaningful adaptations in muscular strength and functional performance, while concurrently attenuating pain and enhancing health-related quality of life in this older cohort.

From a mechanistic perspective, the targeted physiological adaptations are postulated to stem from a complementary triad, including low-load vascular occlusion, multi-planar closed-kinetic-chain demands, and the intracellular bioavailability of methyl donors. Low-load blood flow restriction elicits cyclic venous outflow occlusion paired with partial arterial inflow restriction, establishing an acute, localized hypoxic and ischemic intramuscular microenvironment. This deliberate metabolic perturbation—marked by intramuscular accumulation of lactate, hydrogen ions, and inorganic phosphate—is hypothesized to accelerate the preferential recruitment of high-threshold, type II (glycolytic) motor units in accordance with Henneman's size principle (12, 13, 15). Consequently, this altered intramuscular milieu may activate the downstream mechanistic target of rapamycin complex 1 (mTORC1) signaling cascade and stimulate satellite cell myogenic progression, mediated in part through autocrine/paracrine growth factor release and acute cellular swelling (12, 14).

Concurrently, betaine (trimethylglycine) serves as an essential organic osmolyte and a vital methyl group donor within hepatic and intramuscular transmethylation pathways. By facilitating S-adenosylmethionine (SAM) resynthesis, betaine supplementation may alleviate cellular oxidative stress, protect macromolecular integrity against osmotic perturbations, and potentially modulate anabolic gene expression (16-18). At the

myokine regulatory level, betaine is hypothesized to potentiate Akt phosphorylation, which may attenuate downstream Smad2/3 signaling—the central intracellular effector within the catabolic TGF- β /myostatin pathway—while facilitating the upregulation of follistatin expression. Accordingly, combining the metabolic challenge of BFR with betaine-mediated biochemical priming is expected to favorably modulate serum follistatin and myostatin levels, thereby counteracting the anabolic resistance characteristic of aging and postmenopausal physiology (7, 8, 15).

Crucially, the multi-joint, closed-kinetic-chain functional paradigm is hypothesized to translate these adaptations into task-specific neuromuscular improvements. The combination of transient vascular occlusion with functional exercise may enhance neuromuscular function and dynamic strength, which are expected to contribute to improved functional mobility and walking performance in older adults, as reflected by TUG and 10MWT outcomes (12, 13, 30, 31). Furthermore, improvements in muscular function and joint stability may contribute to reduced musculoskeletal pain and, consequently, better overall quality of life (32, 33).

3.1. Expected Scientific Contributions

From a translational and physiological perspective, this protocol will address important gaps in the evidence regarding combined exercise and nutritional strategies for preserving musculoskeletal health in older women. Although blood flow restriction (BFR) training and betaine supplementation have each been investigated as potential modulators of muscle adaptation, their combined effects on circulating follistatin and myostatin, together with functional outcomes, remain insufficiently characterized in older women. By employing a four-arm randomized design, this study will allow the effects of functional BFR training, betaine supplementation, and their combination to be examined separately, thereby providing a more comprehensive evaluation of their potential independent and combined effects. The concurrent assessment of circulating biomarkers, muscle strength, functional mobility, pain, and health-related quality of life will further help clarify the potential relationship between biological adaptations and clinically relevant functional outcomes in aging clinical and practical

Implications

3.2. Clinical and Practical Implications

From a clinical and translational perspective, this study may provide preliminary evidence on the potential utility of functional BFR training and betaine supplementation as accessible strategies for supporting muscle function and physical performance in older women. If shown to be safe and beneficial, the combined intervention may help inform future exercise and nutritional approaches aimed at maintaining mobility, reducing pain, and preserving quality of life and functional independence in aging populations.

3.3. Limitations

Several limitations should be considered. The recruitment of community-dwelling older women from Tehran may limit generalizability to other populations. The 8-week intervention without long-term follow-up will also limit conclusions regarding the persistence of changes in circulating biomarkers and functional outcomes. In addition, plasma follistatin and myostatin reflect systemic rather than tissue-specific responses, and the absence of muscle biopsies will preclude direct assessment of local molecular adaptations. The fixed betaine dose will prevent evaluation of dose-response relationships, while variations in habitual physical activity and dietary intake may introduce residual confounding despite standardized study procedures.

Nevertheless, the randomized four-arm design, allocation concealment, placebo-controlled supplementation, and standardized intervention and outcome assessment will enhance the methodological rigor of the study and provide preliminary evidence to support future, larger and longer-term trials in older women.

4. Conclusions

This study protocol describes a randomized controlled trial designed to investigate the effects of low-load functional BFR training and betaine supplementation, alone and in combination, on circulating follistatin and myostatin and key functional outcomes in older women. By integrating molecular, functional, pain, and quality-of-life measures, the study will provide preliminary

evidence on exercise–nutrition interactions in aging skeletal muscle and may inform future non-pharmacological strategies for preserving muscle health and functional independence in older women.

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Declaration of AI

The authors acknowledge the use of AI-assisted tool (Grammarly) for editing the language. All content was manually reviewed and edited by the authors to ensure its accuracy and academic integrity; the authors take full responsibility for the final content of the manuscript.

Authors' Contribution

Faezeh Aliakbari Jam: Contributions to the conception and design of the work; acquisition and interpretation of data; software application and visualization; drafting the work. Elham Shakoore: Contributions to the conception and design of the work; formal analysis and interpretation of data; project administration and supervision; drafting the work and reviewing it critically for important intellectual content. Farzaneh Zeynali: Contributions to the conception and design of the work; formal analysis and interpretation of data; reviewing the work critically for important intellectual content. Ghorbangol Ashabi: Contributions to the conception, design, and methodology of the work; acquisition, analysis, and validation of data; drafting the work and reviewing it critically for important intellectual content. Samaneh Seyedi: Contributions to data acquisition, curation, and investigation; methodology implementation and visualization; drafting the work and reviewing it critically for important intellectual content. Katsuhiko Suzuki:

Contributions to formal analysis, methodology, and interpretation of data; reviewing the work critically for important intellectual content. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring that any questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interests: None declared.

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Ethical Approval

The Ethics Committee of Alzahra University, Tehran, Iran approved the protocol with the code of IR.ALZAHRA.REC.1404.010. Also, the protocol was registered in the Iranian Registry of Clinical Trials with the code of IRCT20250418065376N1 (registered on May 15, 2025).

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