New Approaches in Exercise Physiology (NAEP)
Vol. 8, No.16, December 2026
www.nass.atu.ac.ir
DOI: 10.22054/nass.2026.93780.1242

ATU PRESS

Original Research
Received: June 28, 2026 Accepted: July 30, 2026

Creatine Supplementation during Suspension Training Reduces Circulating Myostatin Without Additional Improvements in Physical Function in Inactive Older Men: A Randomized Placebo-Controlled Trial

Armita Beigi — Department of Exercise Physiology, Faculty of Physical Education and Sports Sciences, Allameh Tabataba'i University, Tehran, Iran.

Bakhtyar Tartibian* — Department of Exercise Physiology, Faculty of Physical Education and Sports Sciences, Allameh Tabataba'i University, Tehran, Iran.

Rasoul Eslami — Department of Exercise Physiology, Faculty of Physical Education and Sports Sciences, Allameh Tabataba'i University, Tehran, Iran.

Noushin Azadpour* — Department of Physiotherapy, Ardabil University of Medical Sciences, Ardabil, Iran.

Leila Fasihi — Department of Exercise Physiology, Faculty of Physical Education and Sports Sciences, Allameh Tabataba'i University, Tehran, Iran.

* Corresponding Authors: ba.tartibian@gmail.com; no_azadpoor@yahoo.com

How to Cite: Beigi, A; Tartibian, B; Eslami, R; Azadpour, N & Fasihi, L (2026). Creatine Supplementation during Suspension Training Reduces Circulating Myostatin Without Additional Improvements in Physical Function in Inactive Older Men: A Randomized Placebo-Controlled Trial, *New Approaches in Exercise Physiology*, 8(16), 146-175.
DOI: 10.22054/nass.2026.93780.1242

Abstract

Purpose: Aging is commonly accompanied by progressive declines in skeletal muscle mass, muscle strength, and physical performance, which are associated with functional decline, disability, and reduced quality of life in older adults. The purpose of this study was to Creatine Supplementation during Suspension Training Reduces Circulating Myostatin Without Additional Improvements in Physical Function in Inactive Older Men: A Randomized Placebo-Controlled Trial.

Method: In this randomized, double-blind, placebo-controlled trial, 22 inactive older men aged 65–85 years were assigned to suspension training plus creatine (TRX+Cr; n = 11) or suspension training plus placebo (TRX+Pla; n = 11). Both groups completed a supervised 6-week suspension training program. The TRX+Cr group received creatine monohydrate at 0.3 $g \cdot kg^{-1} \cdot day^{-1}$ throughout the intervention, whereas the TRX+Pla group received placebo. Outcomes included appendicular skeletal muscle mass index (ASMI), serum myostatin, follistatin, insulin-like growth factor-1 (IGF-1), vitamin D, handgrip strength, five-times sit-to-stand (5xSTS), and Short Physical Performance Battery (SPPB). Between-group differences were analyzed using ANCOVA adjusted for baseline values.

Results: Creatine supplementation produced a greater reduction in circulating myostatin compared with placebo ($p < 0.001$). A significant between-group difference was also observed for follistatin ($p = 0.002$); however, this reflected a decrease in the creatine group and should not be interpreted as a favorable anabolic response. ASMI did not differ significantly between groups ($p = 0.051$). No significant between-group differences were observed for IGF-1, vitamin D, handgrip strength, 5xSTS, or SPPB.

Conclusion: Creatine supplementation during 6 weeks of suspension training reduced circulating myostatin in inactive older men, but this biomarker response was not accompanied by statistically significant additional improvements in ASMI, physical function, IGF-1, or vitamin D.

Keywords: Creatine supplementation; suspension training; inactive older men; myostatin; muscle-related biomarkers; physical function.

Introduction

Aging is commonly accompanied by progressive declines in skeletal muscle mass, muscle strength, and physical performance, which are associated with functional decline, disability, and reduced quality of life in older adults (Cruz-Jentoft et al., 2019; Cruz-Jentoft & Sayer, 2019; Landi et al., 2018). At the severe end of this age-related decline, sarcopenia is clinically defined by the European Working Group on Sarcopenia in Older People (EWGSOP2), in which low muscle strength is the primary diagnostic parameter, low muscle quantity confirms the diagnosis, and poor physical performance indicates severity (Cruz-Jentoft et al., 2019; Cruz-Jentoft & Sayer, 2019).

Given the rapid growth of the aging population worldwide and the substantial health consequences of age-related declines in muscle mass and function, identifying effective, accessible, and nonpharmacological strategies to mitigate functional decline in older adults has become a major public health priority (Beaudart, Zaaria, Pasleau, Reginster, & Bruyère, 2017; Cruz-Jentoft et al., 2019; Dent et al., 2018).

Resistance training is widely recognized as a cornerstone intervention for improving muscle strength and physical function in older adults (Liu & Latham, 2009; Peterson, Sen, & Gordon, 2011). However, suspension training should not be considered equivalent to traditional resistance training with free weights or machines. Unlike conventional resistance training, in which external load can be precisely prescribed and progressively increased, TRX suspension training relies primarily on body mass, body angle, leverage, instability, and exercise complexity to manipulate training intensity (Byrne et al., 2014; Mok et al., 2015; Snarr & Esco, 2013). Therefore, although TRX may provide a lower degree of precision for applying heavy mechanical loading, it offers several practical advantages for inactive older adults, including low equipment requirements, scalability, supervised safety, and the ability to adjust exercise difficulty according to individual tolerance (Gaedtke & Morat, 2015; Pierle, McDaniel, Schroeder, Heijnen, & Tseh, 2022). These characteristics justify the use of TRX as a feasible

functional resistance-training modality in older populations, while acknowledging that its loading characteristics differ from traditional machine- or free-weight-based resistance training.

Moreover, randomized trials in older men suggest that suspension training may elicit favorable changes in body composition and handgrip strength compared with non-exercise controls (Campa et al., 2021). More broadly, structured exercise programs in older adults have been shown to improve balance and muscle strength, with supervised training often producing greater gains than unsupervised approaches (Lacroix, Hortobagyi, Beurskens, & Granacher, 2017; Thomas et al., 2019).

Although suspension-based training has been investigated in older adults (Campa et al., 2021; Pierle et al., 2022) and clinically vulnerable older populations (Rezaei, Eslami, & Tartibian, 2024), evidence remains limited regarding its combination with creatine supplementation in physically inactive older men, particularly when muscle-related biomarkers, body composition, and functional performance are evaluated concurrently.

In addition to exercise, nutritional interventions play a critical role in supporting muscle health and physical function in older adults (Dent et al., 2018). Creatine monohydrate is among the most extensively studied ergogenic supplements (Candow, Chilibeck, & Forbes, 2014; Candow et al., 2019; Devries & Phillips, 2014) and has demonstrated beneficial effects on muscle mass, strength, and functional performance in older adults (Chilibeck, Kaviani, Candow, & Zello, 2017), particularly when combined with resistance training.

Creatine supplementation increases intramuscular phosphocreatine availability, supports adenosine triphosphate resynthesis during high-intensity contractions, and may augment training-induced neuromuscular and hypertrophic adaptations (Candow et al., 2014; Candow et al., 2019).

Despite substantial evidence supporting the efficacy of creatine in conventional resistance training settings, limited research has examined

its role when combined with functional or suspension-based training modalities in older populations. At the molecular level, muscle mass regulation is strongly influenced by the balance between anabolic and catabolic signaling pathways (Hughes, Ellefsen, & Baar; Trendelenburg et al., 2009). Myostatin, a member of the transforming growth factor-β family, is a potent negative regulator of skeletal muscle growth (Han & Mitch, 2011; McPherron, Lawler, & Lee, 1997), whereas follistatin acts as an endogenous antagonist of myostatin and promotes muscle hypertrophy (Han & Mitch, 2011). Age-related alterations in these myokines may contribute to impaired muscle remodeling and functional decline in older adults.

Vitamin D was also included as an exploratory circulating outcome because vitamin D status is closely related to musculoskeletal health in older adults and may influence muscle function, weakness, and sarcopenia-related risk. In the present trial, vitamin D was not considered a direct mechanistic target of creatine supplementation; rather, it was assessed to characterize whether the combined intervention was accompanied by changes in a clinically relevant muscle-health-related biomarker in inactive older men (Girgis, Clifton-Bligh, Hamrick, Holick, & Gunton, 2013).

Resistance training can modulate myostatin (Walker, Kambadur, Sharma, & Smith, 2004) and increase circulating follistatin in older adults (Mafi, Biglari, Ghardashi Afousi, & Gaeini, 2019). Creatine supplementation may further influence pathways linked to training adaptations; however, evidence in older adults—particularly in response to functional training interventions—remains limited (Candow et al., 2014; Candow et al., 2019; Chilibeck et al., 2017; Devries & Phillips, 2014). Collectively, these considerations highlight the need to evaluate practical training modalities alongside nutritional strategies and to examine both functional outcomes and relevant biomarkers in inactive older adults.

Therefore, the purpose of this study was to examine the effects of creatine supplementation combined with a 6-week suspension training

program on serum muscle-related biomarkers, body composition, and physical function in inactive older men.

We hypothesized that creatine supplementation would produce more favorable changes in selected muscle-related biomarkers, particularly myostatin, and might support training-related adaptations in body composition and physical performance compared with suspension training plus placebo.

Methods

Study Design

This randomized, double-blind, placebo-controlled, parallel-group study examined the effects of a 6-week suspension training program combined with creatine supplementation on serum muscle-related biomarkers, body composition, and physical function in physically inactive older men. Assessments were conducted 48 hours before the first training session and 48 hours after the final training session. The main outcome of interest was circulating myostatin concentration. Secondary outcomes included follistatin, insulin-like growth factor-1 (IGF-1), vitamin D, appendicular skeletal muscle mass index (ASMI), handgrip strength, five-times sit-to-stand (5xSTS), and Short Physical Performance Battery (SPPB) score. The protocol was approved by the Ethics Committee of Allameh Tabataba'i University (IR.ATU.REC.2506-1339), and all participants provided written informed consent. The trial was not prospectively registered; therefore, the findings should be interpreted as exploratory.

Participants and Recruitment

Community-dwelling older men from Isfahan, Iran, were recruited through public advertisements. Eligible participants were men aged 65–85 years who were physically inactive, defined as no participation in

structured exercise training during the previous 12 months, and were able to safely participate in suspension-based resistance training.

Eligibility Criteria

Participants were eligible for inclusion if they met all of the following criteria: (a) men aged 65–85 years; (b) physically inactive status, defined as no structured exercise training during the previous 12 months; (c) ability to safely perform suspension-based resistance exercises; and (d) willingness to comply with all study procedures. Additional eligibility requirements included the absence of clinically diagnosed cardiovascular, respiratory, metabolic, renal, or hematological diseases; no uncontrolled hypertension; non-smoking status; a regular sleep–wake rhythm; and no use of nutritional supplements or medications known to affect muscle metabolism or physical performance during the study period.

Exclusion Criteria

Participants were excluded if they met any of the following criteria: (a) presence of, or development during the study of, cardiovascular, pulmonary, metabolic, or neurological disease; (b) initiation of medications or dietary supplements that could influence study outcomes; (c) history of major surgery within the previous 2 years; (d) musculoskeletal injury or disorder limiting safe participation in exercise; (e) adherence to a special or therapeutic diet or alcohol consumption during the intervention; (f) nonadherence to the exercise training protocol; (g) failure to attend blood sampling or functional testing sessions; or (h) occurrence of any adverse event requiring withdrawal from the study.

Sample Size

The sample size was estimated using G*Power software based on an expected large effect derived from previous resistance-training and creatine-supplementation research in older adults (Devries & Phillips, 2014). With an assumed effect size of 0.65, $\alpha = 0.05$, and statistical power of 0.95, a total sample size of 22 participants was estimated to be sufficient. Accordingly, 22 participants were enrolled and randomly allocated equally to the TRX+Cr and TRX+Pla groups. Given the small sample size and the absence of adjustment for multiple comparisons, the findings should be interpreted as exploratory and hypothesis-generating.

Randomization and Blinding

After baseline testing, participants were randomly allocated in a 1:1 ratio to the TRX plus creatine group (TRX+Cr) or a TRX plus placebo group (TRX+Pla) using a computer-generated random sequence prepared by an independent researcher who was not involved in outcome assessment. Allocation was concealed using sealed, opaque envelopes. Supplement allocation was double-blinded. Participants and outcome assessors were unaware of whether creatine or placebo was provided. All participants were aware that they were participating in the same supervised suspension training program. Creatine and placebo were provided in identical sachets with similar appearance, and supplement codes were not disclosed until completion of data collection and analysis.

Intervention

TRX Suspension Training

The TRX suspension training protocol was designed according to established principles of suspension-based resistance exercise, with

progression achieved by adjusting body position and exercise complexity (Byrne et al., 2014; Gaedtke & Morat, 2015).

Both groups completed a 6-week TRX program (3 sessions per week on nonconsecutive days), with sessions scheduled between 09:00 and 10:30. Each session included a 10-minute warm-up, approximately 40 minutes of TRX exercises, and a 5-minute cool-down.

Exercises included functional multi-joint movements, including squat, suspended lunge, chest press, hamstring curl, and row (Byrne et al., 2014; Mok et al., 2015). Participants performed two sets per exercise, with 2 minutes of rest between sets (Table 2).

Training intensity was monitored using the Borg rating of perceived exertion (RPE), targeting a range of 12–15, which corresponds to moderate-to-vigorous exercise intensity (Borg, 1998). Progression was implemented every 2 weeks based on individual tolerance and correct technique by increasing repetitions and/or adjusting body position/leverage, including the working angle and distance relative to the TRX anchor point.

Participants completed two familiarization sessions prior to the intervention. To standardize progression, each participant's stance/position relative to the TRX anchor point was marked and recorded for each exercise, and position was gradually adjusted over time to increase difficulty, consistent with established TRX training control principles (Gaedtke & Morat, 2015). All sessions were supervised, and participants were monitored for safety and any adverse symptoms during training.

Creatine and Placebo Supplementation

Participants in the TRX+Cr group consumed creatine monohydrate (Bulk Supplements, USA) at a dose of 0.3 $g \cdot kg^{-1} \cdot day^{-1}$ throughout the 6-week intervention period. For an average participant of approximately 72 kg, this corresponded to approximately 21–22 g per day. The supplement was ingested once daily at lunchtime and mixed with water. This weight-based dosing strategy was selected to provide

a consistent relative creatine dose across participants and was informed by previous creatine supplementation research in older adults undergoing resistance training (Candow et al., 2019; Pinto, Botelho, Carneiro, & Mota, 2016). No separate loading and maintenance phases were used. Participants in the TRX+Pla group consumed maltodextrin as the placebo, provided in identical sachets with similar appearance. Supplement compliance was monitored through regular follow-up and collection of unused sachets. Participants were asked about possible supplement-related symptoms throughout the intervention. No clinically relevant adverse events were reported.

Dietary Monitoring

Dietary intake was monitored using three 24-hour dietary recalls, including two weekdays and one weekend day, with the aid of portion-size guides (Azadpour, Tartibian, & Koşar, 2017). Participants were instructed to maintain their habitual diet throughout the intervention and not to initiate any new nutritional supplements or major dietary changes during the study period. The dietary recalls were reviewed to monitor overall dietary consistency, with particular attention to major changes in total energy and protein intake. However, no individualized dietary prescription was provided, dietary protein and caloric intake were not strictly standardized, and habitual dietary creatine intake was not specifically quantified.

Outcomes

Anthropometrics and Body Composition

Height was measured without shoes using a stadiometer. Body mass and body composition were assessed using bioelectrical impedance analysis (InBody 770, Korea) (Buch et al., 2022). Appendicular skeletal muscle mass index was obtained from device output, and appendicular skeletal muscle mass index (ASMI; kg/m^2) was calculated as

appendicular skeletal muscle mass divided by height squared. Body mass index was calculated as body mass divided by height squared. Body fat percentage and other body composition variables were obtained from the body composition assessment.

Resting Blood Pressure and Heart Rate

Resting systolic and diastolic blood pressure and heart rate were measured after 15 minutes of seated rest using an automated upper-arm monitor (OMRON M2, Omron Healthcare, Kyoto, Japan), in accordance with established clinical guidelines(Carey, Whelton, & Committee*, 2018).

Blood Sampling and Biomarkers

Venous blood samples (5 mL) were collected from the antecubital vein after a 12-hour overnight fast between 08:00 and 09:00 at baseline, 48 hours before the first training session, and post-intervention, 48 hours after the final training session. Samples were centrifuged to obtain serum, and serum aliquots were stored at −80°C until analysis. Serum concentrations of myostatin, follistatin, IGF-1, and vitamin D were determined using commercially available ELISA kits (ZellBio, Germany) according to the manufacturer's instructions. Intra-assay and inter-assay coefficients of variation were <5% and <8%, respectively, as reported in the kit documentation.

Physical Function Tests

To assess physical function, the following standardized tests were performed:
Handgrip strength was measured using a Lafayette hand dynamometer. Participants were seated with the shoulder adducted, elbow flexed at 90°, forearm in a neutral position, and wrist in 0–30° extension. Three maximal trials were performed for each hand with 20 seconds of rest

between trials; standardized verbal encouragement was provided, and the highest value (kg) across all trials was recorded (Mehmet, Yang, & Robinson, 2020).

Five-times sit-to-stand (5xSTS) performance was assessed using a standard chair (~43 cm) placed against a wall for stability. Participants sat with arms crossed over the chest and were instructed to stand up fully and sit down five times as fast as possible; the time to complete the test (seconds) was recorded (Whitney et al., 2005).

The Short Physical Performance Battery (SPPB) included standing balance (side-by-side, semi-tandem, and tandem; each held for up to 10 seconds), 4-m gait speed assessed at habitual pace, and a chair stand test. Each component was scored from 0 to 4, yielding a total score ranging from 0 to 12 (Guralnik, Ferrucci, Simonsick, Salive, & Wallace, 1995).

Compliance and Safety Monitoring

Training attendance was recorded at each session by the supervising staff, and missed sessions or protocol deviations were documented. Supplement compliance was monitored through regular follow-up and collection of unused supplement sachets. Participants were reminded to maintain their usual lifestyle and to report any discomfort, symptoms, injuries, or supplement-related complaints during the intervention. At each training session, participants were asked about possible adverse events, and any events were documented by the supervising staff. Blood sampling was performed by trained personnel under standard safety procedures. No clinically relevant adverse events requiring withdrawal from the study were reported.

Statistical Analysis

Statistical analyses were performed using SPSS software version 27.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation (SD). The normality of data distribution was

assessed using the Shapiro–Wilk test, and homogeneity of variances was examined using Levene's test. Baseline differences between the TRX+Cr and TRX+Pla groups were assessed using independent-samples t-tests. To compare post-intervention outcomes between groups, analysis of covariance (ANCOVA) was performed with the post-intervention value as the dependent variable, group as the fixed factor, and the corresponding baseline value as the covariate. Before interpretation, ANCOVA assumptions were examined, including normality of residuals, homogeneity of variances, and homogeneity of regression slopes. Partial eta squared (ηp^2) was reported as the effect size for ANCOVA models. The level of statistical significance was set at $p < 0.05$. Because this was a small exploratory trial with multiple secondary outcomes, no formal adjustment for multiple comparisons was applied; therefore, p-values should be interpreted cautiously.

Table 1. Baseline anthropometric and physiological characteristics of participants

Variable	TRX + Creatine (n = 11) Mean ± SD	TRX + Placebo (n = 11) Mean ± SD	P value
Age (years)	68.73 ± 1.68	68.91 ± 1.45	0.788
Height (cm)	170.09 ± 1.92	170.00 ± 1.41	0.901
Body mass (kg)	72.64 ± 2.38	72.00 ± 1.41	0.454
Diastolic blood pressure (mmHg)	82.45 ± 1.51	82.55 ± 1.04	0.871
Systolic blood pressure (mmHg)	135.45 ± 1.51	135.55 ± 1.04	0.871
Body mass index (kg/m^2)	25.05 ± 0.22	25.01 ± 0.14	0.648
Waist-to-hip ratio	0.936 ± 0.009	0.941 ± 0.008	0.257

Body fat (%)	26.39 ± 0.38	26.61 ± 0.25	0.126
Resting heart rate (beats/min)	72.27 ± 1.27	72.73 ± 1.01	0.364
ASMI (kg/m²)	7.78 ± 0.10	7.80 ± 0.08	0.635

Values are presented as mean ± SD. Between-group comparisons were performed using independent-samples t-tests.
TRX = suspension training; ASMI = appendicular skeletal muscle mass index.

Table 2. TRX suspension training protocol (6 weeks)

Exercise Type	**Weeks 1–2 (RPE; sets×reps / angle)**	**Weeks 3–4 (RPE; sets×reps / angle)**	**Weeks 5–6 (RPE; sets×reps / angle)**	**Rest between sets**
Squat	12–13; 2×10 / 30°	13–14; 2×10 / 30°	14–15; 2×12 / 50°	2 min
Suspended lunge	12–13; 2×10 / 30°	12–13; 2×10 / 30°	12–13; 2×12 / 50°	2 min
Chest press	12–13; 2×10 / 30°	12–13; 2×10 / 30°	12–13; 2×12 / 50°	2 min
Hamstring curl	12–13; 2×10 / 30°	12–13; 2×10 / 30°	12–13; 2×12 / 50°	2 min
Row	12–13; 2×10 / 30°	12–13; 2×10 / 30°	12–13; 2×12 / 50°	2 min

Angle (°) reflects body lean relative to the anchor point (leverage). Intensity was monitored using Borg RPE (6–20), targeting 12–15.
Progression was applied every 2 weeks by increasing reps/angle and advancing exercise variations. Warm-up: 10 min; Cool-down: 5 min;
Rest: 2 min

Table 3. Baseline and post-intervention body composition, serum biomarkers, and physical function outcomes after 6 weeks of suspension training combined with creatine supplementation or placebo

Outcome	TRX+Cr Pre (n=11)	TRX+Cr Post (n=11)	TRX+Pla Pre (n=11)	TRX+Pla Post (n=11)	Between-group p (ANCOVA) †	Partial η_p^2
ASMI (kg/m²)	7.78 ± 0.10	8.05 ± 0.36	7.80 ± 0.08	7.80 ± 0.08	0.051	0.186
Myostatin (ng/mL)	61.56 ± 8.53	55.86 ± 2.84	61.27 ± 3.81	60.89 ± 2.78	<0.001	0.604
Follistatin (ng/mL)	6.30 ± 0.40	5.34 ± 0.24	5.57 ± 0.37	5.79 ± 0.56	0.002	0.396
IGF-1 (ng/mL)	116.81 ± 3.66	118.00 ± 3.71	116.90 ± 3.38	117.90 ± 3.38	0.182	0.097
Vitamin D (ng/mL)	23.73 ± 1.68	24.73 ± 1.68	23.70 ± 1.49	24.70 ± 1.49	0.838	0.002
Handgrip strength (kg)	29.42 ± 0.64	29.62 ± 1.64	28.45 ± 1.04	29.41 ± 1.11	0.956	<0.001
5xSTS time (s)	11.12 ± 0.75	10.39 ± 1.15	9.67 ± 1.28	10.15 ± 1.43	0.157	0.108
SPPB score (0–12)	8.00 ± 1.10	8.00 ± 2.05	8.00 ± 1.25	7.80 ± 1.23	0.793	0.004

Values are presented as mean ± SD. TRX+Cr = suspension training plus creatine; TRX+Pla = suspension training plus placebo; ASMI = appendicular skeletal muscle mass index; IGF-1 = insulin-like growth factor-1; 5xSTS = five-times sit-to-stand; SPPB = Short Physical Performance Battery; η_p^2 = partial eta squared.

† Between-group p-values and partial ηp² values were obtained from ANCOVA models with post-intervention values as the dependent variable, group as the fixed factor, and the corresponding baseline value as the

covariate. Because multiple secondary outcomes were examined in this small exploratory trial, p-values should be interpreted cautiously.

Results

Participant Flow and Baseline Characteristics

Twenty-two older men were randomized and completed the study (TRX+Cr, n = 11; TRX+Pla, n = 11), with no dropouts or clinically relevant adverse events requiring withdrawal. All participants completed the scheduled training sessions, resulting in 100% training attendance in both groups. Supplement compliance was confirmed through regular follow-up and inspection of returned sachets. Baseline anthropometric and physiological characteristics were not significantly different between groups (all $p > 0.05$), indicating general comparability at baseline. Baseline ASMI values were also similar between groups (TRX+Cr: 7.78 ± 0.10 kg/m²; TRX+Pla: 7.80 ± 0.08 kg/m²; $p = 0.635$). However, because numerical baseline differences were observed for some outcome variables, particularly follistatin and 5xSTS, post-intervention between-group comparisons were interpreted using baseline-adjusted ANCOVA.

Body Composition

After adjustment for baseline values using ANCOVA, the between-group difference in ASMI did not reach statistical significance ($p = 0.051$; partial $\eta p^2 = 0.186$). ASMI increased from 7.78 ± 0.10 to 8.05 ± 0.36 kg/m² in the TRX+Cr group, whereas it remained unchanged in the TRX+Pla group (7.80 ± 0.08 to 7.80 ± 0.08 kg/m²). Although the direction of change favored the creatine group, this finding should be interpreted cautiously because the adjusted between-group difference was not statistically significant.

Serum Biomarkers

After adjustment for baseline values, a significant between-group difference was observed for myostatin ($p < 0.001$; partial $\eta p^2 = 0.604$). Myostatin decreased in the TRX+Cr group from 61.56 ± 8.53 to 55.86 ± 2.84 ng/mL, whereas it remained relatively unchanged in the TRX+Pla group from 61.27 ± 3.81 to 60.89 ± 2.78 ng/mL. These findings indicate that creatine supplementation was associated with a greater reduction in circulating myostatin compared with placebo.

A statistically significant between-group difference was also observed for follistatin ($p = 0.002$; partial $\eta p^2 = 0.396$). However, follistatin decreased in the TRX+Cr group from 6.30 ± 0.40 to 5.34 ± 0.24 ng/mL and increased slightly in the TRX+Pla group from 5.57 ± 0.37 to 5.79 ± 0.56 ng/mL. Therefore, although the adjusted between-group difference was statistically significant, the direction of change does not support a favorable anabolic response to creatine supplementation and should be interpreted cautiously.

No significant between-group differences were observed for IGF-1 ($p = 0.182$; partial $\eta p^2 = 0.097$) or vitamin D ($p = 0.838$; partial $\eta p^2 = 0.002$). These findings indicate that creatine supplementation did not significantly alter circulating IGF-1 or vitamin D compared with placebo over the 6-week intervention period.

Physical Function

After adjustment for baseline values, no significant between-group differences were observed for handgrip strength, 5xSTS performance, or SPPB score. Handgrip strength changed from 29.42 ± 0.64 to 29.62 ± 1.64 kg in the TRX+Cr group and from 28.45 ± 1.04 to 29.41 ± 1.11 kg in the TRX+Pla group ($p = 0.956$; partial $\eta p^2 < 0.001$). For 5xSTS, completion time decreased from 11.12 ± 0.75 to 10.39 ± 1.15 seconds in the TRX+Cr group and changed from 9.67 ± 1.28 to 10.15 ± 1.43 seconds in the TRX+Pla group; however, the adjusted between-group

difference was not statistically significant ($p = 0.157$; partial $\eta p^2 = 0.108$). SPPB score remained unchanged in the TRX+Cr group and changed slightly in the TRX+Pla group, with no significant between-group difference ($p = 0.793$; partial $\eta p^2 = 0.004$).

Discussion

The present randomized, double-blind, placebo-controlled trial examined whether creatine supplementation provides additional benefits during a 6-week suspension training program on serum muscle-related biomarkers, body composition, and physical function in inactive older men. The main finding was that creatine supplementation was associated with a greater reduction in circulating myostatin compared with placebo. However, this biomarker response was not accompanied by statistically significant additional improvements in handgrip strength, 5xSTS performance, SPPB score, IGF-1, vitamin D, or ASMI. A significant between-group difference was also observed for follistatin, but the direction of change was not consistent with a favorable anabolic response and should therefore be interpreted cautiously.

Because both groups completed the same supervised suspension training program, the between-group comparisons primarily reflect the added effect of creatine supplementation rather than the overall effect of exercise training. Therefore, the present findings should be interpreted as evidence of a selective biomarker response, particularly for myostatin, rather than as evidence of broad functional or body-composition benefits of creatine during this 6-week intervention.

Creatine supplementation is one of the most extensively investigated nutritional strategies for supporting skeletal muscle function in older adults. Previous reviews and meta-analyses have reported that creatine combined with resistance training can enhance lean tissue mass and muscular strength, particularly when interventions are sufficiently long and training volume is progressive (Candow et al., 2014; Candow et al., 2019; Chilibeck et al., 2017; Devries & Phillips, 2014).

Mechanistically, creatine increases intramuscular phosphocreatine availability and supports adenosine triphosphate resynthesis during repeated high-intensity contractions, which may improve training tolerance and adaptive responses (Candow et al., 2014; Candow et al., 2019; Kreider et al., 2017). However, the absence of significant between-group differences in ASMI and physical function in the present study suggests that a 6-week suspension-training intervention may have been insufficient to translate the observed myostatin reduction into measurable functional or body-composition benefits.

Suspension training is a functional resistance-training modality that uses body mass, leverage, and instability to provide scalable loading and neuromuscular challenge. Previous studies have suggested that suspension-based exercise is feasible in older adults and may improve selected components of fitness and functional performance (Gaedtke & Morat, 2015; Pierle et al., 2022). However, because both groups in the present study completed the same supervised suspension training program, the absence of significant between-group differences in physical function should be interpreted as a lack of additional benefit from creatine supplementation rather than as evidence that suspension training itself was ineffective.

A particularly important finding of the present study was the greater reduction in circulating myostatin in the creatine group compared with placebo. Myostatin is a member of the transforming growth factor-β family and is recognized as a negative regulator of skeletal muscle growth through inhibitory effects on myogenic signaling and protein synthesis pathways (Han & Mitch, 2011; McPherron et al., 1997; Trendelenburg et al., 2009). Previous studies have shown that resistance training may reduce circulating myostatin or modify myostatin-related signaling in older adults and sarcopenic populations (Mafi et al., 2019; Walker et al., 2004). In the present study, the greater reduction in circulating myostatin suggests that creatine supplementation may have influenced a catabolic regulator of muscle adaptation during suspension training. However, because intramuscular signaling pathways, muscle

biopsy markers, and muscle protein synthesis were not directly assessed, this interpretation should be considered hypothesis-generating rather than direct mechanistic evidence.

In contrast to the myostatin response, the follistatin finding should be interpreted with caution. Follistatin is generally considered an endogenous antagonist of myostatin and has been proposed to support muscle hypertrophy by inhibiting myostatin-related signaling (Han & Mitch, 2011; Mafi et al., 2019). Although the adjusted between-group difference was statistically significant in the present study, follistatin decreased in the creatine group and increased slightly in the placebo group. Therefore, this result does not support a favorable anabolic response to creatine supplementation. Possible explanations include the small sample size, baseline imbalance in follistatin values, biological variability, timing of blood sampling, and assay-related variation. Accordingly, the follistatin result should be considered exploratory and should not be interpreted as evidence of enhanced anabolic signaling.

No significant between-group difference was observed for IGF-1. This finding is consistent with evidence suggesting that circulating IGF-1 does not always change in parallel with resistance-training adaptations and may not fully reflect local intramuscular anabolic signaling (McKendry et al., 2020; Phillips, 2014). Similarly, vitamin D did not differ significantly between groups after the intervention. Because vitamin D supplementation was not part of the intervention and baseline vitamin D values were comparable between groups, the absence of a between-group effect was expected. Overall, these findings indicate that creatine supplementation did not provide additional measurable effects on circulating IGF-1 or vitamin D during the 6-week suspension-training program.

Creatine supplementation did not produce statistically significant additional improvements in handgrip strength, 5xSTS performance, or SPPB score compared with placebo. This finding should not be interpreted as evidence that suspension training was ineffective; rather, it indicates that the added effect of creatine over the same supervised

training program was not statistically supported for these functional outcomes in the present sample. Because handgrip strength, chair-stand performance, and SPPB are clinically relevant indicators of physical function and independence in older adults (Bohannon, 2019; Guralnik et al., 1995), future studies with larger samples, longer intervention periods, and more detailed monitoring of training load and dietary intake are needed to determine whether creatine can meaningfully augment functional adaptations to suspension-based training.

From a practical perspective, suspension training is an accessible and scalable resistance-training modality for older adults because it does not require heavy machinery and can be adjusted through body position and leverage (Gaedtke & Morat, 2015; Pierle et al., 2022). Creatine is also inexpensive and widely studied as a nutritional supplement in exercise and aging research (Candow et al., 2019; Kreider et al., 2017). However, under the conditions of this small 6-week trial, adding creatine to suspension training was not associated with statistically significant additional improvements in physical function or ASMI. Therefore, the present findings support further investigation of creatine as a potential biomarker-modulating supplement during suspension training, but they do not provide sufficient evidence to recommend it for additional functional benefits in inactive older men.

Although training attendance and supplement compliance were complete in the present supervised trial, this finding should be interpreted within the context of a small, closely monitored research setting. Participants were carefully screened, all training sessions were supervised, and supplement use was regularly followed through inspection of returned sachets. Therefore, the observed 100% attendance and compliance may not be directly generalizable to routine clinical or community settings. This issue is particularly relevant because the weight-based creatine dose used in the present study corresponded to approximately 21–22 g/day for an average participant, which is relatively high for prolonged use in older adults. Although no clinically relevant adverse events requiring withdrawal were reported,

the feasibility and tolerability of this high-dose protocol should be interpreted cautiously, and future pragmatic studies should evaluate adherence, gastrointestinal tolerance, renal safety markers, and lower or maintenance-dose strategies in real-world settings.

Several limitations should be considered when interpreting these findings. First, the sample size was small, which may have limited statistical power and contributed to numerical baseline imbalance in some outcomes, particularly follistatin and 5xSTS. Second, the intervention lasted only 6 weeks, and a longer training and supplementation period may be required to observe clearer functional or body-composition adaptations in older adults. Third, the trial was not prospectively registered; therefore, the findings should be interpreted as exploratory. Fourth, although no clinically relevant adverse events were reported, renal function biomarkers were not assessed; therefore, safety conclusions regarding the relatively high creatine dose used in this study should be made cautiously. Fifth, body composition was assessed using bioelectrical impedance analysis rather than dual-energy X-ray absorptiometry or imaging-based methods.

Sixth, dietary intake was monitored using 24-hour dietary recalls; however, total energy and protein intake were not strictly standardized, and habitual dietary creatine intake was not specifically quantified. These factors may have influenced body-composition outcomes and individual responsiveness to creatine supplementation.

Finally, multiple secondary outcomes were analyzed without formal adjustment for multiple comparisons, and the study included only inactive older men; therefore, the findings may not be generalizable to women, physically active older adults, or clinically diagnosed sarcopenic populations.

Conclusion

Creatine supplementation during 6 weeks of supervised suspension training reduced circulating myostatin compared with suspension training plus placebo in inactive older men. However, this biomarker response was not accompanied by statistically significant additional benefits in ASMI, handgrip strength, 5xSTS performance, or SPPB score. No additional between-group effects were observed for IGF-1 or vitamin D, and the significant follistatin finding should be interpreted cautiously because the direction of change was not consistent with a favorable anabolic response. Therefore, under the conditions of this small exploratory trial, creatine supplementation combined with suspension training should not be interpreted as providing additional improvements in muscle mass or physical function beyond suspension training plus placebo. Larger, longer-duration, prospectively registered trials with more rigorous dietary control, direct measures of muscle mass, and safety biomarkers are needed before therapeutic claims regarding sarcopenia prevention can be made.

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of Allameh Tabataba'i University ([IR.ATU.REC.2506-1339]). All participants provided written informed consent before participation.

Trial registration:

The trial was not prospectively registered.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests:
The authors declare that they have no competing interests.

Funding:
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions:
All authors contributed to the conception and design of the study, data collection, analysis and interpretation of data, and drafting or revising the manuscript. All authors read and approved the final manuscript.

Acknowledgments:
The authors of this study would like to thank the subjects who diligently and honestly participated in this study.

Conflicts of Interest:
The authors declare no conflict of interest related to this study.

ORCID

Armita Beigi		**https://orcid.org/**
Bakhtyar Tartibian		**https://orcid.org/0000-0002-4653-5620**
Rasoul Eslami		**https://orcid.org/0000-0002-8493-2053**
Noushin Azadpour		**https://orcid.org/0000-0003-4005-6300**
Leila Fasihi		**https://orcid.org/0000-0002-9557-9152**

Reference

Azadpour, N., Tartibian, B., & Koşar, Ş. N. (2017). Effects of aerobic exercise training on ACE and ADRB2 gene expression, plasma angiotensin II level, and flow-mediated dilation: a study on obese postmenopausal women with prehypertension. Menopause, 24(3), 269–277.

Beaudart, C., Zaaria, M., Pasleau, F., Reginster, J.-Y., & Bruyère, O. (2017). Health outcomes of sarcopenia: a systematic review and meta-analysis. PloS one, 12(1), e0169548.

Bohannon, R. W. (2019). Grip Strength: An Indispensable Biomarker For Older Adults. Clin Interv Aging, 14, 1681–1691. doi:10.2147/cia.S194543

Borg, G. (1998). Borg's perceived exertion and pain scales: Human kinetics.

Buch, A., Ben-Yehuda, A., Rouach, V., Maier, A. B., Greenman, Y., Izkhakov, E., . . . Eldor, R. (2022). Validation of a multi-frequency bioelectrical impedance analysis device for the assessment of body composition in older adults with type 2 diabetes. Nutrition & Diabetes, 12(1), 45.

Byrne, J. M., Bishop, N. S., Caines, A. M., Crane, K. A., Feaver, A. M., & Pearcey, G. E. (2014). Effect of using a suspension training system on muscle activation during the performance of a front plank exercise. The Journal of Strength & Conditioning Research, 28(11), 3049–3055.

Campa, F., Schoenfeld, B. J., Marini, E., Stagi, S., Mauro, M., & Toselli, S. (2021). Effects of a 12-Week Suspension versus Traditional Resistance Training Program on Body Composition, Bioimpedance Vector Patterns, and Handgrip Strength in Older Men: A Randomized Controlled Trial. Nutrients, 13(7). doi:10.3390/nu13072267

Candow, D. G., Chilibeck, P. D., & Forbes, S. C. (2014). Creatine supplementation and aging musculoskeletal health. Endocrine, 45(3), 354–361.

Candow, D. G., Forbes, S. C., Chilibeck, P. D., Cornish, S. M., Antonio, J., & Kreider, R. B. (2019). Effectiveness of creatine supplementation on aging muscle and bone: focus on falls prevention and inflammation. Journal of clinical medicine, 8(4), 488.

Carey, R. M., Whelton, P. K., & Committee, A. A. H. G. W. (2018). Prevention, detection, evaluation, and management of high blood pressure in adults: synopsis of the 2017 American College of Cardiology/American Heart Association Hypertension Guideline. Annals of internal medicine, 168(5), 351–358.*

Chilibeck, P. D., Kaviani, M., Candow, D. G., & Zello, G. A. (2017). Effect of creatine supplementation during resistance training on lean tissue mass and muscular strength in older adults: a meta-analysis. Open access journal of sports medicine, 213–226.

Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., . . . Sayer, A. A. (2019). Sarcopenia: revised European consensus on definition and diagnosis. Age and ageing, 48(1), 16–31.

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

Dent, E., Morley, J., Cruz-Jentoft, A., Arai, H., Kritchevsky, S., Guralnik, J., . . . Cesari, M. (2018). International clinical practice guidelines for sarcopenia (ICFSR): screening, diagnosis and management. The Journal of nutrition, health and aging, 22(10), 1148–1161.

Devries, M. C., & Phillips, S. M. (2014). Creatine supplementation during resistance training in older adults—a meta-analysis. Medicine & Science in Sports & Exercise, 46(6), 1194–1203.

Gaedtke, A., & Morat, T. (2015). TRX suspension training: A new functional training approach for older adults–development,

training control and feasibility. International journal of exercise science, 8(3), 224.

Girgis, C. M., Clifton-Bligh, R. J., Hamrick, M. W., Holick, M. F., & Gunton, J. E. (2013). The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocrine reviews, 34(1), 33–83.

Guralnik, J. M., Ferrucci, L., Simonsick, E. M., Salive, M. E., & Wallace, R. B. (1995). Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. New England Journal of Medicine, 332(9), 556–562.

Han, H., & Mitch, W. E. (2011). Targeting the myostatin signaling pathway to treat muscle wasting diseases. Current opinion in supportive and palliative care, 5(4), 334–341.

Hughes, D., Ellefsen, S., & Baar, K. Adaptations to endurance and strength training. Cold Spring Harb Perspect Med. 2018; 8: a029769. In.

Kreider, R. B., Kalman, D. S., Antonio, J., Ziegenfuss, T. N., Wildman, R., Collins, R., . . . Lopez, H. L. (2017). International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. J Int Soc Sports Nutr, 14, 18. doi:10.1186/s12970-017-0173-z

Lacroix, A., Hortobagyi, T., Beurskens, R., & Granacher, U. (2017). Effects of supervised vs. unsupervised training programs on balance and muscle strength in older adults: a systematic review and meta-analysis. Sports medicine, 47(11), 2341–2361.

Landi, F., Calvani, R., Cesari, M., Tosato, M., Martone, A. M., Ortolani, E., . . . Picca, A. (2018). Sarcopenia: an overview on current definitions, diagnosis and treatment. Current Protein and Peptide Science, 19(7), 633–638.

Liu, C. j., & Latham, N. K. (2009). Progressive resistance strength training for improving physical function in older adults. Cochrane database of systematic reviews(3).

Mafi, F., Biglari, S., Ghardashi Afousi, A., & Gaeini, A. A. (2019). Improvement in Skeletal Muscle Strength and Plasma Levels of Follistatin and Myostatin Induced by an 8-Week Resistance Training and Epicatechin Supplementation in Sarcopenic Older Adults. J Aging Phys Act, 27(3), 384–391. doi:10.1123/japa.2017-0389

McKendry, J., Currier, B. S., Lim, C., McLeod, J. C., Thomas, A. C. Q., & Phillips, S. M. (2020). Nutritional Supplements to Support Resistance Exercise in Countering the Sarcopenia of Aging. Nutrients, 12(7). doi:10.3390/nu12072057

McPherron, A. C., Lawler, A. M., & Lee, S.-J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-p superfamily member. Nature, 387(6628), 83–90.

Mehmet, H., Yang, A. W., & Robinson, S. R. (2020). Measurement of hand grip strength in the elderly: A scoping review with recommendations. Journal of bodywork and movement therapies, 24(1), 235–243.

Mok, N. W., Yeung, E. W., Cho, J. C., Hui, S. C., Liu, K. C., & Pang, C. H. (2015). Core muscle activity during suspension exercises. Journal of science and medicine in sport, 18(2), 189–194.

Peterson, M. D., Sen, A., & Gordon, P. M. (2011). Influence of resistance exercise on lean body mass in aging adults: a meta-analysis. Medicine and science in sports and exercise, 43(2), 249.

Phillips, S. M. (2014). A brief review of critical processes in exercise-induced muscular hypertrophy. Sports Med, 44 Suppl 1(Suppl 1), S71–77. doi:10.1007/s40279-014-0152-3

Pierle, C., McDaniel, A. T., Schroeder, L. H., Heijnen, M. J. H., & Tseh, W. (2022). Efficacy of a 6-Week Suspension Training Exercise Program on Fitness Components in Older Adults. Int J Exerc Sci, 15(3), 1168–1178. doi:10.70252/gpeb7735

Pinto, C. L., Botelho, P. B., Carneiro, J. A., & Mota, J. F. (2016). Impact of creatine supplementation in combination with

resistance training on lean mass in the elderly. Journal of cachexia, sarcopenia and muscle, 7(4), 413–421.

Rezaei, S., Eslami, R., & Tartibian, B. (2024). The effects of TRX suspension training on sarcopenic biomarkers and functional abilities in elderlies with sarcopenia: a controlled clinical trial. BMC Sports Sci Med Rehabil, 16(1), 58. doi:10.1186/s13102-024-00849-x

Snarr, R. L., & Esco, M. R. (2013). Electromyographic comparison of traditional and suspension push-ups. Journal of human kinetics, 39, 75.

Thomas, E., Battaglia, G., Patti, A., Brusa, J., Leonardi, V., Palma, A., & Bellafiore, M. (2019). Physical activity programs for balance and fall prevention in elderly: A systematic review. Medicine, 98(27), e16218.

Trendelenburg, A. U., Meyer, A., Rohner, D., Boyle, J., Hatakeyama, S., & Glass, D. J. (2009). Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. American Journal of Physiology-Cell Physiology, 296(6), C1258–C1270.

Walker, K. S., Kambadur, R., Sharma, M., & Smith, H. K. (2004). Resistance training alters plasma myostatin but not IGF-1 in healthy men. Medicine & Science in Sports & Exercise, 36(5), 787–793.

Whitney, S. L., Wrisley, D. M., Marchetti, G. F., Gee, M. A., Redfern, M. S., & Furman, J. M. (2005). Clinical measurement of sit-to-stand performance in people with balance disorders: validity of data for the Five-Times-Sit-to-Stand Test. Physical therapy, 85(10), 1034–1045.

* Corresponding Authors: ba.tartibian@gmail.com; no_azadpoor@yahoo.com
How to Cite: Beigi, A; Tartibian, B; Eslami, R; Azadpour, N & Fasihi, L (2026). Creatine Supplementation during Suspension Training Reduces Circulating Myostatin Without Additional Improvements in Physical Function in Inactive Older Men: A Randomized Placebo-Controlled Trial, New Approaches in Exercise Physiology, 8(16),146-175 .

DOI: 10.22054/nass.2026.93780.1242