



Comparison of three types of exercise methods on some factors of sarcopenia and inflammation in elderly women

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Abstract

Background: Sarcopenia and systemic chronic inflammation are hallmark features of biological aging, contributing significantly to functional decline in geriatric populations. The present study aimed to evaluate and compare the efficacy of eight-week endurance, resistance, and concurrent (Combined) training protocols on key biomarkers of sarcopenia (C-terminal agrin fragment, CAF) and inflammatory profiles (Cortisol and interleukin-6, IL-6) in elderly women.

Methods: Forty-eight elderly female volunteers (Mean Age: 65.24 ± 3.14 years; Weight: 82.76 ± 5.89 kg; Height: 162.06 ± 4.40 cm; BMI: 31.45 ± 3.21 kg/m²) were recruited and randomly allocated into four homogeneous groups (n = 12 per group): Endurance Training, Resistance Training, Concurrent Training, and Control. The experimental groups participated in their respective exercise regimens for eight weeks (Three sessions per week). To measure serum variables (Cortisol, CAF, and IL-6), fasting blood samples were collected 48 hours before the intervention and 48 hours after the final training session. Data were analyzed using one-way ANOVA, ANCOVA, and Bonferroni post-hoc tests.

Results: Post-intervention analysis demonstrated significant reductions in serum cortisol (P = 0.001), CAF (P = 0.001), and IL-6 (P = 0.001) in all training groups compared to baseline. Significant differences were observed between the exercise groups and the control group, particularly for cortisol levels (P = 0.001); however, intergroup comparisons among the three exercise modalities showed no statistically significant differences (P > 0.05).

Conclusion: The findings suggest that, regardless of modality, an eight-week exercise intervention effectively reduces biomarkers associated with neuromuscular junction degradation and systemic inflammation in elderly women. Therefore, these training strategies may be recommended as viable non-pharmacological approaches to counteract sarcopenic progression and age-related metabolic dysfunction.



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Introduction

The process of biological aging is intrinsically linked to a progressive decline in skeletal muscle integrity, manifested by reductions in muscle mass, fiber size, and contractile strength. Concurrently, there is an elevation in deleterious biological markers, including specific hormones and peptides. This complex syndrome, termed sarcopenia, typically begins during the fifth decade of life and progresses at an annual rate of approximately 0.8% (1). Sarcopenia comprises two distinct pathological components: myopenia, defined as the loss of muscle mass, and dynapenia, referring to the decline in muscle strength (2). Furthermore, age-related physiological changes lead to elevated plasma concentrations of catabolic hormones, such as cortisol. This hormonal imbalance exacerbates tissue degradation and induces significant alterations within the neuromuscular junction (NMJ) (3).

In the context of sarcopenia, beyond the reduction in fiber number and increased heterogeneity in fiber size, significant postsynaptic modifications also occur. Notably, degeneration of the neuromuscular junction impairs the efficient transmission of neural impulses from central motor pathways to muscle fibers. This disruption in signal transduction reduces muscle activation, thereby contributing to functional weakness (4). Under healthy physiological conditions, NMJ structural integrity is maintained by agrin, a nerve-derived proteoglycan. However, during neuromuscular remodeling, the serine protease neurotrypsin cleaves agrin into the C-terminal agrin fragment (CAF). Excessive neurotrypsin activity and subsequent agrin degradation compromise NMJ stability, representing a potential mechanism underlying sarcopenia pathogenesis (5). As agrin cleavage accelerates, circulating CAF levels increase in parallel with neurotrypsin activity, indicating weakening of the synaptic connection. Elevated systemic CAF reflects NMJ destabilization, which promotes denervation, muscle

atrophy, and neuromuscular dysfunction (6). At present, effective pharmacological or nutritional interventions to halt the progression of sarcopenia are limited; however, physical exercise has emerged as a powerful non-pharmacological strategy to attenuate the adverse effects of this condition (7). Senescence is associated with diminished functional capacity in the nervous, muscular, and endocrine systems, leading to reduced force production and cardiovascular output. Cortisol, synthesized by the adrenal cortex, is widely recognized as a primary stress hormone. Elevated cortisol levels promote protein catabolism, inhibit glucose uptake, and stimulate lipolysis (8). In addition, chronic low-grade inflammation is a key contributor to age-related pathologies and serves as a predictive biomarker for various morbidities (9).

Interleukin-6 (IL-6), a pleiotropic cytokine, stimulates the production of acute-phase proteins and activates the hypothalamic-pituitary-adrenal axis. Given its involvement in cardiovascular and metabolic disorders, reducing pro-inflammatory cytokines such as IL-6 is crucial for maintaining health. Evidence indicates that exercise protocols of sufficient intensity and duration may effectively reduce these inflammatory markers (10). In this context, Sohaili et al. (2014) demonstrated that resistance training significantly decreased inflammatory mediators such as IL-6, while also reducing body weight and fat mass in sedentary, overweight women (11).

Conversely, Prophetzadeh et al. (2019) investigated the effects of a 12-week combined training program (Resistance-speed) on CAF levels in an elderly population. Their findings showed that this specific intervention did not produce statistically significant changes in circulating CAF levels among the elderly participants (12).

The principle of specificity in exercise physiology emphasizes that physiological adaptations are limited to the muscle fibers actively engaged during physical activity. Consequently, muscle fibers exhibit distinct adaptive responses depending on the exercise modality.

Endurance training primarily promotes mitochondrial biogenesis and capillarization, whereas resistance training stimulates the synthesis of contractile proteins. From a hormonal perspective, resistance training protocols typically increase both testosterone and cortisol concentrations, whereas endurance training tends to suppress testosterone levels while simultaneously elevating cortisol secretion (13).

In the context of concurrent training, which combines endurance and resistance modalities, the endurance component may promote a predominance of catabolic processes, potentially attenuating gains in muscle strength. Concurrent training protocols have been shown to elevate cortisol levels, particularly during the later phases of exercise sessions, thereby altering the anabolic-catabolic hormonal balance. To date, the mechanisms underlying skeletal muscle adaptation to simultaneous resistance and endurance stimuli remain incompletely understood (14). Moreover, empirical findings regarding adaptive responses and performance outcomes following concurrent training have been inconsistent. Much of the existing literature has focused on younger populations or animal models, resulting in a lack of studies examining these effects specifically in geriatric populations. Therefore, there is a critical need for comparative investigations of hormonal responses and growth factor adaptations to resistance, endurance, and concurrent training in older adults.

Accordingly, the present study aimed to determine whether an eight-week intervention of resistance, endurance, and concurrent training produces significant effects on sarcopenia-related markers and inflammatory profiles in elderly women.

Methods

Subjects

The present study was a semi-experimental applied research design. Inclusion criteria were as follows: Full consciousness; age between 60 and 70 years; absence of cardiorespiratory diseases, diabetes, arthritis, musculoskeletal disorders, severe orthopedic conditions, neurological diseases, or hypertension; not taking medications; non-smokers; and no regular exercise participation during the previous year. Based on these criteria, 48 eligible participants were selected and assigned to three experimental groups and one control group.

Prior to and following the intervention, blood samples and anthropometric measurements of body composition were collected. The required sample size was calculated using G*Power 3.1 software, considering a statistical power of 99%, an effect size of 95%, and a significance level of 0.05. According to the software recommendation, a total sample of 48 participants was deemed sufficient. Participants were recruited voluntarily and purposively to take part in the study.

The subjects were randomly allocated using a simple random sampling method into four groups: resistance training ($n = 12$), endurance training ($n = 12$), concurrent (Combined) training ($n = 12$), and control ($n = 12$). At baseline, participants were homogenized according to age, height, weight, body fat percentage, and body mass index. Written informed consent was obtained from all participants prior to the study. Before initiating the exercise protocols, two to three familiarization sessions were conducted to explain the potential benefits and risks of participation and to provide instructions on exercise procedures. Baseline anthropometric variables (Height, weight, BMI) were also measured.

Exclusion criteria included the presence of neurological disorders (e.g., stroke, Parkinson's disease, paralysis), cardiovascular disorders, acute heart failure, absence from more than three consecutive sessions or four sessions overall during the intervention, uncontrolled hypertension, unstable chronic diseases (e.g., diabetes or malignancies), and severe congenital or musculoskeletal disorders limiting exercise participation.

The study was conducted in accordance with ethical standards and was approved under the code IR.IAU.Mashhad.REC.1401.019, with clinical trial registration number IRCT20220316054315N2.

Measurement of anthropometric indicators

Anthropometric indices, including height, weight, body fat percentage, and body mass index, were measured by the researcher. Height was measured while participants stood barefoot on a flat surface using a stadiometer (Seca, Japan) with an accuracy of 1 mm. Body weight was

assessed using a digital scale (YAGMI, Japan) with an accuracy of 0.1 kg, with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (m^2). To assess body composition, a body composition analyzer (InBody 720, South Korea) was used to determine body fat percentage and skeletal muscle mass percentage.

Exercise protocols

The exercise protocols included resistance, endurance, and concurrent (Combined) training performed over eight weeks. Resistance training was conducted three times per week and consisted of a 10-minute warm-up, upper- and lower-body resistance exercises, and a 10-minute cool-down. Training intensity began at 60–65% of one-repetition maximum (1RM) and progressively increased to 70–80%, with four sets of 8–10 repetitions per exercise.

Endurance training was volume-matched and performed on a treadmill at a speed of 5.5 mph for 20 minutes, beginning at 60–65% of maximum heart rate and progressing to 65–70%. Each session included a 10-minute warm-up and a 10-minute cool-down. In the concurrent training group, resistance training was followed by aerobic exercise performed at half the duration of the endurance protocol to equalize total training volume. Heart rate and 1RM were monitored and adjusted every two weeks.

The control group did not participate in any structured exercise program, and all participants were instructed to maintain their usual diet and refrain from using medications throughout the study period (13).

Measurement of blood biomarkers

Blood sampling was performed by an experienced laboratory specialist in the Sports Physiology Laboratory. Blood samples were collected 24 hours before the start of the training programs and 48 hours after the final training session. A 5-cc blood sample was drawn from the brachial vein by a laboratory specialist. Immediately after collection, the samples were centrifuged at 3000 rpm for 10 minutes, and the separated serum was stored in microvials at -20°C until analysis.

At the end of the study, serum levels of cortisol (Pars Azmoon kit), CAF (ZellBio, Germany), and IL-6 (ZellBio, Germany) were measured using the ELISA method with an ELISA reader. CAF and IL-6 were measured within a range of 1.5 pmol/L to 48 pmol/L, with a sensitivity of 1 pmol/L, using specific ELISA kits manufactured by ZellBio (Germany).

Statistical analysis

All data were analyzed using SPSS version 26 software. Descriptive statistics (Mean and standard deviation) were calculated. The Shapiro-Wilk test was used to assess the normality of data distribution. One-way analysis of covariance (ANCOVA) was applied to compare differences between groups. The Bonferroni post-hoc test was used for pairwise comparisons. A significance level of $P < 0.05$ was considered for all analyses.

Results

The results of the one-way ANOVA for anthropometric and physical indicators are presented in Table 1. As shown in Table 2, after eight weeks of resistance, endurance, and combined training, all three exercise programs significantly affected blood cortisol, CAF, and IL-6 levels in elderly women compared with the control group ($P \leq 0.001$). Post-hoc analysis indicated that cortisol levels increased by 12.66% in the resistance training group, 25.97% in the endurance training group, and 21.59% in the combined training group, whereas the control group showed only a 2.47% increase. No significant differences were observed among the exercise groups for most comparisons.

For CAF, resistance training reduced levels by 23.43%, endurance training by 29.90%, and combined training by 11.74%, while the control group showed an increase of 7.07%. IL-6 levels decreased by 18.69% in the resistance training group, 11.42% in the endurance training group, and 4.95% in the combined training group, whereas the control group showed a 0.84% increase. These findings indicate that all exercise programs improved sarcopenia-related and inflammatory markers in elderly women. Endurance training generally produced the greatest changes in cortisol and CAF, whereas resistance training resulted in the largest reduction in IL-6. Although most differences among the exercise groups were not statistically significant, some minor differences in specific indicators were observed (Table 3).

Table 1. Mean distribution and standard deviation of physiological variables

Group	Indicator	Endurance training group	Resistance training group	Combined training group	Control group	P-value
	Age (Year)	65.41±2.87	65.25±3.74	65.25±2.70	64.91±3.39	0.96
	Height (cm)	163.42±5.18	162.12±4.09	162.7±5.32	160.02±5.19	0.68
	Weight (kg)	78.94±4.15	83.5±5.75	82.48±6.32	84.13±6.01	0.004
		After training	75.33±3.03	80.24±5.26	79.03±6.19	
	Fat mass (Percentage)	29.17±1.99	29.51±1.74	28.80±1.29	29.08±1.47	0.001
		After training	27.13±1.56	28.10±3.04	26.06±2.12	
	Body mass index (kg*2 square meters)	30.83±2.29	31.78±2.42	31.48±2.60	31.74±2.48	0.001
		After training	29.42±3.45	30.62±4.26	30.16±2.09	

Table 2. The results of analysis of covariance of cortisol, CAF and IL6 hormone levels

Indicator (Unit)	Groups	Mean ± Standard deviation		Percent changes	F	P-value
		Before eight weeks	After eight weeks			
Cortisol (mg/dl)	Resistance training	25.41±4.26	28.66±5.08	%12.66	6.85	0.001*
	Endurance training	19.25±1.58	24.25±7.32	%25.97		
	Combined training	22.00±3.93	26.75±4.28	%21.59		
	Control	21.00±6.23	21.52±2.53	%2.47		
CAF (pg/ml)	Resistance training	3.20±0.38	2.45±0.44	-%23.43	24.86	0.001*
	Endurance training	4.28±0.73	3.00±0.45	-%29.90		
	Combined training	2.98±0.57	2.63±0.77	-%11.74		
	Control	3.11±0.81	3.33±0.27	-%7.07		
IL-6 (pg/ml)	Resistance training	18.35±1.70	14.95±1.85	-%18.69	19.09	0.001*
	Endurance training	18.38±2.27	16.28±2.22	-%11.42		
	Combined training	16.35±2.07	15.54±2.14	-%4.95		
	Control	18.86±2.88	19.02±2.78	-%0.84		

Table 3. The results of the post-hoc test of the indicators cortisol, CAF and IL6

Indicator (Unit)	Group	Groups	Difference of means	P-value
CAF (pg/ml)	Resistance training	Control	7.14	0.001*
		Endurance training	4.41	0.60
		Combined training	1.91	0.95
	Endurance training	Control	2.73	0.001*
		Combined training	2.50	0.32
	Combined training	Control	5.23	0.001*
IL-6 (pg/ml)	Resistance training	Control	0.88	0.001*
		Endurance training	-0.55	0.52
		Combined training	-0.18	0.20
	Endurance training	Control	-0.33	0.001*
		Combined training	-0.37	0.10
	Combined training	Control	-0.7	0.001*
Cortisol (mg/dl)	Resistance training	Control	-4.07	0.001*
		Endurance training	-1.33	0.006*
		Combined training	-0.59	0.001*
	Endurance training	Control	-2.74	0.001*
		Combined training	0.74	0.65
	Combined training	Control	-3.48	0.001*

Discussion

The results of the present study indicate a significant effect of eight weeks of endurance, resistance, and combined training on CAF levels in elderly women. According to the findings, the hypothesis related to CAF was confirmed, as a significant difference was observed between each of the three training groups and the control group. However, no significant differences were found among the training groups themselves.

In a study by Karaghan et al. (2021), the effect of eight weeks of dual training with and without blood flow restriction (BFR) on the CAF index in elderly women was examined. The results demonstrated that resistance-type exercises combined with blood flow restriction led to significant changes in CAF, body mass, BMI, and body fat percentage (15). In contrast, Farjala et al. (2014) reported a non-significant increase in blood CAF levels in elderly individuals following a 6-week resistance training program (16). Similarly, Banitalebi et al. (2020) did not observe significant changes in CAF levels after 12 weeks of resistance training using moderate-intensity elastic bands (17). Differences in exercise duration and intensity, participant characteristics (Including age), and exercise protocols may explain the discrepancies between these studies and the present findings.

Moreover, previous research has identified an inverse relationship between CAF levels and muscle strength. Higher circulating CAF concentrations have been reported in older individuals with lower muscle strength and reduced lean mass. These findings support the notion that circulating CAF may serve as a negative biomarker associated with impaired muscle quality and strength in the elderly (16). In contrast to the present results, Prophetzadeh et al. (2019) found that 12 weeks of combined training did not significantly affect blood CAF levels in elderly individuals (12). Among the reasons cited for the lack of significant findings in studies involving elderly participants, researchers have noted differences in physical activity levels and variations in the type of combined training protocols. These factors may explain the discrepancies between previous findings and the results of the present study. For example, differences in outcomes may be attributed to the type of combined exercise used (Resistance-speed training in the study by Prophetzadeh et al. versus aerobic-resistance training in the present study). Bondoc and colleagues also reported no significant changes in CAF following a 12-month sports activity program (18). To justify their findings, several explanations were proposed, including the absence or reduced activity of neurotrypsin. Elevated neurotrypsin activity leads to agrin degradation, which increases CAF release as a result of neuromuscular junction destruction and denervation; however, exercise may prevent denervation and preserve neuromuscular junction integrity. Other contributing factors include baseline CAF levels, the specific exercise protocol used (Low-intensity training may have a more favorable effect on the length and number of neuromuscular junction branches compared with high-intensity training, resulting in lower CAF levels), motor unit recruitment induced by low-intensity exercise, changes in muscle mass, and the precision of measurement techniques (19).

Overall, CAF alterations appear to reflect neuromuscular adaptations. It is well established that significant neuromuscular adaptations occur during the early phases of training programs, particularly aerobic training (20). Given that neurons and muscle fibers are highly metabolically active, it is reasonable to hypothesize that CAF levels may be influenced by even minor impairments in mitochondrial function. Therefore, the type and intensity of exercise, through their effects on mitochondrial activity at the axon terminal, neurotransmitter production and processing, and subsequent strengthening of neuromuscular junction structure, play a crucial role in modulating CAF levels (21).

The results also showed a significant difference in the effects of eight weeks of endurance, resistance, and combined exercise on IL-6 levels in elderly women. According to the findings, the hypothesis related to cortisol was confirmed, and based on the results of the present study, eight weeks of resistance, endurance, and combined exercise led to a significant reduction in serum IL-6 levels in the training groups compared with the control group. However, no significant differences were observed among the training groups. A review of the literature indicates that the response of inflammatory cytokines to exercise and physical activity can vary. Inflammatory cytokines are secreted by

various cells, including neutrophils, activated macrophages, and muscle cells (22). A multifold increase in IL-6 secretion from skeletal muscle following exercise may serve as a hormonal signal of blood glucose utilization and glycogen depletion, and a positive correlation has been reported between plasma IL-6 levels, exercise intensity, muscle glucose uptake, and plasma adrenaline. In contrast, regular exercise, particularly aerobic exercise, may lead to a reduction in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines in elderly individuals (23). Ogawa et al. (2010) also reported that serum IL-6 levels did not change following 12 weeks of resistance training (24).

The conflicting results reported across studies may be attributed to differences in training protocols, participant characteristics, and changes in body composition. Accordingly, the response of inflammatory markers appears to depend on factors such as age, fitness level, and pathological conditions, including obesity and diabetes. Nevertheless, some studies have also reported no changes in inflammatory markers following exercise (3). One consistent finding is that serum IL-6 levels may decrease with weight loss. Conversely, increases in plasma IL-6 have been observed following intense and prolonged exercise in some studies (25). Endurance, resistance, and combined exercise modalities may alter the production of inflammatory cytokines. These exercise forms may reduce the production of inflammatory cytokines by T cells through various mechanisms, including changes in circulating factors such as lactate and catecholamines, alterations in growth factors such as IGF-1, stimulation of lymph nodes, and mobilization of a greater number of natural killer cells into the circulation compared with T cells (26). In addition, increased sympathetic stimulation enhances cytokine release from adipose tissue, and findings have shown that endurance, resistance, and combined exercise can reduce sympathetic stimulation (27). Analysis of covariance revealed a significant difference in the effects of eight weeks of endurance, resistance, and combined exercise on cortisol levels in elderly women. Bagheri et al. (2015) reported that three combined exercise methods had no significant effect on cortisol levels in elderly women. Cortisol levels are influenced by factors such as exercise intensity and duration, exercise conditions (e.g., competition), environmental temperature, psychological stress, rest intervals, and circadian rhythms (28). The lack of change in cortisol levels in the study by Bagheri et al., considering these factors, suggests that the metabolic conditions induced by exercise favored anabolic processes and protein synthesis. In the present study, adaptations resulting from resistance, aerobic, and combined exercise training performed with appropriate intensity and duration may have increased cortisol secretion in all three exercise groups, accompanied by neuromuscular adaptations and improved muscle blood flow.

Conclusion

Resistance, endurance, and combined exercise programs can effectively reduce sarcopenia in elderly individuals, with the use of light weights minimizing injury risk. When performed with appropriate intensity and duration, these exercise modalities also contribute to reductions in inflammatory factors and improvements in neuromuscular function, serving as effective non-pharmacological strategies to counteract age-related problems. Elderly individuals are encouraged to incorporate aerobic, resistance, or combined exercise into their daily routines to enhance physical and mental health.

Research limitations

There are limited studies examining the effects of combined exercise modalities (Aerobic-resistance), largely due to small sample sizes and the presence of multiple comorbidities in this age group. Future research should provide clearer insights into the optimal intensity, duration, and volume of exercise, as well as lifestyle factors, for this population. However, definitive conclusions require further investigation. Therefore, large-scale studies with greater sample sizes in individuals aged 60 to 70 years are recommended.

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Ethical Statement

This study was conducted in accordance with the ethical standards of the institutional research committee.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the present research.

Author Contributions

All authors contributed equally to the conception and design of the study, data collection, data analysis, and manuscript preparation. All authors have read and approved the final version of the manuscript.

Data Availability Statement

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

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