

# Comparing the Effects of Eight Weeks of Combined Training (Endurance and Resistance) in Different Orders on Inflammatory Factors and Adipokines Among Elderly Females

Ebrahim Banitalebi,<sup>1\*</sup> Zahra Mardanpour Shahrekordi,<sup>1</sup> Abdol Reza Kazemi,<sup>2</sup> Laleh Bagheri,<sup>1</sup> Sadegh Amani Shalamzari,<sup>1</sup> and Mohammad Faramarzi<sup>1</sup>

<sup>1</sup>University of Shahrekord, Shahrekord, IR Iran

<sup>2</sup>Vali-e-Asr University of Rafsanjan, Rafsanjan, IR Iran

\*Corresponding author: Ebrahim Banitalebi, University of Shahrekord, Shahrekord, IR Iran. Tel: +98-9132818216, E-mail: Banitalebi@lit.sku.ac.ir

Received 2015 June 24; Revised 2015 September 23; Accepted 2015 December 30.

## Abstract

**Background:** Aging is a process in which the body's physiological capacity continuously decreases after the age of 30. However, interventions such as physical activity can play an important role in the prevention of aging.

**Objectives:** The purpose of this study was to compare the effects of eight weeks of combined training (endurance and resistance) in different orders on inflammatory factors and adipokines among elderly females.

**Patients and Methods:** The design of this study was quasi-experimental. Forty healthy females were selected purposely and randomly assigned to one of four groups including strength after endurance training (E + S, n = 9), strength prior to endurance training (S + E, n = 10), circulation combined (CI, n = 12), and control (n = 9) groups. The training program was performed for eight weeks, three times per week. Within-group differences were analyzed using a paired samples t-test and between-group differences were analyzed using one-way analysis of variance (ANOVA).

**Results:** The data analysis showed that the between group comparison did not influence the adaptive response of tumor necrosis factor (TNF)- $\alpha$  (P = 0.80), high sensitivity-C reactive protein (hs-CRP) (P = 0.55), adiponectin (P = 0.33), chemerin (P = 0.56), omentin (P = 0.51), leptin (P = 0.10) and vaspin (P = 0.70) levels, yet the within group comparison showed a significant difference in the chemerin concentration in the E+S group (P = 0.03).

**Conclusions:** The results indicated that three different combined trainings (strength and endurance) had no positive effect on inflammatory factors and adipokines among aged females. However, the different combined trainings, independent of order, were effective training methods in reducing body fat and body mass index (BMI) in aged females.

**Keywords:** Combined Training, Immunity, Adipocytokines, Elderly, Women

## 1. Background

Aging is a complex medical condition that leads to many unfavorable and inevitable changes in the body. The physiological capacity of the body continuously declines after the age of 30. The shift in population demographics shows increased numbers of elderly individuals above the age of 60 in Iran and the structure of the age pyramid is reversing (1). Moreover, numerous theories, such as free radical, neuroendocrine, pineal gland and immunological theories, have attempted to explain the decline in body function observed during the aging process (2).

Inflamm-aging also refers to a low-grade chronic inflammatory status with advancing age, which is common in most age-related disorders such as infectious diseases, coronary heart disease, stroke, and type 2 diabetes (3). Inflamm-aging is characterized by increased serum, high sensitivity C-reactive protein (hs-CRP), and

pro-inflammatory cytokines such as interleukin 6 and tumor necrosis factor alpha (TNF- $\alpha$ ) (4). Inflamm-aging is a strong independent risk factors for morbidity, mortality and cardiovascular complications among aged people (3). On one hand, overwhelming evidence demonstrates that aging is accompanied by increased adipose tissue, particularly visceral adipose mass (5). On the other hand, aging is accompanied by adipokine dysregulation, and adipokines play a crucial role in the pathobiology of aging and age-related diseases (6).

Leptin and adiponectin are far more studied than other adipokines (7). Aged females have approximately three times higher plasma leptin levels than males (8). Females also have higher plasma adiponectin concentrations than males (9).

Omentin, chemerin and vaspin are identified as novel adipokines, which are predominantly produced by visceral fat tissue and are closely associated with visceral

obesity (10). Visceral adipose tissue-derived serine protease inhibitor (vaspin) is a novel adipokine with insulin-sensitizing effects and is related to metabolic disorders (11). Chemerin, known as retinoic acid receptor responder protein 2, acts as a regulator of adipogenesis, inflammation and glucose metabolism (12). Omentin/intelectin-1 is a newly identified secretory protein that is expressed in visceral adipose tissue more than subcutaneous adipose tissue and may play a paracrine or endocrine role in modulating insulin sensitivity (13). Among the various strategies to improve immune function in the elderly, exercise training is the best non-invasive intervention with no negative side effects (14).

Previously published articles illustrate the potential effects of physical exercises on immunosenescence, through the secretion of anti-inflammatory cytokines, which may contribute to improvements in the metabolic syndrome (15).

Furthermore, it has been shown that weight loss is a useful intervention for reducing TNF- $\alpha$  levels because adipose tissue releases large amounts of these factors (16). Moreover, age-related loss of muscle strength and mass is often associated with problems such as mobility impairment, falls, arthritis, fractures and impaired functional ability (17). Thus, strengthening exercises are particularly important for the elderly, and aged-related disorders may be improved by appropriate resistance training (18). Despite the safety and efficacy of resistance training, and the leisure time and facilities available to older adults, the prevalence of reported strength training is still lower among the elderly (19).

Rall et al. found that 12 weeks of resistance training did not reduce TNF- $\alpha$  and IL-6 levels in elderly subjects (20). It has been shown that endurance exercise improves immune function and risk factors for chronic diseases in elderly individuals (21).

The administration of both resistance and aerobic exercise training to improve functional capacity and body composition in the elderly has been recommended (22). Simultaneous combined endurance and resistance training in regular exercise programs, is called combined training (concurrent training) (23). Research has shown that combined training is both safe and effective for postmenopausal females (24). The exercise order of concurrent training (in which endurance and strength training are carried out) indicates which one (resistance or aerobic training) should be performed first and which one should be done next (25).

It has been hypothesized that performing endurance training immediately before or after resistance training may diminish strength gains because of residual muscle fatigue resulting from the preceding training and, the in-

ability of the muscle to optimally adapt to two different stimuli with different energy pathways during the same session (26, 27). Based on the results of Cutts and Burns performing aerobic exercise prior to resistance training has a greater impact on total energy consumption in females versus the reverse sequence (28). In another study, Ho et al. demonstrated that in a 12-week concurrent training with an aerobic + resistance order for five days/week, body weight and body mass index (BMI) in the concurrent training group were significantly lower than the control and resistance groups. They also observed a significant increase in maximum oxygen uptake (VO<sub>2max</sub>) in the concurrent training group (29).

In a 12-week low-frequency concurrent exercise program, Chtara et al. investigated the effect of manipulating the order of resistance and endurance training on the pattern of physiological functions' adaptation. They showed that despite the effect of endurance + resistance and resistance + endurance training on maximal muscular strength, strength endurance and explosive strength, there were no differences between groups with different order sequences (30). Chtara et al. (30) illustrated that performing endurance training prior to strength training when compared with the inverse order led to greater increases in young male's endurance capacity. However, greater VO<sub>2max</sub> increases were shown in strength training prior to endurance training (31). Recently, Cadore et al. showed that resistance training followed by endurance training resulted in greater lower-body strength as well as greater changes in neuromuscular economy in the elderly (32).

Finally, the compatibility of concurrent training is not independent of order sequences. For many years scientists have been interested in how much endurance and resistance training, and in what order, is most beneficial for achieving health-related physical fitness in elderly people. However, not much is known about the effect of combined training in different orders on inflammation markers and adipokines in elderly people. Libardi et al. illustrated that 16 weeks of combined training in middle-aged males had no effect on IL-6, TNF- $\alpha$ , and CRP levels. They also demonstrated that training did not decrease inflammatory biomarkers (33). Conraads et al. illustrated that four months of combined endurance/resistance training did not alter IL-6 and TNF- $\alpha$  levels (34). Recently, Stefanov et al. in a six-month combined exercise program reported a significant decrease in the hs-CRP of middle-aged females (35). In addition, Jorge et al. in an experimental study, compared the effects of three different modalities of exercise training on inflammatory markers. They showed that there were no differences in hs-CRP levels following aerobic, resistance and combined exercise interventions (36).

Recently, Miyatake et al. reported that the serum levels of vaspin were independently associated with physical activity in females (37). Furthermore, Oberbach et al. showed that, following four weeks of exercise aerobic training, serum vaspin concentrations significantly decreased in healthy young males (38). To establish the source of exercise-induced changes in obesity-related chemerin expression, Keslacy et al. illustrated the effect of exercise on obesity in that it increased chemerin expression specific to adipose tissue (39). Chakaroun et al. (2012) demonstrated that, after 12 weeks of exercise intervention in obese females, chemerin serum concentrations decreased significantly (40). Furthermore, in a long-term exercise intervention, Neuparth et al. illustrated that active females presented a trend towards lower levels of chemerin than sedentary females with type 2 diabetes mellitus (41). Saremi et al. (2010) illustrated that 12 weeks of aerobic training decreased the chemerin levels in overweight and obese males (42). In another study, Saremi et al. (2010) showed that 12 weeks of aerobic exercise was accompanied by increased omentin-1 concentrations in overweight and obese males (43). In the recent years, Malin et al. illustrated that in older adults, 12 weeks of aerobic training for five days/week reduced chemerin concentrations, which was correlated with decreased visceral fat (44).

However, very little is known regarding the interaction between combined training, aging, and the immune system, particularly for combined training in different orders. Researchers were interested to determine the order of strength and endurance training that was most beneficial for regaining health-related physical fitness in elderly females. To the best of our knowledge, there have been no systemic published scientific studies that have examined the effects of different types of combined training on pro-inflammatory factors TNF- $\alpha$ , hs-CRP, and adipokines in aged females.

## 2. Objectives

It was hypothesized that performing combined training in different orders could affect the inflammatory and adipokine profiles of elderly females. Therefore, the purpose of this study was to compare the effects of eight weeks of combined training (endurance and resistance) in different orders on inflammatory factors and adipokines among elderly females.

## 3. Patients and Methods

### 3.1. Participants

The design of the present study was quasi-experimental with three experimental groups and a

control group, with a pretest and a posttest. The sample population of this study consisted of elderly females retired from the ministry of education, area one of Shahrekord Province branch, during year 2013. In this study, the population consisted of 80 elderly females; after the census, 14 individuals were removed due to the inclusion criteria. Since at the beginning of the study, six subjects withdrew for personal reasons, 60 individuals (mean: 60.34) were chosen and were randomly assigned to three experimental groups and a control group (each consisted of 15 individuals). In addition, during the time of the study, 20 individuals could not finish the schedule for different reasons, and the number of subjects was reduced to 40.

The four groups were strength after endurance training (E + S, n = 9), strength prior to endurance training (S + E, n = 10), circulation combined (CI, n = 12) and control (n = 9) groups.

For the homogeneity of groups, the Lon test was administered at the pretest; the P value was higher than 0.05 and groups were homogenous. The inclusion criteria were as follows, not taking any medication, having no chronic disease and no physical activity a year before the beginning of the study, and finally, having an appropriate level of physical and mental health. The exclusion criteria consisted of a history of cardiovascular diseases, cancer, blood pressure, diabetes, thyroid disorders, addiction to tobacco, alcohol and drugs, hormonal disorders, kidney and liver diseases, surgery, and any intervention affecting the laboratory results. All the subjects received information about the research and after reviewing this information, they were asked to sign the written consent. The present study was conducted under the supervision of specialists and experts in exercise physiology. All the subjects completed the related questionnaires and had no blood pressure, diabetes, and kidney and liver diseases. In addition, in one session the subjects became familiar with the methods of exercise.

### 3.2. Anthropometric Measures

Body fat percentage was calculated from the value of a three-site skin fold test (triceps, thigh, and suprailiac), measured with a Lafayette Skinfold Caliper II (45). The BMI was calculated for each subject using the following formula: BMI = weight (kg)/height<sup>2</sup> (m). The waist circumference (WC) was measured using a flexible two-meter standard tape measure at the maximal narrowing of the waist from the anterior view. The hip circumference was measured at the point of maximal gluteal protuberance from the lateral view. The waist/hip ratio was calculated through dividing the waist circumference by the hip circumference. The modified Bruce protocol treadmill test was used

to measure the aerobic capacity of subjects; a method that begins with a lower work-load. The modified protocol test is also a multi-stage test. The initial speed of the treadmill is set to 2.74 km/h, and the inclination is set to 0%. The second and third stages have the same speed, but the gradient increases by 5%. In the second stage, the inclination is increased to 5%, but the speed of the treadmill remains at 2.74 km/h. In the third stage, the speed of the treadmill is set to 1.7 mph and inclination is set to 10% (46). The 1-RM leg press test (47) was used to measure the lower and upper limb strength capabilities(48).

### 3.3. Exercise Training Protocols

After the preliminary tests, the exercise intervention was eight weeks of combined (resistance plus endurance) training (49). Experimental groups underwent training three times per week. Each session consisted of 10 minutes of general warm up, 50 minutes of exercise training, and 10 minutes for cooling-down processes. All subjects performed a familiarization session to become familiar with the training procedures, intensity and equipment. The training program for strength-endurance (S + E) and endurance-strength (E + S) groups was similar but in a different order. Sixteen minutes of endurance training was performed at 45%  $VO_{2max}$  on an ergometer for the first two weeks and continued for 30 minutes until the eighth week. Two minutes after endurance training, resistance training was performed as follows: bench press, leg press, bent over lateral pull down, bilateral biceps curl and bilateral triceps push down. Resistance training was performed at 40% of (one-repetition maximum) 1-RM for the first week and increased to 75% of 1RM until the eighth week. The CI protocol begun with five minutes of warm-up on the ergometer followed by one-third of endurance exercise time duration in E + S alternated with one-third of resistance training volume in the E + S training group (50, 51).

### 3.4. Blood Analysis

To examine serum TNF- $\alpha$ , hs-CRP, leptin, adiponectin, chemerin, omentin and vaspin levels, blood samples (10 cc) were collected 24 hours before the exercise protocol and 48 hours after the last session of the training program in a 12-hour fasting state from the antecubital vein in a sitting position. Blood samples were then centrifuged for 10 minutes at 40°C, 500 × g to separate the serum. A human TNF- $\alpha$  sandwich enzyme linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm Co, cat number CK-E11629), human hs-CRP sandwich ELISA kit (Hangzhou Eastbiopharm Co, cat CK-E10968), human omentin sandwich ELISA kit (Hangzhou Eastbiopharm Co, cat number CK-E11629), human chemerin sandwich ELISA

kit (Hangzhou Eastbiopharm Co, cat number CK-E11406), and human vaspin sandwich ELISA kit (Hangzhou Eastbiopharm Co, cat CK-E10968) were used to measure serum TNF- $\alpha$  and hs-CRP levels, respectively. Serum insulin was estimated using a Monobind insulin ELISA kit (catalog number 2425-300, Monobind Co).

### 3.5. Statistical Analyses

All values are represented as means (SD). To test the normality of distribution, the Kolmogorov-Smirnov test was used. Data were analyzed using a paired t-test to compare pre-test and post-test scores in each group. The one-way analysis of variance (ANOVA) test was used to compare the amount of changes in the experimental and control training groups after eight weeks. When a significant F value ( $P < 0.05$ ) was achieved, Tukey's test was used to examine differences between various groups.

## 4. Results

The results were based on the observations of nine people in the control, nine people in the E + S, ten people in the S + E, and twelve people in the CI group. The p-values for comparisons, before and after training in all groups and between all groups are reported in Tables 1 - 3.

The effects of an eight-week combined endurance/resistance training program on inflammatory markers and adipokines are shown in Tables 1 - 3. The paired t-test showed that in all groups the TNF- $\alpha$  concentration did not significantly change during the eight weeks of training in the E + S ( $P = 0.849$ ), S + E ( $P = 0.674$ ) and CI ( $P = 0.097$ ) groups. The same test revealed that, after eight weeks of combined training, differences were not found for the hs-CRP levels in all groups; E + S ( $P = 0.057$ ), S + E ( $P = 0.131$ ), CI ( $P = 0.112$ ) and control ( $P = 0.694$ ).

The paired t-test conducted on the data from the experimental groups showed that the chemerin levels did not significantly change, during the eight weeks, in the S + E ( $P = 0.25$ ) and CI ( $P = 0.11$ ) groups, yet there was a significant increase in the E + S group ( $P = 0.03$ ) following exercise training. The same test revealed that after eight weeks of combined training, significant differences were not found for the serum omentin levels in the E + S ( $P = 0.15$ ), S + E ( $P = 0.18$ ), CI ( $P = 0.13$ ) and control ( $P = 0.27$ ) groups. In addition, a paired t-test for the vaspin variable in all groups showed no significant differences in the means in the E + S ( $P = 0.34$ ), S + E ( $P = 0.59$ ), CI ( $P = 0.16$ ) and control ( $P = 0.28$ ) groups. The paired t-test for comparing the adiponectin concentrations before and after the eight-week exercise training showed that there was no significant difference in the E + S ( $P = 0.22$ ), S + E ( $P = 0.16$ ), CI ( $P = 0.90$ ) and control ( $P = 0.22$ ), S + E ( $P = 0.16$ ), CI ( $P = 0.90$ ) and control ( $P = 0.22$ ).

**Table 1.** The Comparison of Changes in the Measured Variables Before and After Eight Weeks of Exercise Interventions (Part 1)<sup>a</sup>

Variables	Mean (SD)		P Value	
	Pretest	Posttest	Within Groups	Between Groups
<b>Body mass, kg</b>				0.017 <sup>b</sup>
E + S	74.66 (4.68)	72.77 (4.67)	0.005 <sup>c</sup>	
S + E	70.80 (3.90)	68.60 (3.86)	0.003 <sup>c</sup>	
CI	66.41 (2.69)	64.41 (2.44)	0.000 <sup>c</sup>	
Control	76.88 (3.78)	76.66 (4.05)	0.51	
<b>E + S</b>	29.89 (1.20)	29.12 (1.21)	0.005 <sup>c</sup>	
<b>BMI, kg/m<sup>2</sup></b>				0.023 <sup>b</sup>
S + E	29.23 (1.71)	28.30 (1.56)	0.003 <sup>c</sup>	
CI	27.57 (0.92)	26.76 (0.86)	0.000 <sup>c</sup>	
Control	31.75 (0.91)	31.63 (1.01)	0.42	
<b>Body Fat, %</b>				0.08
E + S	30.49 (1.0)	26.90 (1.47)	0.000 <sup>c</sup>	
S + E	31.66 (1.35)	27.77 (1.30)	0.000 <sup>c</sup>	
CI	30.65 (1.05)	27.88 (0.95)	0.000 <sup>c</sup>	
Control	28.50 (0.92)	27.50 (1.0)	0.08	
<b>WC, cm</b>				0.006 <sup>c</sup>
E + S	98.33 (3.08)	93.44 (3.03)	0.000 <sup>c</sup>	
S + E	95.40 (3.08)	92.50 (3.18)	0.008 <sup>c</sup>	
CI	93.50 (2.64)	90.25 (3.08)	0.003 <sup>c</sup>	
Control	97.44 (4.36)	97.00 (4.53)	0.22	
<b>WHR</b>				0.55
E + S	0.91 (0.01)	0.89 (0.01)	0.17	
S + E	0.88 (0.01)	0.88 (0.01)	0.80	
CI	0.92 (0.01)	0.91 (0.02)	0.32	
Control	0.88 (0.02)	0.88 (0.02)	0.83	

<sup>a</sup>Control group, subjects who not participated in exercise training; E + S, Resistance after aerobic training; S + E' Resistance prior to aerobic training.

<sup>b</sup>Significant difference between two groups (P < 0.05).

<sup>c</sup>Significant difference between two groups (P < 0.01).

= 0.93) groups. The one-way ANOVA test showed that no significant differences were seen among groups in TNF- $\alpha$  (P = 0.803), hs-CRP (P = 0.553), adiponectin (P = 0.33), chemerin (P = 0.56), omentin (P = 0.51), leptin (P = 0.10) and vaspin (P = 0.70) levels.

## 5. Discussion

There have been several studies on the effect of combined training (endurance and resistance) on various inflammatory factors, adipokines and metabolic syndrome. However, to the best of our knowledge, this is the first study that has examined the influence of manipulating the

order of combined training on adaptations of inflammatory factors and adipokines in elderly females.

The results of the current study indicate that serum TNF- $\alpha$  and hs-CRP concentrations did not change after eight weeks of combined training in different orders in aged females. The above results may be related to unchanged waist-to-hip ratio (WHR), as it has been shown that serum TNF- $\alpha$  was related to the WHR and TNF- $\alpha$  polymorphisms affect the WHR in obese people (52).

It seems that the beneficial effect of combined training was not related to hs-CRP and TNF- $\alpha$  reduction, which is in line with other studies (33). A longer period that reduces visceral fat is likely required to lower systemic inflamma-

**Table 2.** The Comparison of Changes in the Measured Variables Before and After Eight Weeks of Exercise Interventions (Part 2)<sup>a</sup>

Variables	Mean (SD)		P Value	
	Pretest	Posttest	Within Groups	Between Groups
<b>VO<sub>2max</sub>, mL/kg/min</b>				0.029 <sup>b</sup>
E + S	29.07 (1.88)	34.01 (2.05)	0.003 <sup>c</sup>	
S + E	24.60 (1.35)	31.81 (1.05)	0.003 <sup>c</sup>	
CI	23.70 (1.78)	27.93 (2.18)	0.024 <sup>b</sup>	
Control	24.77 (3.03)	24.25 (3.01)	0.43	
<b>TNF-<math>\alpha</math>, pg/mL</b>				0.803
E + S	36.80 (2.66)	35.60 (4.88)	0.849	
S + E	37 (2.31)	38.42 (2.06)	0.674	
CI	36.28 (1.67)	39.16 (1.07)	0.097	
Control	40.26 (3.30)	43.53 (3.75)	0.092	
<b>hs-CRP, mg/L</b>				0.553
E + S	9.94 (3.84)	6.95 (3.20)	0.057	
S + E	9.61 (3.41)	6.12 (2.40)	0.131	
CI	6.21 (1.83)	3.39 (8.26)	0.112	
Control	4.3 (1.25)	4.69 (7.25)	0.694	
<b>Adiponectin</b>				0.33
E + S	13.92 (0.89)	16.04 (1.93)	0.22	
S + E	14.76 (1.64)	16.10 (1.81)	0.16	
CI	15.70 (1.05)	15.77 (1.03)	0.90	
Control	14.50 (0.69)	14.44 (0.87)	0.93	
<b>Chemerin</b>				0.56
E + S	412.12 (49.52)	433.37 (47.17)	0.03 <sup>c</sup>	
S + E	409.10 (28.46)	426.55 (21.12)	0.25	
CI	398.54 (37.42)	421.58 (40.23)	0.11	
Control	396.51 (37.49)	398.33 (37.52)	0.11	

<sup>a</sup>Control group, subjects who not participated in exercise training; E + S, Resistance after aerobic training; S + E, Resistance prior to aerobic training.

<sup>b</sup>Significant difference between two groups ( $P < 0.05$ ).

<sup>c</sup>Significant difference between two groups ( $P < 0.01$ ).

tion. In addition, Balducci et al. illustrated a reduction in hs-CRP after 12 months of exercise training in groups undergoing high-intensity aerobic and aerobic + resistance exercise, whereas no significant changes were found in the first three months of training (53). Of course one of the reasons is that the subjects in Balducci et al.'s study were subjects with diabetes, who had low-intensity physical activity and supervised high-intensity aerobic and aerobic plus resistance training during 12 months. Perhaps other factors that significantly change with exercise training are more powerful determinants of hs-CRP concentrations than of other cytokines like TNF- $\alpha$ .

This finding is inconsistent with the results obtained

by Jorge et al. (36), who concluded that combined training significantly reduced the hs-CRP concentration in patients with type 2 diabetes. Furthermore, Touvra et al. found that combined resistance and endurance training improved the hs-CRP concentration in patients with type 2 diabetes without altering the levels of IL-6 and TNF- $\alpha$  (54). Libardi et al. also verified that 16 weeks of combined training increased functional capacity, yet did not improve inflammatory biomarkers (IL-6 and TNF- $\alpha$ ), except the serum hs-CRP concentration, in middle-aged males (33).

Beavers et al. found that one year of combined aerobic, strength, balance and flexibility training did not improve body mass, hs-CRP and TNF- $\alpha$  in elderly males and

**Table 3.** The Comparison of Changes in the Measured Variables Before and After Eight Weeks of Exercise Interventions (Part 3)<sup>a</sup>

Variables	Mean (SD)		P Value	
	Pretest	Posttest	Within Groups	Between Groups
<b>Leptin</b>				0.10
E + S	169.40 (8.68)	176.24 (6.44)	0.21	
S + E	164.14 (3.87)	174.28 (6.34)	0.18	
CI	168.23 (5.49)	177.41 (3.77)	0.10	
Control	157.57 (6.13)	149.70 (7.39)	0.09	
<b>Omentin</b>				0.51
E + S	134.63 (34.17)	128.55 (30.91)	0.15	
S + E	134.80 (21.08)	106.45 (17.84)	0.18	
CI	116.08 (20.42)	109.20 (16.45)	0.13	
Control	155.35 (38.37)	140.69 (30.15)	0.27	
<b>Vaspin</b>				0.70
E + S	4.00 (0.95)	3.66 (0.68)	0.34	
S + E	3.15 (0.26)	3.10 (0.24)	0.59	
CI	2.83 (0.23)	2.75 (0.17)	0.16	
Control	3.31 (0.42)	3.06 (0.54)	0.35	

<sup>a</sup>Control group, subjects who not participated in exercise training; E + S, Resistance after aerobic training; S + E, Resistance prior to aerobic training.

females (55). As shown in the present study, there were no significant reductions in WHR and chronic inflammatory factors. It is possible that exercise interventions effectively improve chronic inflammation only in association with concomitant body fat loss, as there is a strong correlation between hs-CRP and body fat percentage (56).

Furthermore, the mechanisms underlying combined training-induced reductions in serum hs-CRP may include a reduction in some cytokines' production by reducing the adipose tissue (57). In the present study, no differences were found in the inflammatory biomarkers between all combined intervention groups.

In addition, the present study showed that following eight weeks of supervised combined training, there were no significant differences between groups regarding serum omentin levels. The results do not support the hypothesis that different orders of combined training lead to different effects on serum omentin levels. Unfortunately, there are no other studies to compare these results with our findings. The above mentioned findings seem to be inconsistent with prior results indicating that aerobic training was accompanied by increased serum omentin-1 levels (43).

Although the eight-week combined training in different orders decreased both BMI and body fat percentage, the changes were not significant enough to affect serum

omentin levels in elderly females. This hypothesis is not supported by the fact that serum omentin-1 levels increase after weight loss-induced regular exercise training (58).

The above findings demonstrated that, following E + S combined training, there was a significant difference in serum chemerin levels between pre- and posttests. Similar results have been found when examining the effects of 12-week training on chemerin concentrations. Chakaroun et al. showed that exercise training would reduce serum chemerin levels and might correlate with improved insulin resistance (40). Furthermore, Saremi et al. showed that 12 weeks of aerobic training decreases circulating chemerin significantly (42). In another study, they showed that 12 weeks of strength training caused a decrease in chemerin levels in subjects with metabolic syndrome (59). In addition, Neuparth et al. illustrated that a period of moderate-intensity walking would be sufficient to reduce chemerin levels in patients with type 2 diabetes (41). Malin et al. demonstrated that decreased chemerin induced-exercise was an important adipokine involved in metabolic syndrome in the elderly (44). It has been shown that a nordic walking intervention reduces chemerin in middle-aged males with impaired glucose regulation (60). The results of this study, regarding chemerin levels, are consistent with the reduction of body fat percentage, BMI, and WC in elderly subjects of our study. Saremi et al. also il-

illustrated that reduced serum chemerin levels were related to changes in abdominal fat after 12 weeks of aerobic exercise, which may play a crucial role in the regulation of macrophage infiltration into adipose tissue and serum inflammatory markers (i.e., chemerin, omentin and vaspin) (42). Generally, the results of the present study indicated that an eight-week course of combined training in E + S order is sufficient to reduce chemerin concentration. On the other hand, no change was seen in the S + E and CI groups.

Our findings showed that combined training with exercises in different orders is not related to a substantial reduction. This study found no reduction of vaspin levels in elderly females, which is inconsistent with the study by Barzegari et al. (61), who recently reported that exercise decreased the levels of vaspin in adult males with type 2 diabetes. On the other hand, the above findings are consistent with those of Kim et al. who reported that 12 weeks of aerobic exercise did not affect the plasma vaspin levels in obese male adolescents (62).

In general, studies dealing with the effect of different combined trainings on inflammation markers and adipokines are still controversial and require further research at molecular levels to clarify the correct effects of combined training in different orders on immunity and the precise cell source of adipokines. The main limitation of the present study was the lack of strength and endurance training groups.

Despite the fact that prior studies have shown that strength and endurance training interventions could improve proinflammatory and adipokine profiles in aged females, the current study did not prove the effectiveness of this kind of intervention to improve chronic inflammatory marker levels. The results highlight that combined training (endurance and resistance), independent of order, is an effective training method to reduce body fat and BMI in aged females.

## Acknowledgments

The authors acknowledge all the subjects, who participated in this study.

## Footnotes

**Authors' Contribution:** Study concept and design: Ebrahim Banitalebi and Mohammad Faramarzi; acquisition of data: Ebrahim Banitalebi, Zahra Mardanpour Shahrekordi and Laleh Bagheri; analysis and interpretation of data: Ebrahim Banitalebi, Zahra Mardanpour Shahrekordi and Laleh Bagheri; drafting of the manuscript: Ebrahim Banitalebi, Zahra Mardanpour

Shahrekordi and Laleh Bagheri; critical revision of the manuscript for important intellectual content: Ebrahim Banitalebi and Mohammad Faramarzi; statistical analysis: Ebrahim Banitalebi, Zahra Mardanpour Shahrekordi and Laleh Bagheri; administrative, technical and material support: Ebrahim Banitalebi, Mohammad Faramarzi, Zahra Mardanpour Shahrekordi, Laleh Bagheri, Sadegh Amani Shalamzari and Abdol Reza Kazemi; study supervision: Ebrahim Banitalebi, Mohammad Faramarzi, Sadegh Amani Shalamzari and Abdol Reza Kazemi.

**Funding/Support:** Funding was provided by the researchers.

## References

- Noroozian M. The elderly population in iran: an ever growing concern in the health system. *Iran J Psychiatry Behav Sci.* 2012;**6**(2):1-6. [PubMed: 24644476].
- Weinert BT, Timiras PS. Invited review: Theories of aging. *J Appl Physiol (1985).* 2003;**95**(4):1706-16. doi: 10.1152/japplphysiol.00288.2003. [PubMed: 12970376].
- Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured?. *Nutr Rev.* 2007;**65**(12 Pt 2):S173-6. [PubMed: 18240544].
- Gomez CR, Boehmer ED, Kovacs EJ. The aging innate immune system. *Curr Opin Immunol.* 2005;**17**(5):457-62. doi: 10.1016/j.coi.2005.07.013. [PubMed: 16084711].
- Pascot A, Lemieux S, Lemieux I, Prud'homme D, Tremblay A, Bouchard C, et al. Age-related increase in visceral adipose tissue and body fat and the metabolic risk profile of premenopausal women. *Diabetes Care.* 1999;**22**(9):1471-8. [PubMed: 10480511].
- Arai Y, Takayama M, Abe Y, Hirose N. Adipokines and aging. *J Atheroscler Thromb.* 2011;**18**(7):545-50. [PubMed: 21551960].
- Bouassida A, Chamari K, Zaouali M, Feki Y, Zbidi A, Tabka Z. Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. *Br J Sports Med.* 2010;**44**(9):620-30. doi: 10.1136/bjsm.2008.046151. [PubMed: 18927166].
- Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab.* 1996;**81**(9):3424-7. doi: 10.1210/jcem.81.9.8784109. [PubMed: 8784109].
- Filkova M, Liskova M, Hulejova H, Haluzik M, Gatterova J, Pavelkova A, et al. Increased serum adiponectin levels in female patients with erosive compared with non-erosive osteoarthritis. *Ann Rheum Dis.* 2009;**68**(2):295-6. doi: 10.1136/ard.2008.095737. [PubMed: 19139213].
- Auguet T, Quintero Y, Riesco D, Morancho B, Terra X, Crescenti A, et al. New adipokines vaspin and omentin. Circulating levels and gene expression in adipose tissue from morbidly obese women. *BMC Med Genet.* 2011;**12**:60. doi: 10.1186/1471-2350-12-60. [PubMed: 21526992].
- Youn BS, Kloting N, Kratzsch J, Lee N, Park JW, Song ES, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes.* 2008;**57**(2):372-7. doi: 10.2337/db07-1045. [PubMed: 17991760].
- Roman AA, Parlee SD, Sinal CJ. Chemerin: a potential endocrine link between obesity and type 2 diabetes. *Endocrine.* 2012;**42**(2):243-51. doi: 10.1007/s12020-012-9698-8. [PubMed: 22610747].
- de Souza Batista CM, Yang RZ, Lee MJ, Glynn NM, Yu DZ, Pray J, et al. Omentin plasma levels and gene expression are decreased in obesity. *Diabetes.* 2007;**56**(6):1655-61. doi: 10.2337/db06-1506. [PubMed: 17329619].



14. Woods JA, Lowder TW, Keylock KT. Can exercise training improve immune function in the aged?. *Ann N Y Acad Sci.* 2002;**959**:117-27. [PubMed: [11976191](#)].
15. Terra R, Silva SAG, Pinto VS, Dutra PML. Efeito do exercicio no sistema imune: resposta, adaptacao e sinalizacao celular. *Revista Brasileira Medicina Sport.* 2012;**18**(3):208-14. doi: [10.1590/s1517-86922012000300015](#).
16. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res.* 2005;**96**(9):939-49. doi: [10.1161/01.RES.0000163635.62927.34](#). [PubMed: [15890981](#)].
17. Buchner DM. Preserving mobility in older adults. *West J Med.* 1997;**167**(4):258-64. [PubMed: [9348757](#)].
18. Seguin R, Nelson ME. The benefits of strength training for older adults. *Am J Prev Med.* 2003;**25**(3 Suppl 2):141-9. [PubMed: [14552938](#)].
19. Winett RA, Williams DM, Davy BM. Initiating and maintaining resistance training in older adults: a social cognitive theory-based approach. *Br J Sports Med.* 2009;**43**(2):114-9. doi: [10.1136/bjism.2008.049361](#). [PubMed: [18628361](#)].
20. Rall LC, Roubenoff R, Cannon JG, Abad LW, Dinarello CA, Meydani SN. Effects of progressive resistance training on immune response in aging and chronic inflammation. *Med Sci Sports Exerc.* 1996;**28**(11):1356-65. [PubMed: [8933485](#)].
21. Drela N, Kozdron E, Szczypiorski P. Moderate exercise may attenuate some aspects of immunosenescence. *BMC Geriatr.* 2004;**4**:8. doi: [10.1186/1471-2318-4-8](#). [PubMed: [15456521](#)].
22. Coffey VG, Hawley JA. The molecular bases of training adaptation. *Sports Med.* 2007;**37**(9):737-63. [PubMed: [17722947](#)].
23. Hickson RC. Interference of strength development by simultaneously training for strength and endurance. *Eur J Appl Physiol Occup Physiol.* 1980;**45**(2-3):255-63. [PubMed: [7193134](#)].
24. Fisher G, McCarthy JP, Zuckerman PA, Bryan DR, Bickel CS, Hunter GR. Frequency of combined resistance and aerobic training in older women. *J Strength Cond Res.* 2013;**27**(7):1868-76. doi: [10.1519/JSC.ob013e31827367e0](#). [PubMed: [22996024](#)].
25. Cadore EL, Izquierdo M, Pinto SS, Alberton CL, Pinto RS, Baroni BM, et al. Neuromuscular adaptations to concurrent training in the elderly: effects of intrasession exercise sequence. *Age (Dordr).* 2013;**35**(3):891-903. doi: [10.1007/s11357-012-9405-y](#). [PubMed: [22453934](#)].
26. Docherty D, Sporer B. A proposed model for examining the interference phenomenon between concurrent aerobic and strength training. *Sports Med.* 2000;**30**(6):385-94. [PubMed: [11132121](#)].
27. Wilson JM, Marin PJ, Rhea MR, Wilson SM, Loenneke JP, Anderson JC. Concurrent training: a meta-analysis examining interference of aerobic and resistance exercises. *J Strength Cond Res.* 2012;**26**(8):2293-307. doi: [10.1519/JSC.ob013e31823a3e2d](#). [PubMed: [22002517](#)].
28. Cutts R, Burns S. Resistance and aerobic training sequence effects on energy consumption in females. *Int J Exerc Sci.* 2010;**3**(3):11.
29. Ho SS, Dhaliwal SS, Hills AP, Pal S. The effect of 12 weeks of aerobic, resistance or combination exercise training on cardiovascular risk factors in the overweight and obese in a randomized trial. *BMC Public Health.* 2012;**12**:704. doi: [10.1186/1471-2458-12-704](#). [PubMed: [23006411](#)].
30. Chtara M, Chaouachi A, Levin GT, Chaouachi M, Chamari K, Amri M, et al. Effect of concurrent endurance and circuit resistance training sequence on muscular strength and power development. *J Strength Cond Res.* 2008;**22**(4):1037-45. doi: [10.1519/JSC.ob013e31816a4419](#). [PubMed: [18545210](#)].
31. Gravelle BL, Blessing DL. Physiological Adaptation in Women Concurrently Training for Strength and Endurance. *J Strength Condition Res.* 2000;**14**(1):5-13. doi: [10.1519/00124278-200002000-00002](#).
32. Cadore EL, Izquierdo M, dos Santos MG, Martins JB, Rodrigues Lhullier FL, Pinto RS, et al. Hormonal responses to concurrent strength and endurance training with different exercise orders. *J Strength Cond Res.* 2012;**26**(12):3281-8. doi: [10.1519/JSC.ob013e318248ab26](#). [PubMed: [2222319](#)].
33. Libardi CA, Souza GV, Gaspari AF, Dos Santos CF, Leite ST, Dias R, et al. Effects of concurrent training on interleukin-6, tumour necrosis factor-alpha and C-reactive protein in middle-aged men. *J Sports Sci.* 2011;**29**(14):1573-81. doi: [10.1080/02640414.2011.609896](#). [PubMed: [21933039](#)].
34. Conraads VM, Beckers P, Bosmans J, De Clerck LS, Stevens WJ, Vrints CJ, et al. Combined endurance/resistance training reduces plasma TNF-alpha receptor levels in patients with chronic heart failure and coronary artery disease. *Eur Heart J.* 2002;**23**(23):1854-60. [PubMed: [12445534](#)].
35. Stefanov T, Vekova A, Bonova I, Tzvetkov S, Kurktschiev D, Bluhner M, et al. Effects of supervised vs non-supervised combined aerobic and resistance exercise programme on cardiometabolic risk factors. *Cent Eur J Public Health.* 2013;**21**(1):8-16. [PubMed: [23741891](#)].
36. Jorge ML, de Oliveira VN, Resende NM, Paraiso LF, Calixto A, Diniz AL, et al. The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. *Metabolism.* 2011;**60**(9):1244-52. doi: [10.1016/j.metabol.2011.01.006](#). [PubMed: [21377179](#)].
37. Miyatake N, Wada J, Nakatsuka A, Sakano N, Teshigawara S, Miyachi M, et al. Serum vaspin levels are associated with physical activity or physical fitness in Japanese: a pilot study. *Environ Health Prev Med.* 2014;**19**(3):200-6. doi: [10.1007/s12199-013-0375-1](#). [PubMed: [24390774](#)].
38. Oberbach A, Kirsch K, Lehmann S, Schlichting N, Fasshauer M, Zarse K, et al. Serum vaspin concentrations are decreased after exercise-induced oxidative stress. *Obes Facts.* 2010;**3**(5):328-31. doi: [10.1159/000321637](#). [PubMed: [20975299](#)].
39. Keslacy S, Gladstone M, Kim Y. Exercise regulates obesity-induced chemerin expression in a tissue-dependent manner. *FASEB Journal (Meeting Abstracts)*; 2013. p. 712.12.
40. Chakaroun R, Raschpichler M, Klotting N, Oberbach A, Flehmig G, Kern M, et al. Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. *Metabolism.* 2012;**61**(5):706-14. doi: [10.1016/j.metabol.2011.10.008](#). [PubMed: [22136911](#)].
41. Neuparth MJ, Proenca JB, Santos-Silva A, Coimbra S. The positive effect of moderate walking exercise on chemerin levels in Portuguese patients with type 2 diabetes mellitus. *J Invest Med.* 2014;**62**(2):350-3. doi: [10.2311/JIM.0000000000000025](#). [PubMed: [24217118](#)].
42. Saremi A, Shavandi N, Parastesh M, Daneshmand H. Twelve-week aerobic training decreases chemerin level and improves cardiometabolic risk factors in overweight and obese men. *Asian J Sports Med.* 2010;**1**(3):151-8. [PubMed: [22375203](#)].
43. Saremi A, Asghari M, Ghorbani A. Effects of aerobic training on serum omentin-1 and cardiometabolic risk factors in overweight and obese men. *J Sports Sci.* 2010;**28**(9):993-8. doi: [10.1080/02640414.2010.484070](#). [PubMed: [20544489](#)].
44. Malin SK, Navaneethan SD, Mulya A, Huang H, Kirwan JP. Exercise-induced lowering of chemerin is associated with reduced cardiometabolic risk and glucose-stimulated insulin secretion in older adults. *J Nutr Health Aging.* 2014;**18**(6):608-15. doi: [10.1007/s12603-014-0459-7](#). [PubMed: [24950152](#)].
45. Davies PS. Body composition assessment. *Arch Dis Child.* 1993;**69**(3):337-8. [PubMed: [8215541](#)].
46. Alkan BM, Ozel S, Sutbeyaz ST, Culha C. The Effects of the Aerobics Exercise Program on the Cardiopulmoner Capacity and Disease Symptoms of the Patients with Primary Fibromialgia Syndrome\*. *Open Journal of Rheumatology and Autoimmune Diseases.* 2013;**03**(04):202-8. doi: [10.4236/ojra.2013.4031](#).
47. Shields N, Taylor NF. A student-led progressive resistance training program increases lower limb muscle strength in adolescents with Down syndrome: a randomised controlled trial. *J Physiother.* 2010;**56**(3):187-93. [PubMed: [20795925](#)].
48. Mayhew JL, Brechue WF, Smith AE, Kemmler W, Lauber D, Koch AJ. Impact of testing strategy on expression of upper-body work capacity

- and one-repetition maximum prediction after resistance training in college-aged men and women. *J Strength Cond Res.* 2011;**25**(10):2796-807. doi: [10.1519/JSC.0b013e31822dcea0](https://doi.org/10.1519/JSC.0b013e31822dcea0). [PubMed: [21904231](https://pubmed.ncbi.nlm.nih.gov/21904231/)].
49. Vilacxa Alves J, Saavedra F, Simao R, Novaes J, Rhea MR, Green D, et al. Does aerobic and strength exercise sequence in the same session affect the oxygen uptake during and postexercise?. *J Strength Cond Res.* 2012;**26**(7):1872-8. doi: [10.1519/JSC.0b013e318238e852](https://doi.org/10.1519/JSC.0b013e318238e852). [PubMed: [21986689](https://pubmed.ncbi.nlm.nih.gov/21986689/)].
  50. Di Blasio A, Gemello E, Di Iorio A, Di Giacinto G, Celso T, Di Renzo D, et al. Order effects of concurrent endurance and resistance training on post-exercise response of non-trained women. *J Sports Sci Med.* 2012;**11**(3):393-9. [PubMed: [24149345](https://pubmed.ncbi.nlm.nih.gov/24149345/)].
  51. Kuusmaa M. Effects of 24 weeks of single session combined strength and endurance training on body composition and fitness: examination of order effect. University of Jyväskylä; 2013 Spring. .
  52. Tsukui S, Kanda T, Nara M, Nishino M, Kondo T, Kobayashi I. Moderate-intensity regular exercise decreases serum tumor necrosis factor-alpha and HbA1c levels in healthy women. *Int J Obes Relat Metab Disord.* 2000;**24**(9):1207-11. [PubMed: [11033992](https://pubmed.ncbi.nlm.nih.gov/11033992/)].
  53. Balducci S, Zanuso S, Nicolucci A, Fernando F, Cavallo S, Cardelli P, et al. Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutr Metab Cardiovasc Dis.* 2010;**20**(8):608-17. doi: [10.1016/j.numecd.2009.04.015](https://doi.org/10.1016/j.numecd.2009.04.015). [PubMed: [19695853](https://pubmed.ncbi.nlm.nih.gov/19695853/)].
  54. Touvra AM, Volaklis KA, Spassis AT, Zois CE, Douda HD, Kotsa K, et al. Combined strength and aerobic training increases transforming growth factor-beta1 in patients with type 2 diabetes. *Hormones (Athens).* 2011;**10**(2):125-30. [PubMed: [21724537](https://pubmed.ncbi.nlm.nih.gov/21724537/)].
  55. Beavers KM, Hsu FC, Isom S, Kritchevsky SB, Church T, Goodpaster B, et al. Long-term physical activity and inflammatory biomarkers in older adults. *Med Sci Sports Exerc.* 2010;**42**(12):2189-96. doi: [10.1249/MSS.0b013e3181e3ac80](https://doi.org/10.1249/MSS.0b013e3181e3ac80). [PubMed: [20421832](https://pubmed.ncbi.nlm.nih.gov/20421832/)].
  56. Vahdat K, Azizi F, Zandi K, Assadi M, Nabipour I. Chronic inflammation is correlated with percentage of body fat independent of the burden of infection. *Inflammation.* 2012;**35**(4):1322-9. doi: [10.1007/s10753-012-9445-6](https://doi.org/10.1007/s10753-012-9445-6). [PubMed: [22382515](https://pubmed.ncbi.nlm.nih.gov/22382515/)].
  57. Steene-Johannessen J, Kolle E, Reseland JE, Anderssen SA, Andersen LB. Waist circumference is related to low-grade inflammation in youth. *Int J Pediatr Obes.* 2010;**5**(4):313-9. doi: [10.3109/17477160903497035](https://doi.org/10.3109/17477160903497035). [PubMed: [20078378](https://pubmed.ncbi.nlm.nih.gov/20078378/)].
  58. Moreno-Navarrete JM, Catalan V, Ortega F, Gomez-Ambrosi J, Ricart W, Fruhbeck G, et al. Circulating omentin concentration increases after weight loss. *Nutr Metab (Lond).* 2010;**7**:27. doi: [10.1186/1743-7075-7-27](https://doi.org/10.1186/1743-7075-7-27). [PubMed: [20380714](https://pubmed.ncbi.nlm.nih.gov/20380714/)].
  59. Saremi A, Moslehabadi M, Parastesh M. Effects of twelve-week strength training on serum chemerin, tnf- $\alpha$  and crp level in subjects with the metabolic syndrome. *Iran J Endocrinol Metabol.* 2011;**12**(5):536-43.
  60. Venojarvi M, Wasenius N, Manderoos S, Heinonen OJ, Hernelahti M, Lindholm H, et al. Nordic walking decreased circulating chemerin and leptin concentrations in middle-aged men with impaired glucose regulation. *Ann Med.* 2013;**45**(2):162-70. doi: [10.3109/07853890.2012.727020](https://doi.org/10.3109/07853890.2012.727020). [PubMed: [23110613](https://pubmed.ncbi.nlm.nih.gov/23110613/)].
  61. Barzegari A, Amouzad Mahdirezaji H. Effects of 8 weeks resistance training on plasma vaspin and lipid profile levels in adult men with type 2 diabetes. *Caspian J Intern Med.* 2014;**5**(2):103-8. [PubMed: [24778786](https://pubmed.ncbi.nlm.nih.gov/24778786/)].
  62. Kim JY, Kim ES, Jeon JY, Jekal Y. Improved Insulin Resistance, Adiponectin and Liver Enzymes without Change in Plasma Vaspin Level after 12 Weeks of Exercise Training among Obese Male Adolescents. *The Korean Journal of Obesity.* 2011;**20**(3):138. doi: [10.7570/kjo.2011.20.3.138](https://doi.org/10.7570/kjo.2011.20.3.138).