

# The Effect of 12 Weeks Endurance, Resistance and Combined Exercise Training on the Levels of Pentraxin 3 and TNF- $\alpha$ in Sedentary Women With Obesity

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**Abstract-** It's reported that different exercise training modalities can exert anti-inflammatory and cardioprotective effects, and present study conducted for investigating the changes in the levels of pentraxin 3 and TNF- $\alpha$  following 12 weeks endurance, resistance and combined training in sedentary women with obesity. The 48 sedentary women aged 20-35 years old with average body mass index  $32.1 \pm 1.26 \text{ kg.m}^2$  selected among voluntaries for take part in this research and randomly divided in four 12-subject groups: combined (endurance and resistance), control, endurance and resistance groups. The 12 weeks (three session weekly) duration protocol, designed for all training modalities. The 60 % of reserve heart rate and 75 % of 1RM, considered for intensity of endurance and resistance training program respectively. Blood sampling performed at the baseline and 2 days after last training session. Following measurement of variables levels, data were analyzed with ANCOVA test and Bonferroni post-hoc test. Pentraxin 3 levels don't change significantly after 12 weeks interventions ( $P=0.402$ ), but TNF- $\alpha$  level significantly decreased in endurance ( $P=0.033$ ) and combined ( $P<0.001$ ) training groups compared to the control group. Although, body fat percentage reduction was observed in all trained groups ( $P<0.001$ ), but its reduction was also significant in endurance and combined training groups compared to the resistance training group ( $P<0.001$ ). Although we don't observe a significant change in the pentraxin 3 levels with different training, it seems that the anti-inflammatory effect of endurance and combined training (indicated by TNF- $\alpha$  reduction) is related to the further effect of these training in decrease in body fat mass, as a main source of inflammation.

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**Keywords:** Exercise training; Pentraxin 3; Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ); Inflammation

## Introduction

In addition to genetic background, due to the sedentary lifestyle and unhealthy diet, obesity prevalence and its related pathologies, remarkably enhanced during previous years (1). Because of overweight and obesity role in the occurrence of various disorders, 5% of all deaths worldwide are attributed to the obesity, and it imposes a huge economic burden on governments, families, and individuals (2). Obesity is not only a state of ectopic accumulation of fat mass, but also is associated with secretion of biologically active molecules (named adipokines) into the blood circulation, which can affect the organs and tissue metabolism (3). Various processes,

including modulation of energy and appetite, metabolism of glucose and lipid, hemostasis, insulin sensitivity, atherosclerosis development, inflammation, angiogenesis, and metabolic syndrome, affected by adipokines (4).

Obesity is associated with inducing inflammation in adipose tissue and altered (dysregulation) secretion of adipokines, which finally cause a systemic inflammation (5). Accordingly, obesity known as chronic low-grade inflammatory condition (6). The inflammatory factors such as interleukin 6 (IL-6), C reactive protein (CRP) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), progressively increase in the blood flow, simultaneously with the enhancing adipose tissue (7). TNF- $\alpha$  is a cytokine with pleiotropic

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effects, which is involved in the various autoimmune and inflammatory disorders (8). Moreover, the TNF- $\alpha$  upregulation can impair the insulin signaling and cause insulin resistance in adipocytes and peripheral tissues. Therefore, anti-TNF- $\alpha$  treatment strategies have been developed to attenuating the enhanced TNF- $\alpha$  related side effects (9). Pentraxin 3 is another adipose tissue secreted protein, which considered as an important adipokine (10). In addition to adipose tissue, pentraxin 3 secreted by various tissues in response to several inflammatory stimuli such as TNF- $\alpha$  and IL-1 (11).

The higher pentraxin 3 level has been reported in the overweight and obese compared to the normal weight subjects, which in turn can increase the risk of cardiovascular diseases (12). Furthermore, pentraxin 3 is introduced as a biomarker for metabolic syndrome and researchers suggested a positive correlation between its levels and severity of metabolic syndrome (13). In addition, higher circulating levels of pentraxin 3 were observed in prediabetes and type 2 diabetes (T2D) compared with normoglycemic persons (14). Therefore, due to pathological effects of inflammatory mediators including TNF- $\alpha$  and pentraxin 3, the reduction or inhibition of these levels can exert a therapeutic effect.

Exercise training is well known strategy for attenuating the inflammation, and reduction of various inflammatory cytokines (such as IL-6, TNF- $\alpha$ , ...) were observed after chronic exercise training specially in obese participants (15). However, the results regarding to pentraxin 3 is contradiction, and no change (16), increase (17) and decrease (18) of pentraxin 3 have been reported after take part in the exercise training program. Probably, contradictory findings about exercise training effect on the levels of pentraxin 3 are related to the difference in the duration, type and intensity of conducted exercise training, participants characteristics, different method for measuring the pentraxin 3 levels, and other variables. Collectively, because of contradictory and limited findings specially in obese subjects, we aimed to compare the effect of endurance, resistance and combined exercise training for 12 weeks on the levels of pentraxin 3 and TNF- $\alpha$  in sedentary women with obesity.

## Materials and Methods

### Subjects

The Iranian (Tehran, region 12) women aged 20-35 years old with BMI higher than 30 kg.m<sup>2</sup> (i.e. women with obesity) recruited for completing the considered protocols. Among attended volunteers, the researchers selected the 48 eligible women randomly as research

subjects, which subsequently assigned in one of the following groups: combined training (endurance and resistance), control, endurance and resistance training groups.

### Procedures

This semi-experimental study conducted based on the pre-test and post-test design. We followed all ethical guidelines and ethical committee of faculty of physical education and sport sciences, university of Tehran (code: R.UT.SPORT.REC.1400.006) and Iranian registry of clinical trials (code: IRCT20210626051720N1) confirmed our protocol. The eligible obese health women (BMI: 30-35 kg.m<sup>2</sup>) recruited for take part in this research protocol. Potential side effects or benefits for combined, resistance and endurance training protocols were explained to all attended volunteers at the baseline. Unqualified volunteers were excluded from the research, and among eligible volunteers, we randomly selected 48 obese women for considered research groups, and they were asked to sign the informed consent. Following the participants selection and few days after, fasting blood samples collected and subsequently, the baseline physical characteristic including weight, height and body fat mass were measured. The 48 participants randomly divided into 4 groups including 3 training (combined, endurance, resistance) groups and one control (continuing their routine lifestyle as before the intervention) group. In the next step, intervention started for 12 weeks in all research groups.

### Inclusion and exclusion criteria

The inclusion criteria: healthy obese women (without T2D, cardiovascular disease, hypertension, etc.), without heart failure and stroke history, inactive lifestyle in the last 12 months, not consumption of medications or supplements during intervention and 2 months before, physical ability to participate in the exercise training programs, and voluntary participation without any force. exclusion criteria: absence from 2 or more training sessions resulted in removal subjects from research, exercise related or other injuries which was associated with subjects' inability to continue training protocol in remained sessions, unwillingness to complete intervention due to personal reasons.

### Training programs

All three exercise training programs (combined, resistance, endurance) are exerted for 12 weeks/ three sessions weekly, at the certain time of day (9 to 11 AM) by exercise specialists. In all training modalities,

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participants first performed 10 min warm-up and finally 5-7 min cool-down. Endurance training sessions conducted on treadmill with 60 percent of reserve heart rate intensity, which each session consists of 30 min walking/ running. Each resistance training session includes the leg press, leg curl, leg extension, bench press and seated rowing. Resistance training intensity was 75% of one repetition maximum (1RM), with 8-12 repetition for each exercise and totally four sets with one-minute rest between sets. For combined training group, resistance and endurance exercise performed respectively in each session. However, training volume was half of endurance and resistance training groups in the combined training group. In fact, combined training consists of resistance exercise (similar intensity and repetitions with resistance training group but in two set) and 15 min endurance training (similar intensity to endurance group) in each session (19). The participants 1RM were measured according to following formula (20).

$$1RM = W / [102.78 - 2.78(R)] / 100,$$

### Blood sampling and assays

Blood samples collected from all subjects in the two stages: 1) before and 2) after (48 hours after final training session) 12 weeks interventions. For this purpose, participants were asked to attend the gym (8-9 AM) after 10-12 hours nigh fasting. After sitting in the environment for 30 min, blood samples (7 ml) were collected by a laboratory technician from forearm venue of right hand. For both blood sampling stages, the participants were avoided from strenuous physical activity for two days before blood sampling. On the other hand, they were asked to have enough rest specially in the last night before

collection of blood samples. Immediately after blood taking, the samples poured into the falcon tube and centrifuged for 10 min at 3000 rpm to remove the serum samples. The ELISA assay was used for measurement of serum levels of pentraxin 3 (Cusabio company, catalog number: CSB-E12926h, sensitivity: 19.5 pg/ml) and TNF- $\alpha$  (Cusabio company, catalog number: CSB-E04740h, sensitivity: 1.95 pg/mL) according to ELISA kits manufacturer instructions. Body fat percentage determined by BOCA-X1 body composition analyzer, made in South Korea.

### Statistical analysis

The SPSS software version 26 was used for data analysis. The Shapiro-Vilk test indicated that data distribution is normal. Whitin and between groups difference determined by paired t test and analysis of covariance (Ancova) test (with Bonferroni post hoc test) respectively. The significance levels were  $P < 0.05$  for all statistical analysis.

### Results

All 48 participants completed the considered interventions and included in the final data analysis. The subject's height, weight, body mass index (BMI), maximal oxygen consumption ( $VO_{2max}$ ) and percent body fat have been reported in table 1 for control (C), endurance (ET), resistance (RT) and combined (CT) training groups. In addition, within groups difference after 12 weeks intervention compared with baseline, reported in table 1.

**Table 1. The levels of variables (Mean $\pm$ SD)**

Groups/ Variables	Stage	C	ET	RT	CT
Age (years)	pretest	27.3 $\pm$ 3.63	26.6 $\pm$ 3.35	27.5 $\pm$ 2.67	27.8 $\pm$ 3.81
Height (cm)	pretest	159.9 $\pm$ 3.76	158.5 $\pm$ 3.13	161.3 $\pm$ 4.38	160.2 $\pm$ 5.17
Body Weight (kg)	pretest	81.4 $\pm$ 4.99	81.9 $\pm$ 5.17	83.1 $\pm$ 4.09	82.7 $\pm$ 4.75
	post test	81.5 $\pm$ 5.01	78.7 $\pm$ 4.41	82.6 $\pm$ 3.90	79.8 $\pm$ 4.48
Paired t test		0.037	<0.001	0.004	<0.001
$VO_{2max}$ (ml.kg)	pretest	30.2 $\pm$ 2.72	32.7 $\pm$ 3.36	31.2 $\pm$ 2.67	30.6 $\pm$ 2.18
	post test	30.9 $\pm$ 3.03	37.2 $\pm$ 3.73	32.9 $\pm$ 2.71	34.7 $\pm$ 2.71
Paired t test		0.027	<0.001	<0.001	<0.001
BMI (kg.m <sup>2</sup> )	pretest	31.81 $\pm$ 1.13	32.58 $\pm$ 1.56	31.97 $\pm$ 1.38	32.23 $\pm$ 0.92
	post test	31.87 $\pm$ 1.11	31.32 $\pm$ 1.35	31.77 $\pm$ 1.32	31.09 $\pm$ 0.86
Paired t test		0.037	<0.001	0.004	<0.001
Percent Body Fat (%)	pretest	36.9 $\pm$ 2.36	39.1 $\pm$ 3.17	38.5 $\pm$ 2.81	38.7 $\pm$ 3.13
	post test	37.1 $\pm$ 2.69	35.0 $\pm$ 2.56	36.9 $\pm$ 2.42	35.4 $\pm$ 2.85
Paired t test		0.302	<0.001	<0.001	<0.001

Control (C), Endurance Training (ET), Resistance Training (RT), Combined Training (CT).

The two-way ANOVA test output indicated a significant difference between groups for body weight,

BMI,  $VO_{2max}$  and percent body fat ( $P<0.001$ ). Significant difference was found for percent body fat between all trained (endurance, resistance, and combined training groups) groups with control group ( $P<0.001$ ). There was a significant difference for body weight and BMI between combined and endurance training groups with control and resistance training groups ( $P<0.001$ ). Moreover, significant differences were found for  $VO_{2max}$  between combined and endurance trainings groups with control and resistance training groups ( $P<0.001$ ) (table 1). The Bonferroni post hoc test findings for body weight, BMI,  $VO_{2max}$  and percent body fat have been reported in table 2.

The two-way ANOVA test results suggested that change in the serum levels of pentraxin 3 wasn't significant between different groups including control and trainings groups after 12 weeks intervention ( $P=0.052$ ). In addition, within groups analysis with paired t test indicated no significant changes for pentraxin 3 levels in the control ( $P=0.430$ ), endurance ( $P=0.095$ ),

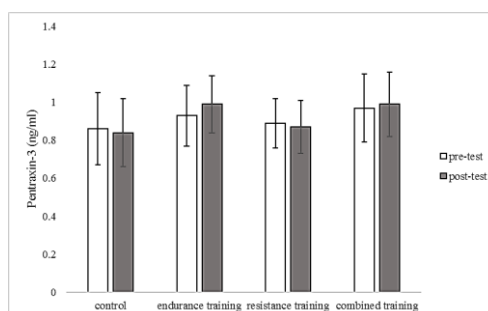
resistance ( $P=0.420$ ) or combined ( $P=0.393$ ) training groups. Pre and post-test levels of pentraxin 3 have been reported in figure 1.

The TNF- $\alpha$  levels data analysis represented a significant difference between groups ( $P<0.001$ ). Based on Bonferroni post hoc test findings, the levels of TNF- $\alpha$  in the endurance ( $P=0.033$ ) and combined ( $P<0.001$ ) training groups significantly decreased compared to control groups. Moreover, TNF- $\alpha$  reduction was also significant in the combined training group compared with resistance training group ( $P=0.012$ ). On the other hand, paired t test indicated a significant reduction in the levels of TNF- $\alpha$  for endurance ( $P=0.004$ ) and combined ( $P<0.001$ ) training groups, but not for resistance ( $P=0.219$ ) training group compared with baseline. Serum levels of TNF- $\alpha$  in the baseline and after training programs, reported in figure 2. In addition, the Bonferroni post hoc test findings for TNF- $\alpha$  levels have been reported in table 3.

**Table 2. The Bonferroni post hoc test findings for body weight, BMI,  $VO_{2max}$  and percent body fat**

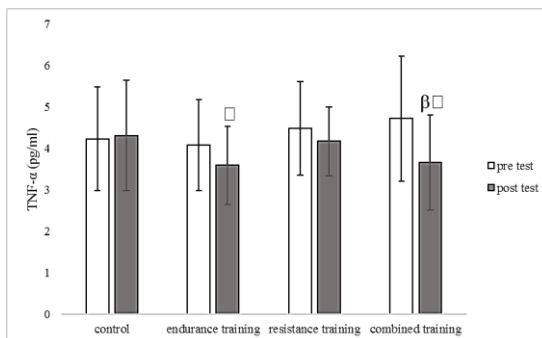
Variable	Group	Group	P
Percent Body Fat	C	ET	<0.001
		RT	<0.001
		CT	<0.001
	ET	RT	<0.001
		CT	0.149
		CT	<0.001
Body Weight	C	ET	0.148
		RT	<0.001
		CT	<0.001
	ET	RT	<0.001
		CT	0.957
		CT	<0.001
BMI	C	ET	0.059
		RT	<0.001
		CT	<0.001
	ET	RT	<0.001
		CT	1.000
		CT	<0.001
$VO_{2max}$	C	ET	<0.001
		RT	0.329
		CT	<0.001
	ET	RT	<0.001
		CT	1.000
		CT	<0.001

Control (C), Endurance Training (ET), Resistance Training (RT), Combined Training (CT).



**Figure 1. Pentraxin 3 levels in the different groups**

**Effect of different exercise training on pentraxin 3 and TNF- $\alpha$  levels**



**Figure 2.** TNF- $\alpha$  levels in the different groups. \* Significant decrease compared with control group.  $\beta$  significant reduction compared with resistance training group

**Table 3. The Bonferroni post hoc test findings for TNF- $\alpha$  levels**

Variable	Group	Group	P
TNF- $\alpha$	C	ET	0.033
		RT	0.706
		CT	<0.001
	ET	RT	1.000
		CT	0.350
	RT	CT	0.012

Control (C), Endurance Training (ET), Resistance Training (RT), Combined Training (CT).

**Discussion**

The present study was aimed to investigate the effect of 12 weeks endurance, resistance and combined exercise training on the levels of pentraxin 3 and TNF- $\alpha$  in obese women. According to the present study findings, the pentraxin 3 levels don't change with different conducted training programs. The contradictory findings have been reported about the changes in the levels of pentraxin 3 with obesity. Significantly higher levels of pentraxin 3 is reported in the overweight and obese persons compared with normal weight persons, and its upregulation considered as a cardiovascular risk factor (12). On the other hand, due to enhanced serum levels of pentraxin 3 in the children and adolescents with metabolic syndrome, researchers concluded that pentraxin 3 can be a new metabolic syndrome biomarker (13). In another study and contrary to the above mentioned findings, researchers observed that pentraxin 3 is negatively correlated with BMI, waist circumference and body fat mass, and weight loss was associated with increasing the pentraxin 3 levels (21). However, we observed that despite BMI and body fat percentage reduction following different types of exercise training (endurance, resistance, combined), there was no significant change in serum pentraxin 3 levels.

Consistent with the present study findings, its reported that eight weeks aerobic training in active and sedentary elderly subjects had no significant effect on plasma levels of pentraxin 3, but pentraxin 3 expression in the peripheral blood mononuclear cells (PBMCs) was

significantly decreased, and reduction of PTX3/TLR4 ratio was observed (22). In another study, Hovsepian *et al.* confirmed our findings and showed that 10 weeks HIIT on an ergometer (four 4-minute intervals with 85-90% of maximum heart rate followed by three-minute active rest intervals) don't has significant effect on pentraxin 3 levels in overweight and obese women (16). In addition, Liu *et al.*, which investigate the effect of 12 weeks combined (aerobic and resistance) training on serum levels of pentraxin-3 in T2D patients, reported no significant changes in pentraxin-3 levels. These researchers suggested that positive effects of combined training in patients with T2D (decrease in insulin resistance and improving lipid profile) can be exerted independent from change in the pentraxin-3 levels (23). Similarly, we observed significant decrease in body fat percentage in obese women after 12 weeks endurance, resistance, and combined trainings, without significant changes in pentraxin-3 levels.

Unlike present findings, Zempo *et al.*, indicated that plasma pentraxin 3 significantly increased after eight weeks of aerobic training in normal weight middle-aged and elderly adults. Comparing the pentraxin 3 levels in different weeks, it showed that its levels increased significantly from the fourth week, and pentraxin 3 upregulation was associated with a decrease in arterial stiffness. The researchers suggested that, due to expression of pentraxin 3 in the various body tissues, including endothelial cells, fibroblasts, hepatocytes, monocytes, heart, skeletal muscles, ovary, kidney, lung,

etc., the origin of enhanced pentraxin 3 levels by exercise training is unknown (17). However, enhancing the pentraxin-3 levels with exercise training can be partly attributed to its secretion from skeletal muscles. In this regard, Slusher *et al.*, reported a significant increase in the levels of pentraxin-3 following acute high intensity interval exercise in obese and normal weight men, which significant further increase were observed in the normal weight men (24). These findings emphasize the participants physical characteristics importance for observed changes in the levels of pentraxin-3 by exercise session. In addition to exercise training, it seems that nutritional status could also influence the pentraxin 3 levels, and its reported that 12 weeks dietary modification alone and combined with aerobic training resulted in a significant increase in plasma pentraxin 3 in overweight and obese men (25). Regarding the importance of subjects' physical characteristics, some researchers observed that despite enhancing pentraxin 3 levels after eight weeks HIIT in healthy and T2D patients, enhancing the levels of pentraxin 3 was statistically significant only in the healthy group (26). Collectively, the results about exercise training effect on the levels of pentraxin 3 are limited and contradictory. Therefore, the mechanism for change in the levels of pentraxin 3 (decrease or increase) by exercise training is remarkably unknown and should be determined in future studies.

Moreover, present study findings indicated that 12 weeks combined and endurance training resulted in significant decrease in TNF- $\alpha$  levels compared to control group, and TNF- $\alpha$  reduction in the combined training group was also significant compared to the resistance training group. TNF- $\alpha$  decreased 12, 7.12 and 22.45 percent respectively in the endurance, resistance and combined training groups. These findings emphasize the further anti-inflammatory (confirmed by reduction of TNF- $\alpha$  in the present study) effect of combined training. The lower impact of resistance training in modulating the TNF- $\alpha$  (as an inflammatory marker) levels compared to endurance and combined training is probably related to less reduction of body fat mass in this group. We observed 10.48, 4.15, and 8.52 percent reduction in the body fat percentage by endurance, resistance, and combined training respectively, which confirms the above hypothesis. Despite body fat percentage reduction with resistance training compared to control group, its reduction in the combined and endurance training groups was also significant compared to the resistance training group. Similar to our research findings, some researchers suggested a lower anti-inflammatory property for resistance training, and for increasing the resistance

training effect in modulating the systemic inflammation, the researchers recommended performing this training simultaneously with aerobic training (27).

Regarding to importance of change in the body fat mass for mediating the inflammatory markers, Mateus *et al.*, found that even long-term (24 weeks) exercise training without weight loss and reduction of body fat percentage, can't change the TNF- $\alpha$  levels (28). Therefore, it seems that without body composition improvement, the levels of inflammatory factors don't change significantly. Obesity and expanded adipose tissue play an important role in the development of systemic inflammation, due to obesity role in decreasing the secretion of anti-inflammatory cytokines such as adiponectin, and increasing the secretion of pro-inflammatory cytokines such as IL-6, TNF- $\alpha$ , and MCP-1 (29). Accordingly, body fat mass reduction can be considered as one of the main mechanisms for exerting the anti-inflammatory effects of exercise training, as confirmed in the present study.

Consistent to the present study results, exercise training introduced as an anti-inflammatory intervention, which its role in the reduction of wide arrays of inflammatory markers including CRP, IL-6 and TNF- $\alpha$  have been confirmed. In addition, improving cardiometabolic risks through exercise training partly exerted by improving inflammatory status (30). Although, exercise training effect in attenuating the inflammation is remarkably due to adipose tissue reduction and changes in the adipose tissue structure (increase angiogenesis, increase blood supply, decrease hypoxia, decrease macrophage infiltration) (31), but other mechanisms are involved in exercise training induced anti-inflammatory effects. It suggested that, skeletal muscles induced cytokine (known as myokine) such as IL-6, play important role in decreasing inflammation by exercise training including in obese people (32). In addition, exercise training related decrease in systemic inflammation can be attributed to the upregulation of anti-inflammatory cytokine including IL-10, decrease M1 (IL-1 $\beta$ , TNF- $\alpha$ ) macrophages and increase M2 (IL-10, IL-1ra) macrophages (33). According to present study findings, it seems that exercise training positive effects in obese participants can be observed without change in the levels of pentraxin 3. Moreover, despite body composition improvement with different types of exercise training, only endurance and combined training groups indicated a TNF- $\alpha$  reduction, with further effect in combined training. Unfortunately, we don't investigate the change in the levels of other inflammatory and anti-inflammatory cytokines, and probably the conducted exercise training

especially resistance training benefits is associated with changes in other cytokines, which should be determined in other researches.

We observed that pentraxin 3 levels don't change with different exercise training, and probably positive effects of exercise training exerted through changing the other factors and signaling pathways. In addition, endurance and combined training was more effective for decreasing inflammation (TNF- $\alpha$ ) compared to resistance training, which is due to further effect of these training in reduction of adipose tissue as main source of inflammation. Therefore, taking part in the endurance and especially combined training for obese and overweight peoples can be effective strategy for decrease in the cardiovascular risk factors.

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