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# The effect of three month combined training on the serum levels of interleukin-6 and C-reactive protein in sedentary obese women

Mahrokh Kooti[1], Rahman Soori[1]✉, Fatemeh Shabkhiz[1], Parisa Pournemati[1]

[1]Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, University of Tehran, Tehran, Iran

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✉**Correspondence to:**
Rahman Soori, Department of Sports Physiology, Faculty of Physical Education and Sport Sciences, North Kargar Street, between 15th and 16th Street, Tehran, Iran.
Postal Code: 14179-35771
Tel: +98 2188351730
Fax: +98 2188021527
Email: Soori@ut.ac.ir

**ABSTRACT**

**Introduction:** Combined training play important role in improving body composition, but less is known about its anti-inflammatory mechanism in obesity. Researcher in the present study investigated the effect of three-month combined exercise training on the serum levels of interleukin-6 and C-reactive protein in sedentary obese women.

**Materials and Methods:** The 24 obese women age ranging 20-35 years old with average body mass index (BMI) 32.02±1.03 kg/m$^2$ randomly allocated in 2 groups (12 participants in each group) including control and combined training (endurance-resistance) groups. Exercise training program conducted for 12 weeks and three session per week. Endurance training intensity was 60 percent of reserve heart rate and resistance training intensity was 75 percent of 1RM. Blood samples collected before and after 12 weeks training program and IL-6 and CRP levels were measured by Elisa method. Data were analyzed by means of SPSS software version 24 with analysis of covariance test.

**Results:** Present study findings indicated that serum levels of IL-6 in combined training group significantly decreased compared to control group ($P < 0.001$). In addition, significant decrease in CRP levels were observed in combined training group compared to control group ($P = 0.0188$), which decrease in inflammatory mediators was associated with significant decrease in percent body fat in combined training group ($P < 0.001$).

**Conclusion:** According to present study, combined training plays an important role in down-regulation of inflammatory mediators and the anti-inflammatory effect may be related to decrease in body fat mass as a main source for secreting the inflammatory mediators including CRP and IL-6.

**Keywords:** Exercise Training, Cytokine, Interleukin-6, Inflammation

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## Introduction

Obesity prevalence has tripled over the last three decades and obesity considered as one of the leading causes of deaths worldwide, because of its role in enhancing the risk of cardiovascular disease, diabetes, cancer and several other systemic disorders (1, 2). The obesity pathological effects have attributed to dysregulation of inflammatory and anti-inflammatory adipose tissue secreted factors, known as adipokines (3). Adipokines play an important role in the interaction of adipose tissue with other body tissues, and now hundreds of adipokines including leptin, fibroblast growth factor 21 (FGF21), endothelin 1 (ET-1), interleukin 6 (IL-6), tumour necrosis factor α (TNF-α), IL-1β, C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), adiponectin, IL-10, omentin-1, and cardiotrophin 1, have been identified (4). Due to the secretion of different types of inflammatory adipokines from adipose tissue and increased its levels in obese individuals, obesity considered as a chronic low grade inflammatory condition (5), and inflammation attracted a lot of attention as an independent risk factor for various types of metabolic and cardiovascular disorders (6).

IL-6 is a pro-inflammatory cytokine with a molecular weight of 25 kDa and consist of 184 amino acids (7), which express and secreted by different cell types including immune cells, endothelial cells, smooth and skeletal muscle cells, thyroid cells, fibroblasts, mesangial cells, keratinocytes, microglial cells, astrocytes, several types of tumor cells and pancreatic beta cells (8). In addition, adipocytes and adipose tissue macrophages have been recognized as main sources of circulating levels of IL-6 (9). Enhancing the expression and circulation levels of IL-6 are associated with the pathogenesis of various diseases, including chronic inflammatory diseases, autoimmune diseases, and tumor development (10), and IL-6 upregulation in different pathological condition including type 2 diabetes, atherosclerosis, depression, rheumatoid arthritis and several types of cancer have been reported, and its inhibition considered as a promising therapeutic target for above mentioned disorders (11). Moreover, IL-6 cause to increase in insulin resistance and risk of type 2 diabetes in obese persons by affecting different signaling pathways (12). In addition to the direct pathological effects of IL-6, it has been reported that IL-6 as a pleiotropic cytokine can induce several immune and physiological processes such as production of acute phase proteins including CRP and hepcidin, inflammation, hematopoiesis, apoptosis, differentiation, as well as affect the cellular metabolism (13, 14).

CRP is another inflammatory cytokine which produced and secreted predominantly by the liver in response to IL-6, and IL-1β synergistically enhanced the IL-6 synthesis (15). Although early studies have suggested that CRP is produced exclusively by the liver, further studies observed the CRP production by atherosclerotic lesions (especially smooth muscle cells), kidneys, neurons, and alveolar macrophages, and especially adipose tissue (16). Despite relatively low levels of CRP in healthy people, this cytokine levels significantly increased in response to infections, tissue damage, and inflammation (17), and higher levels of CRP result in incidence of different cardiovascular diseases in healthy men and women (18). Despite the pathological effects of inflammation, calorie restriction and physical exercise determined as effective treatment strategies for attenuate systemic inflammation (19). Some researchers suggested that exercise training and cardiorespiratory fitness are negatively correlated with inflammatory markers such as CRP (20). Although exercise training is known as effective anti-inflammatory intervention (21), the effects of different modalities of exercise training on the levels of inflammatory mediators are

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contradictory. Some researchers have reported a significant decrease in IL-6 and TNF-α levels after eight weeks resistance training (22), but others observed that despite significant decrease in CRP levels by aerobic and combined training in type 2 diabetic patients, resistance training don't have a significant effect on CRP level and further improvement were observed in combined training compared to resistance or aerobic training (23).

In contrast, in another study researchers indicated that eight weeks combined training in obese men with type 2 diabetes don't has a significant effect on inflammatory cytokines (TNF-α and IL-6) levels (24). Due to exercise training importance in management of obesity and inflammation, and contradictory findings regarding the effect of different modalities of exercise training such as combined training on the levels of inflammatory markers, the researcher in the present study investigated the effect of 12 weeks combined training on the serum levels of IL-6 and CRP in sedentary obese women.

## Materials and Methods

### Participants

Obese women (BMI: >30 kg/m$^2$) aged ranging 20 to 35 years old from Tehran, Iran, participated in the present study. The subjects were selected after recruitment and public call in the region 12 of Tehran. The 24 subjects randomly chosen among recruited obese women for take part in the considered intervention. All subjects participated in the present study voluntarily.

### Study design

The present study was a semi experimental research conducted as pre-test and post-test design using laboratory and field tests. This research protocol approved by ethical committee of faculty of physical education and sport sciences, university of Tehran with the following code: R.UT.SPORT.REC.1400.006. In addition, the present randomized clinical trial, under the registration number IRCT20210626051720N1 was conducted. Firstly, the study protocol, intervention properties and duration, and potential advantages and disadvantages of combined exercise training were explained to participants and all of them signed the informed consent. In the next step, the subject's height, weight and body fat percentage were measured and pre-test blood samples collected. Subsequently, they were allocated into control and combined training group randomly and considered intervention (control or combined training) started for 12 weeks. Over 12 weeks research period, the subjects in control group don't take part in any regular physical exercise and continued their daily routine life. The subjects in both groups were asked to don't change their habitual diet until completed 12 weeks intervention.

The inclusion criteria herein were: not having obesity-related diseases (such as cardiovascular disease, hypertension, type 2 diabetes), no history of stroke and heart failure, lack of malignant disorders (cancer), no regular participation in training program in the last year, not taking any dietary supplements or medication in eight weeks before and within 12 weeks of intervention, having no physical limitation for completing the exercise sessions, voluntary participation in the present study and signing the informed consent. On the other hand, the exclusion criteria included the following; not regularly participating in exercise training sessions, subject injury and inability for completing the exercise sessions, don't take part in pre-test or post-test blood sampling, subject's unwillingness to continue considered intervention, and having to take medication within the intervention period.

### Combined Training Program

The combined training program conducted over 12 weeks, and three sessions per week

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(on alternate days), and simultaneously the control group participants hasn't regular physical activity and continue their routine lifestyle. The combined training program was performed in the morning (9–11 am) under the guidance of an exercise physiologist. Before and after each combined training session, 10- and 7-8-minute warm-up and cool-down performed respectively. In each training session, firstly resistance training was performed which consisting of five exercises: leg press, leg curl, leg extension, bench press and seated rowing, which each resistance exercise performed with 8-12 repetitions with intensity of 75% repetition maximum (1RM), and subjects rested for one minute between sets. The participants 1RM was determined again after 4 weeks and training was continued according to new 1RM. Subsequently, endurance part of training program was performed on the treadmill. Endurance training program consist of 15 minutes walking or running in each session with the intensity of 60% of the reserve heart rate. In order to measurement of subjects 1RM, the following formula have been used (25):

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

Blood Sampling

Due to conducting present study according to pre-test and post-test design, blood samples collected before and after 12-weeks intervention. Both blood sampling stages performed in similar conditions, and after 12 hours night fasting. Post-test blood samples collected 48 hours after last session of combined training program, in order to avoid the immediate (acute) effects of combined exercise. The 24 hours before blood sampling, the subjects were asked to avoid strenuous physical exercise and to have adequate rest specially in the last night before collecting blood samples. In each sampling stages, 7 ml blood samples collected in the seated position (after 30 minutes resting in the testing environment) from forearm venous (right hand). Collected blood samples poured into the falcon tube, subsequently were centrifuged at 3000 rpm for 10 minutes, and serum samples removed. Obtained serum samples transferred to a microtube, and was stored in a freezer for subsequent laboratory analysis.

Biochemical Analysis

The participants height and weight were measured, and body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meter) squared (26). Body fat percentage determined by BOCA-X1 body composition analyzer, made in South Korea. Serum levels of IL-6 (Cusabio company, catalog number: CSB-E04638h, sensitivity: 2.453 pg/mL) and CRP (Cusabio company, catalog number: CSB-E08617h, sensitivity: 0.156 ng/mL) was measured by ELISA method. It should be noted that, all the measurements steps were conducted according to the kit manufacturer instructions.

**Statistical Analysis**

All data analysis steps were performed with SPSS software version 24. The Shapiro-Vilk test were used for determined the data distribution, and between group difference (control and combined training) was assessed by parametric tests. Analysis of covariance (Ancova) test used for comparing the between group difference, and within group difference were investigated through paired t test. The significance level was considered at $P<0.05$ for all of data analysis test.

**Results**

The physical characteristics of participants including age, height, body weight, BMI and body fat percentage in the pre-test and post-test stages in the control and combined training groups have been indicated in the Table 1. According to Ancova test findings, there was a significant between group (control and combined training) difference for body weight, BMI and body fat

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percentage, and significant decrease in body weight, BMI and body fat percentage in combined training group compared to control group after 12 weeks intervention was observed.

**Table 1.** Physical characteristics of participants in the control and training groups at the baseline and following 12 weeks combined training.

| Variables | | Control group | Training group |
|---|---|---|---|
| Age (years) | | 27.36 ± 3.63 | 27.86 ± 3.81 |
| Height (cm) | | 159.91 ± 3.76 | 160.21 ± 5.17 |
| Body weight (kg) | Pre-test | 81.43 ± 4.99 | 82.75 ± 4.75 |
| | Post-test | 81.58 ± 5.01 | 79.84 ± 4.40* |
| BMI (kg/m$^2$) | Pre-test | 31.82 ± 1.13 | 32.23 ± 0.92 |
| | Post-test | 31.87 ± 1.12 | 31.10 ± 0.86* |
| Body fat percentage | Pre-test | 36.92 ± 2.36 | 38.74 ± 3.13 |
| | Post-test | 37.13 ± 2.69 | 35.43 ± 2.85* |

* Significant decrease compared to control group. Data shown as Mean ± SD.

Between group analysis by means of Ancova test indicated a significant difference between control and combined training groups for IL-6 level, and serum levels of IL-6 in combined training group significantly decreased compared to control group ($P < 0.001$). Paired t test represented a significant decrease in IL-6 level after 12 weeks combined training ($P = 0.003$) and non-significant changes in control group ($P = 0.812$) (Figure 1).

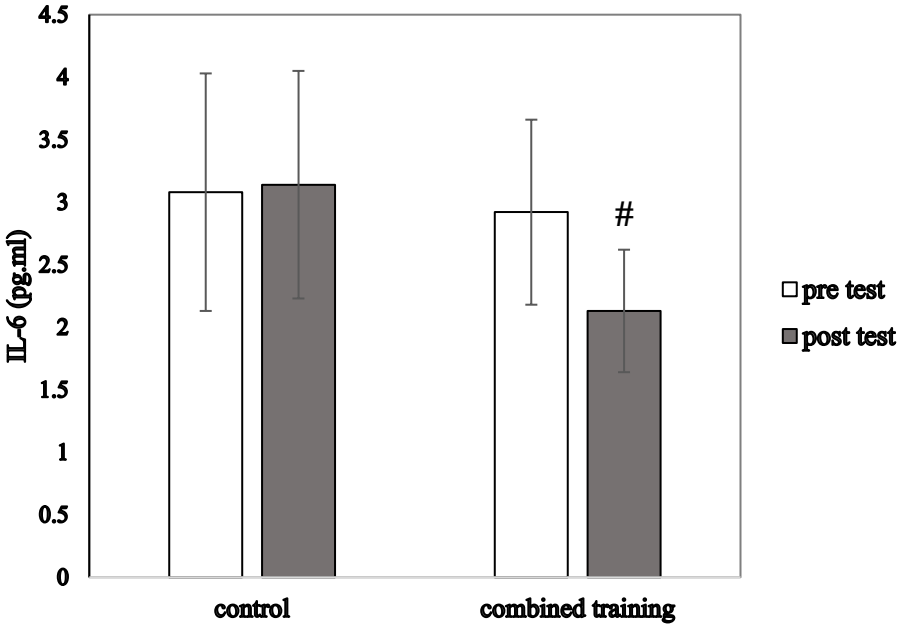



**Figure 1.** Serum IL-6 level in the control and training groups before and after 12 weeks combined training. #Significant decrease compared to the control group.

Analysis of covariance test findings for serum level of CRP showed a significant difference between combined training group with control group, and significant decrease in serum levels of CRP in the combined training group compared to control group were observed ($P = 0.006$). In addition, intra-group analysis of CRP level by means of paired t-test showed a significant decrease in CRP levels in the combined training group ($P = 0.021$) and no significant change in the control group ($P = 0.494$). Changes in the serum levels of CRP after 12 weeks intervention indicated in Figure 2.

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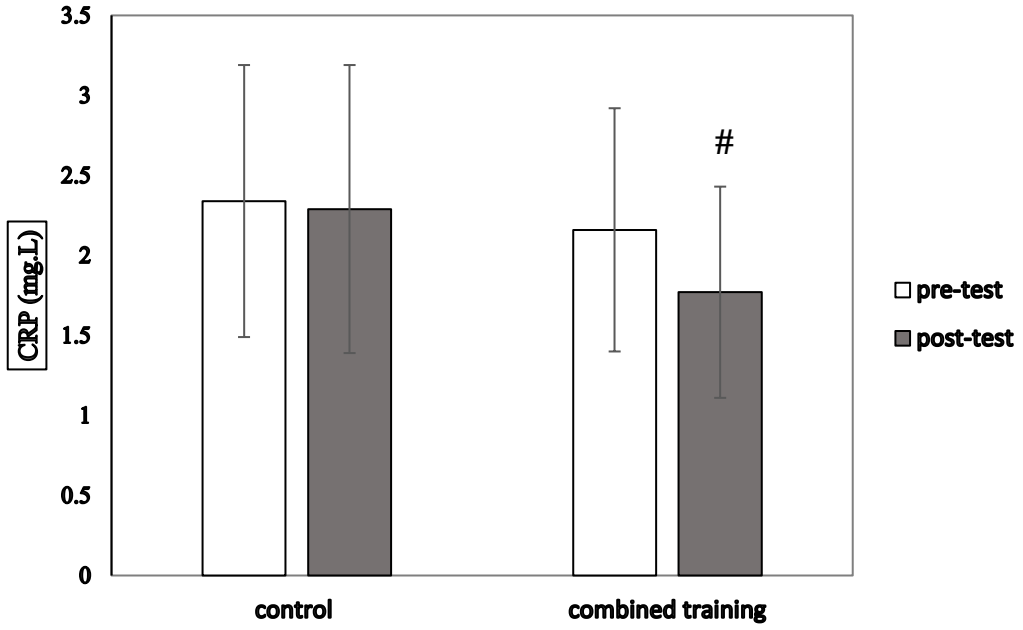



**Figure 2.** Serum CRP level in the control and training groups before and after 12 weeks combined training. #Significant decrease compared to control group.

## Discussion

The present study main finding was that 12 weeks combined training resulted in a significant decrease in serum levels of inflammatory mediators including IL-6 and CRP compared to the control group. Adipokine imbalance (upregulation of pro-inflammatory and downregulation of anti-inflammatory adipokines) in adipose tissue or circulation known as a potential and major risk factor in the pathogenesis of insulin resistance, nonalcoholic fatty liver disease, cardiovascular disease and dyslipidemia (27). In contrast, exercise training plays an important role in the improvement and prevention of various disorders, and exercise training beneficial effects are attributed to the anti-inflammatory effects in the central and peripheral organs (28). Different factors consist of duration (acute, short term, and long term), calorie consumption, type (resistance, endurance, and combined training), method (cycling, water training, etc), volume (sessions per week), and intensity (low, moderate, and high) of exerted exercise training can affected the exercise training effects on immunity-related metabolic dysfunction (29). Regarding the importance of exercise training intensity and duration in modulating the levels of inflammatory factors, its suggested that although different intensities of exercise training cause to decrease in inflammatory factors, higher-intensity exercise training with longer duration (more than eight weeks) is associated with further anti-inflammatory effects (30), which the present study findings supported this hypothesis.

Although acute physical exercise result in significant increases in IL-6 level (up to 100-fold), but long-term regular exercise training significantly lowers the levels of inflammatory factors such as IL-6 (31). Consistent with these statement and present study findings, researchers indicated that eight weeks resistance training in sedentary men leads to a significant downregulation of serum levels of IL-6 and CRP (22). Salamat et al (2016) investigated the effect of different modalities of exercise training in overweight men, and suggested that eight weeks aerobic and combined training was associated with a significant decrease in the levels of IL-1β and IL-6, but resistance training had no significant effect on the IL-1β and IL-6 levels, and researchers concluded that aerobic training is more

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effective compared to other exercises training for reducing inflammatory factors (32). In addition, comparing the effect of 12 weeks high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) in the type 2 diabetic women, showed a statistically significant decrease in the levels of IL-6 and TNF-α in both MICT and HIIT groups, but there was no significant difference between HIIT and MICT groups for observed changes in the levels of IL-6 and TNF-α, which emphasizes the effectiveness of either HIIT and MICT in modulating inflammatory pathways, and researchers attributed the anti-inflammatory effect of exercise training to decrease in body fat mass (33).
In another study, investigating the effect of eight weeks weight loss intervention (diet), resistance and aerobic training in overweight women, indicated that serum levels of IL-6 were significantly reduced in all intervention groups, and downregulation of IL-6 were associated with the reduction of body weight and adipose tissue, and decrease in other inflammatory cytokines such as TNF-α and CRP (34). Similarly, we observed simultaneous decrease in the serum levels of IL-6 and CRP after 12 weeks combined training in sedentary obese women. In a review article, researchers suggest that engaging in exercise training is associated with a decrease in CRP levels regardless of the age or sex of the individual; however, greater improvements in CRP level occur with a decrease in BMI or fat percent, although changes in the CRP levels independent of changes in the body weight and fat mass have been observed (35). Okita et al (2004) reported that eight weeks aerobic training in pre or postmenopausal women cause to significant decrease in CRP levels, and researchers considered the decrease in body fat mass, weight loss, increased antioxidant capacity and improved endothelial function as potential mechanisms for reduction of CRP levels following conducted exercise training (36).

Despite the above-mentioned studies, contradictory findings regarding the effect of exercise training on the CRP levels have been reported. Several conducted researches suggested that 16 weeks of moderate and high intensity aerobic training, despite a decrease in body fat mass and BMI, don't have a significant effect on CRP levels (37), representing the positive effects of exercise training independent of changes in the CRP levels. The changes in the CRP levels following regular exercise training may differ depending on subjects' characteristics, and despite a significant reduction in inflammatory cytokines levels such as CRP and TNF-α after seven months of endurance training in obese women, the observed changes in the levels of CRP and TNF-α wasn't significant statistically in the normal weight group, although significant decrease in body fat mass was observed in both obese and normal weight groups (38). Exercise training type can also affect the observed changes in the levels of inflammatory mediators, despite no significant change in the CRP levels by resistance training, significant decrease in CRP levels after 10 weeks aerobic and combined training were observed in type2 diabetic women (23). Therefore, performing resistance training combined with aerobic training for amplify the anti-inflammatory effects of resistance training have been suggested (39). The present study findings confirmed the combined training importance in attenuate the systemic inflammation. In addition, contrary to this study's findings, different types of exercise training, including aerobic, resistance and combined training for 16 weeks in sedentary men did not have a significant effect on IL-6, CRP and TNF-α levels, which non-significant changes in the levels of inflammatory mediators was associated with no significant change in the body weight and BMI in all trained groups, and researchers attributed the lack of changes in the levels of inflammatory mediator by different exercise training to don't changes in the body weight (40). In

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accordance with this hypothesis, decrease in inflammatory factors such as MCP-1 after eight weeks combined training in obese women was associated with decrease in body weight, BMI, and body fat percentage (41).

Some researchers attributed the anti-inflammatory effect (decrease in IL-6 and TNF-α levels) of 12 weeks aerobic training in women with metabolic syndrome to decrease in body fat mass and upregulation of IL-10 level as anti-inflammatory cytokine (42). Exercise training-induced improvements in inflammatory status may also result from the modulation of intracellular signaling pathways and cellular function that are mediated by nitric oxide (NO) and reactive oxygen species (ROS) (43). Exercise training through inhibiting the macrophages infiltration and accelerating the change of macrophages phenotype from M1 to M2, leads to inhibition of adipose tissue inflammation and subsequently cause to decrease in systemic inflammation (44). In addition, inducing skeletal muscle by exercise training to release different anti-inflammatory cytokines (peptides originating from skeletal muscle are known as myokines), increased angiogenesis and blood supply in adipose tissue, reduced vasoconstriction and hypoxia in adipose tissue, decreased adhesion molecules and increased cell regeneration resulting in attenuating the vascular inflammation, as well as increasing regulatory T cells and decreasing inflammatory monocytes and Toll-like receptors (TLRs) are other mechanisms by which exercise training exerts its anti-inflammatory effects (45). Unfortunately, in the present study we don't investigate the changes in the above-mentioned signaling pathways, and simultaneous measurement of anti-inflammatory cytokines like IL-10, can provide a better understanding for improving systemic inflammation by combined training, which should be evaluate in the future studies. However, according to present study findings, losing body fat mass as a main source for secreting different inflammatory adipokines (such as IL-6 and CRP) can be considered as a major potential mechanism for attenuating inflammation by combined training.

## Conclusion

Based on the present study findings, it can be concluded that combined training plays an important role in reducing inflammatory factor in obese women, which the anti-inflammatory effects of combined training are associated with decreasing body fat mass as a major secretory site for inflammatory mediators including CRP and IL-6. However, further studies should be conducted in order to identify the other anti-inflammatory mechanisms of combined training.

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## Conflict of Interest

The authors declare that no conflict of interest exists.

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