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The Effect of CLA Supplementation and High-Intensity Interval Training on Plasma Neuregulin 4, Interleukin 6, and TNF-α in Obese Men

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Abstract

Background Despite extensive research, the effects of conjugated linoleic acid (CLA) supplementation in treating obesity are still unclear and equivocal. Adipokines play an important regulatory role in the activation of adipose tissue function. The purpose of the present research is to investigate the efficacy of CLA supplementation during high-intensity interval training (HIIT) on plasma neuregulin 4 (NRG-4), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) in obese men.

Methodology Forty-four nonactive, obese males (age = 26.29 ± 0.97 y, body mass index [BMI] = 32.29 ± 1.27 kg/m^2) participated in this research. Participants were randomly divided into four groups: control, without training or CLA supplementation; supplement, CLA supplementation without training; exercise, HIIT without CLA supplementation; supplement + exercise, HIIT with CLA supplementation. HIIT was performed for 12 weeks, three sessions per week, with a training intensity ranging from 60 to 75% of the Vo2 max on a treadmill. Subjects in the CLA supplement groups consumed two 1-g CLA capsules daily in two meals with breakfast and dinner. The amount of plasma NRG-4, IL-6, and TNF-α was measured using an ELISA (enzyme-linked immunosorbent assay) kit. Various variables were collected at two time points (pretest and posttest).

Results The study found that both the HIIT and HIIT + CLA groups showed greater improvements in weight and BMI compared with the other groups. Notably, posttest levels of NRG-4 were significantly higher in the CLA, HIIT, and HIIT + CLA groups compared with the control group ($p < 0.001$). Additionally, following the intervention

Keywords
- conjugated linoleic acid
- immune functions
- therapeutic window
- obesity-related inflammation

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period, IL-6 levels in the HIIT + CLA group and TNF-α levels in the HIIT and HIIT + CLA groups were significantly lower than in the control group ($p < 0.001$).
Conclusion CLA supplementation, in conjunction with HIIT, emerges as a highly effective approach and more benefits on the amount of adipokines secreted from fat tissue in obese people than either of them separately.

Introduction

Overweight and obesity are considered as major health concerns all around the world; in addition, they play a great role in the progression of several noncommunicable diseases, including diabetes, cardiovascular diseases, and cancer.[1] Obesity is a significant public health challenge associated with chronic inflammation and metabolic dysregulation[2]. Physical activity is recommended for primary prevention or adjunctive treatment for various types of metabolic diseases, especially obesity. Some studies show that NRG-4 and proteins involved in regulating metabolism can be changed by nutritional interventions and physical activity.

Many naturally occurring food substances possess functional properties, such as conjugated linoleic acid (CLA). CLA is a set of various fatty acids, including geometric and positional isomers of linoleic acid.[3] CLAs are a family of positional and geometrical dienoic isomers of linoleic acid. The two double bonds are conjugated (separated by a single bond) and can be either cis or trans, which leads to a wide variety of CLA isomers.[4] Positive effects of CLA on human health have been reported. CLA has shown potent anticarcinogenic and antiatherogenic effects, may modulate immune activity, and improve body composition and anthropometric parameters in overweight or obese subjects.[3]

In obesity, the high amount of nutrients in fat tissues stimulates the release of inflammatory adipokines such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and chronic inflammation.[5] This cytokine causes many metabolic and cellular changes in patients with critical condition. TNF-α increases in insulin resistance caused by obesity, which indicates that this factor can play a role in the occurrence of insulin resistance.[6] IL-6 is also a cytokine that is produced in large amounts by adipose tissue and its circulating amount is related to body mass, insulin sensitivity, and glucose tolerance.[7] These two cytokines can lead to local or general inflammations by affecting immune cells, and as a result, by affecting the function of the endothelial system, they can play a role in the occurrence of obesity-related disorders such as diabetes.[8] Baikpour et al showed that there was a positive and significant correlation between IL6 levels and serum body mass index (BMI) in healthy obese people.[9]

Nevertheless, previous clinical studies reported inconsistent data regarding effects of CLA supplementation on obesity-related indexes. Research has shown that CLA supplementation leads to weight loss and improved body composition in obese people.[10] A previous study on animal models by Ennequin et al showed that low-intensity endurance training and a balanced diet activate the neuregulin 1 (NRG-1) pathway.[11] Similarly, various studies show that the reduction of NRG-4 levels is associated with obesity, insulin resistance, diabetes mellitus, dyslipidemia, metabolic syndrome, nonalcoholic fatty liver disease, inflammation, oxidative stress, coronary artery disease, myocardial infarction, and various cardiovascular diseases.[12,13] As an endocrine factor, NRG-4 reduces hepatic lipogenic signaling and maintains glucose stability and lipid homeostasis.[14]

However, certain authors have reported conflicting outcomes. The research conducted by Froozandeh et al on the effect of 8 weeks of resistance training and intermittent aerobic training on NRG-1 levels in women with diabetes showed that the changes of NRG-1 in two training groups were not significant.[15] In another research showed that 4 weeks of aerobic training had no effect on the serum and liver levels of neurolol 4 in diabetic and healthy rats.[16] Also, CLA can be effective as an antiobesity agent to reduce energy consumption, reduce lipogenesis, and increase energy expenditure, lipolysis and fat oxidation.[17] The effects of CLA supplement in improving the complications of obesity have been reported in some studies.[18] In addition, in a study by Sadeghi et al has been reported that the use of supplements in the short term does not lead to an improvement in the rate of fat burning in obese women.[10]

Disruption of white adipose tissue can cause changes in the metabolism of adipose and whole body. Understanding the pathways related to adipose tissue dysfunction can help identify pharmaceutical or nutritional interventions, thereby increasing biogenesis or adipose tissue function. High-intensity periodic exercises (HIIT) include high-intensity exercise activity with low-intensity active rest. HIIT is a very efficient method that creates different metabolic adaptations.[19] It appears that these exercises can be effective and efficient in preventing and treating obesity.

We hypothesized that supplementation with CLA during 12 weeks will result in a significant improvement in variables in obese men who are engaged in high-intensity periodic training. This study therefore aimed to examine the effect of CLA supplement and on NRG-4, interloon 6, and TNF-α plasma in obese men during HIIT.

Materials and Methods

Study Design and Settings

This semiexperimental research study employed purposive sampling to select participants based on specific criteria. Subsequently, the participants after preliminary clinical assessments such as history, cardiovascular disease history, and clinical and diagnostic examinations were allocated into four groups using the systematic random grouping method, ensuring an equal distribution of male subjects across each group. The subjects in the CLA supplement group completed the training program within the allotted time after ingesting two 1-g CLA capsules twice a day with breakfast and dinner.[20]

Obtaining written consent from study participants was a crucial step, involving a comprehensive explanation of the research's objectives and methods. This process aimed to ensure participants' thorough comprehension of the study, enabling an informed decision regarding their involvement. Participants were familiarized with various aspects, including concurrent training, research characteristics, variable measurement, training protocol, pretest, midtest, and posttest procedures, as well as instructions, possibilities, and limitations related to the research's time and location.[21]

Subjects engaged in training programs on the same day. Variable measurements were conducted for all groups before the study initiation at the conclusion of the 12-week period. The maximum oxygen consumption was determined by using the modified Bruce test. The study protocol consisted of three sessions per week with warming up for ~10 minutes, including stretching, walking, and running. They ran on the treadmill for 30 minutes at three sessions per week, during the first week at 65% and second week at 75% of their Vo2 max. Active recovery for 4 minutes at 75% Vo_2 max and 4 minutes at 75% VO2 max was performed on the subjects during the third and fourth weeks. The subjects exercised for a maximum of 85% of their maximum VO2 max for 4 minutes during the fifth, sixth, and seventh weeks, they performed active recovery for 4 minutes with a maximum of 20% of Vo2 max, and then proceeded to use a treadmill for 4 minutes. In the eighth, ninth, and tenth weeks, subjects performed four 4-minute exercise sessions on the treadmill with 90% Vo2 max along with active recovery intervals with 30% Vo2 max, which lasted 4 minutes. For the past 2 weeks, they have completed four 4-minute stages with 95% Vo2 max on the treadmill, using active rest with 50% Vo2 max for a total of 4 minutes. The intensity of the exercise was based on the maximum oxygen consumption, and the heart rate of the subjects was monitored in each session using a heart rate monitor during the exercise.[22]

Sampling Method and Sample Size Calculation

Initially, 51 sedentary males were recruited based on the sampling technique and criteria. However, seven individuals withdrew from the study for reasons unrelated to the research. Consequently, a cohort of 44 nonactive, obese males, with a mean of BMI of $32.3 \pm 1.27\,kg/m^2$, and an average age of 26.29 ± 2.97 years, were enrolled. The participants were randomly allocated into four equally sized groups of 11 subjects: (1) control, (2) CLA supplementation without training, (3) HIIT without CLA supplementation, and (4) HIIT + CLA supplementation. Participant demographics and characteristics are detailed in ►**Fig. 1**.

Inclusion and Exclusion Criteria

The study employed the purposive sampling technique to select participants based on predetermined criteria. Inclusion criteria specified sedentary males aged between 25 and 30 years with a BMI within the range of 30 to $35\,kg/m^2$. Recruitment occurred in the Tehran city area in Iran through direct outreach and advertising. Exclusion criteria included allergies to CLA supplementation, a lack of recent physical activity, chronic illnesses, medication use, diabetes, digestive disorders, sleep disorders, no smoking and no alcohol consumption, and caffeine and drug treatment supplementation. Prospective participants were also required to have refrained from engaging in conditioning exercises exceeding 2 hours per week for the 6 months preceding the study.

Procedure of Experimental Measurements

Laboratory tests offer a multifaceted advantage, providing a noninvasive, easily attainable, and economical approach to assessment, while also playing a pivotal role in diagnosing various medical conditions within routine clinical practice, with notable emphasis on screening procedures and accurate disease diagnostics. Diagnostic biomarkers, including specific molecules, genes, or other biological substances, are critical tools in modern medicine, aiding in the early detection, diagnosis, and management of diseases. They offer objective, quantifiable information that assists clinicians in making informed decisions about patient care, enabling insights into underlying pathology, early disease identification, monitoring illness progression, and assessing treatment effectiveness. Additionally, the development of novel diagnostic biomarkers has the potential to enhance diagnostic accuracy, reduce the need for invasive procedures, and guide personalized treatment plans. As a result, ongoing research in this area continues to shape the landscape of modern medicine, with the ultimate goal of improving patient outcomes and enhancing overall healthcare quality.[23]

Blood pressure is the force of blood pushing against the walls of the arteries as the heart pumps blood. It is usually measured in millimeters of mercury (mmHg) and recorded with two numbers: systolic pressure (the higher number) and diastolic pressure (the lower number). Subsequently, systolic blood pressure and diastolic blood pressure were determined millimeters of mercury through examination of the brachial artery, utilizing a mercurial sphygmomanometer (blood pressure monitor model BP380A), in accordance with established clinical procedures.[1] Blood pressure measurements were taken after 12 hours and in two phases, 48 hours prior to and after 12 weeks, to assess metabolic factors.[24]

A laboratory professional took blood samples from five complete blood count (CBC) sites and a suitable vein while the patient was at rest. After obtaining the blood sample and separating the serum, it was kept at a negative temperature of 80°C. To avoid rhythm effects on blood indicators, blood was collected between 8 and 10 AM. Serum is the liquid fraction of whole blood that is collected after the blood is allowed to clot. The clot is removed by centrifugation and the resulting supernatant, designated serum, is carefully removed using a

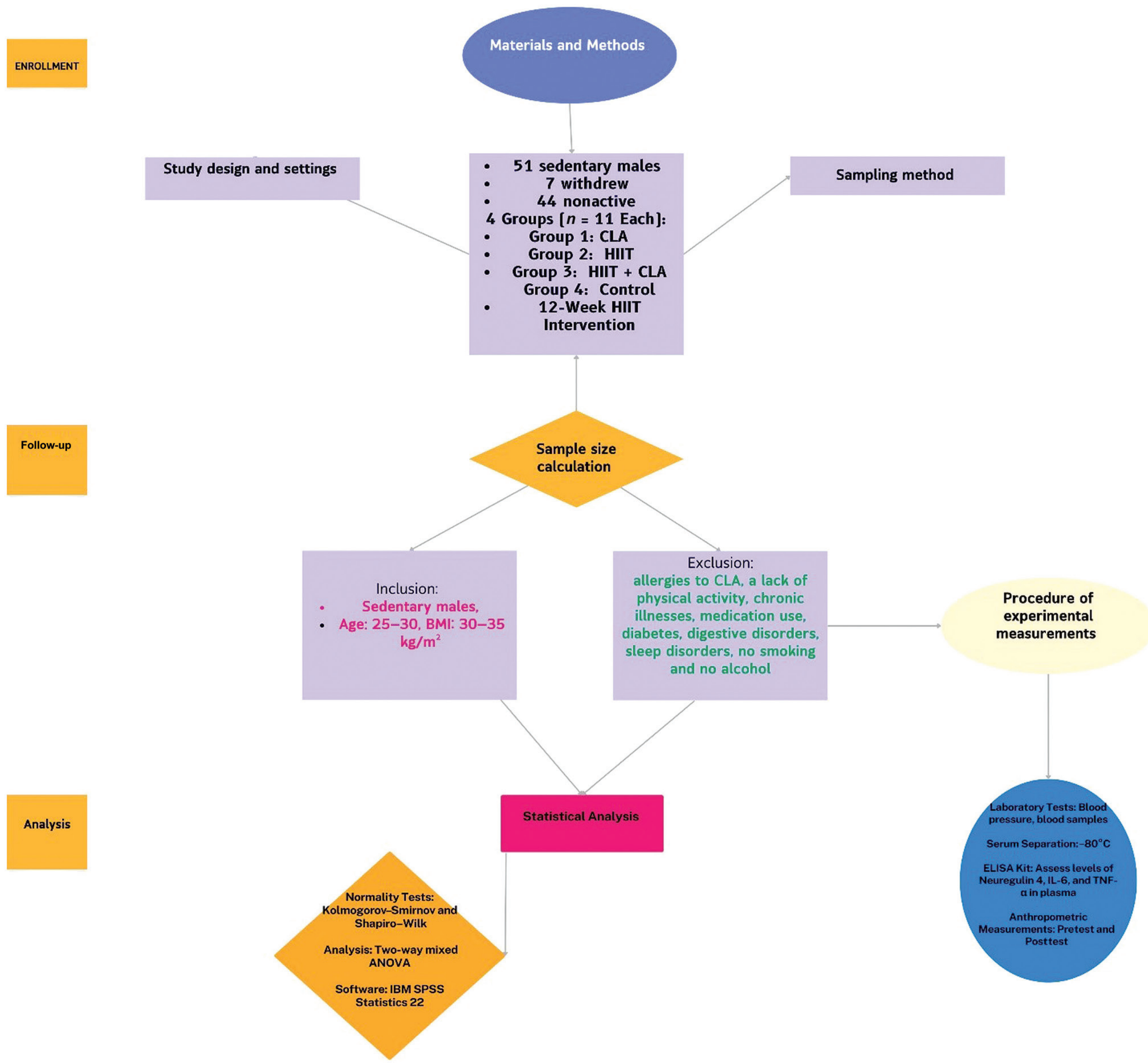

Fig. 1 Flow diagram of study. CLA, conjugated linoleic acid; HIIT, high-intensity interval training; IL, interleukin; TNF-α, tumor necrosis factor α.

Pasteur pipette. Plasma is produced when whole blood is collected in tubes that are treated with an anticoagulant. The blood does not clot in the plasma tube. The cells are removed by centrifugation. The supernatant, designated plasma is carefully removed from the cell pellet using a Pasteur pipette.[25] Whole blood was collected in a covered test tube. If commercially available tubes are to be used, the researcher should use the red topped tubes. These are available from Becton Dickinson (BD). BD's trade name for the blood handling tubes is Vacutainer. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15 to 30 minutes. Remove the clot by centrifuging at 1,000 to 2,000× g for 10 minutes in a refrigerated centrifuge. The resulting supernatant is designated serum. Following centrifugation, it is important to immediately transfer the liquid component (serum) into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2 to 8°C while handling. If the serum is not analyzed immediately, the serum should be apportioned into 0.5 mL aliquots, stored, and transported at –20°C or lower. It is important to avoid freeze–thaw cycles because this is detrimental to many serum components. Samples which are hemolyzed, icteric, or lipemic can invalidate certain tests.[26]

The enzyme-linked immunosorbent assay (ELISA) method was employed to measure the plasma using the kit. The ELISA method is a widely used laboratory technique to detect and quantify substances such as peptides, proteins, antibodies, and hormones. After a 3-day period, the subjects were taken to a laboratory for blood collection to assess their levels of neuroglin-4, IL-6, and TNF-α plasma. After that, the supplement groups began to practice taking the CLA. Repeated anthropometric measurements and blood samples were taken at the end of the training sessions.[27]

Statistical Analysis
All data were set as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to examine if variables were normally distributed. To confirm that the variables were distributed, the Shapiro–Wilk test was employed. Following the data's natural distribution, the biochemical variables were compared using two-way mixed analysis of variance, with time (before and post) acting as a within-subject factor and group (control, supplement, exercise, and supplement-exercise) acting as a between-subject factor, at significance level set on $p \leq 0.05$. All data were processed using SPSS software (IBM SPSS Statistics 22).[28]

Results

Personal characteristics of the subjects for pretest and posttest based on the mean and standard deviation are shown in ►**Table 1**. Research finding has shown that exercise without and with supplementation in HIIT without CLA and HIIT + CLA groups improved weight and BMI more than CLA and control group at both pretest and posttest in obese men. These variables were not significant for supplement group and control group ($p \leq 0.05$).

The results of the two-way analysis of variance test showed that 12 weeks of HIIT with CLA supplementation has a significant effect on plasma neuroglin 4 in obese men ($p = 0.000$, $F = 163.485$). The results of Tukey's post hoc test showed that the values of neuroglia 4 in the exercise, supplement, and exercise-supplement groups were significantly higher than the control group ($p < 0.001$). Also, neuroglin 4 was significantly higher in the exercise group than the supplement group ($p = 0.001$). Also, the results of the correlated *t*-test showed that neuroglin 4 in the effect of exercise ($p = 0.001$), supplementation ($p = 0.009$), and exercise-supplement ($p = 0.001$) increased significantly compared with the pretest, but decreased in the control group, although this decrease was not significant ($p = 0.681$; ►**Table 2** and ►**Fig. 2**).

The results of the two-way analysis of variance show that 12 weeks of HIIT with CLA supplementation has a significant effect on plasma IL-6 levels in obese men ($p = 0.000$, $F = 163.485$). The results of Tukey's post-test showed that in the post-test, IL-6 in the exercise-supplement group was significantly lower than the exercise, supplement, and control groups ($p < 0.001$), but there was no significant difference between other groups ($p < 0.05$). Also, the results of the correlated *t*-test showed that the interaction of training and supplementation on IL-6 decreased the level of IL-6 compared with the pretest ($p < 0.001$). In the exercise and supplement group, despite the decrease in the level of IL-6, this decrease was not statistically significant ($p < 0.05$). In the control group, the level of IL-6 increased, but it was not statistically significant ($p < 0.05$; ►**Table 3** and ►**Fig. 3**).

The results of two-way analysis of variance show that 12 weeks of HIIT with CLA supplementation has a significant effect on plasma TNF- α levels in obese men ($p = 0.000$, $F = 19.965$). The results of Tukey's posttest showed that in the posttest, TNF-α was significantly lower in the exercise

Table 1 Characteristics of study participants

Variable	Group	Pretest	Posttest
Weight (kg)	Supplement	92.92 ± 2.85	90.85 ± 1.81
	Exercise	92.41 ± 1.93	88.82 ± 1.65[a,b]
	Supplement + exercise	93.77 ± 1.91	87.07 ± 2.15 [ab]
	Control	93.87 ± 2.05	93 ± 2.20
BMI (kg/m^2)	Supplement	32.48 ± 1.40	31.74± 0.83
	Exercise	33.03 ± 1.28	31.76 ± 1.27[a,b]
	Supplement + exercise	30.77 ± 0.99	26.54 ± 2.73[a,b]
	Control	32.90 ± 1.44	32.60 ± 1.49
Age (Y)	Supplement	25.81 ± 2.67	
	Exercise	26.36 ± 4.15	
	Supplement + exercise	26.54 ± 2.73	
	Control	26.45 ± 2.33	
Height (cm)	Supplement	169.1 ± 2.76	
	Exercise	167.2 ± 2.61	
	Supplement + exercise	168.2 ± 1.71	
	Control	168.9 ± 3.17	

[a]Significant differences compared with the control group.
[b]Significant difference compared with pretest.
Abbreviations: supplement, conjugated linoleic acid supplementation without training; exercise, high-intensity interval training without conjugated linoleic acid supplementation; supplement + exercise, high-intensity interval training with conjugated linoleic acid supplementation; BMI, body mass index; all values are presented as mean ± standard deviation.

Table 2 Two-way analysis of variance test in NRG

NRG4	Type III sum of squares	df	Mean square	*F*	Sig.
Corrected model	50.085	4	12.521	163.485	0.000
Intercept	18.810	1	18.810	245.59	0.000
NRG.pre	0.042	1	0.042	0.552	0.462
Supplement	7.845	1	7.845	102.434	0.000
Exercise	40.344	1	40.344	526.746	0.000
Supplement + Exercise	0.744	1	0.744	9.71	0.003
Error	2.987	39	0.077		

Abbreviations: supplement, conjugated linoleic acid supplementation without training; exercise, high-intensity interval training without conjugated linoleic acid supplementation; NRG4, neuregulin4; supplement + exercise, high-intensity interval training with conjugated linoleic acid supplementation.

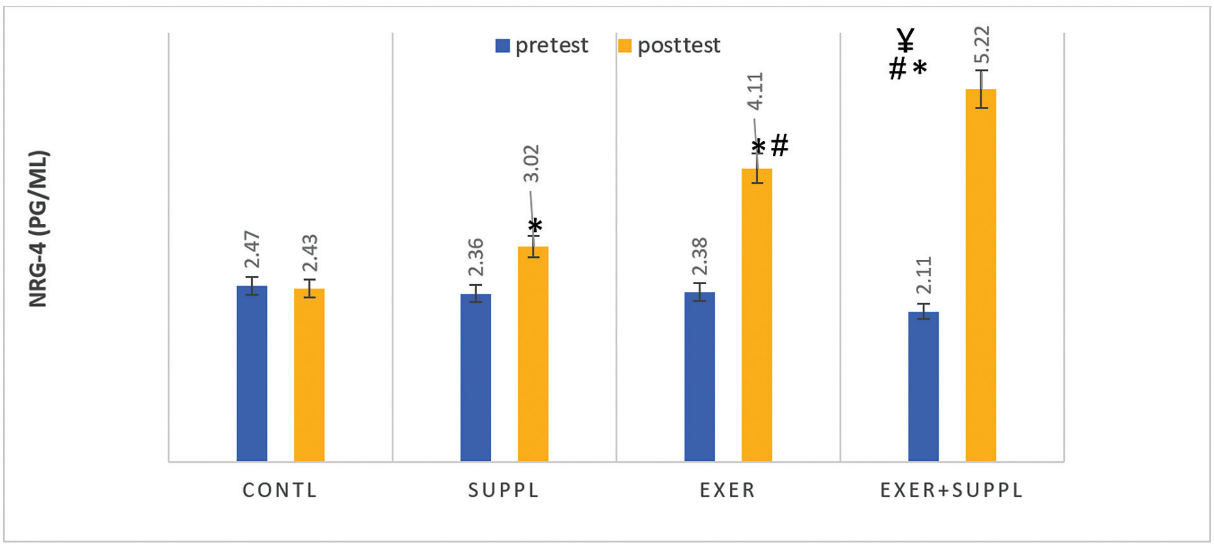

Fig. 2 Changes in plasma neuroglin-4 levels in obese men according to different groups. *: significant difference compared with the control group, #: significant difference compared with the supplement group; ¥: significant difference compared with the training group ($p < 0.05$). CONTL, without training or CLA supplementation; SUPPL, conjugated linoleic acid supplementation without training; EXER, high-intensity interval training without conjugated linoleic acid supplementation; EXER + SUPPL, high-intensity interval training with conjugated linoleic acid supplementation; NRG-4, neuregulin 4.

Table 3 Two-way analysis of variance test in IL-6

IL-6	Type III sum of squares	df	Mean square	*F*	Sig.
Corrected model	50.085	4	12.521	163.485	0.000
Intercept	18.810	1	18.810	245.59	0.000
IL-6	0.042	1	0.042	0.552	0.462
Supplement	7.845	1	7.845	102.434	0.000
Exercise	40.344	1	40.344	526.746	0.000
Supplement + Exercise	0.744	1	0.744	9.71	0.003
Error	2.987	39	0.077		

Abbreviations: supplement, conjugated linoleic acid supplementation without training; exercise, high-intensity interval training without conjugated linoleic acid supplementation; IL-6, interleukin 6; supplement + exercise, high-intensity interval training with conjugated linoleic acid supplementation.

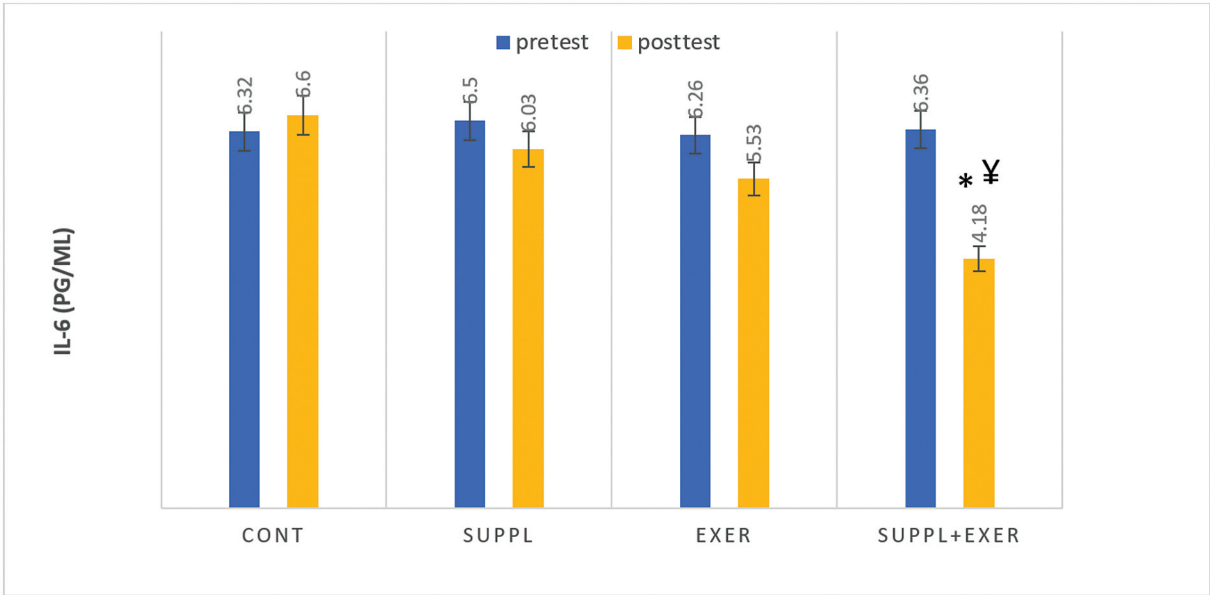

Fig. 3 Changes in plasma IL-6 levels in obese men according to different groups. *: significant difference compared with the control group; ¥: significant difference compared with the supplement and exercise group ($p < 0.05$). CONTL, without training or conjugated linoleic acid supplementation; SUPPL, conjugated linoleic acid supplementation without training; EXER, high-intensity interval training without conjugated linoleic acid supplementation; EXER + SUPPL, high-intensity interval training with conjugated linoleic acid supplementation; IL-6, interleukin 6.

and exercise-supplement groups than the control group ($p < 0.001$), but there is no significant difference between other groups ($p < 0.05$). Also, the results of the correlated *t*-test showed that TNF-α decreased significantly as a result of training, supplementation, and training and supplementation compared with the pretest ($p < 0.001$), but the reduction was not significant in the control group ($p < 0.05$; ►**Table 4** and ►**Fig. 4**).

Discussion

This research explored the impact of CLA supplementation in combination with HIIT training on plasmaNRG-4, IL-6 and TNF-α among obese males. The primary outcome of this investigation revealed that a 12-week regimen of CLA supplementation paired with HIIT training led to substantial improvements in some of cytokines. Contrastingly, in the experimental group that consumed CLA supplements without engaging in physical exercise, no significant alterations were observed across the measured variables. These results highlight the critical role of physical exercise in eliciting beneficial changes in NRG-4, IL-6, and TNF-α, reinforcing the synergy between dietary supplementation and physical training. In this research, the interaction of exercise and supplementation had a significant effect on IL-6 and TNF-α and caused a decrease in IL-6 and TNF-α levels in obese men. These results suggest that the interaction of exercise and CLA supplementation may have additional benefits for adipose tissue-secreted inflammatory markers in obese men.[17]

In our results, CLA supplementation significantly reduced body weight and BMI, in experimental group 1 after 12 weeks but the reducing effect was not identified for other groups. This kind of training could help reduce body weight and fat mass by increasing lean mass and basal metabolic rate. This

Table 4 Two-way analysis of variance test in TNF-α

TNF-α	Type III sum of squares	df	Mean square	*F*	Sig.
Corrected model	52.458	4	13.115	19.965	0.000
Intercept	23.846	1	23.846	36.303	0.000
TNF-α	0.162	1	0.162	0.246	0.623
Supplement	12.217	1	12.217	18.599	0.000
Exercise	32.770	1	32.770	49.887	0.000
Supplement + Exercise	7.038	1	7.038	10.714	0.002
Error	2.987	39	0.657		

Abbreviations: supplement, conjugated linoleic acid supplementation without training; exercise, high-intensity interval training without conjugated linoleic acid supplementation; IL-6, interleukin 6; supplement + exercise, high-intensity interval training with conjugated linoleic acid supplementation; TNF-α, tumor necrosis factor α.

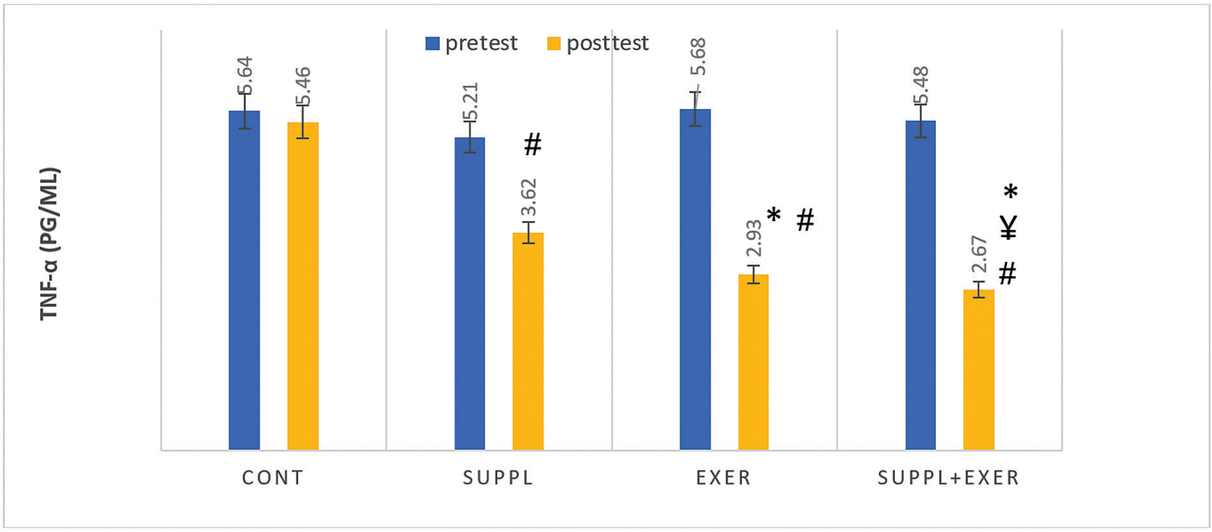

Fig. 4 Changes in plasma TNF-α levels in obese men according to different groups. *: significant difference compared with the control group; ¥: significant difference compared with the supplement and exercise group ($p < 0.05$). CONTL, without training or conjugated linoleic acid supplementation; SUPPL, conjugated linoleic acid supplementation without training; EXER, high-intensity interval training without conjugated linoleic acid supplementation; EXER + SUPPL, high-intensity interval training with conjugated linoleic acid supplementation; TNF-α, tumor necrosis factor α.

decrease could be one of the causes of the increase in neuroglin 4 and ultimately leads to the improvement of fat tissue function. The results of this research show that HIIT may lead to an increase in adipokines secreted from adipose tissue and improve metabolic homeostasis during obesity through the effect on NRG-4. Also, the results of our study show that 12 weeks of HIIT led to a nonsignificant decrease in IL-6 and a significant decrease in TNF-α in obese subjects. This finding of the current research is consistent with the results of some previous studies.[29–31] In agreement with this, Nyman et al showed that CLA supplementation can modulate inflammatory markers and metabolic parameters, while HIIT is known to improve cardiovascular fitness and metabolic health. However, our study uniquely explores their combined impact, particularly in the context of obesity.[32] Physical activity seems to be an important component of lifestyle interventions for weight loss and maintenance. Although the effects of physical activity on weight loss may seem to be modest, there seems to be a dose–response relationship between physical activity and weight loss. Physical activity also seems to be a critically important behavior to promote long-term weight loss and the prevention of weight regain. The benefits of physical activity on weight loss are also observed in subjects with severe obesity (BMI $\geq$ 30 kg/m^2)[33]. Previous publications showed exercise is a strong factor in the treatment of obesity, and when performed with adequate intensity and frequency, it could provide protection against comorbidities of obesity. These findings were also shown in a study by Medeiros et al, who demonstrated a reduction in weight, body fat percentage, and waist circumference in overweight adults who performed concurrent training three times per week with an intensity of 65 to 80% V_{O2} peak. However, in this same study, two other groups that performed strength training or aerobic training only had a decrease in waist circumference, showing the relevance of the type of training[34]. CLA supplementation can affects many processes, including reducing the size and number of fat cells, modifying adipokines and cytokines, increasing lipase-sensitive hormone activity, or increasing fatty acid oxidation in obese people.[35] Consumption of unsaturated fatty acids, including CLA, reduces inflammatory responses through various mechanisms.[36]

The results of this study showed that 12 weeks of HIIT with CLA supplement led to a significant increase in neuroglin 4 in obese men. The response of neuroglin to exercise is not well defined, especially in obese subjects. In a study on diabetic women after 8 weeks of intermittent training with an intensity of 60 to 75% maximal heart rate (MHR) and resistance training with an intensity of 30 to 75% One-repetition maximum (1RM), the changes of NRG-1 were not significant.[15] The type of exercises performed may bring different adaptations. Also, the type of research subjects can be the cause of the difference between the results and the above findings. To our knowledge, so far only one animal study has investigated NRG-4 levels after exercise. In this context, Rouhani Doost and Fathi showed that 4 weeks of aerobic training had no effect on the serum and liver levels of neuroglin 4 in diabetic and healthy rats.[16] The results of this researches showed that obesity is associated with functional changes in NRG-4 levels and causes an increase in metabolic disorders. NRG-4 levels significantly improve metabolic parameters and homeostasis disruption caused by diet.[14,37] These results show that the amounts of adipose tissue can help increase the level of neuroglin 4 and consequently improve the function of adipose tissue.

In this study, the weight was reduced in the subjects of the exercise groups. This weight loss can reduce TNF-α. But the research of Yadav and Yadav found that 12 weeks of yoga exercises can make a significant difference in the level of TNF-α in overweight people.[38] Our analysis showed that 12 weeks of HIIT can be associated with the adaptation of different tissues, especially muscle tissue. Therefore, there was no significant change in the level of IL-6. Obesity and increased body mass lead to higher production of TNF-α. The increase in lipolysis due to aerobic exercise can reduce the TNF mechanism through the activities of sensitive lipase. Also, the potential mechanism of exercise training in the reduction of TNF-α, of course, includes the reduction of the mass increase, the increase of macrophages in the potential tissue and the reduction of the production of IL-6, which causes TNF-α.[39] Various studies show that a period of long-term sports activity reduces IL-6 levels, although the changes in IL-6 levels during exercise are affected by the type of subjects. Untrained subjects are likely to experience more muscle damage, and the response of cytokines to exercise shows more changes in them.[40] It seems that the intensity, duration, and type of sports activity is the main factor in determining the level of inflammatory response.[41] Of course, IL-6 is a cytokine that is produced during intense exercise more than other proinflammatory or anti-inflammatory cytokines and may be involved in metabolic changes associated with exercise.[42]

To our knowledge, this is the first reported case that simultaneously analyses for factors of CLA supplementation, HIIT, NRG-4, IL-6, and TNF-α and also it would be a reduced cost and effect factor in nonpharmacological. Therefore, the consumption of CLA in appropriate doses may potentially be a useful therapeutic strategy for obesity and related metabolic diseases. The results of our study show that CLA supplementation and exercise led to a significant increase in neuroglin 4 in obese men. The present study's results cannot be generalized due to the low sample size and participant characteristics. The main limitation of this study was the small number of subjects and dietary factors. It is important to be cautious when generalizing the results to other populations since the present study focused on obese men with BMIs above 30 kg/m^2. Another limitation of our study was the lack of measuring stress levels or sleep quality. It could have provided insight into the potential neurophysiological impact of CLA supplementation. So future research should consider the synergistic effects of combining CLA supplementation with other interventions, such as dietary modifications, pharmacotherapy, or different exercise modalities. Investigating combination therapies may offer enhanced benefits for improving metabolic health and reducing inflammation in obese individuals.

Conclusion

CLA supplementation, in conjunction with HIIT training, emerges as a highly effective approach for improving the inflammatory indicators of adipose tissue in obese adult men. CLA supplementation, in conjunction with HIIT, emerges as a highly effective approach and more benefits on the amount of adipokines secreted from fat tissue in obese people than either of them separately. The interaction effect of CLA supplement and HIIT can have more benefits on the amount of adipokines secreted from fat tissue in obese people than either of them separately. Intensity, type and duration of physical activity, level of initial preparation of subjects, gender, age, place and time of sampling, type of nutrition, heredity, characteristics and sensitivity of measuring instruments are the determining factors in the production of adipokines, which can be used as determining factors.

Conflict of Interest

None declared.

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