

Original Article

The Effect of Conjugated Linoleic Acid Supplementation on Body Composition, Serum Insulin and Leptin in Obese Adults

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Background: Studies have reported contradictory findings regarding the effect of a mixture of 2 conjugated linoleic acid (CLA) isomers on body weight and some serum indices. This study aims to investigate the effect of daily supplementation of these 2 isomers on body composition and serum leptin and insulin levels in obese adults.

Methods: This randomized, double-blind clinical trial was performed on 54 adults with class I obesity. The subjects were randomly assigned into 2 groups of 27 and were followed for 3 months so that a total of 3000 mg of CLA supplement and placebo were administered in 3 daily doses in the intervention and control groups, respectively. Body composition indices as well as fasting serum levels of insulin and leptin were also measured. The paired t-test was used for evaluating within-group effects from baseline. The independent t-test was used to compare between-group differences for variables with normal distribution.

Results: Although body weight and body mass index (BMI) were not significantly decreased during intervention in groups, but the body fat mass (BFM) ($P=0.034$), body fat percentage ($P=0.022$) and trunk fat ($P=0.027$) decreased significantly during intervention with CLA. The fasting plasma sugar ($P=0.042$) and Homeostatic model assessment for insulin resistance (HOMA/IR) ($P=0.044$) in the intervention group declined during 12 weeks of intervention. Serum leptin was associated with a significant decrease during the intervention period ($P=0.039$).

Conclusion: CLA supplementation could reduce body fat and serum leptin. Hence, it could be taken into account as a factor for weight loss but not to control or prevent diabetes.

Keywords: Body composition, Conjugated linoleic acid, Insulin, Leptin

Cite this article as: Esmaeili Shahmirzadi F, Ghavamzadeh S, Zamani T. The effect of conjugated linoleic acid supplementation on body composition, serum insulin and leptin in obese adults. Arch Iran Med. 2019;22(5):255–261.

Received: April 15, 2018, Accepted: December 23, 2018, ePublished: May 1, 2019

Introduction

Obesity refers to excessive accumulation of fat in the body, which is not only known as a fitness problem but as a multifactorial disease. Genetic factors, unlimited access to high-energy foods, industrial and urban lifestyles associated with reduced physical activity are among many factors that contribute to development and increasing incidence of obesity.¹ The danger of growing prevalence of obesity as an epidemic has long been highlighted in Iran and its prevalence has been indeed raising in recent decades in this country.^{2,3} Lifestyle changes such as modifying the pattern of dietary intake and physical activity are recognized as the fundamentals of weight management.^{4,5}

However, along with these changes, researchers have sought benefits from complementary therapies such as the use of a variety of dietary supplements and medications in weight loss. The biological active isomers of conjugated linoleic acid (CLAs) are a category of supplements used in weight loss. Since the discovery of the CLA, many studies have focused on the traits of CLA in animal models and cell culture environments (humans and animals) because it has been shown that CLA could have beneficial

effects on health including anti-adipogenic,^{5,6} anti-carcinogenic,^{6,7} anti-atherogenic,^{8,9} anti-diabetic¹⁰ and also anti-inflammatory properties.^{11,12} It has also been reported that CLA could stimulate apoptotic mechanisms as well as regulate lipolytic pathways¹³ and, therefore, it could have beneficial effects on body composition and weight loss in humans and animals.¹⁴ Some studies have suggested that CLA leads to weight loss by reducing the size of fat cells and altering the evolution of fat cells.¹⁵ Yet numerous studies have been carried out regarding the health effects of CLA on body composition changes and related factors in human and animal models.

Nevertheless, due to methodological differences, similar results have not been achieved in this context. For example, these studies have been conducted on populations with different genetic backgrounds and various inclusion and exclusion criteria. Different anthropometric and biochemical variables have been measured with various instruments with different accuracy. Also, a type of CLA isomer from the point of view of geometry structure (cis, trans and the ratio of the combination of these isomers), supplementation duration and dosage, and of the form of

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supplementation (as capsules or as dairy products enriched with CLA) were different from each other.¹⁶⁻²⁰ For instance, some studies have shown that supplementation with CLA leads to a decrease in fat mass and an increase in muscle mass in humans,¹⁷⁻¹⁹ while in other studies, such effects have not been observed.²⁰⁻²³ Due to increasing prevalence of obesity, serum leptin could be explored as a predictive risk factor for T2DM.²⁴ Although most surveys indicated a positive relationship of leptin and insulin resistance in their populations,²⁵ others showed inconsistent results.²⁶

Therefore, the present study aimed to investigate the effect of complementary conjugated linoleic acid on body composition, blood glucose, insulin, leptin and serum lipid profiles in obese adults.

Materials and Methods

Study Subjects

One hundred twenty obese persons who had returned to Weight Loss Clinic for weight reduction since January 2015 became candidates for inclusion to this study. After coordination with Nikan hospital administrators, 64 people were assigned to this study based upon exclusion criteria. All of the eligible participants were allocated into 2 groups using the random allocation rule method as follows: the intervention group (CLA group) (n = 32) and the placebo group (n = 32).

Randomization occurred by selection of a total study size of 64. Afterwards, 2 groups of cards, named A and B, were placed in a hat drawn randomly without replacement. The sequence generation would randomly order 32 subjects in the CLA group and 32 subjects in the placebo group.

From a statistical viewpoint, using formula suggested

for clinical trials, having 15 subjects in each group were adequate while considering a type 1 error (α) 0.05 and type 2 error (β) of 0.05 (power = 95%) according to Blankson et al study¹⁷ in which the mean \pm SD of changes in body fat mass (BFM) in CLA (3.4 g) and placebo groups during 12 weeks of intervention were 1.73 ± 1.9 and 1.47 ± 2.43 respectively. However with regard to subjects who dropped out the study as well as to enhance the validity of the study, we decided to increase the sample size as far as possible (n = 32). Finally, it was occurred 5 drop-outs for each group (Figure 1) and we completed the study with 27 people in each of the studied groups.

These individuals were included to study if they were 18 and 45 years' old and being at grade 1obesity (body mass index [BMI] of 30–34.9 kg/m²). The exclusion criteria were having any illnesses e.g. diabetes and cardiovascular diseases, history of cigarette smoking, use of any medications, use of CLA supplement and having a weight loss diet in 3 months ago.

It was asked all participants in this study for completing informed consent form approved by the Ethics Committee of Urmia University of Medical Sciences. All information collected from the subjects will remain confidential at all stages of the investigation. Also, as the research protocol states, the participant was asked to quit the research in case of any problem. All stages of the study were conducted by a trained expert.

Data Collection

In this randomized double-blind clinical trial study, the subjects were divided into 2 groups: CLA group receiving a daily dose of 3000 mg of CLA supplements (1000 mg t.d.s

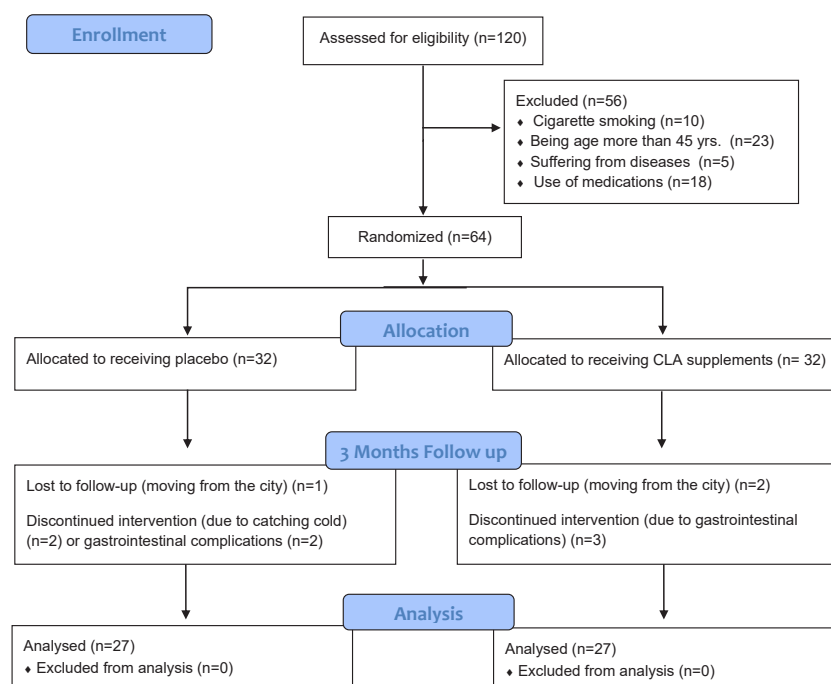


Figure 1. Flowchart Sample Selection in 2 Groups According to the Criteria for Entering and Leaving and Dropping Samples.

containing 50:50 mixture of cis-9, trans-11 and trans-10, cis-12 CLA isomers), and the placebo group receiving daily the same number of placebos (500 mg t.d.s paraffin oil) for 12 weeks. The CLA capsules used in this study were prepared by Nutricentury®/Canada and placebo capsules by the Zahravi Co. Pharmaceutical Company in Iran. Blinding was applied at 2 levels: the participants and the data gatherer co-worker. Both CLA and placebo capsules were completely similar in size and color. The encoded boxes of the both capsules were presented to participants by an assistant aware to the boxes contents. They were instructed to take 3 capsules before eating any meals. Individuals who reported that they had taken less than 80% of capsules based on self-reports or counting the number of capsules in the package delivered at the end of the study, were excluded from the final analysis of the data. Participants were asked not to change their physical activity, diet or lifestyle during the study and report any abnormal feelings quickly. Also, at beginning and end of the study, general information questionnaires, dietary intake, and International Physical Activity Questionnaire were completed and collected. The checklist for body composition measurements and biochemical tests was completed for each of the subjects.

In order to measure physical activity, a Persian translated international physical activity questionnaire²⁷ was used. The validation and reliability of the Persian translated questionnaire had been confirmed by Vasheghani-farahani et al.²⁸ The questionnaire was completed by self-administering the samples themselves at the beginning and end of the study. They were asked to not change their activity until the end of the study.

Dietary Assessment

Dietary assessment was fulfilled by 3-day written food record form. For this purpose the participants were trained at 2 public sessions by an experienced fieldworker. Accordingly, energy and macronutrients intakes were estimated as 3 consecutive days (one day off and 2 normal days). A modified version of Nutritionist IV software for Iranian community was used to analyze nutritional data. The energy and macronutrients intake as well as vitamin D and calcium intake were reported based on the kilocalories, grams and milligrams per day respectively.

Laboratory Tests

Fasting venous blood samples (5 mL) were collected from the participants by a trained laboratory technician. Blood samples were moved to anticoagulant tubes of EDTA and non-anticoagulant tubes, and transferred to the Nikan hospital clinical laboratory. The EDTA-containing tubes were closed by parafilm and slowly mixed with anticoagulants to prevent clotting. To prepare the serum, the tubes were centrifuged for 10 minutes at 1500 rpm. Serum samples were then stored in encoded microtubes

for each patient and stored at -80°C to retain if needed.

Blood glucose was measured by a procedure based upon the enzyme glucose oxidase (Mindray BS-380 Clinical Chemistry Analyzer) using the Biosystem Kit (Spain). Fasting plasma insulin levels were measured by radioimmunoassay (RIA) using the insulin assay kit (Germany), using the SIEMENS IMMULITE® 2000/2000 Xpi System. Insulin resistance was estimated based on HOMA/IR using the following formula²⁹: Fasting glucose (mg / dL) × Fasting insulin (mIU /L)/405

Serum leptin was measured using Human Leptin Sandwich EIA-2395 kit (DRG® Diagnostics- Germany). The basis of the serum leptin measurements is based on the Solid Phase Enzyme Immunoassay and Sandwich method. In order to greater reliability, biochemical tests, food intake and anthropometric measurements are performed by a certain person and/or device.

Anthropometric Measurements

The weight of the body was measured using a Seca 725 scale manufactured in Germany with 100 g accuracy, with light clothing and after urination and bowel movement. The height measurement was made using the mechanical telescopic height rod, with a precision of 0.1 cm in which the person stood with no shoes in a vertical position without the curvature of the back so that the heel and back of the leg was tangent to the wall and the head was on the Frankfurt horizontal plane.³⁰ BMI was calculated using the following formula: Weight to kilogram divided by Height to meter to the power of two.³¹

It was measured the waist circumference with a precision of 0.1 cm in the narrowest waist region between the last rib and the iliac crest, when the patient was at the end of the tail phased using a non-stretch tape.³⁰ The body composition measurement was performed by bioelectrical impedance analysis technique (model 770, Inbody Co., LTD, Seoul, Korea).

Statistical Analysis

Data were reported as mean ± standard deviation. The statistical significance level in all analyzes was considered less than or equal to 0.05. Chi-square test and/or Fisher exact test was used to examine the statistical relationships between qualitative variables. Normality of data was examined by Kolmogorov-Smirnov test and if data distribution were normal, paired *t* test was used to compare the values before and after the intervention in each of the CLA and placebo groups. If not, Wilcoxon Signed Rank Test was used. Mann-Whitney U test was used to compare the mean of data in the CLA and placebo groups, if they were normal; independent *t*-test was used. Analysis of between group differences of changes was also done using independent *t* test. Data were analyzed by SPSS software version 20.0.

Results

Of the 120 individuals participating in this study, the subjects were not suffering from any addiction to cigarettes, alcohol and narcotics, and did not suffer from any other illnesses, including endocrine, liver, diabetes, renal, cardiac or respiratory diseases as well as they did not follow any diet during the last 3 months. We included 120 participants with grade 1 obesity totally; however, 56 subjects were excluded in the eligibility phase because of not meeting inclusion criteria. Five subjects in each group were dropped out due to some reasons reflected in Figure 1. Therefore, the rate of compliance ranged 85% for both groups.

The general characteristics considered are shown in Table 1. The age of all subjects was between 29 and 64 years with the mean \pm SD of 36.72 ± 5.78 for placebo group and 38.22 ± 7.74 for CLA group. No significant difference in literacy levels, occupational or marital status was found among the groups at the beginning of the study

Table 1. General Characteristics of the Participants at the Baseline (n = 54)

Studied Variables	Placebo Group n = 27	CLA Group n = 27	P
Age (year)	36.72 \pm 5.78	38.22 \pm 7.74	0.232
Gender, No. (%)			
Male	13 (48.2)	12 (44.4)	0.784
Female	14 (51.8)	15 (55.6)	
Literacy levels, No. (%) ^a			
Illiterate	0 (0)	1 (3.7)	0.231
Under-Diploma	8 (29.7)	10 (37.0)	
Diploma	9 (33.3)	7 (26.0)	
Academic literacy	10 (37.0)	9 (33.3)	
Occupational Status, No. (%)			
Business	9 (33.3)	10 (37.0)	0.458
Governmental employee	6 (22.3)	9 (33.3)	
Housekeeper	12 (44.4)	8 (29.7)	
Marital status ^b , No. (%)			
Married	21 (77.7)	23 (85.2)	0.879
Divorced	1 (3.7)	0 (0.0)	
Single	5 (18.6)	4 (14.8)	

The values of age were shown as mean \pm SD and student *t* test was used to compare of data of 2 studied groups. The rest of the data were reported as absolute frequency and percent (in parentheses) using chi-square test (* Fisher exact test) for statistical analyses.

Table 2. The Comparison of the Body Composition Indicators at the 2 Studied Stages in CLA and Placebo Groups (n = 54)

Studied Variables	Placebo Group			CLA Group			P ^c	P ^d	P ^e
	Week 0 (n = 27)	Week 12 (n = 27)	Δ 12-0 (n = 27)	Week 0 (n = 27)	Week 12 (n = 27)	Δ 12-0 (n = 27)			
Weight (kg)	88.83 \pm 5.50	88.33 \pm 6.68	-0.50 \pm 2.21	89.38 \pm 5.31	87.29 \pm 5.56	-2.09 \pm 2.32	0.411	0.169	0.169
Height (cm) ^a	159.68 \pm 6.74	—	—	163.85 \pm 9.27	—	—	0.573	—	—
BMI (kg/m ²) ^b	32.52 \pm 1.27	31.89 \pm 1.32	-0.63 \pm 1.02	32.87 \pm 1.58	30.36 \pm 1.79	-2.51 \pm 0.98	0.678	0.184	0.089
WC (cm)	98.61 \pm 8.73	97.95 \pm 8.74	-0.66 \pm 4.22	99.12 \pm 7.47	96.34 \pm 7.14	-2.78 \pm 4.36	0.631	0.137	0.073
BFM (kg)	36.46 \pm 7.92	35.88 \pm 4.92	-0.58 \pm 4.32	37.42 \pm 8.19	34.49 \pm 5.64	-2.93 \pm 6.23	0.476	0.246	0.034
PBF (%)	37.34 \pm 6.46	36.43 \pm 5.75	-0.91 \pm 3.26	38.14 \pm 7.26	35.59 \pm 6.29	-2.55 \pm 4.26	0.382	0.263	0.022
TF (%)	40.71 \pm 3.01	40.15 \pm 4.59	-0.56 \pm 3.12	41.46 \pm 4.17	38.12 \pm 8.65	-3.34 \pm 5.29	0.364	0.031	0.027

Abbreviations: WC, waist circumference, BFM body fat mass, PBF percent of body fat, TF trunk fat
Data are presented as mean \pm standard deviation.

^a Height was not measured at the post intervention stage; ^b Body Mass Index for post intervention stage was calculated based on the height at the first stage; ^c *P* value of independent *t* test for placebo and CLA groups at baseline stage; ^d *P* value of independent *t*-test for placebo and CLA groups at post intervention stage; ^e *P* value of independent *t* test for changes of the studied variables in CLA and placebo groups.

(*P* > 0.05).

Table 2 shows the findings of the study on the body composition of the subjects. The weight for subjects in baseline were not differ significantly (88.83 ± 5.50 kg for placebo group vs. 89.38 ± 5.31 kg for CLA group, *P* = 0.411). As we can be concluded from the table, the BFM (*P* = 0.034), body fat percentage (*P* = 0.022) and trunk fat (*P* = 0.027) were decreased significantly during intervention with CLA.

The findings of the study on laboratory variables are presented in Table 3. The fasting plasma glucose (FPG) (*P* = 0.042) and HOMA/IR (*P* = 0.044) in intervention group were declined during 12 weeks of intervention. Serum leptin was associated with a significant decrease during the intervention period (*P* = 0.039). In the other words, serum leptin showed a statistical significant decrease in the intervention group (*P* = 0.039) as well as it was seen a significant improvement in HOMA/IR in CLA group (*P* = 0.044).

Tables 4 and 5 illustrate the data on dietary intake and physical activity. As shown in these tables, among the variables studied, the only increase in dietary intake of vitamin D in the CLA group was statistically significant during the study period (*P* = 0.042). The most subjects were at the minimally inactive category in both groups and in all of the studied phases within a range of 38% to 48%.

Discussion

Up to now various aspects of the beneficial effects of CLA, especially its anti-obesity and anti-diabetic effects have been studied.^{5,10} The main finding CLA supplementation could have useful effects on BFM reduction including trunk fat. The study of Steck et al was similar to our study in terms of dose and complementary CLA and supplemental length. This clinical trial study was performed on obese subjects with a BMI of 30–35 kg/m² for 12 weeks with CLA supplementation (50:50 ratios of cis-9, trans-11 and trans-10, cis-12 isomers). The supplements were given in 2 doses of 3.2 and 6.4 g/d

Table 3. The Comparison of the Laboratory Indicators at the 2 Studied Stages in CLA and Placebo Groups (n = 54)

Studied Variables	Placebo Group			CLA Group			<i>P</i> ^a	<i>P</i> ^b	<i>P</i> ^c
	Week 0 (n = 27)	Week 12 (n = 27)	Δ12-0 (n = 27)	Week 0 (n = 27)	Week 12 (n = 27)	Δ12-0 (n = 27)			
FPS (mg/dL)	101.09 ± 11.82	100.50 ± 9.12	-0.59 ± 7.23	102.62 ± 10.18	94.73 ± 8.41	-7.89 ± 6.98	0.622	0.239	0.042
Insulin (mIU/L)	14.81 ± 7.27	15.07 ± 8.61	0.26 ± 4.26	14.82 ± 8.04	14.22 ± 7.64	-0.60 ± 5.24	0.987	0.221	0.269
HOMA/IR	1.92 ± 0.76	2.17 ± 1.02	0.25 ± 0.92	2.03 ± 0.92	1.41 ± 0.92	-0.62 ± 0.89	0.473	0.164	0.044
Leptin (ng/mL)	25.67 ± 16.13	26.91 ± 16.21	1.27 ± 15.36	26.39 ± 16.14	23.96 ± 16.29	-2.43 ± 12.11	0.704	0.048	0.039

Data are presented as mean ± standard deviation.

^a*P* value of independent *t*-test for placebo and CLA groups at baseline stage; ^b*P* values of independent *t*-test for placebo and CLA groups at the post intervention stage (*P* values for Leptin were tested using Wilcoxon Signed Rank Test); ^c*P* value of independent *t*-test for changes of the studied variables in CLA and placebo groups. FPS fasting plasma glucose, HOMA/IR homeostasis model assessment of insulin resistance.

Table 4. The Comparison of the Dietary Intake Data at the 2 Studied Stages in CLA and Placebo Groups (n = 54).

Studied Variables	Placebo Group			CLA Group			<i>P</i> ^a	<i>P</i> ^b	<i>P</i> ^c
	Week 0 (n = 27)	Week 12 (n = 27)	Δ12-0 (n = 27)	Week 0 (n = 27)	Week 12 (n = 27)	Δ12-0 (n = 27)			
Energy (kcal/d)	1853 ± 279	1813 ± 318	-40 ± 259	1890 ± 350	1820 ± 379	-70 ± 316	0.389	0.342	0.229
Protein (g/d)	64 ± 17	62 ± 13	-2 ± 14	66 ± 22	64 ± 18	-2 ± 18	0.572	0.166	0.326
Carbohydrate (g/d)	234 ± 59	228 ± 57	-6 ± 49	241 ± 58	236 ± 53	-5 ± 46	0.488	0.454	0.499
Fat (g/d)	66 ± 19	66 ± 17	0 ± 14	67 ± 24	65 ± 20	-2 ± 17	0.676	0.811	0.123
Vitamin D (μg/d)	3.16 ± 0.82	2.89 ± 0.73	-0.27 ± 0.64	3.06 ± 0.58	4.22 ± 0.64	1.16 ± 0.66	0.546	0.036	0.042
Calcium (mg/d)	689 ± 62	629 ± 73	-60 ± 64	636 ± 78	654 ± 59	18 ± 52	0.371	0.496	0.493

Data are presented as mean ± standard deviation.

^a*P* value of independent *t* test for placebo and CLA groups at baseline stage; ^b*P* value of independent *t* test for placebo and CLA groups at post intervention stage; ^c*P* value of independent *t*-test for changes of the studied variables in CLA and placebo groups.

Table 5. The Comparison of the Physical Activity of the 2 Studied Stages in CLA and Placebo Groups (n = 54)

Studied Variables	Baseline		<i>P</i>	Post Intervention		<i>P</i>
	Placebo Group (n = 27)	CLA Group (n = 27)		Placebo Group (n = 27)	CLA Group (n = 27)	
Inactive	7 (26)	8 (30)	0.491	6 (22)	9 (33)	0.180
Minimally active	12 (44)	10 (37)		13 (48)	11 (41)	
HEPA* active	8 (30)	9 (33)		8 (30)	7 (26)	

Data were reported as absolute frequency and their percentages (in the parentheses) using chi-square test for statistical analyses. * Health-enhancing physical activity.

CLA. The results showed that CLA supplementation may lead to a significant increase ($P < 0.05$) in body weight or lean body mass in the receiving group of 6.4 g/d CLA, but the fat mass was alleviated in 2 intervention groups whereas it was raised in the placebo group, although none of them was not statistically significant³² The Dual-energy x-ray absorptiometry method was used to determine the body composition components in this study. Although our findings confirmed boosting effect of CLA in body fat loss, this influence was shown at a dose of 3 g/d while in the Steck study, it was achieved at a dose of 6.4 g/d. The study population of the survey, unlike our sample population, belongs to the different racial groups as well as there were differences in sex ratio between 2 studies too. Besides, it was not fulfilled an exact dietary intake assessment; so that it was only reported a point estimation of the resting energy expenditure. It was possible the participants could not achieve to a stable dietary intake of effective nutrients in adiposity status during the study period. Whereas our findings showed the groups studied were not achieve to

a significant difference in terms of the above mentioned nutrients ($P > 0.05$).

It should be noted that the results of dietary intake assessment (Table 4) shows the participants of CLA group received significantly more vitamin D intake during the study period ($P = 0.042$), and this may lead to boosting effect of CLA on the body composition. It could play a role in serum leptin levels too.³³⁻³⁵ Hence, our findings may reflect the synergistic effects of CLA and vitamin D.

As shown in Table 4, CLA supplementation alike to some other studies has no effect on macronutrient intake as well as energy intake of the diet. Similar results have been found in some studies^{22,37,38} implying the mechanism of the CLA effects on body adiposity should be searched at the other areas beyond the food intake. The findings of the studies on animal models demonstrated more obvious effects of CLA on reducing the fat mass that it could be attributed to higher doses given for laboratory animals and differences in CLA consumption isomers.³⁹

The effects of CLA supplementation on insulin

resistance and serum leptin have been investigated in both animal models and in human models. In a review article, Wang et al. stated that the addition of CLA did not have any advantage over conventional drugs for increasing insulin sensitivity.⁴⁰

Moloney et al in a randomized, double-blind, placebo-controlled trial has also suggested an increase in insulin resistance due to CLA use ($P = 0.05$).⁴¹ Based upon our findings, serum leptin and HOMA/IR that have significantly decreased in the CLA group ($P = 0.044$); although the fasting plasma insulin did not show a remarkable change, FPG showed a significant ($P = 0.042$) decrease during the period of intervention (Table 4). The reduction of serum leptin levels in this study seems logical due to a significant decrease in the adipose tissue particularly in the trunk area ($P = 0.027$).^{33,42} In a cross-sectional study, the authors pointed larger trunk fat mass as well as larger trunk lean mass were associated with higher fasting glucose.⁴³ The study population of the study has been formed from diabetic, impaired glucose tolerance and normal glucose tolerance subjects. Our results regarding serum leptin were confirmed by the findings of a meta-analysis study²⁴ in which it was asserted CLA supplementation might be able to decrease serum leptin concentration in studies with duration of less than 8 weeks particularly among male and overweight subjects.

It should be noted that in our research we did not measure serum thyroid hormones and resting energy expenditure. We also did not have access to the DXA technique to determine the composition of the body. We also could not measure serum 25OH vitamin D as a possible confounding variable.

The authors concluded that the use of CLA supplementation to help reduce the amount of adipose tissue, especially in the trunk area, along with the use of dietary regimens, could be beneficial. Furthermore, this supplementation may have a positive effect on insulin resistance in individuals with grade 1 obesity. To prove these findings, authors suggest that future studies can give a more compelling answer to this issue in the larger sample, especially if the complementary combination of CLA and vitamin D could be further efficacy in this context.

Authors' Contribution

FES and SG wrote the manuscript. FES, SG and TZ contributed to the study design and data analysis. FES performed/ analyzed experiments. SG performed statistical analysis and helped with drafting the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

This study was conducted in accordance with the Helsinki Declaration and approved by Urmia University of Medical Sciences Ethics Committee (umsu.rec.1392.202, Jan. 2014). It was also registered at Iran Clinical Trials Center (identifier: IRCT2014052413678N2;

<https://www.irct.ir/trial/13459>).

Acknowledgments

This study was conducted with the research budget of Urmia University of Medical Sciences. We are grateful to this university. We also thank Mr. Naser Sheikhi for statistical analysis of the data.

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