



Effects of Conjugated Linoleic Acid Intake in the Form of Dietary Supplement or Enriched Food on C-Reactive Protein and Lipoprotein (a) Levels in Humans: A Literature Review and Meta-Analysis

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What's Known

- Conjugated linoleic acid (CLA), prescribed as a safe nutraceutical, has shown efficacy against obesity, carcinoma, diabetes mellitus, and cardiovascular disease and boosts the immune system.

What's New

- However, we found that CLA administered as a supplement increases C-reactive protein as a risk factor for cardiovascular disease.

Abstract

Background: C-reactive protein (CRP) and lipoprotein (a) (Lp[a]) play essential roles in cardiovascular disease incidence. This study aimed to review the association between the intake of conjugated linoleic acid (CLA) in the form of dietary supplement or enriched food with different treatment durations and the levels of Lp(a) and CRP in human studies.

Methods: All the articles published in Cochrane Library, ProQuest, Scopus, and Google Scholar from November 2014 to October 2015 were searched and the clinical trials on the effects of CLA on Lp(a) and CRP levels were assessed. Of the 2249 articles initially retrieved, 21 eligible randomized clinical trials were enrolled in this systematic review. The publication dates of the eligible articles ranged from 2005 to 2013. The mean difference and the standard deviation of changes in CRP and Lp(a) levels in intervention and control groups were used as effect-size measures for meta-analysis. The obtained data from the eligible randomized controlled trials were meta-analyzed using Stata, version 13.

Results: The intake of CLA as a dietary supplement led to a significant increase in CRP levels (standardized mean difference [SMD]=0.41, 95% CI: 0.28 to 0.54; P=0.001). Subgroup analysis based on the duration of CLA consumption showed that CLA consumption more than 24 weeks resulted in a significant increase in the levels of CRP (SMD=0.52, 95% CI: 0.36 to 0.68; P=0.001) and Lp(a) (SMD=0.24, 95% CI: 0.01 to 0.47; P=0.04).

Conclusion: The current systematic review and meta-analysis showed that the long-term consumption of CLA increases the levels of CRP and Lp(a).

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Keywords • Conjugated linoleic acid • C-reactive protein • Lipoprotein (a) • Meta-analysis

Introduction

Recent studies have shown that functional foods have the potential to positively affect and contribute to public health. Conjugated linoleic acid (CLA), a fatty acid with 2 isomers, namely cis-9,

trans-11 (*c9,t11*) and trans-10, cis-12 (*t10,c12*), is considered a functional food¹ and is produced through the hydrogenation of vegetable oils in industry, by bacteria residing in the rumen of animals, or via bioconversion in the mammary gland of ruminants.² The isomers of CLA have demonstrated anti-obesity characteristics.³ Furthermore, according to the latest studies on animals, CLA has proven to be advantageous both for the prevention of carcinoma, diabetes mellitus, and cardiovascular disease (CVD) and for the furtherance of body fat decrement, lean body mass increment, and the immune system.⁴ Research on the possible adverse effects of the long-term consumption of CLA remains to be done. CLA belongs to a subgroup of fatty acids entitled “trans fatty acids (TFAs)”⁵ and randomized controlled trials (RCTs) have indicated that TFAs are detrimental to CVD risk factors.⁶

C-reactive protein (CRP) and lipoprotein (a) (Lp[a]) as nontraditional risk factors have been known to play essential roles in CVD incidence.⁷ A review of previous work on this subject reveals that there are serious discrepancies and controversies regarding the association between CLA consumption and these CVD risk factors.⁹⁻¹¹ Some of these investigations have indicated a significant increment in Lp(a) and CRP levels following CLA consumption.¹²

Since the RCTs on the effects of CLA consumption on the levels of CRP and Lp(a) have yielded discrepant findings, a systematic review and meta-analysis is necessary to settle the controversies and offer more reliable findings, especially given that commercial CLA is used as a safe dietary supplement and can be easily purchased everywhere.¹³ Accordingly, in the present study, we aimed to systematically review the association between CLA consumption in 2 distinct forms of dietary supplement and enriched food, with their respective different treatment durations, and the levels of Lp(a) and CRP in human studies.

Methods

Literature Search

A thorough search was conducted on all the articles published from November 2014 to October 2015 in Cochrane Library, ProQuest, Scopus, and Google Scholar. Additional records were identified through other sources, namely PubMed, Science Direct, and Ovid. The keywords consisted of “trans-10, cis-12-conjugated linoleic acid”, “cis-9, trans-11-conjugated linoleic acid”, “CLA fatty acid”, “CLA”, “conjugated linoleic acid”, “trans fatty acid”,

“TFA”, “C-reactive protein”, “CRP”, “lipoprotein (a)”, and “Lp(a)” with the medical subject heading [MeSH]. No restrictions were applied with regard to age, gender, and language during the search. The clinical trials investigating the effects of CLA (in the form of either dietary supplement or enriched food) on CRP or Lp(a) levels in healthy adults were objectively and meticulously assessed. The criteria for the inclusion of studies in the meta-analysis were RCT design, research on humans, healthy adult populations, intervention by using CLA dietary supplements or CLA enrichment in foods, and determination of serum CRP or Lp(a) levels as the outcome variable. Animal studies, studies with any design other than CRT design, studies on unhealthy or non-adult individuals, studies on the combined effects of CLA along with other factors such as creatine monohydrate, and studies on sport were excluded. Also excluded were studies examining the effects of CLA on other outcomes or studies lacking sufficient data (e.g., those without control groups or baseline information). As the first step, the titles and abstracts of the articles were examined and relevant papers were selected. Thereafter, the papers were screened, sorted according to their titles and abstracts, and carefully reviewed.

Data Extraction

The data of 27 articles which investigated the effects of CLA intake in either dietary supplement form or enriched food on Lp(a) and CRP in healthy adult populations were entered in the meta-analysis. However, the data of the following RCTs were not extracted: a study without complete data for analysis,¹⁴ a study without a placebo group,¹⁵ and 4 studies with unsuitable forms of CLA.¹⁶⁻¹⁹ Tables 1 and 2, respectively, provide summaries of the RCTs on the association between the intake of CLA in either dietary supplement form or enriched food and CVD risk factors in human studies.^{2-9, 11-12, 14, 17-36} All the information regarding the excluded RCTs is presented in figure 1. The mean differences and standard deviations (SDs) for changes in Lp(a) and CRP levels before and after the intervention for each of the intervention groups and control groups were extracted. In addition, the mean differences and SDs reported for the changes in Lp(a) and CRP levels within each group of intervention and control were extracted.

Based on the consumption of CLA in either dietary supplement form or enriched food, 2 subgroups were defined. The 2 subgroups were meta-analyzed separately. Additionally, in order to account for and evaluate the effects of other factors

Table 1: Eligible randomized controlled trials on supplementation with the CLA isomeric mixture with different proportions

Reference	Population	Age (mean±SD)	Duration (wk)	CLA Dose and Form	Isomers	Placebo Dose and Form	Results
Mullen and colleagues, ² 2007	30 healthy men	49.4±1.8	8	2.2 g/d of CLA	(50:50) c9,t11 t10,c12	2.2 g/d of placebo	CLA supplementation significantly changed CRP levels.
Gaullier and colleagues, ¹¹ 2004	180 men and women: healthy overweight	45.83±10.3	48	3.4 g/d of CLA-TAG 3.6 g/d of CLA-NEFA	(50:50) c9,t11 t10,c12	4.5 g/d of olive oil	CLA supplementation increased the level of Lp(a).
Gaullier and colleagues, ¹² 2007	118 men and women: healthy overweight or obese	47.25±9.6	24	3.4 g/d of CLA-TAG	(37.5:38) c9,t11 t10,c12	4.5 g/d of olive oil	Six months' CLA supplementation marginally but significantly increased Lp(a) in both the CLA and placebo groups. CRP levels were significantly increased in the CLA group compared with the placebo group.
Tholstrup and colleagues, ¹⁴ 2008	75 healthy postmenopausal women	60.16±4.46	16	4.6 g/d of CLA-mix 5.1 g/d of CLA-TAG	(41.17:39.90:1.79) c9,t11-t10,c12-other CLA (85.03:7.11:0.47) c9,t11-t10,c12-other CLA	5.5 g/d of olive oil	CRP was significantly higher in women supplemented with the CLA mixture than in those supplemented with CLA milk and the placebo.
Ormsbee and colleagues, ¹⁷ 2014	22 men and women: inactive healthy overweight or obese	35±3.45	8	1 g of fat, 99 mg of caffeine, 1510 of mg/d blend of: (green tea, CLA, and BCAA)		soybean oil	CLA supplementation had no effect on hs-CRP concentrations.
Tarnopolsky and colleagues, ¹⁶ 2007	39 community-dwelling older adults	71.1±5	24	5 g/d of CrM + 6 g/d of CLA-mix	(50:50) c9,t11 t10,c12	7 g/d of dextrose + 6 g/d of safflower oil	CrM+CLA supplementation had no significant effect on CRP levels.
Yonei and colleagues, ¹⁹ 2007	17 men and 18 women	48.3±6.6	8	700 mg/d of CLA + 200 mg/d of L-carnitine	(33.1 : 33.9) c9, t11 t10, c12	placebo	A small increase in serum levels of CRP was noted in the study group.
Watrass and colleagues, ²⁰ 2007	40 men and women: healthy overweight	33±7.5	24	3.2 g/d of CLA-mix	(39.2:38.5) c9,t11 t10,c12	4 g/d of safflower oil	Within the CLA group, the CRP level was increased.
Steck and colleagues, ²¹ 2007	48 men and women: healthy obese	34.50±4.85	12	3.2 g/d of CLA-TAG 6.4 g/d of CLA-TAG	(50:50) c9,t11 t10,c12	8 g/d of safflower oil	CLA increased the CRP level, although the absolute mean values remained within the normal limits.
Joseph and colleagues, ²² 2011	27 overweight hyperlipidemic men	18-60 (range)	8	3.5 g/d of CLA-mix 3.5 g/d of c9,t11	(50:50) c9,t11 t10,c12	3.5 g/d of safflower oil	CRP was not significantly different in men supplemented with the CLA mixture than in those supplemented with c9, t11 or the placebo.
Pfeuffer and colleagues, ²³ 2011	85 men: healthy overweight or obese	45-68 (range)	4	3.4 g/d of CLA-TAG	(50:50) c9,t11 t10,c12	4.5 g/d of safflower oil	No significant treatment-dependent changes in Lp(a) and hs-CRP were noted.

Gaullier and colleagues, ²⁴ 2005	134 men and women: healthy overweight	46.26±9.96	96	3-4 g/d of CLA-TAG 3-4 g/d of CLA-NEFA	(50:50) c9,t11 t10,c12 (50:50) c9,t11 t10,c12	3-4 g/d of placebo	Within-group comparisons showed that Lp(a) levels were increased significantly.
Blankson and colleagues, ²⁵ 2000	60 men and women: healthy overweight or obese	44.35±12.95	12	1-7 g/d of CLA-TAG 3-4 g/d of CLA-TAG 5-1 g/d of CLA-TAG 6-8 g/d of CLA-TAG	(50:50) c9,t11 t10,c12	9 g/d of olive oil	Changes in Lp(a) were not significantly different from the baseline values.
Berven and colleagues, ²⁶ 2000	60 men and women: healthy overweight or obese	47.05±3.9	12	3-4 g/d of CLA-TAG	(50:50) c9,t11 t10,c12	4-5 g/d of olive oil	Blood Lp(a) remained unchanged during the study.
Kim and colleagues, ²⁷ 2008	51 women: healthy overweight Korean women	28.24±20.3	12	2-25 g/d of CLA-NEFA	(37:95:38:84:0.96:1:35) c9,t11-CLA-t10, c12-CLA-c9,c11-CLA-t9, t11-CLA	3 g/d of olive oil	Lp(a) changes between the treatment groups had no significant difference and the values were in the normal range.
Smedman and colleagues, ²⁸ 2001	27 men and 26 women	45.5±11.45	12	2-25 g/d of CLA-TAG 4-2 g/d of CLA	(37:83:38:55:0.98:1:86) c9,t11-CLA-t10, c12-CLA-c9,c11-CLA-t9,t11-CLA (50:50) c9,t11 t10,c12	4-2 g/d of olive oil	CLA supplementation increased levels of CRP.
Taylor and colleagues, ²⁹ 2006	40 men: healthy overweight or obese	46±7	12	4-5 g/d of CLA-mix	(35:36) c9,t11-t10,c12 (1-2%) c9,c11-c10,c12 (1-5%) t9,t11-t10,t11 (<1%) t8,c10-c11,t13	4-5 g/d of olive oil	There was no change in the estimated CRP concentration.

CLA: Conjugated linoleic acid; CRP: C-reactive protein; Lp(a): Lipoprotein (a); CrM: Creatine monohydrate

Table 2: Eligible randomized controlled trials on foods enriched with CLA

Reference	Population	Age (mean±SD)	Duration (wk)	CLA Dose and Form	Isomers	Placebo Dose and Form	Results
Desroches and colleagues, ⁹ 2005	16 men: healthy overweight or obese	36.6±12.4	8	butter- CLA (4.22 g/d of CLA)	(80:20) C9,t11/ other isomers	butter (0.38 g/d of CLA)	CRP levels did not change significantly between the 2 groups.
Raff and colleagues, ³⁰ 2008	38 healthy young men	25.9±3.9	5	butter-CLA (4.6 g/d of CLA)	(39.4: 38.5) C9,t11 t10,c12	butter (0.3 g/d of CLA)	The CRP concentration did not differ between the groups either at baseline or after the intervention.
Naumann and colleagues, ³¹ 2006	92 men and women: healthy overweight or obese with LDL phenotype B	52.33±7.66	13	drinkable dairy product-CLA (3 g/d of CLA) drinkable dairy product -CLA (3 g/d of CLA)	(>80: <5) c9,t11 t10,c12 (>80: <5) t10, c12 c9,t11	drinkable dairy product (3 g/d of high-oleic-acid sunflower oil)	Plasma concentrations of CRP did not change differently between the groups.
Ramackers and colleagues, ³² 2005	42 men and women: healthy moderately overweight or subjects with LDL-phenotype B	55.6±6	13	drinkable yogurt-CLA (3 g/d of c9,t11) (3 g/d of t10,c12)	(>80: <5) c9,t11 t10,c12 (>80: <5) t10,c12 c9,t11	drinkable yogurt (3 g/d of high-oleic acid sunflower oil)	CRP did not change during CLA supplementation.
López-Plaza and colleagues, ³³ 2013	38 men and women: healthy overweight	44±8	24	skimmed milk-CLA (3 g/d of CLA)	(50: 50) c9,t11 t10,c12	skimmed milk (3 g/d of olive oil)	CLA-enriched skimmed milk did not lead to a significant variation in CRP levels.
Penedo and colleagues, ³⁴ 2013	29 healthy normal-weight young adults	25.9±6.24	8	butter-CLA (20 g/d of c9,t11 CLA)	c9,t11 CLA	low-fat dairy products (skimmed milk, fat-free yogurt and low-fat cheeses)	CLA-enriched butter had no effects on the serum levels of CRP.
Lopes and colleagues, ³⁵ 2013	28		16	milk-CLA (4 g/d of CLA)		milk (4 g/d of canola oil)	There was no significant difference between CRP levels obtained at months 0 and 4.
Smit and colleagues, ³⁶ 2011	61 men and women: healthy	31±14	3	margarines and yogurt drinks -CLA (50 g/d of CLA)	(80:20) c9,t11 t10,c12	margarines and yogurt drinks (50 g/d of high oleic acid sunflower oil)	There was no effect of c9, t11 CLA supplementation on CRP.

CLA: Conjugated linoleic acid; CRP: C-reactive protein; LDL: Low-density lipoprotein; NEFA: Non esterified fatty acids

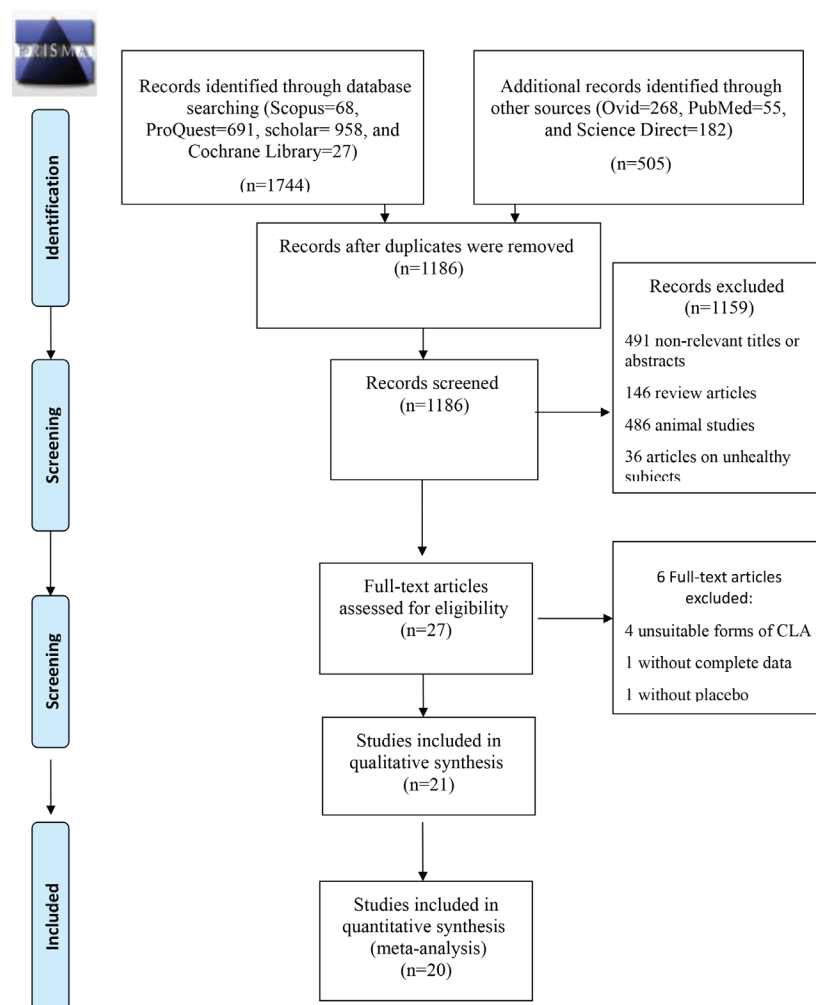


Figure 1: PRISMA flowchart shows the literature search and review.

such as the length of CLA dietary supplementation on CVD risk factors (CRP and Lp[a]), we devised 2 other subgroups. Furthermore, heterogeneity was diminished by determining and subsequently deleting the outlier studies based on the funnel plot. Therefore, 2 separate sets of data (with elimination and without elimination of the outlier studies) were included in the meta-analysis.

The process for study selection (i.e., screening and eligibility), method of data extraction (i.e., piloted forms, independently, and in duplicate), listing and defining the variables, and processes for data obtaining and confirmation were all selected and done in complete accordance and compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 checklist.

Risk of Bias Assessment

The qualitative Critical Appraisal Skills Program (CASP) for RCTs was used to assess the risk of bias in the studies included in the review. Based on this tool, 2 screening questions

were first used to decide whether it was worth proceeding to the following 8 detailed questions: selection bias, allocation bias, confounding, blinding, data collection method, withdrawal and dropouts, statistical analysis, and intervention integrity. The detailed questions were used to make a qualitative decision as to whether or not to include the study in the review. In addition, the quantitative 5-Point Jadad Score was applied to assess the quality of the relevant studies based on randomization, concealment of the treatment allocation, blinding, completeness of follow-up, and use of intention to treat analysis. Each item was given a score with a possible score of 0 (lowest level of quality) to 5 (highest level of quality). Studies with higher scores were considered to possess higher qualities.

Statistical Analysis

All the outcomes were recorded as continuous variables, and pooled meta-analyses were completed for the studies which reported the same outcomes. The mean differences and SDs

were combined with a Cohen random-effect model. The mean difference was calculated by subtracting the mean values before and after the intervention for the experimental and control groups. If it was not directly reported, the SD of the mean differences was calculated using,

$$SD = \sqrt{SD_b^2 + SD_a^2 - 2 * r * SD_b * SD_a}$$

where SD_b =SD before, SD_a =SD after, and r =correlation between the scores before and after the intervention. The correlation was estimated using,

$$r = \frac{SD_b^2 + SD_a^2 - SD_c^2}{2SD_bSD_a}$$

where SD_b , SD_a , and SD_c represent the SD for before, after, and change scores, respectively. The median correlation for change from the baseline among the trials included in the systematic reviews was 0.66 (interquartile range=0.40, 0.90).^{11, 21, 24, 30, 37}

It was found that a simple formula:

$$\bar{x} = \frac{a + 2m + b}{4}$$

could be used to estimate the mean by using the values of the median (m) and the low end and high end of the range (a and b , respectively). Additionally, the variance can be estimated via the formula:

$$s^2 = \frac{1}{12} \left\{ \frac{(a - 2m + b)^2}{4} + (b - a)^2 \right\} \quad 38$$

The I^2 and χ^2 tests were used to assess heterogeneity between the studies. All the meta-analyses were done with the Cohen random-effect analysis model. Forest plots were applied to illustrate the results of the meta-analyses as well as heterogeneity in the results. In addition, 95% confidence intervals (95% CIs) were calculated for the standardized mean difference (SMD), and all the results were reported at a significance level of 0.05. The presence of publication bias was explored by constructing a funnel plot in which the estimate of the reported effect size was plotted against a measure of precision. Because an inevitable degree of subjectivity exists in the interpretation of funnel plots, the corresponding Egger's tests were used to reassuringly assess the potential existence of publication bias. All the statistical analyses were conducted with Stata, version 13 (College Station, TX, USA).

Results

Of the 2249 articles initially identified, the titles and abstracts of all the articles were reviewed to identify and select appropriate articles for inclusion. A total of 27 eligible RCTs were selected for inclusion in the analysis. Next, the full text of each article was reviewed and the findings were re-evaluated. The flowchart depicting the literature search and review is provided in figure 1.

Of the eligible articles, 4 studies were excluded due to the use of non-eligible forms of CLA.¹⁶⁻¹⁹ One article¹⁴ lacked inclusive data, and another study¹⁵ did not use a placebo control. Twenty-one articles were left to be included in the meta-analysis. Out of all the studies evaluating the effects of CLA intake in the form of either dietary supplement or enriched food on CRP,^{12, 14, 17-23, 31, 32, 39, 40} the results of 7 studies indicated no significant change in CRP.^{2, 17, 18, 22, 23, 31, 32} Out of all the studies included in the present review on the effects of the CLA isomeric mixture on Lp(a), with the exception of 2 investigations,^{11, 24} none reported a significant change in Lp(a). The characteristics and main outcomes of the RCTs are depicted in tables 1 and 2.

CLA Intake in the Form of Dietary Supplement or Enriched Food and CRP

The findings of the human studies and the overall summary results for 15 RCTs are shown in figure 2. First, the analysis of the 15 RCTs demonstrated a significant increase in CRP (SMD=0.43, 95% CI: 0.22 to 0.65; $P=0.001$) with the highest heterogeneity (I^2 [variation in the SMD attributable to heterogeneity]=67.4%, heterogeneity $\chi^2=52.09$ [df. =17]; $P<0.001$). Based on the funnel plot, the outlier studies^{2, 21, 30} were eliminated (figure 3; funnel plot) and consequently the heterogeneity was reduced to $I^2=16.4\%$. However, the results obtained following the elimination, confirmed that CLA intake significantly increased CRP levels (SMD=0.30, 95% CI: 0.16 to 0.44; $P<0.001$). Further, there was no significant heterogeneity between the studies ($I^2=16.4\%$, heterogeneity $\chi^2=16.74$ [df.=14]; $P=0.27$) (figure 4). The studies were categorized based on CLA sources. The subgroup analysis based on CLA sources showed that CLA dietary supplement led to a significant increase in CRP levels (SMD=0.41, 95% CI: 0.28 to 0.54; $P=0.001$). The subgroup analysis based on CLA-enriched foods showed that the consumption of these food items led to a nonsignificant increase in CRP levels (SMD=0.05, 95% CI:-0.22 to 0.33; $P=0.7$).

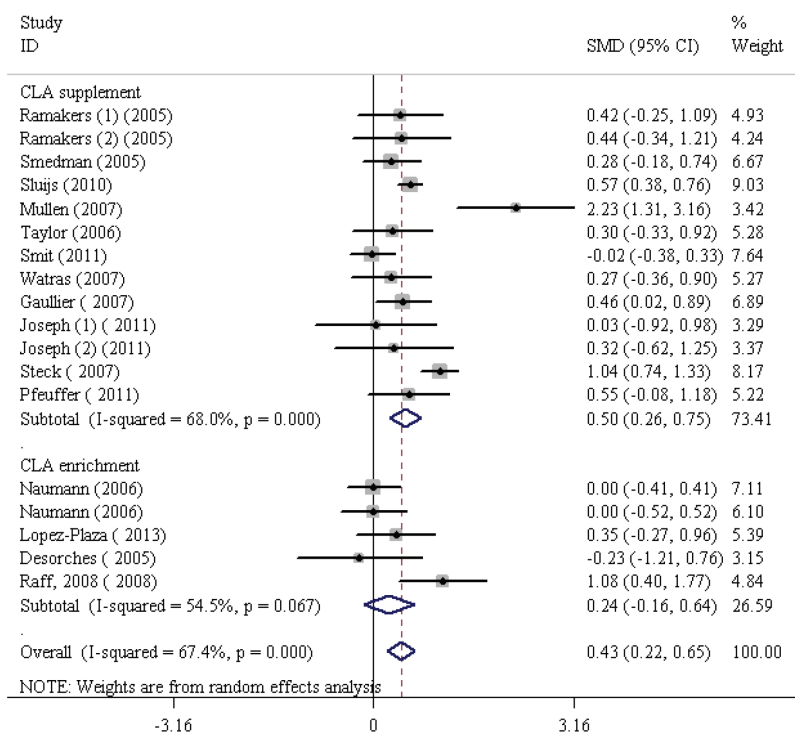


Figure 2: Meta-analysis shows a significant increase in C-reactive protein following conjugated linoleic acid supplementation. The black squares show study-specific standardized differences (Std diff) in means, and the horizontal lines show 95% CIs. The area of the black squares is proportional to the specific-study weight in the overall meta-analysis. The center of the black diamonds indicates the pooled standardized difference in means, and their width represents the pooled 95% CI.

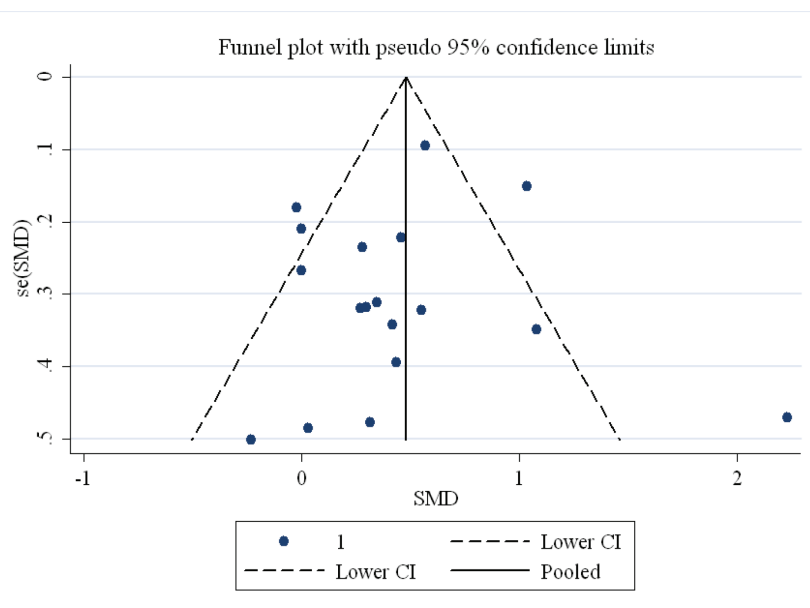


Figure 3: Funnel plot for the included studies on the effects of conjugated linoleic acid on C-reactive protein demonstrates outlier studies.

The test for heterogeneity was not statistically significant for the CLA dietary supplement subgroup ($I^2=3\%$, heterogeneity $\chi^2=10.03$ [df.=10]; $P=0.43$) (figure 4) and nonsignificant for the CLA-enriched food subgroup ($I^2=0.00\%$, heterogeneity $\chi^2=1.30$ [df.=3]; $P=0.73$) (figure 4). Moreover, publication bias was found not to be significant (Egger’s test $P=0.199$).

CLA Dietary Supplementation and Lp(a)

The findings of the human studies and the inclusive summary results for 8 RCTs are shown in figure 4. CLA dietary supplementation led to a nonsignificant change in Lp(a) levels (SMD=0.17, 95% CI: 0.02 to 0.32; $P=0.15$); the test result for heterogeneity was not statistically significant ($I^2=32.1\%$, heterogeneity $\chi^2=16.21$ [df.=11];

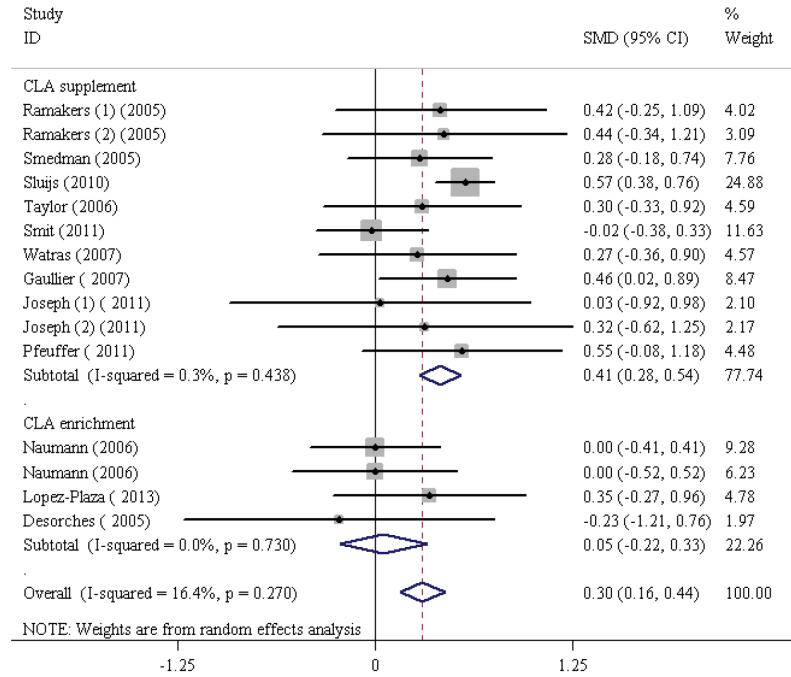


Figure 4: Meta-analysis shows a significant increase in C-reactive protein following conjugated linoleic acid supplementation after dropping the outlier studies. The black squares show study-specific standardized differences (Std diff) in means, and the horizontal lines show 95% CIs. The area of the black squares is proportional to the specific-study weight in the overall meta-analysis. The center of the black diamonds indicates the pooled standardized difference in means, and their width represents the pooled 95% CI.

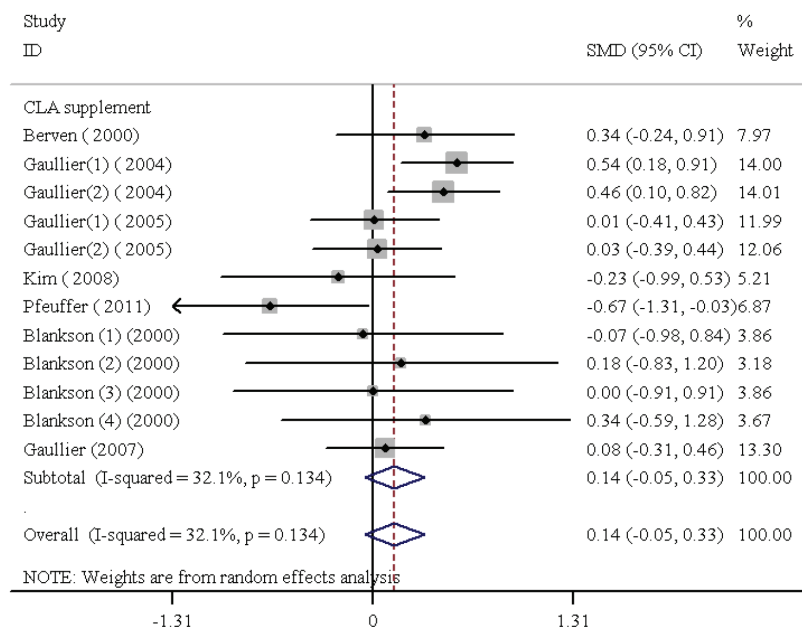


Figure 5: Meta-analysis shows no significant change in lipoprotein (a) following conjugated linoleic acid supplementation. The black squares show study-specific standardized differences (Std diff) in means, and the horizontal lines show 95% CIs. The area of the black squares is proportional to the specific-study weight in the overall meta-analysis. The center of the black diamonds indicates the pooled standardized difference in means, and their width represents the pooled 95% CI.

P=0.134) (figure 5). In addition, publication bias was found not to be weighty (Egger's test P=0.446).

CLA Consumption Duration and CRP

The SMDs and 95% CIs for all the 12 studies on the effects of CLA, irrespective of its

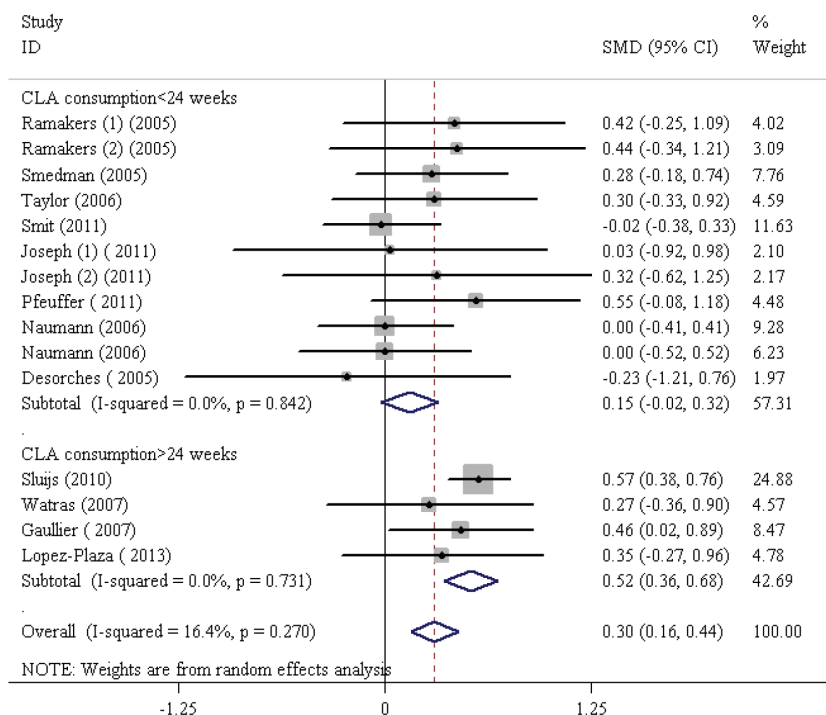


Figure 6: Meta-analysis shows a significant increase in C-reactive protein after conjugated linoleic acid supplementation for more than 24 weeks. The black squares show study-specific standardized differences (Std diff) in means, and the horizontal lines show 95% CIs. The area of the black squares is proportional to the specific-study weight in the overall meta-analysis. The center of the black diamonds indicates the pooled standardized difference in means, and their width represents the pooled 95% CI.

consumption in the form of dietary supplement or enriched food, on CRP are presented in figure 5. CLA consumption greatly increased CRP levels (SMD=0.30, 95% CI: 0.16 to 0.44; P=0.001). There was significant heterogeneity between the studies ($I^2=16.4%$, heterogeneity $\chi^2=16.74$ [df.=14]; P<0.27) (figure 6). The studies were categorized based on the duration of CLA consumption. The subgroup analysis based on CLA consumption periods of less than 24 weeks indicated a nonsignificant rise in CRP levels (SMD=0.15, 95% CI: -0.02 to 0.32; P=0.08). The subgroup analysis based on the duration of CLA consumption demonstrated that CLA intake with regard to time intervals longer than 24 weeks led to a consequential increase in CRP levels (SMD=0.52, 95% CI: 0.36 to 0.68; P=0.001). The test for heterogeneity was not statistically notable for the subgroup with CLA consumption durations of more than 24 weeks ($I^2=00.0%$, heterogeneity $\chi^2=1.29$ [df.=3]; P=0.73) and nonsignificant for CLA consumption durations of less than 24 weeks ($I^2=0.00%$, heterogeneity $\chi^2=5.67$ [df.=10]; P=0.84) (figure 5). In addition, publication bias was found not to be considerable (Egger's test P=0.199).

CLA Consumption Duration and Lp(a)

The SMDs and 95% CIs for all the 7 studies

on the effects of CLA consumption in the form of dietary supplement on Lp(a) are outlined in figure 7. CLA consumption did not confer a significant boost to Lp(a) levels (SMD=0.14, 95% CI: -0.05 to 0.26; P=0.15). In addition, there was no significant heterogeneity among the studies ($I^2=32.1%$, heterogeneity $\chi^2=16.21$ [df.=11]; P=0.13) (figure 7). The studies were categorized based on the duration of CLA consumption. Next, the subgroup analysis done on the basis of the duration of CLA consumption demonstrated that CLA supplementation for periods longer than 24 weeks led to a considerable increase in Lp(a) levels (SMD=0.24, 95% CI: 0.01 to 0.47; P=0.04). The test for heterogeneity was not statistically significant for the subgroups with CLA consumption for longer than 24 weeks ($I^2=41.9%$, heterogeneity $\chi^2=6.88$ [df.=4]; P=0.14). On the other hand, the subgroup analysis based on CLA consumption periods of less than 24 weeks showed that the consumption of these food items resulted in a nonsignificant decrease in Lp(a) levels (SMD=-0.04, 95% CI: -0.35 to 0.26; P=0.37). Additionally, there was no noteworthy heterogeneity for subgroups with CLA consumption periods of less than 24 weeks ($I^2=6.7%$, heterogeneity $\chi^2=6.43$ [df.=6]; P=0.37) (figure 7). Finally, publication bias was found not to be consequential (Egger's test P=0.446).

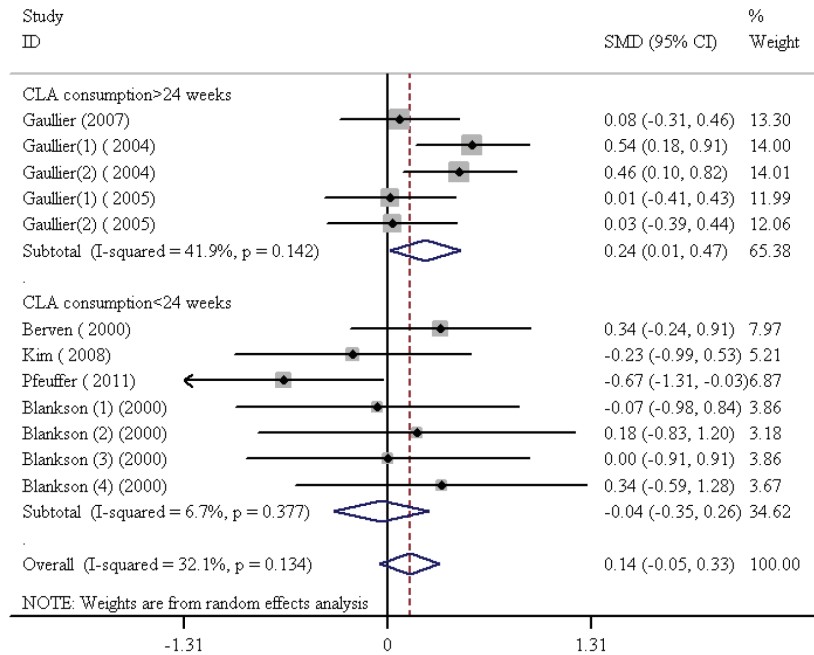


Figure 7: Meta-analysis shows a significant increase in lipoprotein (a) after conjugated linoleic acid supplementation for more than 24 weeks. The black squares show study-specific standardized differences (Std diff) in means, and the horizontal lines show 95% CIs. The area of the black squares is proportional to the specific-study weight in the overall meta-analysis. The center of the black diamonds indicates the pooled standardized difference in means, and their width represents the pooled 95% CI.

Discussion

The present systematic review and meta-analysis of RCTs concluded that CLA intake and particularly CLA dietary supplements notably increased CRP levels. In accordance with the findings of the present meta-analysis, the results of several studies have asserted and justified the significant increases in CRP levels following CLA consumption, particularly in the form of commercial dietary supplements.^{12, 14, 19-21, 39, 40} Nonetheless, there are some RCTs in the existing literature reporting no considerable impact.^{7, 18, 22, 23, 31, 32} The findings of some studies have exhibited significant gains in CRP levels following *t10,c12* CLA isomer supplementation.^{41, 42} Moreover, the findings of some of the reviewed studies showed that the differences in the proportion of the isomers of CLA (*c9,t11* and *t10,c12*) did not control the impact of CLA consumption on CRP. Numman and colleagues³¹ and Remark and colleagues³² examined 2 different proportions of 2 of the most abundant isomers of CLA (*c9,t11* and *t10,c12*) on CRP in a group of participants and found no notable variations. We assume that other factors such as genetic predisposition, gender, and duration of CLA administration might account for and explain the incongruous findings having been reported in different studies. Abdelmagid and colleagues⁴³ showed that the plasma

concentration of CLA was influenced by stearoyl-CoA desaturase 1 (SCD1) genetic variations and hormonal status. It has been reported that the circulating CLA concentration is significantly higher in women than in men.⁴³ It could be concluded that even a modest amount of *t10,c12* CLA induces considerable inflammatory effects in humans. The duration of CLA administration might also explain the inconsistencies in findings and address the posited discrepancies. Our subgroup meta-analysis based on the duration of the intervention illustrated that CLA consumption for periods of 24 weeks or longer increased CRP levels.

The specific intricate mechanism which accounts for the impact of CLA consumption on CRP is still ambiguous and requires further research. Steck and colleagues²¹ reported that higher dosages of CLA dietary supplements might give rise to inflammatory markers in healthy obese adults. Consistently, CLA dietary supplementation in human subjects has increased the 15-keto-dihydro-PGF2a urine level, a marker of cyclooxygenase-catalyzed lipid peroxidation. It should be borne in mind that increases in CRP and 15-keto-dihydro-PGF2a are correlated.³⁰ On the other hand, CLA has at least a trans double bond and belongs to the TFA group.⁴⁴ The relationship between TFA and inflammation is clear and can be found elsewhere.⁴⁵ CRP is an acute-phase protein

produced by the liver up to 1000 times through inflammation. It is more sensitive to inflammatory cytokines than other acute-phase proteins.⁴⁶ Therefore, a rise in CRP levels after an increase in CLA levels in circulation is expectable.

We observed no significant increase in Lp(a) concentration following CLA dietary supplementation in RCTs. However, when we categorized the studies based on the duration of CLA consumption, the subgroup analysis demonstrated that CLA supplementation for periods longer than 24 weeks led to a consequential increase in Lp(a) levels. The results obtained through our analysis proved to be in complete accordance with the findings of 5 other studies^{12, 23, 25-27} and in discordance with 2 others.^{11, 24} The duration of CLA consumption can explain the discrepancy between the 2 groups of studies. Gaullier and colleagues,^{11, 24} in 2004 and 2005, used various forms of CLA mixtures in healthy overweight subjects for 1 and 2 years, respectively, and concluded that CLA supplementation was able to increase the level of Lp(a). Nevertheless, in another study conducted in 2007, Gaullier and colleagues¹² used CLA-TAG over a 6-month interval in healthy overweight and obese subjects and found no differences in Lp(a) levels between the CLA group and the placebo group. Similarly, other studies with durations of CLA supplementation for 4 weeks,²³ 12 weeks,^{25, 26} and 24 weeks¹² found no effect on Lp(a). In the studies conducted by Gaullier and colleagues, which took place over a period longer than 1 year, Lp(a) increment was accompanied by increases in leukocytes and thrombocytes, suggesting an inflammatory and immunological response to CLA supplementation. However, the precise process and functioning are still not fully clear and require further research.

In humans, no limits on safety parameters (duration, dosage, variety, etc.) have been reported with CLA consumption. Studies on overweight or obese adults, with different doses of CLA (i.e., 1.7, 3.4, 5.1, and 6.8 g/d) and durations (i.e., 4, 8, 12, 36, 48 weeks and more), have been conducted.^{28-30, 33-36} A consensus should emerge on the usage of CLA and the upper limit for this functional food.

The major strength of the present study lies in its analysis of CLA dietary supplements and CLA-enriched foods in separation of one another. Moreover, consideration and examination of biases, with specific focus on publication bias, can be pointed out as another decisive and differentiating strength of the current study.

As for the potential limitations or shortcomings of the present study, we note that the current meta-analysis strives to reconcile the conflicting

results previously reported and merely reflects the existing evidence. Furthermore, deficiencies which have their roots in the design, conduct, analysis, and interpretation of the research, along with the ability to identify sources of diversity across various types of studies have served as another source of limitation.

It is important to bear in mind, however, that in general, not only is meta-analyses unable to improve the quality of the original studies or address the variability of patient populations, but also they are incapable of enhancing the quality of the data and diminishing the potential for the underlying biases.

Conclusion

The current systematic review and meta-analysis of randomized placebo-controlled trials illustrated that CLA in the form of dietary supplement greatly affects some CVD risk factors such as CRP and Lp(a). Nonetheless, the length of intervention is a determining factor in the safety of the CLA consumer. We conclude that the long-term consumption of CLA increases CRP and Lp(a) levels.

Since CLA has long been widely present in various diets and also in commercial dietary supplements, long-term trials with different doses of CLA on various patient populations are deemed essential to attest the safety of commercial CLA dietary supplements, endorse their use, and ensure the absence of any adverse impact or danger to human health.

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