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Review Article

Adverse effects of conjugated linoleic acids supplementation on circulating lipoprotein (a) levels in overweight and obese individuals: results of a systematic review and meta-analysis of randomized controlled trials

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Abstract: Conjugated Linoleic Acids (CLA) may have beneficial effects on the prevention of atherosclerosis, but their net effects on circulating levels of lipoprotein (a) [Lp (a)] are unclear. The present study aimed to systematically review and analyze the Randomized Clinical Trials (RCTs) assessing the effects of CLA on circulating Lp (a) concentrations. A literature search of SCOPUS, PubMed-Medline, ISI, Web of Science, and Cochrane library databases was conducted for the relevant RCTs investigating the effects of CLA supplementation on circulating Lp (a) levels, which had been published up to 20 August 2020. Weighted Mean Difference (WMD) and 95% Confidence Intervals (CI) were reported as the summary statistics. Statistical analysis were done with Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ). Totally, six studies with 13 treatment arms including 752 subjects were included in the meta-analysis. The results showed a significant increase in circulating Lp (a) levels after CLA supplementation (WMD: 16.68 mg/L, 95% CI: 5.43-27.93; P=0.004) with no evidence of heterogeneity across the studies. In the subgroup analysis, a more significant elevation of Lp (a) levels was observed in the trials lasting for six months or more (WMD: 21.61 mg/L, 95% CI: 9.85-33.37, P<0.001) as well as in those with a supplementation dosage of ≥3.5 g/d (WMD: 26.13 mg/L, 95% CI: 7.02-45.24, P=0.007). These findings were sensitive to one study. It can be concluded that CLA supplementation with a dose of ≥3.5 g/d over a six-month period might significantly increase the circulating Lp (a) concentrations.

Keywords: Trans-10, cis-12-conjugated linoleic acid, cis-9, Trans-11-conjugated linoleic acid, lipoprotein (a)

Introduction

Lipoprotein (a) [Lp (a)], an atherogenic particle, is similar to Low-Density Lipoprotein (LDL) [1] with a different metabolic activity [2]. The atherogenicity of Lp (a) has been well documented in the previous studies [3, 4]. In addition, a large body of evidence has shown a relationship between high Lp (a) concentrations and increased risk of coronary artery disease [5, 6], myocardial infarction [7], venous thromboembolism [1], aortic valve calcification in patients with familial hypercholesterolemia [8], stroke [9], inflammation, and foam cell formation [10, 11]. Several possible mechanisms have been proposed for the association between Lp (a) and cardiovascular disease, including binding to the extracellular matrix of arterial intima and growth plaques of atherosclerosis [12], a prothrombotic effect due to its similarity to plasminogen, a fibrinolytic enzyme [13], and binding to oxidized phospholipids that promote its atherogenicity [14].

Although Lp (a) levels are genetically regulated, some reports have shown that dietary interventions, specifically a pharmacological dose of niacin or nicotinic acid, might help to decrease them [15]. Various clinical trials have assessed the effects of Conjugated Linoleic Acids (CLA) supplementation on circulating Lp (a) concentrations in different populations [16-20]. Some

studies have demonstrated the positive effects of CLA supplementation on circulating Lp (a) levels [20], while others have shown that CLA supplementation increased Lp (a) concentrations [17, 19]. Gaullier et al. [18] showed a significant increase in circulating Lp (a) levels in the overweight people who had received CLA. However, Pfeuffer et al. [20] indicated that 3.5 g/d CLA supplementation could decrease Lp (a) levels.

Based on what was mentioned above, the available published Randomized Controlled Trials (RCTs) demonstrated a substantial amount of uncertainty regarding the net effect of CLA supplementation on plasma Lp (a) levels. In order to resolve the controversy, this meta-analysis aims to investigate the effect of CLA supplementation on plasma Lp (a) levels. It is worth mentioning that this is the first meta-analysis conducted on this issue.

Materials and methods

Search strategy

This meta-analysis was designed based on the guidelines of the PRISMA statement [21]. SCOPUS (http://www.scopus.com), Medline (http://www.ncbi.nlm.nih.gov/pubmed), ISI, Web of Science, and Cochrane Library databases were searched up to 20 August 2020 in order to find the studies investigating the influence of CLA supplementation on Lp (a) concentrations using the following MeSH terms and keywords: (((((“trans-10, cis-12-conjugated linoleic acid” [Supplementary Concept]) OR “cis-9, trans-11-conjugated linoleic acid” [Supplementary Concept]) OR “conjugated linoleic acid” [Title/Abstract]) OR CLA [Title/Abstract])) AND (((((“Lipoprotein (a)” [Mesh]) OR “lipoprotein (a)” [Title/Abstract]) OR “Lp (a)” [Title/Abstract]) OR “LP (a)” [Title/Abstract])). The reference lists of the included articles and the related reviews and meta-analyses were handsearched, as well.

Study selection

Two independent investigators (K.L. and A.K.) reviewed the titles and abstracts of all identified studies to ascertain whether these studies were eligible for the meta-analysis based on the inclusion criteria. In case of discrepancies, the third investigator (M.MS.) was involved. The studies were chosen for analysis if they met the following criteria: (i) being an RCTs with either parallel or crossover design conducted among adults (age ≥18 years), (ii) having an intervention duration of at least two weeks, (iii) having a suitable controlled design; i.e., if CLA was supplemented as an adjunct to another drug/supplement, the control group contained that drug/supplement, and (4) presenting sufficient data on Lp (a) concentrations and their corresponding Standard Deviations (SDs) in CLA and control groups at baseline and at the end of the follow-up.

Data extraction

The inclusion-exclusion screening form was used to select the eligible articles. After selecting the eligible articles, the data of RCTs were reviewed independently by two authors (K.L. and A.K.) and the following data were abstracted using a standardized electronic form: first author’s name, publication year, study location, study design, duration of the intervention, sample size in each group, form and administered dose of CLA, type of placebo, age, Body Mass Index (BMI), and percentage of females. The mean values and SDs of the intended outcome were also extracted at the study baseline, post-intervention, and between baseline and post-intervention. For the studies that reported data at multiple measurements or multiple doses, only the measures of the longest durations of treatment at the end of the trials as well as each dose of supplementation were used in the meta-analysis.

Quality assessment

The quality of the eligible studies was evaluated using the Jadad scale [22], which assigned zero or one point to each of the following five criteria: 1) reporting randomization, 2) describing a suitable method of randomization, 3) reporting double-blinding, 4) describing a suitable method of double-blinding, and 5) reporting the explanations and reasons for withdrawals and dropouts. The trials were considered high-quality if they obtained a score of three or higher [23]. Moreover, two authors (AK and ZS) have performed the quality assessment of RCTs by using Cochrane Collaboration risk of bias Tool. In this assessment, studies were evaluated according to the following domains: random sequence generation, concealment of

allocation to conditions, inhibition of awareness of the allocated intervention, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. The status of each domain was defined as "Low", "High" and "Unclear" risk of bias. RevMan V5.3 was executed to draw a proper graph.

Quantitative data synthesis and statistical analysis

The mean Lp (a) changes from baseline were used to assess the effects of CLA in both intervention and placebo groups. Net changes were calculated as follows: value at the end of the trial - the value at baseline. The effect sizes were defined as Weighted Mean Difference (WMD) and 95% Confidence Interval (CI). The following formula was used for calculating the SDs of the mean differences: SDs = square root [(SD pre-intervention)^2 + (SD post-intervention)^2 - (2 R × SD pre-intervention × SD post-intervention)], assuming a correlation coefficient of 0.5 because this is a conservative estimate between zero and one [24]. If Standard Errors (SEs) were reported instead of SDs, they were converted to SDs for analysis using the following formula: SDs = SEs × square root (*n*), where *n* was the sample size in each group. The meta-analysis (heterogeneity, sensitivity analysis, meta-regression, and publication bias) was performed by Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) [25]. *P-values less than 0.05 were considered statistically significant.* Heterogeneity was assessed by Cochran's Q-test (with significance set at P< 0.1). In addition, I-squared (I^2) statistic was used for calculating the percentage of heterogeneity (I^2 value ≥50% was assumed to indicate substantial heterogeneity among the studies). Moreover, the pooled effect size was calculated using a random effects model in the presence of heterogeneity; otherwise, a fixed effects model was applied. The pre-defined subgroup analyses were conducted based on the dose of supplementation and trial duration. Besides, sensitivity analysis was done via the leave-one-out method (i.e., removing a single trial each time and repeating the analysis) to assess the impact of each study on the overall effect size. Furthermore, meta-regression was performed using the unrestricted maximum likelihood method to explore the association among the net effect size, CLA dose, duration of supplementation, and the participants' baseline BMIs. The potential publication bias was detected using the funnel plot, Begg's rank correlation, and Egger's weighted regression tests. For adjusting the analysis in the effects of the publication bias, use was made of the Duval and Tweedie "trim and fill" and "fail-safe N" methods [26].

Results

Search results and study selection

The flowchart of the selection process in the meta-analysis has been presented in **Figure 1**. Out of the 28 studies initially identified, 12 articles were excluded for duplication. Thus, 12 articles were included for title and abstract screening. However, eight articles were excluded because they were either not RCTs on humans or unrelated to the present meta-analysis according to the inclusion criteria after a careful review of the titles and abstracts. Among the four articles remaining, one was excluded because of the lack of a control group. On the other hand, three articles were found during the hand-search. Finally, six studies were included in the meta-analysis [16-20, 27].

Characteristics of the included studies

The characteristics of the studies that met all inclusion criteria of the meta-analysis have been presented in **Table 1**. The six eligible studies contained 13 treatment arms, which included 752 participants (392 in the CLA arm and 360 in the control arm). The sample size in these trials ranged from 47 to 130 participants. The included studies were published between 2000 and 2011 and were conducted in Norway (four studies) and Germany. The CLA doses administered in the included studies ranged from 1.7 to 6.8 g. All trails were conducted among overweight and obese individuals. Out of the six trials used in the meta-analysis, one was conducted exclusively on males and the rest were conducted on both sexes. Duration of supplementation with CLA ranged from four weeks to 12 months. All the studies used the parallel design. The demographic and baseline parameters of the included studies have been presented in **Table 1**.

Data quality

The quality of the eligible studies ranged from three to five (maximum score). Hence, all the

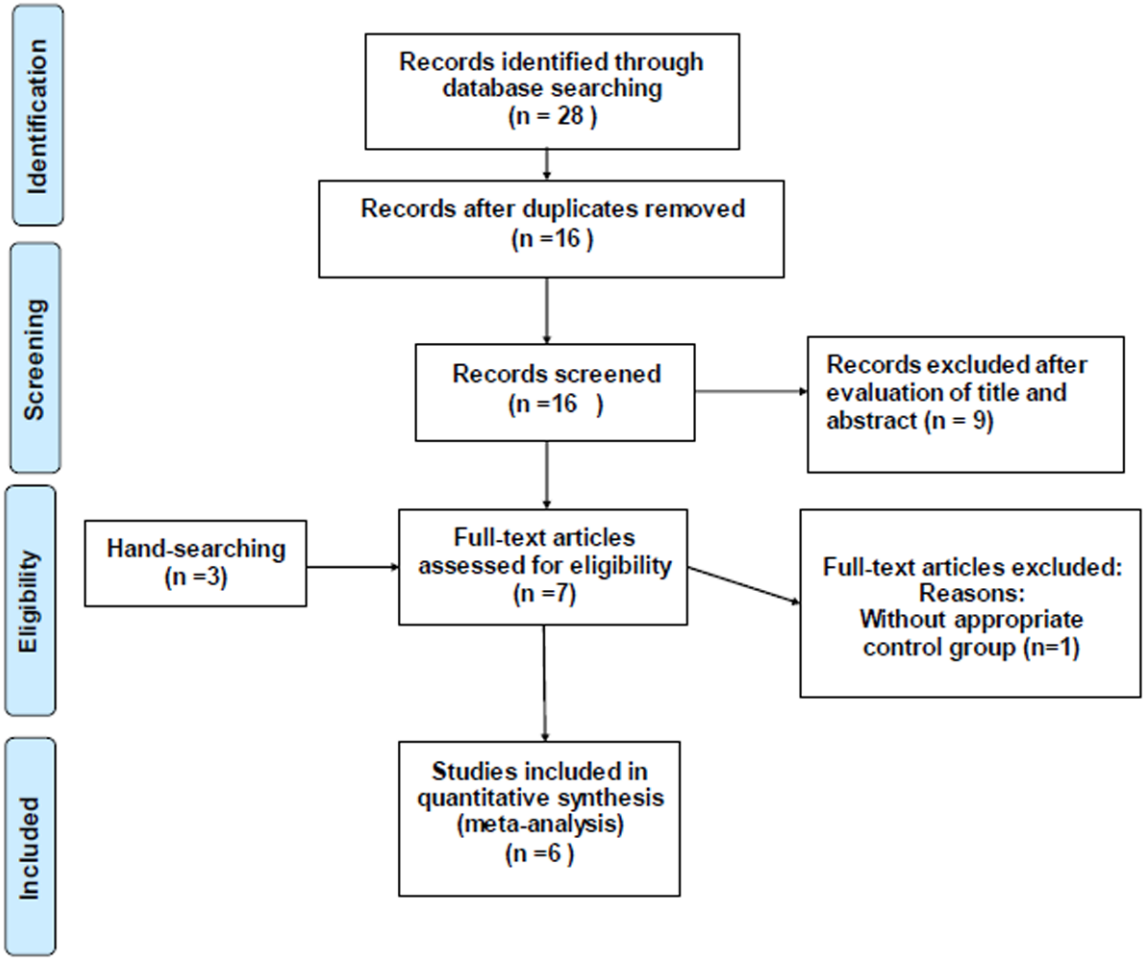


Figure 1. Flowchart of the number of studies identified and included in the meta-analysis.

studies were high quality as assessed by the Jadad scale (**Table 1**). In addition, all the studies had a parallel design and were randomized, double-blind, and placebo-controlled. However, three studies had not adequately explained the method of randomization [19, 20] and one had not explained the blinding procedure [20]. All the studies had reported the details of dropouts and the reasons for withdrawal. Methodological quality of RCTs based on authors' judgments according to Cochrane Collaboration risk of bias Tool is shown in **Figure 2**.

Pooled effects of CLA on circulating Lp (a) levels

The effect of CLA on circulating Lp (a) levels was reported in six trials with 13 treatment arms. The forest plots of the RCTs evaluating the effects of CLA supplementation on circulating Lp (a) levels have been illustrated in **Figure 3**. The net change of Lp (a) concentrations showed a significant increase in circulating Lp (a) levels following CLA supplementation (WMD: 16.68 mg/l, 95% CI: 5.43-27.93, P=0.004) (**Figure 3A1**, upper plot). The result was obtained using a fixed effects model since no significant between-study heterogeneity was observed (P=0.282, Q=14.29, and I^2= 16.05%). Sensitivity analysis showed that removing the study performed by Gaullier et al. could change the effect of CLA on serum Lp (a) levels to non-significant (WMD: 14.04 mg/L, 95% CI: -1.10-29.18, P=0.069 (**Figure 3A2**, lower plot).

Sub-group analysis

After stratifying the trials according to their duration, the results revealed a significant elevation of the circulating Lp (a) levels in the trials

Table 1. Characteristics of the included studies

Author	Year	Country	BMI (kg/m^2)	Sample size (n)	Age (years)	Females (%)	Duration (weeks)	Type of CLA	Placebo	CLA dose (mg/d)	Jadad score
Berven	2000	*Norway*	27.5-39.0	55	>18 y	36	12	CLA isomeric mixture	Olive oil	3.4	5
Blankson	2000	*Norway*	25-35	47	>18 y	NR	12	CLA isomeric mixture	Olive oil	1.7 a, 3.4 b, 5.1 c or 6.8 d	5
Gaullier	2004	Norway	25-30	120	18-65 y	82.7	48	CLA-FFA a CLA-triacylglycerol b	Olive oil	3.6 a 3.4 b	5
Gaullier	2005	Norway	25-30	130	18-65 y	82	48	CLA-FFA a CLA-triacylglycerol b	Olive oil	3.4	4
Gaullier	2007	Norway	28-32	105	18-65 y	80	24	CLA isomeric mixture	Olive oil	3.4	4
Pfeuffere	2011	Germany	25-35	85	45-68 y	0	4	CLA isomeric mixture	Safflower oil, heated safflower oil, olive oil	3.4	3

CLA, conjugated linoleic acids; FFA, free fatty acid; NR, not reported.

Figure 2. The methodological quality of RCTs based on authors' judgments.

lasting for ≥6 months (WMD: 21.61 mg/L, 95% CI: 9.85-33.37, P<0.001) and a near to significant reduction in those with lower durations (WMD: -36.70 mg/L, 95% CI: -75.40-1.99, P=0.063) (**Figure 3F1**, **3F2**). Another sub-group analysis according to the dose of supplementation showed a significant Lp (a)-increasing effect in the trials using ≥3.5 g/day doses (WMD: 26.13 mg/L, 7.02-45.24, P=0.007), but not in those with <3.5 g/day dosages (WMD: 11.67 mg/L, -2.24-25.59, P=1.00) (**Figure 3G1**, **3G2**).

The effect of CLA on other lipid indices

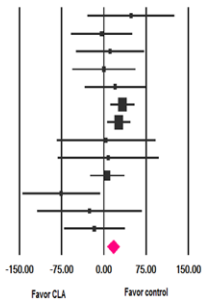
The included studies were analyzed in order to determine the influence of CLA supplementation on lipid profiles. The results revealed no significant changes in the concentrations of plasma Triacylglycerols (TAGs) (WMD: 0.018 mmol/l, 95% CI: -0.06-0.10, P=0.662), LDL cholesterol (WMD: -0.075 mmol/l, 95% CI: -0.224-0.073, P=0.320), and High-Density Lipoprotein (HDL) cholesterol (WMD: -0.027, 95% CI: -0.059-0.005, P=0.092) after CLA sup-

A1

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Bervena., 2000	48.000	39.274	1542.421	28.975-	124.975	1.222	0.222
Blankson., 2000 a	4.000-	27.683	766.327	58.257-	50.257	0.144-	0.885
Blankson., 2000 b	11.000	30.869	952.890	49.502-	71.502	0.356	0.722
Blankson., 2000 c	0.000	28.371	804.937	55.607-	55.607	0.000	1.000
Blankson., 2000 d	20.000	27.677	766.026	34.246-	74.246	0.723	0.470
Gaullier., 2004 a	32.900	11.068	122.504	11.207	54.593	2.972	0.003
Gaullier., 2004 b	26.500	10.554	111.377	5.815	47.185	2.511	0.012
Gaullier., 2005 a	4.000	44.847	2011.243	83.898-	91.898	0.089	0.929
Gaullier., 2005 b	8.000	45.710	2089.364	81.589-	97.589	0.175	0.861
Gaullier., 2007	6.000	15.587	242.970	24.551-	36.551	0.385	0.700
Pfeuffer., 2015 a	75.600-	35.248	1242.453	144.686-	6.514-	2.145-	0.032
Pfeuffer., 2015 b	25.200-	47.412	2247.860	118.125-	67.725	0.532-	0.595
Pfeuffer., 2015 c	16.800-	27.572	760.211	70.840-	37.240	0.609-	0.542
	16.686	5.740	32.950	5.435	27.937	2.907	0.004

Difference in means and 95% CI

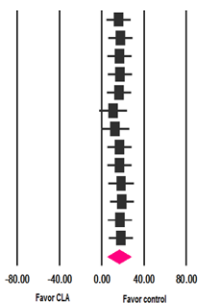


A2

Statistics with study removed

Author	Point	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Bervena., 2000	16.003	5.803	33.670	4.630	27.375	2.758	0.006
Blankson., 2000 a	17.616	5.868	34.431	6.115	29.116	3.002	0.003
Blankson., 2000 b	16.890	5.842	34.131	5.439	28.340	2.891	0.004
Blankson., 2000 c	17.398	5.861	34.357	5.910	28.887	2.968	0.003
Blankson., 2000 d	16.537	5.868	34.431	5.036	28.038	2.818	0.005
Gaullier., 2004 a	10.720	6.714	45.074	2.436-	23.879	1.597	0.110
Gaullier., 2004 b	12.563	6.841	46.794	0.845-	25.970	1.837	0.066
Gaullier., 2005 a	16.897	5.788	33.499	5.553	28.241	2.919	0.004
Gaullier., 2005 b	16.825	5.786	33.478	5.485	28.166	2.908	0.004
Gaullier., 2007	18.363	6.174	38.120	6.262	30.464	2.974	0.003
Pfeuffer., 2015 a	19.200	5.818	33.848	7.797	30.603	3.300	0.001
Pfeuffer., 2015 b	17.309	5.783	33.441	5.975	28.643	2.993	0.003
Pfeuffer., 2015 c	18.203	5.869	34.443	6.701	29.706	3.102	0.002
	16.686	5.740	32.950	5.435	27.937	2.907	0.004

Difference in means (95% CI) with study removed

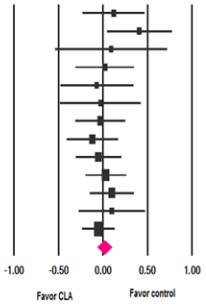


B

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Pfeuffer., 2011 a	0.120	0.178	0.032	0.229-	0.469	0.674	0.500
Pfeuffer., 2011 b	0.410	0.186	0.035	0.045	0.775	2.203	0.028
Pfeuffer., 2011 c	0.090	0.322	0.103	0.540-	0.720	0.280	0.780
Blankson., 2011 a	0.020	0.168	0.028	0.310-	0.350	0.119	0.905
Blankson., 2011 b	0.070-	0.209	0.044	0.481-	0.341	0.334-	0.738
Blankson., 2011 c	0.030-	0.232	0.054	0.484-	0.424	0.129-	0.897
Blankson., 2011 d	0.030-	0.142	0.020	0.309-	0.249	0.211-	0.833
Gaullier., 2005 a	0.120-	0.147	0.022	0.408-	0.168	0.816-	0.415
Gaullier., 2005 b	0.050-	0.131	0.017	0.307-	0.207	0.381-	0.703
Gaullier., 2004 a	0.030	0.117	0.014	0.199-	0.259	0.257	0.798
Gaullier., 2004 b	0.100	0.127	0.016	0.149-	0.349	0.786	0.432
Berven., 2000	0.100	0.192	0.037	0.275-	0.475	0.522	0.602
Gaullier., 2007	0.050-	0.094	0.009	0.234-	0.134	0.534-	0.593
	0.018	0.042	0.002	-0.063	0.100	0.438	0.662

Difference in means and 95% CI

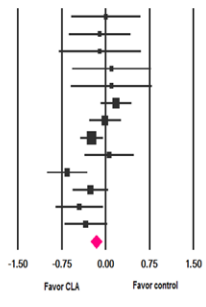


C

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Berven., 2000	0.000	0.302	0.091	0.592-	0.592	0.000	1.000
Blankson., 2011 a	0.100-	0.269	0.072	0.627-	0.427	0.372-	0.710
Blankson., 2011 b	0.100-	0.358	0.128	0.801-	0.601	0.280-	0.780
Blankson., 2011 c	0.100	0.343	0.118	0.573-	0.773	0.291	0.771
Blankson., 2011 d	0.100	0.354	0.125	0.594-	0.794	0.283	0.778
Gaullier., 2004 a	0.180	0.134	0.018	-0.083	0.443	1.343	0.179
Gaullier., 2004 b	0.010-	0.138	0.019	0.280-	0.260	-0.072	0.942
Gaullier., 2007	0.240-	0.101	0.010	0.438-	-0.042	2.375-	0.018
Gaullier., 2011 a	0.060	0.214	0.046	0.359-	0.479	0.281	0.779
Gaullier., 2011 b	0.660-	0.177	0.031	1.007-	0.313-	3.729-	0.000
Pfeuffer., 2011 a	0.260-	0.154	0.024	0.562-	0.042	1.685-	0.092
Pfeuffer., 2011 b	0.450-	0.207	0.043	0.856-	-0.044	2.170-	0.030
Pfeuffer., 2011 c	0.340-	0.187	0.035	0.707-	0.027	1.814-	0.070
	0.162-	0.050	0.002	0.260-	0.065-	3.256-	0.001

Difference in means and 95% CI



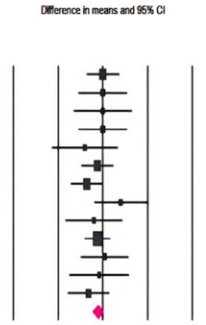
Triglycerides

D

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Berven., 2000	0.000	0.047	0.002	-0.092	0.092	0.000	1.000
Blankson., 2011 a	0.000	0.069	0.005	0.136-	0.136	0.000	1.000
Blankson., 2011 b	0.000	0.084	0.007	0.164-	0.164	0.000	1.000
Blankson., 2011 c	0.000	0.069	0.005	0.136-	0.136	0.000	1.000
Blankson., 2011 d	0.100-	0.095	0.009	0.286-	0.086	1.054-	0.292
Gaullier., 2004 a	0.030-	0.047	0.002	0.121-	0.061	0.644-	0.520
Gaullier., 2004 b	0.090-	0.046	0.002	0.180-	0.000	1.959-	0.050
Gaullier., 2005 a	0.100	0.075	0.006	-0.048	0.248	1.325	0.185
Gaullier., 2005 b	0.050-	0.081	0.007	0.209-	0.109	0.617-	0.537
Gaullier., 2007	0.030-	0.036	0.001	0.101-	0.041	0.829-	0.407
Pfeuffer., 2011 a	0.010	0.068	0.005	0.123-	0.143	0.147	0.883
Pfeuffer., 2011 b	0.020-	0.086	0.007	0.188-	0.148	0.233-	0.816
Pfeuffer., 2011 c	0.080-	0.060	0.004	0.197-	0.037	1.337-	0.181
	-0.027	0.016	0.000	-0.059	0.005	1.683-	0.092

Difference in means and 95% CI



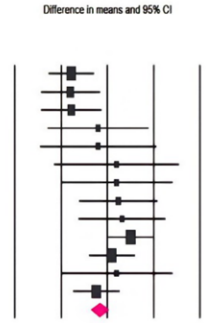
Total cholesterol

E

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Pfeuffer., 2011 a	0.390-	0.127	0.016	0.638-	0.142-	3.082-	0.002
Pfeuffer., 2011 b	0.400-	0.163	0.027	0.719-	0.081-	2.456-	0.014
Pfeuffer., 2011 c	0.390-	0.168	0.028	0.719-	-0.061	2.327-	0.020
Blankson., 2011 a	0.100-	0.278	0.077	0.645-	0.445	0.360-	0.719
Blankson., 2011 b	0.100-	0.322	0.103	0.730-	0.530	0.311-	0.756
Blankson., 2011 c	0.100	0.345	0.119	0.576-	0.776	0.290	0.772
Blankson., 2011 d	0.100	0.308	0.095	0.503-	0.703	0.325	0.745
Gaullier., 2005 a	0.120	0.217	0.047	0.305-	0.545	0.553	0.580
Gaullier., 2005 b	0.160	0.239	0.057	0.309-	0.629	0.668	0.504
Gaullier., 2004 a	0.250	0.122	0.015	0.011	0.489	2.047	0.041
Gaullier., 2004 b	0.050	0.127	0.016	0.199-	0.299	0.394	0.694
Berven., 2000	0.100	0.308	0.095	0.504-	0.704	0.324	0.746
Gaullier., 2007	0.120-	0.129	0.017	0.372-	0.132	0.932-	0.351
	-0.083	0.049	0.002	0.179-	0.013	1.700-	0.089

Difference in means and 95% CI



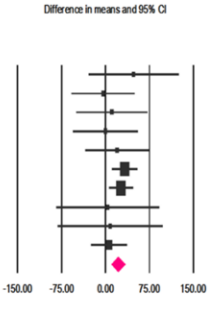
HDL-cholestrol

F1

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-pulse	P-value
Bervena., 2000	48.000	39.274	1542.421	28.975-	124.975	1.222	0.222
Blankson., 2000 a	4.000-	27.683	766.327	58.257-	50.257	0.144-	0.885
Blankson., 2000 b	11.000	30.869	952.890	49.502-	71.502	0.356	0.722
Blankson., 2000 c	0.000	28.371	804.937	55.607-	55.607	0.000	1.000
Blankson., 2000 d	20.000	27.677	766.026	34.246-	74.246	0.723	0.470
Gaullier., 2004 a	32.900	11.068	122.504	11.207	54.593	2.972	0.003
Gaullier., 2004 b	26.500	10.554	111.377	5.815	47.185	2.511	0.012
Gaullier., 2005 a	4.000	44.847	2011.243	83.898-	91.898	0.089	0.929
Gaullier., 2005 b	8.000	45.710	2089.364	81.589-	97.589	0.175	0.861
Gaullier., 2007	6.000	15.587	242.970	24.551-	36.551	0.385	0.700
	21.616	5.999	35.993	9.857	33.374	3.603	0.000

Difference in means and 95% CI



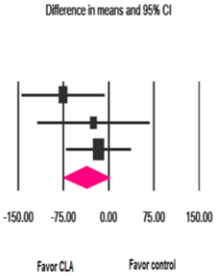
LDL-cholestrol

F2

Statistics for each study

Author	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value
Pfeuffer., 2015 a	75.600-	35.248	1242.453	144.686-	6.514-	2.145-	0.032
Pfeuffer., 2015 b	25.200-	47.412	2247.860	118.125-	67.725	0.532-	0.595
Pfeuffer., 2015 c	16.800-	27.572	760.211	70.840-	37.240	0.609-	0.542
	36.706-	19.744	389.841	75.405-	1.992	1.859-	0.063

Difference in means and 95% CI



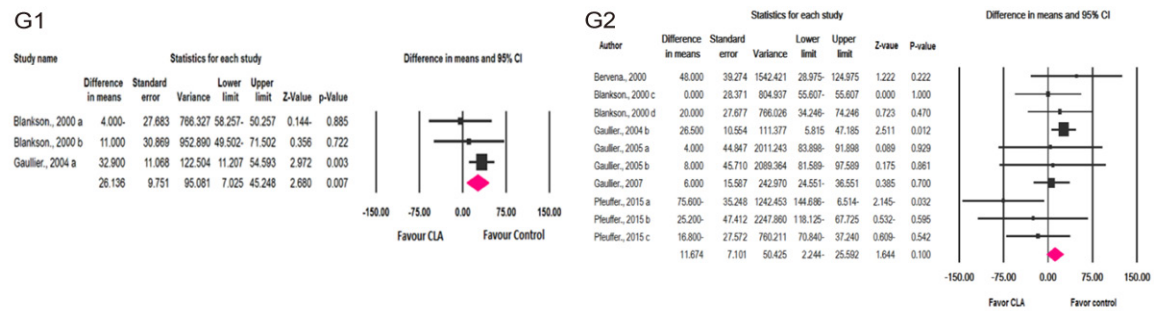


Figure 3. The forest plot detailing the weighted mean differences and 95% confidence intervals for the effect of CLA on plasma Lp (a) level (A1-upper plot). The lower plot shows the leave-one-out sensitivity analysis (A2-lower plot), and the forest plot detailing the weighted mean differences and 95% confidence intervals for the effect of CLA on plasma lipids (B-E), and the forest plot displaying the mean differences and 95% confidence intervals for the impact of CLA supplementation on plasma Lp (a) concentrations in the trials lasting for six months or more (upper plot F1) and those lasting for less than six months (lower plot F2), and in the trials using CLA doses ≥3.5 g/day (upper plot G1) and those using <3.5 g/day dosages (lower plot G2). HDL, high-density lipoprotein; LDL, low-density lipoprotein.

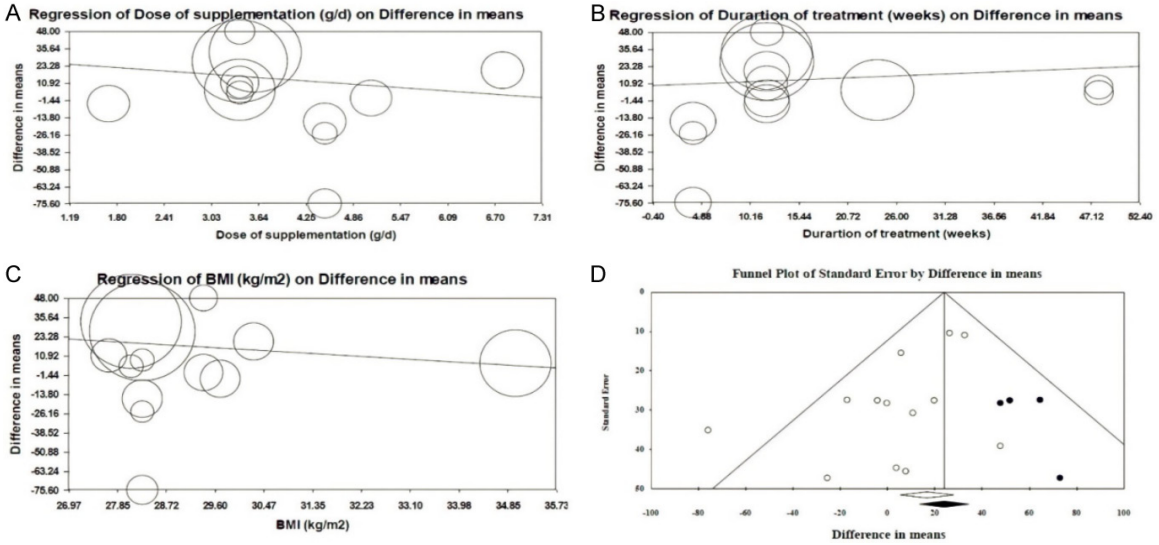


Figure 4. The meta-regression plots of the association between the mean changes in plasma Lp (a) concentrations after CLA treatment and dose of CLA, duration of treatment, and baseline BMI (A-C), and The funnel plot detailing publication bias in the studies reporting the impact of CLA on plasma Lp (a) concentrations (D).

plementation. However, a significant reduction was found in the level of total cholesterol (WMD: -0.160, 95% CI: -0.30-0.01, P=0.029) (**Figure 3B-E**).

Meta-regression

The potential associations between the Lp (a)-lowering effects of CLA and dose of supplementation, duration of intervention, and change in baseline BMI were evaluated using unrestricted maximum likelihood meta-regression analysis. The results suggested that the pooled estimate was independent of the CLA dose (slope: -4.23, 95% CI: -17.06-8.59, P=0.517), duration of supplementation (slope: 0.10, 95% CI: -1.32-1.52, P=0.889), and changes in BMI (slope: -2.13, -6.99-2.73, P=0.390) (**Figure 4**).

Publication bias

The visual inspection of the funnel plot was asymmetric, indicating a significant potential publication bias in the meta-analysis of the effect of CLA on circulating Lp (a) levels (**Figure 4**). Although the results of Egger's linear regression (intercept =-1.26, standard error =0.50, 95% CI =-2.37-0.14, *t*=2.49, df=11, two-tailed

P=0.029) confirmed the publication bias, Begg's rank correlation test (Kendall's Tau with continuity correction =-0.19, *z*=0.91, two-tailed *p*-value =0.360) did not show any significant publication bias. After adjusting the effect size for the potential publication bias using the "trim and fill" correction, five potentially missing studies were imputed in the funnel plot, yielding a corrected effect size of 24.00 mg/L (95% CI: 13.59-34.41). The "fail-safe N" test showed that no studies would be needed to bring the effect size down to a non-significant value (P>0.05).

Discussion

To the best of the authors' knowledge, the current systematic review and meta-analysis was the first study to analyze the evidence available from the RCTs regarding the effects of CLA supplementation on circulating Lp (a) levels. The findings of this meta-analysis showed that CLA supplementation had a significant increasing effect on circulating Lp (a) levels. However, in the subgroup analysis, this significant increasing effect was observed only in the trials lasting for six months or more and using a dose of ≥3.5 g/d CLA. Furthermore, the influence of CLA on circulating Lp (a) levels was found to be independent of duration, dose of supplementation, and baseline BMI. High circulating Lp (a) levels have been known to be an independent risk factor for cardiovascular disease [28]. Therefore, reducing the Lp (a) concentration would be of great clinical interest although its levels are believed to be largely under genetic control.

Up to now, conflicting findings have been obtained on whether or not Lp (a) changes occur after CLA supplementation, with some RCTs suggesting a slight reduction [17] and others reporting an increase (as in the current study) [18]. In line with the current study findings, Gaullier et al. [19] conducted a research with a similar method but a longer follow-up period and demonstrated that Lp (a) levels increased in both triglyceride and Free Fatty Acid (FFA) forms. Similar results were also reported in the study performed by Berven et al. [16], in which CLA was supplemented for 12 weeks. However, Blankson et al. [17] examined different doses of CLA among overweight or obese adults for 12 weeks and observed no significant effects. Given the contradictions in the literature, the authors reviewed the relevant study designs. Based on the findings, such inconsistencies might be related to the type of CLA (triglyceride or FFA), different dosages (1.7 to 6.8 g/d), duration of treatment (from four weeks to 12 months), biological variability in Lp (a) measurement, high variability in baseline Lp (a) levels (from 34.17 to 364.3 mg/L), and specificity effects of isomers and their proportions across the reviewed studies. These differences, along with the low number of trials, should be considered when determining whether or not CLA supplementation actually increases Lp (a) levels. Overall, it might be concluded that CLA supplementation had damaging effects. However, the sensitivity analysis in the current study indicated that by disregarding a study performed by Gaullier et al. [18], the effect of CLA on serum Lp (a) level was changed to non-significant. Therefore, the results of the current study must be interpreted with caution.

An important finding of the present meta-analysis was the significant increasing effect of CLA, which was seen only in the CLA dosages equal to or higher than 3.6 g/d as well as in the supplementation durations over six months. In other words, a high dose of CLA per day over six months might increase Lp (a) levels. With respect to the duration of supplementation, the study findings were consistent with those of the trials that had used CLA for a period greater than six months and up to one year [18, 19, 27]. Considering the supplementation dose, the findings were in line with those of most previous studies [17, 18].

The mechanism by which CLA supplementation increases Lp (a) levels is unclear. It has been reported that the oxidized LDL (oxLDL) concentration was positively correlated to the Lp (a) concentration [29]. However, CLA could elevate LDL-cholesterol and apoB levels [30]. Therefore, it was hypothesized that the adverse effects of CLA might increase in long-term as a result of the utilization of high dose supplementation.

Although CLA has garnered more attention for its possible positive effects on cardiovascular health [31], no evidence exists to support the protective effects of CLA against cardiovascular risk factors in humans, because there is inconsistency among studies regarding its effects on blood lipids. The main objective of

the present meta-analysis was not to evaluate the effects of CLA supplementation on lipid profiles; thus, the studies investigating such effects were not included. However, the findings of the current meta-analysis were consistent with those of the previous human studies [32], which showed no significant effects of CLA supplementation on plasma lipid concentrations. In contrast, another study [33] indicated significant elevations in total cholesterol and LDL cholesterol levels and a decrease in HDL cholesterol levels, but no significant effects on TAGs concentrations. One meta-analysis [34] exploring the effects of CLA on profiles reported a significant decrease in LDL cholesterol concentration, a non-significant decrease in HDL cholesterol and TAGs levels, and a non-significant increase in the total cholesterol concentration. These findings indicated that some lipid indices were improved following supplementation, while others were worsened. Due to the significant inconsistences regarding blood lipids and the significant increase in Lp (a) levels observed in the current meta-analysis, it is impossible to predict the real effects of CLA consumption on cardiovascular health. To determine whether CLA has protective, deleterious, or no effects against atherogenesis, further investigations are required. In particular, the definitive association between CLA and Lp (a) level and the mechanisms by which CLA affects Lp (a) levels must be determined. Furthermore, there is no agreement on the recommended dosage of CLA. Based on the evidence, 3 g/d appeared to be the most appropriate dose [34-36], which was consistent with the subgroup analysis in the current study.

The present analysis had several strengths. All trials were double-blind, which strengthened the inference for a cause-and-effect relationship. Moreover, all the RCTs were high quality as assessed by the Jadad scale, and most of them had large sample sizes. The limitations of this analysis must also be considered while interpreting the results. Firstly, there were few eligible studies. Secondly, changes in Lp (a) levels were not among the primary outcomes of the eligible studies.

Conclusion

CLA supplementation might significantly increase circulating Lp (a) levels, especially after six months of supplementation with doses higher than 3.6 g/day. However, more high-quality investigations are needed to confirm these findings.

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Disclosure of conflict of interest

None.

Abbreviations

CMA, Comprehensive meta-analysis; Lp (a), Lipoprotein (a); LDL, low-density lipoprotein; CLA, Conjugated linoleic acids; SDs, Standard deviation; BMI, Body mass index; WMD, Weighted mean difference; CI, Confidence interval; SEs, Standard errors; CMA, Comprehensive meta-analysis; FFA, Free fatty acid.

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