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The Effects of Conjugated Linoleic Acid Supplements on Biomarkers of Oxidative Stress in Human Studies: A Systematic Review and Meta-Analysis

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SYSTEMATIC REVIEW and META-ANALYSIS

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ABSTRACT

Background: Oxidative stress is the leading cause of chronic disorders. The aim of the present study is to assess the effects of conjugated linoleic acid (CLA) supplements on oxidative stress biomarkers in adults. **Methods:** PubMed, Web of Science, Google Scholar, ProQuest, Scopus, and Embase were searched up to December 2020. All clinical trials that evaluated the effect of CLA on malondialdehyde (MDA), GSH-peroxidase (GPX), and 8-Isoprostanes$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) were included. **Results:** Twelve eligible studies were included in the meta-analysis. A significant increase was observed in 8-iso-$PGF_{2\alpha}$ level (SMD=1.48 nmol/mmol of creatinine; 95% CI: 1.11 to 1.85) with low heterogeneity level (I^2= 31.5%, and *P*=0.199). This effect was also significant in both subgroups of healthy and metabolic disorder individuals. Moreover, after Hartung-Knapp adjustment, the results remained significant. No significant changes were found in MDA (SMD=-0.34 µmol/l; 95% CI: -0.82 to 0.14) and GPX (SMD=0.31 U/gHb; 95% CI: -0.03 to 0.66) levels. However, after Hartung-Knapp adjustment, the results became significant for GPX level (SMD=0.31, 95% CI: 0.04 to 0.59). **Conclusion:** CLA supplementation could significantly increase some markers of oxidative stress such as 8-iso-$PGF_{2\alpha}$ level and GPX level, without any significant effect on MDA level.

Keywords: *Conjugated linoleic acid; Oxidative stress; Malondialdehyde; Isoprostanes*

Introduction

Conjugated Linoleic acid (CLA) is a natural trans fatty acid produced via bioconversion of Vaccenic acid in mammary glands, or rumen of ruminants (Derakhshandeh-Rishehri *et al.*, 2019). It is also generated synthetically by the hydrogenation of linoleic acid (Derakhshandeh-

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Rishehri *et al.*, 2019). Dairy products and ruminant meat are the two main sources of CLA (Mirzaii *et al.*, 2016). It has various isomers, among which trans-10, cis-12 (t10, c12), cis-9, and trans-11 (c9, t11) are the most abundant ones (Laso *et al.*, 2007). CLA isomers in the natural form or CLA supplements have positive effects on increasing lean body mass, decreasing fat mass, bolstering the immune system, and decreasing the risk of chronic diseases such as carcinoma, diabetes mellitus, and cardiovascular disease (Diaz *et al.*, 2008, Whigham *et al.*, 2007).

Through the production of Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS), oxidative stress plays a crucial role in the development of numerous chronic diseases such as neurodegenerative diseases, cancers, and diabetes (Tan *et al.*, 2018). According to the evidence, ROS over-production leads to oxidative damage of macromolecules, which causes neuronal death, and affects the health span of several organ systems (Mazon *et al.*, 2017, Wang *et al.*, 2017). According to some dose-response meta-analysis, antioxidants intake has favorable effects on the health status of humans (Ghaedi *et al.*, 2020, Ghaedi *et al.*, 2019, Hadi *et al.*, 2019, Kord-Varkaneh *et al.*, 2018). On the other hand, studies have demonstrated that under different models of oxidative stress, the regular intake of dietary fat is able to attenuate or increase free radical production at the mitochondrial level (Mataix *et al.*, 2006). Diet especially fatty acids have different effects on cell oxidation (Mataix *et al.*, 2006). Poly-unsaturated fatty acids can increase the risk of oxidation, while monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) can cause more protection (Mataix *et al.*, 2006).

According to previous studies many benefits of CLA supplementation have been described in both animals and humans, as anti-carcinogenic, oxidative stress modulating, anti-atherogenic, bone health, anti-obesity, anti-diabetes and bolstering the immune system (Silveira *et al.*, 2007). Results of some other studies showed that trans10 and cis12 CLA consumption was effective in weight management, but some negative outcomes were observed such as an increase in plasma insulin and glucose, reduction of insulin sensitivity, and increase in urinary prostaglandin 8-iso-$PGF_{2\alpha}$, and C-reactive protein (CRP) levels as markers of oxidative stress and inflammation (Risérus *et al.*, 2002a, Risérus *et al.*, 2002b, Risérus *et al.*, 2004a). It is reported that supplementation with 2.5 g/d CLA-mixed isomers for 12 weeks caused GPX increment, but MDA levels reduction (Aryaeian *et al.*, 2009). In a clinical trial study, 3g/d CLA supplementation for 8 weeks caused a significant reduction in GPX and MDA levels. However, another study showed that 4.2g/d CLA supplementation for 12 weeks had no effect on MDA levels but a significant increase in 8-iso-$PGF_{2\alpha}$ level (Basu *et al.*, 2000a, Eftekhari *et al.*, 2013).

Regarding to the effects of CLA supplements on oxidative stress biomarkers, different studies have had different results. So, the present systematic reviews and meta-analysis were conducted to examine the effects of CLA supplements on biomarkers of oxidative stress in adults.

Materials and Methods

The present systematic review and meta-analysis were registered in PROSPERO, https://www.crd.york.ac.uk/PROSPERO, [PROSPERO registration number: CRD42021224325], and it was conducted in accordance with PRISMA checklist 2009 (Shuster 2011).

Search strategy: PubMed, Web of Science, Google Scholar, ProQuest, Scopus, and Embase were searched. The following Medical Subjects and Headings (MeSH) terms and keywords were used to find relevant papers: 1) "cis-9, trans-11-conjugated linoleic acid" [Supplementary Concept] OR "trans-10, cis-12-conjugated linoleic acid" [Supplementary Concept] OR "Linoleic Acids, Conjugated" OR "CLA fatty acid" [Supplementary Concept] OR "CLA" OR "conjugated linoleic acid" OR "Trans Fatty Acids" OR "TFA"; 2) "Malondialdehyde" OR "MDA" OR "Oxidized low-density lipoprotein" OR "OX-LDL" OR "Thiobarbituric acid reactive substances" OR "TBARS" OR "Total Antioxidant Capacity" OR

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"TAC" OR "oxidative stress" OR "selenium-independent glutathione peroxidase" [Supplementary Concept] OR "GSH-peroxidase" OR "GPX" OR "glutathione peroxidase" OR "catalase" OR "SOD" OR "Superoxide Dismutase" OR "Isoprostanes"; 3) 1 & 2. To find more relevant papers, a hand search was performed on the references of related papers. All studies published at any time till December 2020 were included with no language restriction. Full electronic search strategy for one database is provided as supplementary material.

Study selection and eligibility criteria: Two different authors (Rajabzadeh-Dehkordi M, Ghobadi S) conducted the search and screening process. The inclusion and exclusion criteria were conducted according to PICOS guideline **(Table 1)**.

Data extraction: Two authors (Rajabzadeh-Dehkordi M, Ghobadi S) were responsible for data extraction. The following information was extracted from each relevant article: first author's name, year of publication, age of participants, sample size, study duration, dose and form of intervention, placebo, and outcomes. Means and standard deviations (SD) or standard errors (SE) of MDA, GPX, and 8-ISO-$PGF_{2\alpha}$ were extracted for the effect size calculation. For uncertainty in data extraction process, the issue was discussed between the three reviewers (SMDR, MRD, and SG). For incomplete data, an email was send to the corresponding author. In case of receiving no response, the study was excluded.

Quality assessment: Cochrane criteria were used for bias assessment of the eligible studies (Higgins *et al.*, 2019). Two authors (Rajabzadeh-Dehkordi M, Ghobadi S) assessed the quality of the studies including random sequence generation, allocation concealment, blinding, blinding of outcome assessor, incomplete outcome data, selective reporting, and risk of other biases. Based on the Cochrane Handbook recommendation, studies were categorized as unclear, low risk, and high risk in each domain. **Suggestion**: If all criteria were met or only one criterion was unclear, the quality of the included studies was considered 'good'. If one criterion was not met or two criteria were unclear, the quality was considered 'fair'. However, if two or more criteria were not met or unclear, the quality was considered 'poor'.

Data analysis: µmol/l, U/gHb, and nmol/mmol of creatinine were used as unit scale for values of MDA, GPX, and 8-ISO-$PGF_{2\alpha}$, respectively. In order to calculate the effect size, changes in mean and standard deviation (SD) were applied for both the intervention and control groups. If they were not reported directly, mean differences were calculated by subtracting the mean value of after the intervention from before the intervention in both groups. Then SDs of mean differences were calculated by the following equation:

$$\mathrm{SD} = \sqrt{\mathrm{SD}_{\mathrm{before}}^2 + \mathrm{SD}_{\mathrm{after}}^2 - 2 * \mathrm{r} * \mathrm{SD}_{\mathrm{before}} * \mathrm{SD}_{\mathrm{after}}}$$

Where "r" refers to the correlation between before and after values.

As the change values were not reported in included articles, the correlation coefficient of 0.5 was considered as the reference correlation coefficient between baseline and endpoint values (r=0.5) and to check the sensitivity of meta-analyses to the correlation coefficient, all analyses were replicated using the correlation coefficient of 0.2 and 0.8. The mean value was calculated by $\times = \frac{a+2m+b}{4}$, where "m" was median and "a" and "b" were low and high end of the range, respectively. The variance was calculated by the following equation (Follmann *et al.*, 1992):

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

Due to the small number of included studies, Hartung-Knapp adjustment was applied (IntHout *et al.*, 2014). The *I*-squared test was used for heterogeneity assessment. According to the *I*-squared test, values <25%, 25% to 50%, and >50% were considered as low, medium, and high amounts of heterogeneity, respectively. Random-effect model (I-V heterogeneity, no standard) was

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used for calculating the pooled effect size. To calculate the weighted mean difference (WMD), a 95% confidence interval was used. The level of significance was considered 0.05 or less. In addition, a funnel plot was used, and Begg and Egger test was conducted for publication bias assessment. All statistical analyses were done using Stata version 11.0 software (Stata Corporation).

Quality of evidence: The quality of evidence for each outcome was assessed by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach which contains the following domains: risk of bias, publication bias, imprecision of the results, inconsistency, indirectness of evidence, effect size, and dose-response relationship (Guyatt *et al.*, 2011a, Guyatt *et al.*, 2011b, Schünemann *et al.*, 2008). Since the included studies in this meta-analysis were randomized trials without important limitations, the baseline quality was considered as high. Then, the baseline score was downgraded or upgraded according to the mentioned domains. The criteria assessed to downgrade the quality included risk of bias, inconsistency, indirectness, imprecision, and publication bias. For risk of bias, the authors assessed the extent to which the high-risk studies contribute towards the estimate of the magnitude of effect through study sample size. Inconsistency was considered as an unserious limitation when I2 was <50%, as serious when I2 was between 50 and 75, and very serious for I2 >75%. Indirectness would be verified if the present research directly compared the interventions in which we were interested and delivered to the populations in which we were interested. For imprecision, it was assessed whether or not the sample size for the analysis met the optimal information size (OIS) criterion. For calculating OIS, 0.05 and 0.2 were considered as α and β error thresholds, and minimally important difference (MID) as the Δ. MID was considered as a one-half standard deviation change in outcome measures (calculated from baseline values of participants included in a given analysis. Publication bias was judged based on Egger or Begg's test. Effect size and presence of dose-response relationship were assessed to upgrade the quality of evidence. Standardized mean difference (SMD) of 0.2 to 0.49 was considered as small effect (0 point); 0.5-0.79 moderate effect (+1 point); and ≥ 0.80 large effect (+2 point). The quality of evidence was categorized as high, moderate, low, and very low.

Results

Study selection: Among 3867 articles, 27 full texts were assessed for inclusion and exclusion criteria (**Figure 1**). Fifteen articles were excluded after full-text screening; 4 articles on CLA mixed with other ingredients, 3 studies with insufficient data, 1 study without control group, two without pdf, and 5 articles on CLA-enriched food products. Finally, 12 studies were included in the systematic review and analysis.

Characteristics of the included studies: Eligible studies are described in **Table 2**. The study duration varied from 4 to 12 weeks and the mean age of the participants ranged from 37 to 62 years. Three studies were conducted on healthy individuals, and nine studies on individuals with metabolic disorders. Doses of CLA ranged from 2 to 4.5 g/d. As a control group, most of the studies used olive oil or oleic acid extracts; some of them used safflower oil, sunflower oil, or soybean oil. Five studies were conducted in Iran, four in Sweden, and one in Germany, Finland, and Korea. In the present review, all the included clinical trials had a parallel design. In total, twelve studies with 684 participants were included in the meta-analysis **(Table 2)**.

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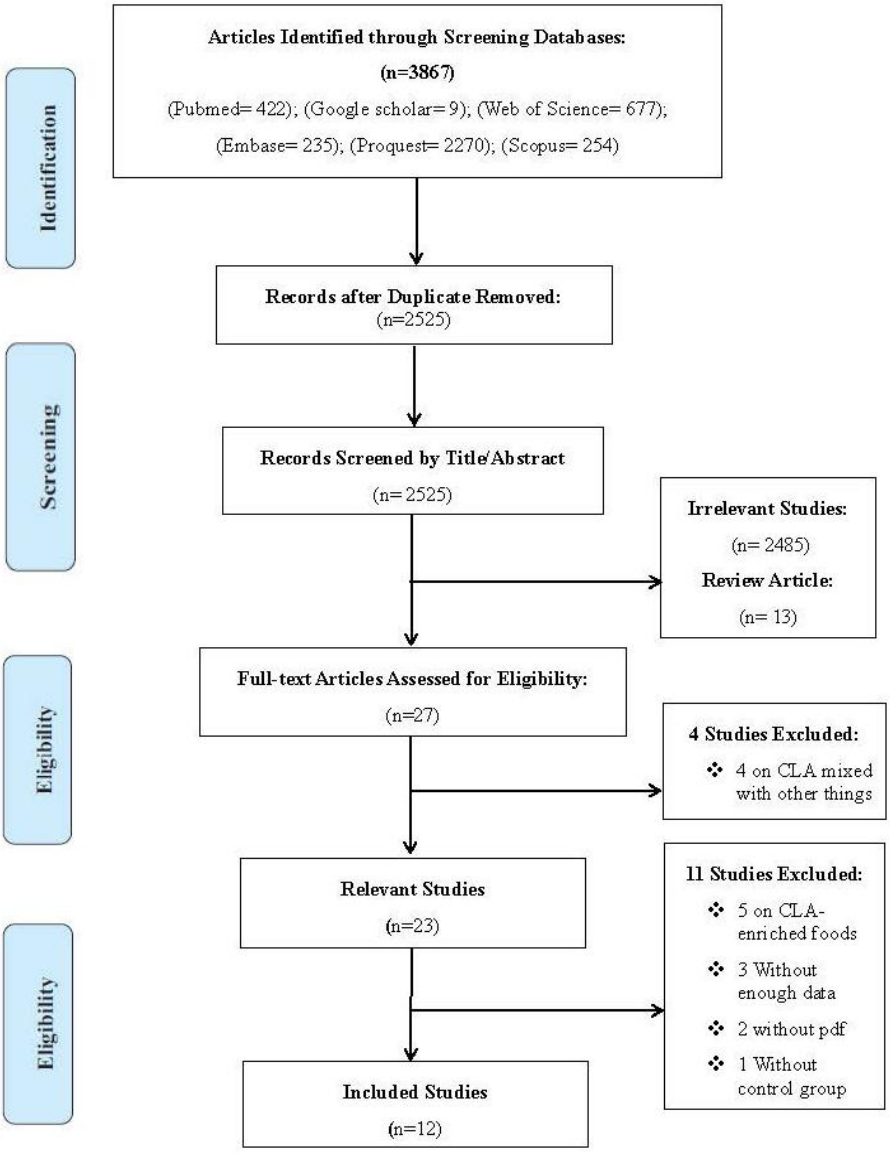

Figure 1. Flow diagram of database search and study selection.

Quality assessment: Three studies had good quality (Aryaeian *et al.*, 2009, Ebrahimi-Mameghani *et al.*, 2016, Matin *et al.*, 2018), seven studies had fair quality (Basu *et al.*, 2000a, Basu *et al.*, 2000b, Eftekhari *et al.*, 2013, Pfeuffer *et al.*, 2011, Risérus *et al.*, 2002b, Risérus *et al.*, 2004b, Shadman *et al.*, 2013), and two of them had poor quality (Kim *et al.*, 2012, Turpeinen *et al.*, 2008). All these studies were included in this systematic review and meta-analysis.

Publication bias: The funnel plot showed no evidence of publication bias for the effects of CLA on oxidative stress biomarkers in human studies (**Figure 2**). The Begg and Egger tests did not show publication bias for the effects of CLA intake on MDA (P=0.34, P=0.30), GPX (P=0.60, P= 0.55), and 8-ISO-$PGF_{2\alpha}$ (P=0.85, P=0.55).

Sensitivity analysis: To evaluate the effects of CLA on oxidative stress biomarkers in human studies, a sensitivity analysis was performed according to the random-effects model. Results of sensitivity analysis showed that one study had no impact regarding the effects of CLA supplementation on oxidative stress biomarkers.

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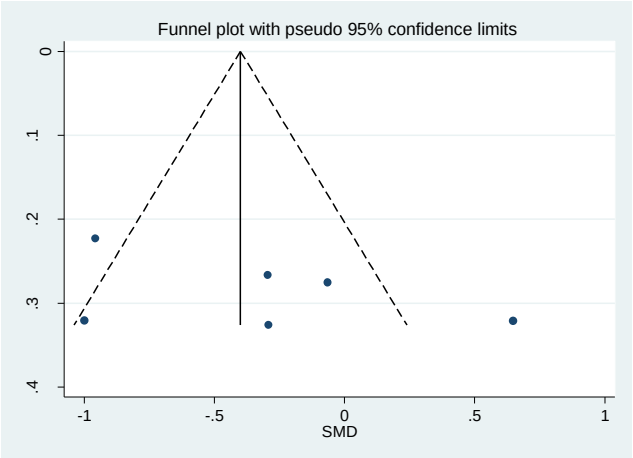
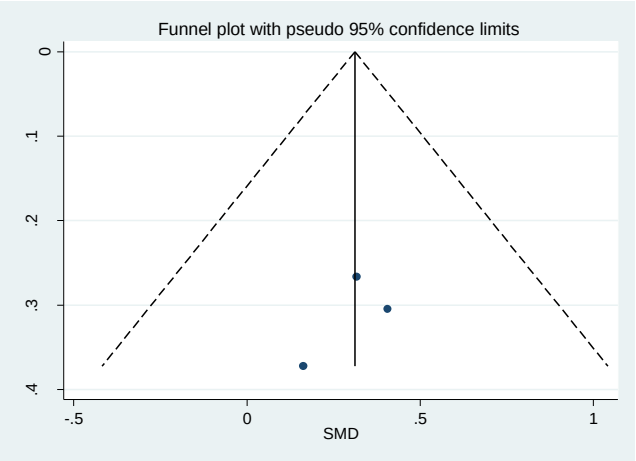
MDA



GPX



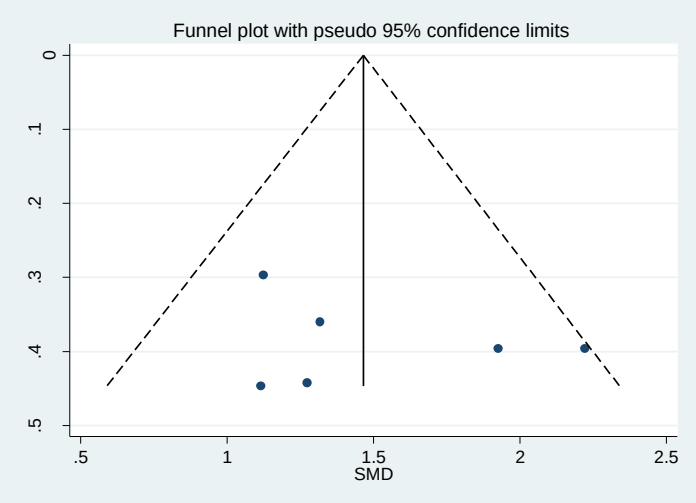


Figure 2. Funnel plot for publication bias estimation.

The effect of CLA supplements on MDA, GPX, and 8-ISO-PGF$_{2\alpha}$: A non-significant reduction was observed in MDA level following consumption of CLA supplements (SMD=-0.34 µmol/l; 95% CI: -0.82 to 0.14) with high heterogeneity level ($I^{2:}$ 77.4%, and P<0.001, **Figure 3**). After Hartung-Knapp adjustment due to few included studies, the results were still non-significant for MDA (SMD=-0.34, 95% CI: -0.98 to 0.30; I^2:77.4%, and P<0.001). Furthermore, the overall effect of CLA supplements on GPX level was not significant (SMD=0.31 U/gHb; 95% CI: -0.03 to 0.66) with very low heterogeneity level (I^2: 0.0%, and P=0.87, **Figure 4**). However, after Hartung-Knapp adjustment, the results became significant for GPX (SMD=0.31; 95% CI: 0.04 to 0.59; I^2: 0.0%, and P=0.87).

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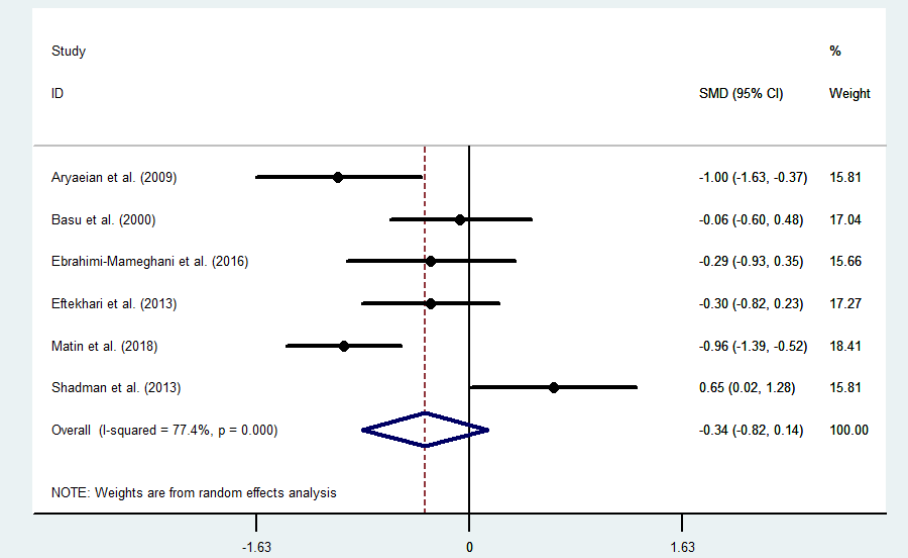

Figure 3. Forest plot of the effects of CLA supplements on MDA level.

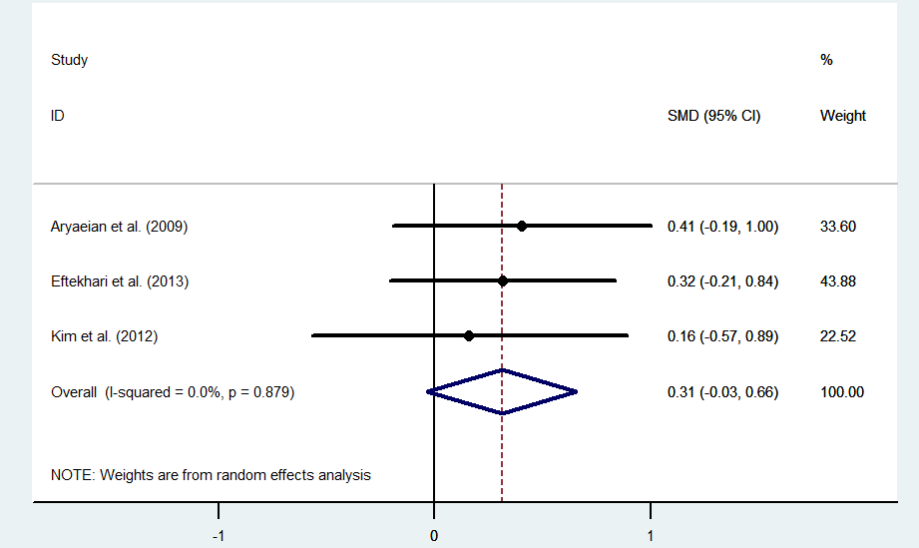

Figure 4. Forest plot of the effects of CLA supplements on GPX level.

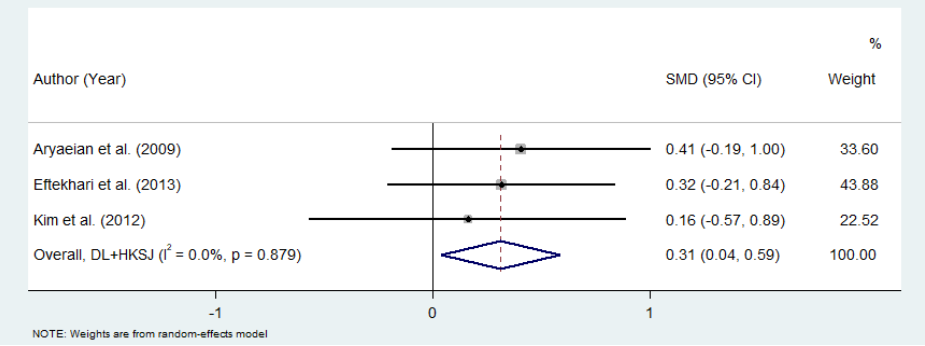

Figure 5. Forest plot of the effects of CLA supplements on GPX level after Hartung-Knapp-Sidik-Jonkman (HKSJ) adjustment.

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A significant increase was observed in 8-ISO-$PGF_{2\alpha}$ level following consumption of CLA supplements (SMD=1.48 nmol/mmol of creatinine; 95% CI: 1.11 to 1.85) with low heterogeneity level (I^2: 31.5%, and *P*=0.19, **Figure 6**). Moreover, in subgroup analysis based on health status of the participants, in both subgroups of healthy and metabolic disorder individuals, CLA supplement consumption caused a significant increase in 8-ISO-$PGF_{2\alpha}$ level (SMD=1.22 nmol/mmol of creatinine; 95% CI: 0.82 to 1.62; and SMD=1.78 nmol/mmol of creatinine; 95% CI: 1.16 to 2.41, respectively, **Figure 7**). After excluding one study with poor quality (Turpeinen *et al.*, 2008), the overall effect of CLA supplement on 8-ISO-$PGF_{2\alpha}$ was still significant (SMD=1.52 nmol/mmol of creatinine; 95% CI: 1.07 to 1.98) with low heterogeneity level (I^2: 43.6%, and *P*=0.13). Based on the subgroup analysis, CLA supplementation had no effect on 8-ISO-$PGF_{2\alpha}$ level of healthy individuals (SMD=1.17 nmol/mmol of creatinine; 95% CI: 0.69 to 1.65) However, in metabolic disorder subgroup it was still significant (SMD=1.78 nmol/mmol of creatinine; 95% CI: 1.16 to 2.41, **Table 3**). After Hartung-Knapp-Sidik-Jonkman (HKSJ) adjustment, the results were still significant for 8-ISO-$PGF_{2\alpha}$ (SMD=1.48, 95% CI: 1.00 to 1.96; I^2: 31.5%, and *P*=1.9).

Quality of meta-evidence: The GRADE meta-evidence rating indicated a very low quality of evidence for MDA, and a moderate quality for GPX, and a high quality for 8-ISO-$PGF_{2\alpha}$.

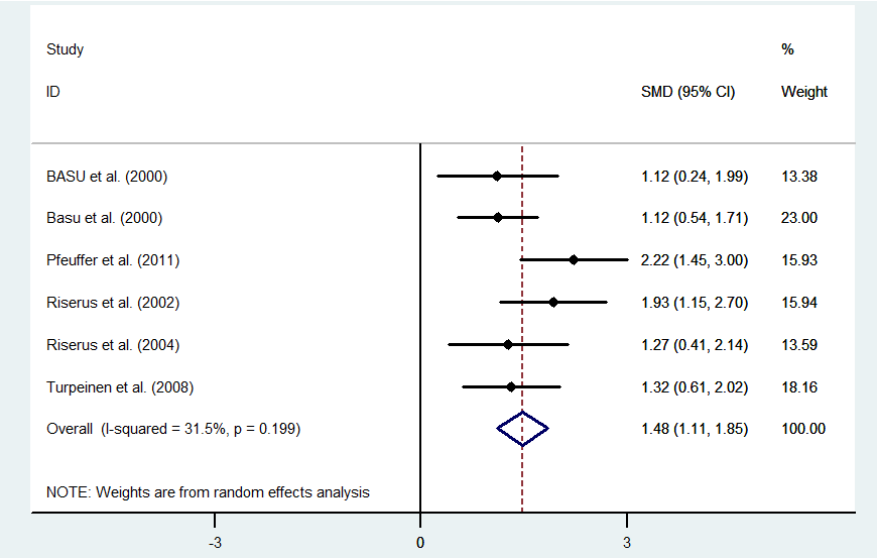

Figure 6. Forest plot of the effects of CLA supplements on 8-iso-$PGF_{2\alpha}$ level

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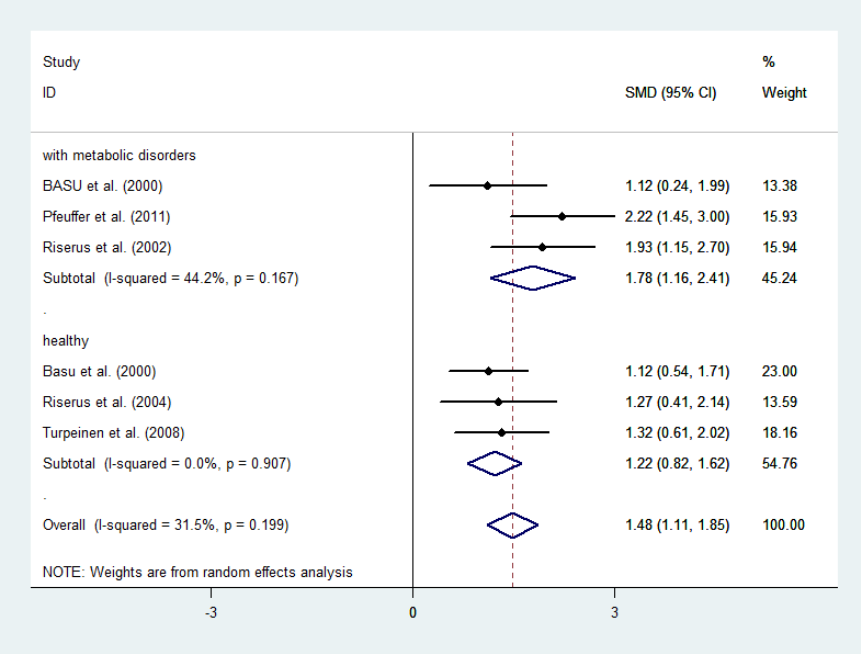

Figure 7. Forest plot of the effects of CLA supplements on 8-iso-$PGF_{2\alpha}$ level, stratified by health status

Table 1. Inclusion and exclusion criteria according to PICOS guideline (population, intervention, comparison, outcomes, study design).

PICOS	Inclusion criteria	Exclusion criteria
Population	Adults either healthy or with metabolic disorders	
Intervention	Supplementation with conjugated linoleic acid	CLA mix with other ingredients such as creatine monohydrate
Comparison	Placebo	Studies without control groups
Outcomes	Oxidative stress biomarkers such as MDA, 8-ISO-$PGF_{2\alpha}$, GPX	Studies with insufficient data
Study design	Controlled trial studies	Review studies, Editorial/letter to editor, Books, Citations, Studies not being published in peer-reviewed journals such as abstracts from conference proceedings, dissertations, and master's thesis

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Table 2. Characteristics and main outcome of the randomized clinical trials (RCTs).

Author and Year	Population	Age (y) mean (SD)	Duration (week)	CLA Dose and Form	Isomers (C9, t11: t10, c12)	Placebo	Results
Aryaeian *et al.* (2009)	87 patients with active RA	46.82 (12.21)	12	2.5 g/d CLA mixed isomers	50:50)	High oleic sunflower oil	GPX level increased in CLA group. MDA decreased in all groups.
Basu *et al*. (2000)	24 middle-aged obese men with signs of metabolic syndrome	53 (6.75)	4	4.2 g/d CLA mixed isomers	(50:50)	Olive oil	8-iso-$PGF_{2\alpha}$ in urine was significantly increased in the CLA-treated patients.
Basu *et al*. (2000)	53 healthy men and women	45.4 (11.7)	12	4.2 g/d CLA mixed isomers	(50:50)	Olive oil	The morning urinary levels and 24 h urinary levels of 8-iso-$PGF_{2\alpha}$ increased significantly. No significant change of the plasma MDA level was seen.
Ebrahimi-Mameghani *et al*. (2016)	38 obese NAFLD patients	37.66 (7.55)	8	3 g/d CLA	(50:50)		Serum MDA levels decreased significantly.
Eftekhari *et al.* (2013)	90 atherosclerotic patients	54.39 (14.48)	8	3 g/d CLA	(50:50)	Olive oil	In CLA group MDA and GPX reduced significantly compared to the baseline.
Kim *et al.* (2012)	29 healthy overweight/obese participants	40.05 (22.61)	8	2.4 g/d CLA mixture	(36.9:37.9)	Olive oil	CLA supplementation had no effect on GPX.
Matin *et al*. (2018)	90 COPD patients	62.62 (10.77)	6	3.2 g/d CLA	(50:50)		There was no significant difference between the 2 groups regarding serum MDA level.
Pfeuffer *et al*. (2011)	85 male (76.5% showed a metabolic syndrome)	45-68y	4	4.5 g/d CLA mixture	(50:50)	Safflower oil	CLA supplementation significantly increased urinary 8 iso PGF2α compared to safflower oil.
Riserus *et al*. (2002)	60 men with metabolic syndrome	53 (8.1)	12	3.4 g CLA mixed isomers 3.4 g/d t10,c12 CLA	(50:50)	Olive oil	T10, c12 CLA supplementation caused a significant increase in 8 iso PGF2α level.
Riserus *et al*. (2004)	25 abdominally obese men	55 (5.75)	12	3 g/d c9,t11 CLA	(83.3:7.3)	Olive oil	c9, t11 CLA supplementation caused a significant increase in 8 iso PGF2α level.

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Shadman *et al*. (2013)	63 participants with type 2 diabetes	46.06 (4.6)	8	3 g CLA/d	(50:50)	Soybean oil	CLA supplementation did not significantly affect MDA. But there was a significant trend to increase in MDA.
Turpeinen *et al.* (2008)	40 subjects with diagnosed birch pollen allergy	20-46	12	2 g CLA/d	(65.3;8.5)	High-oleic acid sunflower-seed oil	Urinary 8-iso-$PGF_{2\alpha}$ increased significantly following CLA supplementation compared to control group.

Table 3. Crude analysis vs. quality adjusted analysis for the effects of CLA supplements on 8-ISO-PGF2α .

Analysis	Serum 8-ISO-$PGF_{2\alpha}$		Effect Size	95%CI	I^2 (%)	P for Heterogeneity
All eligible studies	Overall effect		1.48	(1.11;1.85)	31.5	0.199
	Subgroup Analysis based on health status	Healthy	1.22	(0.82;1.62)	0.0	0.907
		With metabolic disorders	1.78	(1.16;2.41)	44.2	0.167
Good and fair quality studies	Overall effect		1.52	(1.07;1.98)	43.6	0.131
	Subgroup analysis based on health status	Healthy	1.17	(0.69;1.65)	0.0	0.778
		With metabolic disorders	1.78	(1.16;2.41)	44.2	0.167

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Discussion

The present systematic review and meta-analysis of RCTs demonstrated that CLA supplementation increased 8-ISO-PGF2α level significantly, and this effect was more prominent in individuals with metabolic disorders compared to the healthy ones. The results for 8-ISO-PGF2α were still consistent after adjustment according to the quality of included studies. CLA supplementation increased GPX level significantly, but CLA supplementation had no effect on MDA level. Moreover, the results of GRADE assessment confirmed the reliability and certainty of the findings for 8-ISO-PGF2α and GPX.

Cornish *et al.* assessed the effects of 6 g/d CLA supplements combined with whey protein and creatine monohydrate (CrM) on sixty-nine participants during 5 weeks of strength training. The results showed that there were no differences in the markers of oxidative stress such as 8-isoprostanes in comparison with other groups consuming CrM and whey protein or placebo (Cornish *et al.*, 2009). In another study, Tarnopolsky *et al*. used 6g/d of CLA plus CrM during resistance training, for 6 months in thirty-nine older adults. They found that the level of 8-isoprostanes was higher in women who consumed CrM+CLA supplements in comparison with other groups (Tarnopolsky *et al.*, 2007). Basu and Smedman observed that 4.2g/d CLA supplementation caused a significant increase in urine concentration of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ after 3 months compared to the control group (Basu *et al.*, 2000b). In another study in 2004, Basu and Smedman reported that 3.5g/d CLA supplementation for 6 weeks resulted in the production of PGF2α (Smedman *et al.*, 2004). Kuhnt *et al.* demonstrated that supplementation with 6g/d 11trans- and 12trans-18:1 for 6 weeks resulted in the augmentation of urinary concentration of 8-ISO-$PGF_{2\alpha}$ in the test group compared to the control groups (Kuhnt *et al.*, 2006).

Oxidative stress takes place as the result of the imbalance between ROS formation and enzymatic and nonenzymatic antioxidants (Marrocco *et al.*, 2017). Improvements in physical activity and diet could be beneficial in reducing oxidative stress (Anderson *et al.*, 2016). Diet could be connected to the oxidative stress via the consumption of various antioxidant nutrients (Tan and Norhaizan, 2019). Hadi *et al*. reported that high doses of curcumin /turmeric (150 and 2400 mg/day) for at least twelve weeks can lead to eNOS protein expression and total antioxidant capacity increase, by saving glutathione (GSH) and decreasing over production of ROS (Hadi *et al.*, 2019). Moreover, supplementation or food fortification with 800 to 4000 mg/d phytosterol for 4 to 24 weeks, could improve atherogenic and anti-atherogenic apolipoproteins in humans (Ghaedi *et al.*, 2020).

On the other hand, dietary fats may also be linked to oxidative stress levels (Anderson *et al.*, 2016). High-fat consumption causes overproduction of circulating free fatty acids, systemic inflammation, and oxidative stress (Tan *et al.*, 2018). High-fat diet (HFD) can promote oxidative stress, since HFD leads to obesity, which induces a permanent state of inflammation. In this situation, white adipose tissue secrets proinflammatory factors, and activated immune cells produce high amounts of ROS mediated by nuclear factor kappa B (NF-ĸB) and proinflammatory cytokines (Knight, 2000, Muñoz and Costa, 2013); Tan and Norhaizan 2019). Moreover, fat content of the diet, different kinds of fatty acids such as SFAs, unsaturated, or trans fatty acids have different effects on oxidative stress (Tan and Norhaizan, 2019). In a recent study among midlife women, higher intakes of trans fats and SFAs were also related to higher oxidative stress (Tomey *et al.*, 2007). Findings from RCTs that assessed the effects of CLA supplements, a trans fatty acid, on oxidative stress are controversial. This can be attributed to differences in the forms of CLA (NEFA or TG), CLA dosage, using different isomers with different proportions, study duration, and using different control groups. On the other hand, the mechanism by which CLA supplements increase the level of 8-isoprostanes could be justified as follows: oxidative stress

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generates numerous isoprostane species as D2-, E2-, and F2- that can be applied in plasma or tissues in the free form or stratified ones (Morrow and Roberts, 1997, Ormezzano *et al.*, 2005). Isoprostanes in the esterified form disturb the integrity of the cell membrane and have a cytotoxic impact on cell growth (Meagher and FitzGerald, 2000). 8-ISO-PGF2α is an established index of non-enzymatic lipid peroxidation, especially arachidonic acid (Basu, 1998). CLA affects prostaglandin production, also it has anti-carcinogenic properties (Kavanaugh *et al.*, 1999). It is unlikely that CLA amplifies the release of isoprostane F2α by augmenting the activity of PON, PAF-AH (Iannone *et al.*, 2009). CLA-induced cytotoxicity is associated with increased lipid peroxidation (Iannone *et al.*, 2009). Moreover, in competition with 8-iso-PGF2a for peroxisomal B-oxidation, CLA blocks degradation of 8-iso-PGF2α and results in 8-iso-PGF2a augmentation (Iannone *et al.*, 2009).

The present systematic review and meta-analysis has some strength. It included all available RCTs regarding the effects of CLA supplements on oxidative stress biomarkers. It is the latest systematic review and meta-analysis on this topic. An important strength of the present review lied in the method of analysis and interpretation of the results, by assessing all studies and high-quality studies separately and detecting the biases like publication bias, or defaults in methods, analysis, and interpretation. Moreover, the authors are moderately confident in the results for GPX and very confident for 8-ISO-PGF2α. However, the present study has some limitations that must be considered in the interpretation of the results. First, in general, meta-analysis cannot improve the quality of the eligible studies. Second, the short period or small sample size of some of the included RCTs, made it difficult to find a significant effect. Third, an insufficient number of CLA-enriched food studies made it impossible to perform any subgroup analysis and compare natural products with supplements of CLA. Meta-evidence for MDA was also very low, so the results for this outcome should be interpreted with caution.

Conclusion

According to the present systematic review and meta-analysis, CLA supplementation greatly increased some markers of oxidative stress such as 8-ISO-PGF2α in both healthy and individuals with metabolic disorders, and GPX levels. However, trials with various CLA dosages, longer duration, and higher sample sizes are necessary to confirm the findings of the present study. On the other hand, more trials with CLA-enriched food products are needed to assess its effect on oxidative stress biomarkers.

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Authors' contributions

Derakhshandeh-Rishehri SM, cooperated in study conduction, data analysis, and writing the paper; Rajabzadeh-Dehkordi M and Ghobadi S, cooperated in study conduction, screening, data extraction, and data analysis; and Faghih S, cooperated in research design, study conduction, and had primary responsibility for final content. All authors read and approved the final manuscript.

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Conflict of interests

The authors declare that they have no conflict of interest.

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