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Original Article

The Effect of Conjugated Linoleic Acids, Vitamin E and Their Combination on Lipid Peroxidation in Active Rheumatoid Arthritis

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Abstract

Background: Reactive oxygen species (ROS) have important role in the etiology and pathogenesis of Rheumatoid Arthritis (RA). We investigated the effect of conjugated linoleic acids (CLAs) and vitamin E on lipid peroxidation.

Methods: In a randomized, double-blind placebo, controlled, clinical trial 87 patients with active RA were enrolled. They were divided into 4 groups, received one of the following daily supplement for 3 months; 1- group C: 2.5gr CLA, that contained 2 gr 50:50 mix of *cis* 9-*trans*11 and trans 10-cis 12 CLAs, 2- group E: 400mg Vitamin E, 3- group CE: CLAs plus vitamin E, 4- group P: placebo. After supplementation Glutathione peroxidase (GSH-Px) level increased in C, E and CE groups, CE group had lower GSH-Px than P group($P \leq 0.05$).

Results: Glutathione reductase (GR) increased in CE, C and P groups. Catalase increased in all groups, but enhancement was significant in C and CE. Superoxide dismutase (SOD) decreased in E and C ($P \leq 0.05$). Catalase and SOD did not show significant differences between groups. Malondialdehyde (MDA) decreased significantly in all groups but this decrease was only significant in CE in comparison with other groups.

Conclusion: CLAs might be useful in reducing oxidative stress in RA

Keywords: *Conjugated linoleic acid, Vitamin E, Lipid peroxidation, Active rheumatoid arthritis*

Introduction

Rheumatoid Arthritis is a relapsing autoimmune disease with predominant synovial proliferation and destruction of articular cartilage. It is the most common inflammatory Arthritis affecting approximately 1% of the general population worldwide (1, 2). The exact etiology of RA remains unknown. Recently, a great number of studies have investigated the possible role of Reactive oxygen species in the etiology and pathogenesis of RA (2-5).

Free radicals from oxygen metabolism destroy antioxidant systems (6). It has been suggested that enzymatic and/or nonenzymatic antioxidant systems are impaired in RA; therefore Patients with RA are exposed to oxidant stress (3).

Under normal conditions, a variety of antioxidant mechanisms serve to control this ROS production (6). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (3). The destructive chain reaction initiated by ROS can be broken by antioxidants, which are able to convert ROS into harmless derivatives (6).

Specific antioxidant enzymes are Superoxide dismutase (SOD), Catalase (CAT) and GSH-Px (7, 8). The role of SOD is to accelerate the dismutation of toxic super oxide radicals, produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. GSH-Px and Catalase detoxify hydrogen peroxide and convert lipid hydroperoxides into non-toxic alcohols (9). Reduced Glutathione (GSH) plays an important role in metabolic processes, transport and cellular protection in nearly all cells of the body by its thiol groups (5, 6). These antioxidants are also essen-

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tial for inhibition of inflammation related to the function of neutrophils (5).

The CLAs fatty acids, a natural component of ruminant meat and dairy products, have been shown to have beneficial effects in delaying the development of various diseases (10-12). There has always been concern over the instability of the very long-chain fatty acids and their susceptibility to oxidation. Consumption of high levels of n-3 PUFAs (poly unsaturated fatty acids) leads to enhanced membrane lipid peroxidation by free radicals (13). Feeding Fish oil adequately supplemented with vitamin E significantly increased the activities and mRNA levels of Catalase, GSH-Px, and Superoxide dismutase in livers of autoimmunity-prone Mice (14).

The role of CLAs in oxidative stress has been investigated in models and biological systems (15-20). CLA inhibited linoleic acid oxidation, (20) suppressed lipid peroxidation in mammary glands of Rats, (21) and directly reacted and quenched stable 2, 2-diphenyl-1-picryhydrazyl (DPPH) radicals (22). Others have noted that some CLA isomers do not form dienes; therefore, such CLA compounds have been described as less capable of undergoing lipid peroxidation (23). Other evidence exists showing that individual CLA isomers differ in their Capacity to modulate oxidative status (24-27). Physiological behavior differences may be due to structural/conformational differences that affect the permeability of lipid structures (membranes) to oxygen and lipid peroxidation.

The objective of this study was to determine the antioxidant efficacy of CLAs compared with α-tocopherol. We used 50: 50 mixes of two CLA isomers (10-trans, 12-cis-CLA and 9-cis, 11-trans-CLA) in glycerinated form because of the reported efficacy in improving Immunity (28-30) and lowering oxidation rate (31).

Material and Methods

Patients and controls

A randomized double blind placebo controlled trial was conducted in a 12 week period in patients with active RA. Patients were between 19-69 yr old and had RA for at least 2 yr. Data on dietary habits, dietary supplements, anthropometric indices including body mass index (BMI), smoking habit, and drug history were obtained by face to face interview.

The exclusion criteria were: abnormal renal and/ or hepatic function, smoking, and history of myocardial infarction, pregnancy, taking vitamin and/ or mineral supplement, and taking drugs such as thyroid hormones, estrogens, progesterone, diuretics or β-blockers.

A written informed consent was obtained from all participants

The research protocol was approved by the Ethics Committee of Tehran University of Medical Sciences. The Vitamin E was used as under tolerable upper intake levels that is 1000mg (32), and the mean intake of Vitamin E in RA patients were 400 mg per day, RA patients assigned to one of the four treatment groups using Random Permuted Block procedure.

Depending upon the treatment groups, each subject received two capsules and one pearl per day for a period of 3 months. Each capsule contained one of the following preparations; group C: CLA (consumed 2 capsules per day that each capsule weight was 1.25 gr and contained 80% CLA, equal 2 gr from 50:50 mix of cis -9, trans-11 and trans-10, *cis*-12 CLA in form of glycerinated (clarinol G-80) and Placebo of vitamin E, E group: Vitamin E pearl and Placebo of CLA; group CE: both of the above CLA and Vitamin supplements; P group (placebo): Corn oil in replace of Vitamin E and HOSF (High oleic sunflower oil) in replace of CLA. The vitamin E pearl and placebo capsules looked identical and were specially prepared for this study by Zahravey Co and CLA and it's placebo by Lipid Nutrition Co. After 12-14 h overnight fasting 10 ml blood was collected from each subject at the beginning and at the end of 3 months trial. Blood samples were collected in 4 trace element free tubes, one for serum separation, two with EDTA one tube for Hb measurement other tube for plasma separation, and one citrated tube for ESR. The plasma

samples were separated from the cells by centrifugation at 3000 rpm for 10 min. The remaining blood was washed three times with 9g/l Nacl solution. Cell membranes were removed by centrifugation at 1200Xg for 5 min at 4º C. The hemolyzate were used for determining Antioxidant Enzyme activity. The Hb concentration was also determined in the hemolysates, which were then stored in -70º C until enzyme activity assays. The Serum was separated by centrifugation tube with coagulated blood for 10 min at 1000g at 4º C. Blood hematological and serum inflammatory markers were measured within 6 h after blood collection. Red blood cells, plasma and serum portions were stored at -70° C.

Serum α-tocopherol was determined by high performance liquid chromatography (33). GSH-Px activity of Erythrocytes was measured spectrophotometrically by Ransod kit (Ransel, Manual) at 37º C and 340 nm according to the method described by Paglia and Valentin. GR Erythrocytes activity was measured spectrophotometrically by Ransod kit (Manual) at 37º C and 340 nm according reduction of Glutathione (GSSG) in the presence of NADPH, which is oxidaised to $NADP^+$due to GR. SOD activity was assayed by kit Ransod (cat.NO.SD 125). This method employs Xanthine and Xanthine oxidase to generate Superoxide radicals which react with 2-(4-iodophenyl) -3-(4-nitrophenol) -5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity then measured by the degree of inhibition of this reaction. CAT activity was determined by enzymatic method according to Hygo Aebi (34). Activity of CAT was determined by following the decomposition of H_2O_2 in Phosphate buffer spectrophotometrically at 230 nm.

MDA was determined by a colorimetric method with an interassay coefficient variation less than 2% concentration. The principle of the method was based on the spectrophotometer measurement of the color produced during the reaction of thiobarbituric acid with MDA (35).

CRP values were determined with Immunoturbidometric assay in this method CRP gives a complex with polyclonal antibody and creates turbidity that has positive relation with CRP in sample. Other Haematological analysys counts were obtained by an automated blood counter (Beckman Coulter, Miami, USA). ESR was measured using an ESR apparatus (Greiner Labor Technic GmbH, Germany).

Nutrients intakes were estimated using 24 h dietary recall questionnaire at the beginning and at the end of the 3 months trial for 2 d and analyzed by Food Processor software. The subjects were asked not to alter their usual diets and physical activities throughout the study, and any changes in their medication were avoided whenever possible. Compliance with the supplementation was assessed by counting number of the capsules used and also by measuring changes in the serum α-tocopherol.

Statistical analysis

All values are expressed as mean±S*D*. Log transformation was used to normalize the distribution of CRP. All other variables were normally distributed. Differences between four groups were compared by one-way analysis of variance (ANOVA) for continuous data and the χ2-test for categorical data. Post hoc comparisons were performed with Tukey test. Adjustment for differences in baselines covariantes and changes in variables during study were performed by analysis of covariance. A value of $P \leq 0.05$ was considered to be statistically significant. All data were analyzed using SPSS Software.

Results

During the follow up five patients withdrew and 10 were excluded from statistical analysis because they interrupted trial treatment or changed their medication. As shown in Table 1, at the beginning of the study, the groups were similar with respect to the sex, age, duration of RA, BMI, daily intake of Vitamin E (Table 1).

There were no significant changes in BMI, physical activity or medication during the study. Table 2 shows the serum α-tocopherol, MDA and Red blood cell activity of CAT, Glutathione

peroxidase, Glutathione reductase and Superoxid dismutase following the study. At the baseline there were no significant differences between groups by ANOVA and ANCOVA. Following 3 months of supplementation, serum levels of CAT in E group increased significantly. SOD decreased in all groups, but in E and C Groups decreased significantly ($P \leq 0.05$). GSH-Px level increased in C, E and CE Groups, but CE group had higher GSH-Px than P group ($P \leq 0.05$). GR increased significantly in the CE, C and P groups ($P \leq 0.01$). Although GR increased 9.7% in E group, but had lower levels than P groups ($P \leq 0.05$). Following the study serum levels of α-tocopherol increased significantly in C, E and CE groups as compared with baseline ($P \leq 0.05$, $P \leq 0.0001$, $P \leq 0.0001$ respectively) and in E and CE groups in comparison with group *P* ($P \leq 0.0001$). MDA decreased in all groups but this decrease was significant only in CE group in comparison with other groups ($P \leq 0.001$). MDA decreased in C, E and CE groups ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively).

Table 3 shows the serum FBS, CRP, ESR, WBC, platelet count, BMI before and after supplementation for subjects who completed the study. At the baseline there were no significant differences between groups by ANOVA and ANCOVA. After 3 months of supplementation ESR levels decreased significantly in the C and E and CE groups as compared with baseline ($P \leq 0.05$ $P \leq 0.05$, $P \leq 0.001$, respectively) and CE group had significantly lower ESR levels than P group ($P \leq 0.05$). After 3 months of supplementation CRP dropped nonsignificantly in P, C, E and CE groups (19%, 24%, 55%and 39% respectively). The reduction of WBC in CE group was significant as compared with other groups ($P < 0.05$). Platelet count nonsignificantly decreased in CE, C and E groups. BMI and FBS did not change significantly in four groups (Table 3).

Table 1: Demographic, Anthropometric and Biological Data for the Four Study Groups before Supplementation (Mean±SD)

Variables	Placebo (n=22)	Conjugated linleic acids (CLAs) (n=22)	Vitamin E (n=21)	CLAs+Vitamin E (n=22)
Male/Female¶	3/19	3/19	4/17	5/17
Age (years)	47.95±11.14	46.23±13.07	49.33±11.89	43.77±12.75
Duration of RA(years)	8.88±9.65	9.95±8.41	7.24±5.82	7.64±6.19
BMI (kg/m2)*	28.48±3.94	27.18±4.63	27.14±4.7	25.65±3.97
Vitamin E intake (mg per day)	7.9± 2.44	8.75± 2.31	7.78± 3.50	8.80± 3.44

There were no significant differences between groups by ANOVA or ¶Chi-square, *BMI =body mass index.

Table 2: Levels of Antioxidants variables in Patients with active RA before and after 3 months supplementation (Mean±SD)

Variables		Placebo (n=22)	Conjugated linleic acids (CLAs) (n=22)	Vitamin E (n=21)	CLAs+ Vitamin E (n=22)
CAT(U/gHb)	before	180.40±96.84	177.26±77.19	165.19±50.33a	168.68±40.15a
	after	188.32±73.07	202.091±59.83	190.13±59.07	195.68±65.17
SOD(U/gHb)	before	804.78±134.14	800.00±111.3b	795.48±150.09a	717.96±188.40
	after	721.26±298.31	693.07±127.05	671.79±148.88	680.05±108.34
GPX(U/gHb)	before	28.42±6.58	28.45±6.94	27.57±6.35	24.07±5.20†
	after	28.03±6.32	31.45±11.32	28.18±10.74	27.24±7.39
GR(U/gHb)	before	3.52±1.05b	3.33±1.05c	3.60±1.46¶	3.61±1.35b
	after	4.61±1.43	4.19±1.34	3.95±1.149	4.31±1.68
MDA§(ng/ml)	before	1.834±1.09	2.25±1.02c	2.36±1.08[a]	2.53±1.3 d‡
	after	2.14±1.31	1.6±0.64	1.7±.0.76	1.29±0.68
Serum Vitamin E (µg/ml)	before	4.83±3.78	4.72±4.41a	5.24±2.87 b‡	6.64±4.64c ψ
	after	5.03±3.72	5.51±4.46	6.63±4.22	7.78±.4.62

There were no significant baseline differences between groups by ANOVA.
Statistically significant differences between before and after: a $P \leq 0.05$, b $P \leq 0.01$, c $P \leq 0.001$. After supplementation † Group CE had significantly Higher levels than P group ($P \leq 0.05$) ,‡ E Group had significantly lower levels than group P ($P \leq 0.001$), § E and C Groups had significantly lower levels than P group ($P \leq 0.05$).¶ E Group has significantly lower levels than P group ($P \leq 0.05$). ψ Group CE had significantly higher levels than P group ($P < 0.05$).

Table 3: Levels of FBS and inflammation variables in Patients with active RA before and after 3 months supplementation (Mean± SD)

Variables		Placebo (n=22)	Conjugated linleic acids (CLAs) (n=22)	Vitamin E (n=21)	CLAs+Vitamin E (n=22)
FBS(mg/dl)	before	90.18±6.41	78.27±1.95	90.85±8.51	85.68±6.15
	after	85.04±5.46	75.86±1.41	78.05±2.41	81.85±3.14
CRP(mg/l)	before	6.44± 1.68	7.18± 2.16	9.06±3.12c	5.23±1.37
	after	5.48± 1.19	5.46± 1.18	4.07±.99	3.17±0.83
ESR(mm/h)	before	28.36± 4.59	26.81±2.38a	40.43±5.72a	28.45±3.68ξb
	after	27.04± 4.04	19.14±2.16	32.28±5.01	17.77±2.60
WBC(/UL)	before	8587.72±483.94	7728.57±551.24	7724.76±400.36	9280.95±513.42a
	after	7752.95±457.03	8533.33±401.86	10276.19±2373.96	8528.57±491.99
BMI(kg/m2)	before	28.48.92±3.94	27.18±4.63	27.14±.4.70	25.65±3.97
	after	28.66±3.97	27.45±4.59	27.97±4.08	25.94±.3.59

There were no significant baseline differences between groups by ANOVA.
Statistically significant differences between before and after: a $P \leq 0.05$, b $P \leq 0.001$, c P= 0.054. After supplementation: ξ Group CE had significantly lower levels than P group ($P \leq 0.05$).

Discussion

Although the exact causes of RA are unknown, involvement of ROS is suspected. Several studies suggest a beneficial effect of antioxidants such as vitamin E in RA. This study investigated the effects of CLAs and Vitamin E supplementation on lipid peroxidation in adults with active RA.
At baseline there were no significant differences in the GSH-Px, CAT, SOD and GR activities in Erythrocytes between groups by ANOVA and ANCOVA. GSH-Px level increased in C, E and CE Groups, but CE Group had significantly higher increase in GSH-Px levels than group P ($P \leq 0.05$).
Following the study CAT increased in all groups in comparison to the baseline, but this increase was significant only in C and CE groups and didn't show significant differences between groups. GSH-Px and CAT activities were higher in the C and CE groups than in control. The low levels in GSH-Px and Catalase activities are accompany by increase levels of oxidative stress. It is known that GSH-Px and Catalase detoxify hydrogen peroxide and convert lipid hydroperoxides into non-toxic alcohols (3-5). The decrease in plasma GSH-Px and CAT activities may be due to inactivation of the enzymes by hydrogen peroxide (6).
Our results show lower levels of the antioxidant (CAT, GSH-Px, SOD and vitamin E) in the P group (Table 2). Several previous studies reported low intake of the vitamin E and vitamin A as a risk factor for RA (36-40). Heliovaara et al. reported elevated risks of RA at low levels of α-tocopherol and beta-carotene (3). Helmy et al. showed that high dose vitamin E treatment decreased disease activity in patients with RA (36). Cerhan et al. hypothesized that consumption of vitamin E and β-carotene was inversely associated with the risk of developing RA in the elderly (37). Low level of vitamin E, GSH-Px, GSH, CAT, SOD and increase in MDA have been shown in patients with RA(38- 42).
Following our study GR increased significantly, but this increase was significant only in E group in

comparison with P group. These data underline the synergic action of these enzymes in the pathogenesis of joint damage. RA patients also exhibit higher levels of GR, which is important for the detoxification pathway of oxygen free radicals.

In our study SOD decreased in all groups, but this decrease was significant in C and E groups and there wasn't significant difference between groups. In Ozkan et al. serum SOD activities did not show any statistical difference between the patients and healthy controls (43). In our study vitamin E and CLA hadn't benefit effect in enhancement of SOD, but because of synergist effect of vitamin E plus CLA, CE group had higher SOD level than P group.

There were no significant differences between groups by ANOVA and ANCOVA in the serum MDA, CRP concentrations, ESR, WBC, platelet count, and BMI. After 3 months of supplementation, ESR levels decreased in the C and E and CE group compared with the baseline ($P \leq 0.05$, $P \leq 0.05$, $P \leq 0.001$ respectively) and ESR levels in CE group was lower than P group ($P \leq 0.05$) (Table 3). CRP dropped in E group as compared with baseline ($P \leq 0.05$). It was found that CRP in patients with RA may be a sensitive inflammation markers for reflecting the presence and activity of the disease (3, 9). The reduction of WBC in CE group was significant compared to other groups ($P \leq 0.05$). Platelet count nonsignificantly decreased in CE, C; E groups (Table 3). In some study similar to our results high-sensitivity CRP concentrations were significantly lowered with α-tocopherol supplementation than with placebo (32%; $P < 0.001$) (44). α-Tocopherol supplementation significantly reduced urinary Isoprostane F2α (44, 45) and, as like our study serum vitamin E concentration increased significantly (45).

Santos Zago et al. studied on Supplementation with Commercial Mixtures of CLA in Association with vitamin E and the Process of lipid autoxidation in 60 Rats. The CLA amount was 2% of feed consumption. Animals were supplemented for 42 d. Hepatic peroxide results indicated that CLA increased oxidation; in contrast, as like our study serum MDA results showed that CLA reduces oxidation. Serum CAT indicated a significantly reduction of oxidation (46).

Kim et al. studied the effect of dietary CLA on lipid peroxidation in Male Sprague-Dawley Rats. They were fed one of the experimental diets; normal diet, vitamin E-deficient control diet, 0.5% CLA vitamin E-deficient diet, or 1.5% CLA vitamin E-deficient diet for 5 weeks. Hepatic TBARS were increased in the vitamin E-deficient control group, but they were significantly lowered in the CLA groups. Similarly, hepatic Glutathion peroxidase activity increased in the vitamin E-deficient diet and reduced by CLA supplementation. In addition, similar to our study CLA caused a significant decrease in SOD activity while had no effect on Catalase activity. Analysis of the fatty acid composition revealed that dietary CLA was incorporated into hepatic microsomal membrane dose-dependently. Compared to the vitamin E-deficient control, CLA resulted in significantly higher saturated and monounsaturated fatty acids and lower levels of oxidation-susceptible PUFA (linoleic, linolenic, and arachidonic acids) in both plasma and hepatic membrane. These results suggested that dietary CLA has antiatherosclerotic and antioxidant activity by increasing oxidative stability in plasma and hepatic membrane in the vitamin E-deficient Rats (47).

Tsuzuki et al. study showed that the oxidation rate of CLAs was reduced by triacylglycerol esterification. The oxidative stability of α-ESA (α-eleostearic acid) was examined compared with linoleic acid (LA), α-linolenic acid (LnA), and CLA. Thin layers of the FA (LA, 9c, 11 t-CLA, 10c, 12t-CLA, LnA, and α-ESA) were auto-oxidized at 37º C, and the FA remaining, the absorbed oxygen volume, the lipid hydroperoxide content, and the TBARS content were determined. The oxidation rate of α-ESA was faster than that of the unconjugated FA and CLA. Furthermore, the oxidative stability of conjugated FA in which the carboxylic acid group esterified with triacylglycerol was greater than that of the FFA. Addition of α-tocopherol also increased the stability of the conjugated FA to a level similar to that of the unconjugated FA (48).

Igarashi et al. studied on each amount of phospholipids hydroperoxide in CLA-supplemented cells, it was one-fourth as much as that in LA-supplemented cells, but the amount of TBARS was not deferent in any group. They recognized that the inhibition of cell growth by CLA was not induced via lipid peroxidation, and CLA might act as an antioxidant in HepG2 (49).

Choi et al. investigated the effects CLA preparations containing different ratios of CLA isomers on fatty acid on Sprague-Dawley Rats. The c9, t11-CLA-mix (80: 20) increased the activities of cytochrome C oxidase, manganese-SOD, GSH-Px, GR, and the level of GSH enhanced mitochondrial function and protection against oxidative stress, all CLA preparations reduced insulin resistance. Among them, the c9, t11-CLA-mix was the most effective based on the parameters reflecting insulin resistance, fat oxidation, and mitochondrial antioxidative enzyme activity in the liver (50). Arab et al. investigated the effects of several fatty acids on the redox status and lipid peroxidation of human fibroblasts. After 48 h, only arachidonic acid and CLA enhanced GSH content through an induction of γ-Glutamylcysteine ligase. CLA was more potent than arachidonic acid in inducing GSH synthesis. Lipoperoxidation was associated with increase haemoxygenase-1, cyclooxygenase-2 mRNA expression and a high level of ROS at 7 d. As demonstrated by a tert-butylhydroperoxide cytotoxicity test, the GSH synthesis obtained with arachidonic acid is not sufficient to protect the cells, whereas this protective effect was obvious with CLA at 48 h as well as at 7 d. The present results show that CLA is the only PUFA able to induce GSH synthesis without any changes in oxidative balance, whereas an upregulation of cyclooxygenase-2 by other PUFA is concomitant with an overproduction of malondialdehyde and ROS (51).

Bergamo et al. study, examined the biological effects of CLA administration in the MRL/MpJ-Fas (lpr) mouse, an animal model of systemic Lupus Erythematosus. They found enhancement GSH content in CLA-treated Rats (52).

Palacios et al. studied effect of CLA and vitamin A on the PUFA composition, chemiluminescence, peroxidizability index of microsomes and mitochondria isolated from Rat liver. The peroxidizability index, a parameter based on the maximal rate of oxidation of fatty acids, showed significant changes in the CLA group compared to vitamin A and control groups. The simultaneous analysis of peroxidizability index, chemiluminescence and fatty acid composition demonstrated that CLA is more effective than vitamin A in protecting microsomes or mitochondria from peroxidative damage (53).

Alan et al. studied GPX4 mRNA levels and activity in human umbilical vein endothelial cells. They were increased optimally by CLA whereas Arachidonic acid had no effect, but enzyme activity was increased by Arachidonic acid (54).In conversely several studies had shown that Supplementation with CLA markedly increased 8-iso-PGF2α (55-57) and CRP compared with placebo (55), while vitamin E Supplementation may be reduce CRP and 8-iso-PGF2α production (46).

It seems that in patients with RA, CLAs decrease lipid Peroxidation and oxidative stress, but the influence of CLA is dependent on supplement type, supplement dosage and determination methodology, and its combination with vitamin E could be helpful to reducing of oxidative stress and exacerbating its anti-oxidative effect in RA active patients. It is concluded that co-supplementation of 2 g CLA 50:50 mix, CLA plus 400 mg vitamin E and vitamin E for 12 weeks altered significantly lipid Peroxidation, antioxidant and antioxidant enzymes so CLAs might be useful in reducing oxidative stress in RA.

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The authors declare that there is no conflict of interests.

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