

Anti-inflammatory effects of conjugated linoleic acid on young athletic males

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Abstract

Objective: To explore the efficacy of conjugated linoleic acid supplementation on some inflammatory factors in young healthy males during exhaustive exercise.

Methods: The randomised double-blind controlled study was conducted at Ardabil University of Medical Sciences, Iran, from December 2012 to March 2013, and comprised healthy male athletes 18-24 years of age. The subjects were randomly distributed into control and intervention groups. About 5.6 g/day conjugated linoleic acid supplement and oral paraffin (placebo) were given to intervention and control groups respectively daily for two weeks. Fasting blood samples were taken at baseline and at the end of the two weeks of intervention. The subjects underwent exhaustive exercise and then fasting blood samples were taken. Serum levels of tumour necrosis factor alpha, interleukin-6, high-sensitivity C-reactive protein, matrix metalloproteinases-2 and 9 were measured.

Results: There were 23 subjects in the study, with 13(56.5%) in the supplemented group and 10(43.4%) in the control group. Serum levels of matrix metalloproteinases-2 and tumour necrosis factor alpha were significantly decreased in the supplemented group ($p < 0.05$). After exhaustive exercise, serum levels of matrix metalloproteinases-2, high-sensitivity C-reactive protein and tumour necrosis factor alpha significantly decreased in the supplemented group compared to the control group ($p < 0.05$).

Conclusion: Two-week administration of conjugated linoleic acid reduced the inflammatory factors following exhaustive exercise in young healthy males.

Keywords: CLA, MMP2, MMP9, hs-CRP, Exercise. (JPMA 66: 280; 2016)

Introduction

Strenuous exercise induces an increase in the pro-inflammatory cytokines.¹ The production of inflammatory and pro-inflammatory mediators² and matrix metalloproteinases (MMPs)³ are increased during exercise activity. Several MMPs exist in muscle, among them MMP2 and MMP9 play main role in skeletal muscle adaptation to contractile demands and injury.^{4,5} It has also been shown that activation of MMP2 and MMP9 is involved in various myopathic and inflammatory-induced changes in skeletal muscle,^{5,6} and intake of natural antioxidants⁷ such as conjugated linoleic acid (CLA) provides an extra measure of protection that slows the process of musculoskeletal depletion.⁸ CLA is important in human nutrition due to its related health benefits such as anti-inflammatory, anti-obesity, anti-cancer, anti-atherosclerosis and anti-diabetic effects.⁹ Dietary CLA reduces the release of pro-inflammatory cytokines^{10,11} and suppresses serum MMP9 and MMP2 in animal models.¹²

The current study was planned to evaluate the efficacy of CLA supplementation on MMP9 and MMP2 and some inflammatory factors in young healthy males during exhaustive exercise.

Subjects and Materials

The randomised double-blind controlled study was conducted at Ardabil University of Medical Sciences, Iran, from
Department of Biochemistry, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.

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December 2012 to March 2013. After approval by the institutional ethics committee, healthy male athletes 18-24 years of age living in the university dormitories were recruited and randomly divided into two groups; supplemented and control. Subjects with alcoholism, certain diseases, like diabetes, cardiovascular and metabolic syndrome etc., professional athletes, non-athletes, body mass index (BMI) $< 18.5 \text{ kg/m}^2$, smokers, and consumers of drugs and dietary supplements were excluded.

After obtaining informed written consent from the subjects, a standardised questionnaire was filled for each subject.

In a healthy dose range,¹³ 5.6g/day CLA supplement in the shape of soft-gel capsules with 80% purified CLA (NCC, 300 Don Park, Markham, Ont. Canada) was given to supplemented group daily for two weeks. The control group was given oral paraffin as placebo daily for two weeks. Anthropometric factors (height and weight), and fasting blood samples were taken at baseline and post-supplementation as well as after exhaustive exercise. Sera were separated from the blood samples immediately and were stored at -80°C until biochemical and inflammatory analysis.

Serum levels of tumour necrosis factor alpha (TNF α), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), MMP2 and MMP9 were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience and Boster) according to the manufacturer's instructions. For accurate assessment, duplicate assays were performed. Serum

concentration levels of hs-CRP were measured by turbidimetric immunoassay method (Parsazmoon, Iran). After the separation of serum, triglycerides (TG) and total cholesterol (TC) were measured using enzymatic kits (BioSystems, Spain), and high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol were measured using photometric method test and Pars automatic analyser using reagents and calibrators as recommended by the manufacturer (Hitachi 911, Japan).

Food intake was estimated for energy and other nutrients by

24-hour recall method for three days in a week during the study. Mean daily dietary intake and food composition were estimated using Nutritionist IV software.

Data were analysed using descriptive statistical tests, paired and independent samples t-test. Level of statistical significance for all tests was $p < 0.05$.

Results

There were 23 subjects in the study, with 13(56.5%) in the

Table-1: Comparison of age, weight, calorie, and nutrient intake in both groups.

Variables	groups		P value	variables	groups		P value
	Supplement M $\pm$ SD	Control M $\pm$ SD			Supplement M $\pm$ SD	Control M $\pm$ SD	
Age (year)	0.97 $\pm$ 18.46	0.42 $\pm$ 18.2	0.43	Zn (mg)	3.85 $\pm$ 9.94	1.61 $\pm$ 9.91	0.97
Weight (kg)	4.63 $\pm$ 71.07	74.12 $\pm$ 6.73	0.18	Se (μ g)	0.03 $\pm$ 0.08	0.04 $\pm$ 0.07	0.65
BMI (kg/m ²)	0.89 $\pm$ 23.13	23.83 $\pm$ 2.18	0.25	Linoleic acid (g)	0.3 $\pm$ 0.56	0.29 $\pm$ 0.51	0.07
Height (cm)	4.08 $\pm$ 175.23	5.87 $\pm$ 176.44	0.54	Omega-3 (g)	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.91
Cal (Kcal/day)	261.37 $\pm$ 2675.7	271.15 $\pm$ 2545.31	0.20	DHA (g)	0.12 $\pm$ 0.07	0.09 $\pm$ 0.06	0.94
Protein (g)	20.44 $\pm$ 99.58	9.11 $\pm$ 91.01	0.14	Oleic acid (g)	7.67 $\pm$ 18.20	8.40 $\pm$ 15.69	0.41
CHO (g)	46.34 $\pm$ 340.07	60.15 $\pm$ 308.59	0.13	V.C (mg)	46.94 $\pm$ 68.93	83.24 $\pm$ 71.95	0.90
Fiber (g)	5.42 $\pm$ 11.99	4.87 $\pm$ 12.82	0.66	V. A (RE)	1331.82 $\pm$ 2905.67	1911.25 $\pm$ 3703.49	0.19
Total fat (g)	32.96 $\pm$ 106.61	20.62 $\pm$ 112.42	0.56	V. E (mg)	18.57 $\pm$ 23.68	18.03 $\pm$ 22.31	0.84
SF (g)	14.69 $\pm$ 38.38	8.83 $\pm$ 36.27	0.8	V. B5 (mg)	9.51 $\pm$ 13.71	8.46 $\pm$ 7.27	0.06
PUFA (g)	12.17 $\pm$ 22.87	10.82 $\pm$ 28.88	0.17	MUFA (g)	10.16 $\pm$ 31.99	6.30 $\pm$ 31.58	0.89

CHO = carbohydrate, RE = retinol equivalent, BMI = body mass index, V = Vitamine, Cal = Calorie, MUFA = Mono unsaturated fat (g), PUFA = Poly unsaturated fat, SF= Saturated fat, , Se = Selenium, Zn = Zinc, DHA=Docosahexaenoic acid.

Table-2: Comparison of inflammatory factors' activities and lipid profile before and after severe physical activity in both groups.

Variables	Measurement stage	supplement group	control group	P value
MMP2(ng/ml)	Before supplementation	6.87 $\pm$ 3.06	7.02 $\pm$ 1.65	0.87
	After supplementation	5.12 $\pm$ 2.81*	7.15 $\pm$ 2.56	0.05
	After severe physical activity	5.23 $\pm$ 1.94	7.18 $\pm$ 2.35	0.02
MMP9(ng/ml)	Before supplementation	1.44 $\pm$ 18.94	1.61 $\pm$ 19.40	0.42
	After supplementation	18.45 $\pm$ 1.64	1.85 $\pm$ 19.07	0.35
	After severe physical activity	19.31 $\pm$ 0.79	19.79 $\pm$ 0.78	0.11
IL-6(pg/ml)	Before supplementation	0.72 $\pm$ 0.81	0.43 $\pm$ 0.75	0.79
	After supplementation	0.70 $\pm$ 0.34	0.83 $\pm$ 0.42	0.38
	After severe physical activity	3.41 $\pm$ 2.12	4.16 $\pm$ 2.17	0.36
TNF α (pg/ml)	Before supplementation	623.25 $\pm$ 1104.10	422.51 $\pm$ 1048.77	0.77
	After supplementation	766.68 $\pm$ 420.58*	1190.49 $\pm$ 561.59	0.03
	After severe physical activity	1731.60 $\pm$ 1043.08	3038.20 $\pm$ 1247.23	0.006
hs-CRP(mg/l)	Before supplementation	0.24 $\pm$ 0.50	0.16 $\pm$ 0.45	0.51
	After supplementation	0.47 $\pm$ 0.31	0.48 $\pm$ 0.26	0.96
	After severe physical activity	1.78 $\pm$ 0.87	2.77 $\pm$ 1.12	0.01
Cholesterol (mg/dl)	Before supplementation	27.55 $\pm$ 184.62	47.13 $\pm$ 170.05	0.33
	After supplementation	186.15 $\pm$ 27.91	195.38 $\pm$ 65.78	0.65
	After severe physical activity	193.31 $\pm$ 25.95	188.80 $\pm$ 64.03	0.82
HDL- Cholesterol (mg/dl)	Before supplementation	8.4 $\pm$ 39.69	6.39 $\pm$ 40.13	0.87
	After supplementation	42.23 $\pm$ 11.16	43.31 $\pm$ 11.35	0.80
	After severe physical activity	42.15 $\pm$ 7.84	28.70 $\pm$ 9.59	0.001
LDL- Cholesterol (mg/dl)	Before supplementation	16.68 $\pm$ 125.08	13.15 $\pm$ 114.95	0.07
	After supplementation	119.85 $\pm$ 23.72	113.69 $\pm$ 19.87	0.45
	After severe physical activity	121.23 $\pm$ 22.80	126.01 $\pm$ 37.31	0.71
Triglyceride (mg/dl)	Before supplementation	45.43 $\pm$ 117.77	38.19 $\pm$ 111.14	0.67
	After supplementation	128.69 $\pm$ 44.28	136.39 $\pm$ 48.24	0.66
	After severe physical activity	157.15 $\pm$ 44.11	137.01 $\pm$ 41.90	0.28

Values are mean $\pm$ SD, *. ($p < 0.05$) Paired-sample t test indicated a significant difference in supplemented group between baseline and after supplementation. hs-CRP: High sensitivity C-reactive protein, MMP2: Matrix metalloproteinases 2, MMP9: Matrix metalloproteinases 9, TNF α : Tumor necrosis factor alpha&IL-6: Interleukin 6, HDL: High-density lipoprotein, LDL: Low-density lipoprotein.

supplemented group and 10(43.4%) in the control group. There was no significant difference in terms of age, weight, calorie, and nutrient intake between the groups ($P>0.05$ each) (Table-1).

The serum levels of MMP2 and $\text{TNF}\alpha$ were significantly decreased after the two-week intervention in the supplemented group ($p<0.05$), but decreased levels of MMP9, IL-6 and hs-CRP were non-significant ($p>0.05$). In the supplemented group the serum levels of $\text{TNF}\alpha$ and MMP2 were significantly less than the control group after severe physical activity ($p<0.05$). Although the serum levels of hs-CRP were increased in both groups after exhaustive exercise, but the increase was significant in control group compared to the supplemented group ($p<0.05$). In the supplemented group the serum level of HDL cholesterol was higher than the control group after severe physical activity ($p<0.05$) (Table-2).

Discussion

The study demonstrated that the CLA is an efficient oral supplementation before and after exhaustive exercise. Intake of CLA supplementation for two weeks significantly reduced serum levels of MMP2 and $\text{TNF}\alpha$. Our findings are similar to a study by Butz et al.¹⁴ CLA has anti-inflammatory properties via inhibiting of cyclooxygenase (COX) and lipoxygenase (LOX) messenger ribonucleic acid (mRNA) expression and can bind to and activate the peroxisome proliferator-activated receptor gamma ($\text{PPAR}\gamma$).¹⁵⁻¹⁷ The decreased serum levels of $\text{TNF}\alpha$ are probably mediated via activation of $\text{PPAR}\gamma$.¹⁴ Plasma $\text{TNF}\alpha$ levels confirmed that the CLA had a biological immune effect.¹⁸ Growing evidence suggests that the resolution phase of inflammatory states underlying many acute and chronic diseases closely involves the action of $\text{PPAR}\gamma$.¹⁹ CLA has the potential effect to bind and activate $\text{PPAR}\gamma$.¹⁹ In vitro and in vivo models studies have shown that CLA modulates soluble factors or mediators of immunity such as eicosanoids, cytokines, immunoglobulin production and another involved factors.^{20,21} Our study showed that the serum levels of MMP2 and $\text{TNF}\alpha$ decreased significantly after 14 days of supplementation. MMP9, IL-6 and hs-CRP serum levels decreased in the test group and their serum levels increased in the control group, but this was not significant. Used dose of CLA (5.6 g/day) has important role probably. CLA decreased MMP2 level because of down-regulation of gene expression via nuclear factor κB ($\text{NF-}\kappa\text{B}$) probably.¹⁰ Inconsistent with our finding, studies of Hubbard et al. in animals models showed CLA increased level of total MMP2 and MMP9,²² and Mohammadzadeh et al. in cancer patients with chemo radiotherapy¹⁸ did not significantly affect blood level of MMP2 and $\text{TNF}\alpha$ in rectal cancer patients with chemo radiotherapy.¹⁸ This difference may be related to the type of sample. Our finding confirms the anti-inflammatory effects of CLA in young healthy males.

Our results reveal significantly decreased serum levels of MMP2, hs-CRP and $\text{TNF}\alpha$ in the supplemented group in comparison with the controls after severe exercise, but decreased serum levels of MMP9 was non-significant. It is shown that exercise induces increases in MMP9 mRNA levels and its activity in the skeletal muscle.²³ Physical exercise is a complex process constituting several factors affecting the expression levels of MMP2 and MMP9 in skeletal muscle in a situation such as local ischaemia and increases in muscle stretching; in shear stress; and in the wall tension of blood vessels.²⁴ Rullman and et al. showed single bout exercise was significantly increased MMP9 and vascular endothelial growth factor-A (VEGF-A)mRNA level of muscle biopsy but no significant effect on MMP2 and MMP-14 mRNA levels of muscle biopsy.^{25,26} In our study, MMP2 and MMP9 non-significantly increased in control group after severe physical activity. That difference may be related to type of exercise and sample types examined. Exercise is associated with temporary changes in the immune system, for example, count of immune cells²⁷ and concentrations of cytokines,²⁸ adhesion molecules²⁹ and MMPs.³⁰ Maximal exercise induces an inflammatory response characterised by MMP concentrations.³¹ It has been reported that the administration of anti-oxidant supplementation may play a positive role on metabolism in exercise.³² The number of controlled clinical study in humans has been published investigating the effects of CLA during exercise,³³ and it is useful in endurance exercise.³⁴ Our results showed that the serum levels of $\text{TNF}\alpha$ and hs-CRP were decreased significantly in the supplemented group in comparison with the controls after severe exercise. Therefore, CLA supplementation may modulate some inflammatory factors during exercise. CLAs have also been reported to suppress the release of pro-inflammatory cytokines, particularly $\text{TNF}\alpha$, in animals, which is similar to the current study.³⁵ In order for CLAs to elicit their anti-inflammatory effects through this pathway, an increase in Prostaglandin E2 (PGE2) or other inhibitory prostanoids would have to occur.³⁶ Previous animal studies have illustrated that muscle pathologies are associated with elevated levels of circulating inflammatory mediators, including $\text{TNF}\alpha$.³⁶ It has been suggested that the anti-proliferative effects of CLA arise from its anti-inflammatory properties by negatively regulating the expression of pro-inflammatory cytokines such as $\text{TNF}\alpha$, IL-1, and IL-6.³⁷ This study indicates that in response to supplementation, CLA attenuates $\text{TNF}\alpha$ and hs-CRP after severe exercise. CLA exerts anti-inflammatory effects by negatively regulating the expression of some pro-inflammatory genes such as $\text{TNF}\alpha$, IL-1 β and IL-6.¹⁰ In fact, expression of these genes in the presence of CLA are mimicked by the inhibitory effect of $\text{TNF}\alpha$ treatment alone.¹⁰ Although it has been postulated that CLA can modulate the expression of $\text{TNF}\alpha$ through the transcription factor $\text{NF-}\kappa\text{B}$,³⁸

but it is possible that CLA may act through different mechanisms/regulatory pathways that are further dependent on the isomeric form of CLA.

Postulated modes of action of CLA include effects on regulation of genes involved in arachidonic acid metabolism, resulting in attenuation of inducible eicosanoids involved in inflammatory events, modulation of genes involved in apoptosis and cell cycle control, or direct modulation of expression of inflammatory genes.³⁹ TNF α an inflammatory cytokine, regulates MMP activation such as MMP2.⁴⁰ Previous reports have documented that TNF α could activate the pro-MMP2 activation in synovial fibroblasts and that collagen could stimulate rat endothelial cells to activate the pro-MMP2.⁴¹⁻⁴³ In our study, the serum levels of MMP2, TNF α and hs-CRP in supplemented group significantly decreased after exhaustive exercise, but reduction of MMP9 and IL-6 weren't significant. Since high levels of TNF α are found in severe exercise, TNF α mediated activation of pro-MMP suggests a mechanism for the destructive role of excessive inflammation on tissue. Accordingly, the observation that high levels of active MMP2 are found in severe exercise could be explained by our proposed linkage of TNF α to a molecular pathway for pro-MMP2 activation.

The small sample size is the main limitation of the study.

Conclusion

Regular intake of CLA significantly modulates TNF α - and then MMP2, and protects against exhaustive exercise -induced oxidative injury in young healthy males. CLA supplementation may reduce MMP2 by decreasing TNF α before and after exhaustive exercise. CLA seems to exert beneficial effects in reducing MMP2 serum levels and some inflammatory factors before and after exhaustive exercise. Further large-scale studies in this area are recommended.

Acknowledgments

We are grateful to Ardabil University of Medical Sciences for financial support; to all the subjects for their participation; and to the Research Committee of Students for help in data collection.

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