NOTA CIENTÍFICA | *RESEARCH NOTE*

Effect of conjugated linoleic acid supplementation on lipoprotein lipase activity in 3T3-L1 adipocyte culture[1]

Efeito da suplementação com ácido linoléico conjugado sobre a atividade da lípase lipoprotéica em cultura de adipócitos 3T3-L1

Adriana Prais BOTELHO[2]
Lilia Ferreira SANTOS-ZAGO[2,3]
Admar Costa de OLIVEIRA[4] (*in memorian*)

ABSTRACT

Supplementation with conjugated linoleic acid may reduce fat body mass and increase lean body mass in various species. Some studies have demonstrated that conjugated linoleic acid reduces body fat, in part, by inhibiting the activity of lipoprotein lipase in adipocytes. The objective of this work was to study the effect of conjugated linoleic acid supplementation on lipoprotein lipase activity in 3T3-L1 adipocyte culture. 3T3-L1 adipocytes received linoleic acid (group C) or conjugated linoleic acid (group AE, supplemented with AdvantEdge® CLA, and group CO, supplemented with CLA One®) in concentrations of 1 mmol/L. Heparin-releasable lipoprotein lipase activity was analyzed by means of a 3T3-L1 adipocyte culture. After 7 days, heparin-releasable lipoprotein lipase activity was lower in the groups AE and CO supplemented with conjugated linoleic acid. These results suggest that one of the mechanisms by which CLA is capable of reducing body fat is by reducing lipoprotein lipase activity.

Indexing terms: Linoleic acid, conjugated. Lipoprotein lipase. Nutrition. Dietary supplements.

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[2] Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Programa de Doutorado em Alimentos e Nutrição, Departamento de Alimentos e Nutrição. R. Monteiro Lobato, 80, Cidade Universitária Zeferino Vaz, 13083-862, Campinas, SP, Brasil. Correspondência para/*Correspondence to*: A.P. BOTELHO. *E-mail*: <abotelho@fea.unicamp.br>.

[3] Pontifícia Universidade Católica de Campinas, Centro de Ciências da Vida, Faculdade de Nutrição. Campinas, SP, Brasil.

[4] Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Departamento de Alimentos e Nutrição. Campinas, SP, Brasil.

RESUMO

A suplementação com ácido linoléico conjugado pode reduzir a gordura corporal e aumentar a massa magra em diferentes espécies. Alguns estudos têm demonstrado que o ácido linoléico conjugado reduz a gordura corporal, por meio da inibição da atividade de lípase lipoprotéica em adipócitos. O objetivo deste estudo foi avaliar o efeito da suplementação com uma mistura de isômeros do ácido linoléico conjugado sobre a atividade da lípase lipoprotéica em cultura de adipócitos 3T3-L1. Os adipócitos 3T3-L1 receberam ácido linoléico (grupo controle) ou ácido linoléico conjugado (grupo AE, suplementado com AdvantEdge® CLA, e grupo CO, suplementado com CLA One®) na concentração de 1 mmol/L. A atividade de lípase lipoprotéica livre de heparina foi analisada pela média da cultura de adipócitos. Após 7 dias, a atividade da lípase lipoprotéica livre de heparina mostrou menores valores nos grupos AE e CO, suplementados com ácido linoléico conjugado. Estes resultados sugerem que um dos mecanismos pelo qual o ácido linoléico conjugado seja capaz de reduzir a gordura corporal é a partir da redução da atividade da lípase lipoprotéica.

Termos de indexação: *Ácido linoléico conjugado. Lípase lipoprotéica. Nutrição. Suplementos dietéticos.*

Conjugated Linoleic Acids (CLA), substances that occur naturally in meats and dairy products, are a group of geometrical and positional isomers of linoleic acid with conjugated double bonds[1,2]. CLA is produced in the rumen by the incomplete biohydrogenation of dietary polyunsaturated fatty acids, and also by the desaturation of C18:1 *trans*-11 fatty acid by the enzyme delta-9-desaturase present in mammary glands and adipose tissue[3]. Numerous studies associate CLA consumption with beneficial health effects in humans, among them anticarcinogenesis, reduction of atherosclerosis, modulation of the immune system, and enhancement of bone mineralization[4,5]. One of the most interesting aspects of CLA is its ability to reduce body fat while increasing lean body mass. The mechanisms proposed to explain these changes are still controversial. CLA ingestion has been associated with decreased preadipocyte proliferation and differentiation, reduced esterification of fatty acids into triacylglycerols, increased energy expenditure, and changes in the activity of the enzymes carnitine palmitoyltransferase, lipoprotein lipase, and the hormone leptin, among others[6,7]. Some studies have demonstrated that CLA reduces body fat, in part, by inhibiting the activity of Heparin-Releasable Lipoprotein Lipase (HR-LPL) in adipocytes[8,9]. The objective of this work was to assess the effect of conjugated linoleic acid supplementation on the activity of the Lipoprotein Lipase (LPL) enzyme in a 3T3-L1 adipocyte culture.

3T3-L1 adipocytes were purchased from Cell Bank (*Rio de Janeiro, RJ, BR*). Dulbecco´s modified Eagle´s medium was obtained from Gibco BRL (Paisley, UK). Both fetal bovine serum and human serum were obtained from Bio Whittaker (Verviers, Belgium). 12-Well cell-culture plates were obtained from Costar (NY, USA). Methylisobutylxanthine, insulin, bovine serum albumin (BSA, essential fatty acid-free), dexamethasone, heparin (10.000U/mg), ^{3}H-triolein, Picofluor scintillation solution, 60% linoleic acid supplement and all other chemicals were obtained from Sigma-Aldrich (St. Louis, USA). The conjugated linoleic acid supplements used in the study were AdvantEdge® CLA 75% obtained from EAS™ (Golden, CO, USA) and CLA One® Free Fatty Acid Oil 75% obtained from Pharmanutrients (Gurnee, IL, USA). CyQuant® Cell Proliferation Assay Kit (C7026) was obtained from Molecular Probes (Oregon, USA). The fatty acid profiles were determined by methylation, according to the Christie method[10].

Total CLA contents, their isomeric distribution, and fatty acid profiles of linoleic acid and conjugated linoleic acid mixtures were analyzed by gas chromatography with a capillary silica column CP SIL 88 (0.25mm x 0.2μm x 100m), as described by Sehat *et al*.[11]. Determination of lipoprotein lipase activity was done in a 3T3-L1 adipocyte culture[12]. The 3T3-L1 adipocytes were cultured in a basic medium composed of Dulbecco's modified Eagle's medium, with 10% added fetal

bovine serum. Temperature was kept constant at 37°C; the atmosphere was humidified, and contained 10% CO_2. After 2 days, cell differentiation was induced by adding 0.5mmol/L of methylisobutylxanthine, 0.25µmol/L of dexamethasone and 1µg/L of insulin to the basic medium. After 2 days the medium was replaced by another one containing only 10% of fetal bovine serum and insulin (1µg/L). Before starting the assay with the experimental medium, cell viability was evaluated with the CyQuant® Cell Proliferation Assay Kit. The experimental medium was prepared using the basic medium and adding bovine serum albumin (BSA), to a final concentration of 0.25mmol/L. The pH of this basic medium-BSA complex was adjusted to 10.0 with a 1mol/L NaOH solution. In the next stage, linoleic acid supplement was added to group C, and commercial conjugated linoleic acid mixtures AdvantEdge® (EAS™) and CLA One® (Pharmanutrients) to groups AE and CO, respectively, until a solution of 1mmol/L of fatty acid concentration was reached. At this point, the pH was adjusted to 7.4 with a 1mol/L HCl solution. The solution containing the basic medium, BSA and fatty acids was sonicated in ultrasound for 1 hour, and diluted with the basic medium-BSA complex to a final fatty acid concentration of 100µmol/L. After 7 days incubating the mature adipocytes in the experimental medium, the latter was discarded, and the cells were rinsed with a 0.5mL phosphate buffer (PBS) (pH 7.4), and cultured for 1 hour in a heparin solution and phosphate buffer (0.3mL per well at 10U/L of heparin).

The culture dishes were then placed in an ice bath, and the heparin-PBS solution was collected from each well for determination of heparin-releasable lipoprotein lipase activity. For this determination, a stock solution was prepared with ^{3}H-triolein (45µCi, 600mg of triolein), phosphatidylcholine (36mg) and glycerol (10mL). The following were added to this stock solution: Tris-HCl buffer (pH 8.2 at 37°C), 3% BSA solution in 0.78M NaCl, and inactivated human serum, in the proportion of 1:4:1:1 (v/v/v/v). For the measurement of lipoprotein lipase activity, 100µL of this solution were added to 100µL of the heparin-PBS solution followed by incubation in a water bath at 37°C for 1 hour. The reaction was interrupted by adding to the mixture 3mL of methanol, dichloromethane and heptane in the proportion of 1.41:1.25:1 (v/v/v). After that, 1mL of the supernatant aqueous phase was collected and transferred to a vial containing 10mL of scintillation liquid (Picofluor). Sample radioactivity was counted in a liquid scintillation counter (Beckman LS 6000TA), and the heparin-releasable lipoprotein lipase (HR-LPL) activity (EC 3.1.1.34, 10U heparin/mL media for 1 hour at 37ºC) was measured as described by Nilson-Ehle et Schotz[13]. Protein was determined as described by Lowry et al[14].

Results are expressed as Means (M) Standard Error (SE). Data were analyzed statistically by ANOVA using the Statistical Analysis System (SAS)[15]. The Tukey test was used to test the differences among groups. Differences were considered significant if $p \leq 0.01$.

The predominant composition of the isomers in the 60% linoleic acid supplement acquired from Sigma and the commercial conjugated linoleic acid mixtures 75% AdvantEdge®, CLA (EAS™), and 75% CLA One® Free Fatty Acid Oil 1CLA1-FFBL-KG (Pharmanutrients), expressed in g/100g of fatty acids, can be seen in Table 1. Supplements used in this research contained 79.27 and 73.08% of CLA for the brands AdvantEdge® CLA e CLA One®, respectively, and the proportion between the predominant isomers *cis-9, trans-11* and *trans-10, cis-12* was approximately 1:1 (Table 1).

LPL activity was analyzed by means of a 3T3-L1 adipocyte culture. After 7 days, the HR-LPL activity of groups AE and CO supplemented with CLA was significantly lower ($p \leq 0.01$) (Figure 1).

Similar results were obtained when 3T3-L1 adipocyte cultures were treated with an increasing amount (20 to 200µmol/L) of a CLA isomer mixture. CLA treatment significantly reduced heparin-releasable lipoprotein lipase activity and intracellular concentrations of triacylglycerol and glycerol[8]. In order to investigate which isomer would be responsible for this alteration, 3T3-L1

Table 1. Fatty acid composition of linoleic acid and commercial conjugated linoleic acid mixtures.

Fatty acids	Linoleic acid 60%	Conjugated linoleic acid 75% AdvantEdge® CLA	CLA One® Free Fatty Acid Oil 75% 1CLA1-FFBL-kg
	(g/100g of fatty acids)		
C18:2 *cis-9, cis-12*	59.64	0.75	0.93
C18:2 *cis-9, trans-11* CLA	0.09	40.12	36.81
C18:2 *trans-10, cis-12* CLA	0.08	39.15	36.27

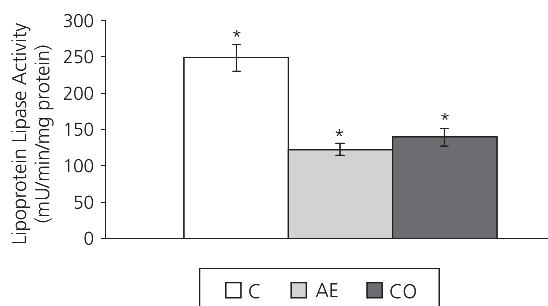

Figure 1. Effects of CLA on heparin-releasable lipoprotein lipase activity in 3T3-L1 adipocytes. Values represent mean ± SE. (n=12, taken from three independent experiments).

Note: *Asterisks indicate statistical difference according to the Tukey test ($p \leq 0.01$).
C: control group; AE: group supplemented with AdvantEdge® CLA; CO: group supplemented with One® CLA.

adipocyte cultures were treated with *trans-10, cis-12* and *cis-9, trans-11* CLA isomers separately. The *trans-10, cis-12* isomer reduced lipoprotein lipase activity, intracellular triacylglycerol and glycerol, and enhanced glycerol release into the medium. By contrast, the *cis-9 trans-11* CLA isomer did not affect these biochemical activities[9]. More recently, CLA significantly reduced LPL activity in 3T3-L1 adipocytes, confirming those results[16]. In order to discover the key structural feature of *trans-10 cis-12* CLA responsible for inhibiting HR-LPL, Park *et al*.[17] tested fatty acids that are structurally related to *trans-10, cis-12* CLA using 3T3-L1 adipocytes. The results demonstrated that *trans-10, cis-12* CLA is directly responsible for reducing HR-LPL activity, and that the *trans-10* double bond may be the key[17].

This experiment, which used a 3T3-L1 adipocyte culture, indicated that CLA reduced LPL activity. Assuming that *in vitro* experiments reflect physiological changes that occur *in vivo*, these data can suggest that the CLA is capable of reducing body fat in different experimental models[8,9]. In order to assess the potential involvement of altered lipoprotein lipase activity in the reduction of body fat, Zabala *et al.*[18] investigated the effects of *trans-10, cis-12* CLA in hamster adipose tissue. After the experimental period (6 weeks), the group receiving *trans-10, cis-12* showed a significant reduction in lipoprotein lipase activity compared with the control group given linoleic acid. The authors concluded that the activity of LPL can be regulated at many levels, including transcription, translation, processing and transport from adipocytes to endothelial cells. Both total and HR-LPL activities were reduced in the CLA-fed groups, indicating that the reduction in mRNA levels leads one to the conclusion that the regulation of LPL by CLA takes place at the transcriptional level. Peroxisome proliferator-activated receptor γ (PPARγ) is a transcriptional factor, highly expressed in adipose tissue, which plays an important role in the regulation of lipid metabolism. Its activation promotes lipid storage by stimulation of LPL. Zabala *et al*[18] found that the expression of this nuclear receptor was significantly reduced by feeding CLA feeding. Similar results were obtained when human primary adipocyte cultures were treated with *trans-10, cis-12* CLA isomer[19].

In conclusion, the results obtained in this study confirm the ability of CLA to reduce LPL activity, indicating that the inhibition of LPL activity seems to be a mechanism underlying body fat reduction. Further research using different experimental conditions is needed to characterize the effects of CLA on lipogenesis, and the role of these effects in the ability of CLA to reduce body fat.

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CONTRIBUTORS

A.P. BOTELHO, L.F. SANTOS-ZAGO and A.C. OLIVEIRA were equally responsible for developing the research project, the tables, discussing the results, and writing the manuscript.

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