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CLA-enriched milk powder reverses hypercholesterolemic risk factors in hamsters

Luis Miguel Rodríguez-Alcalá [a], Javier Fontecha [a], Lucia de la Hoz [b], Vera Sonia Nunes da Silva [b], João Ernesto Carvalho [c], Maria Teresa Bertoldo Pacheco [b,*]

[a] Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), Food Analysis and Bioactivity, Department of Lipids. Universidad Autónoma de Madrid (UAM), Nicolás Cabrera, 9, 28049, Madri.Espanha, Madrid, Spain
[b] Instituto de Tecnologia de Alimentos. Food Chemistry and Applied Nutrition Center, Av. Brasil, 2880, Campinas, SP, CEP 13070-178, P.Box 139, Brazil
[c] Chemical, Biological and Agricultural Multidisciplinary Research Center, CPQBA/UNICAMP, Rua Alexandre Cazelatto, 999, P.Box 6171, CEP 13081, 970, Vila Betel, Paulínia, SP, Brazil

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ABSTRACT

Conjugated linoleic acid isomers (CLA) have been reported to exert anticarcinogenic effects, protection against atherosclerosis and decrease of body fat among others effects, in both animals and humans. However the mechanism of action of CLA remains still unknown, with various proposed pathways. Moreover previous works have reported ambiguous results and contradictory effects. The C18:2t10,c12 has been associated elsewhere to deleterious bioactivities. According to this, further data are needed to unravel the biological activities of CLA. The aim of this study was to evaluate the effects of CLA as part of the diet of adult hamsters in reversing hypercholesterolemia, a risk factor associated with atherosclerosis. The hypercholesterolemic condition was induced in male Syrian Golden hamsters, then divided into three groups receiving CLA premixed in the diet (diet CLA1), administered separately (through gavage) as CLA oil, (diet CLA2), or not added (CD, control diet). All diets contained 0.1% cholesterol and were equivalent in lipid content.
Blood physiological parameters, lipid profile, glucose, liver enzymes and body weight were monitored weekly. After 35 days, hamsters fed CLA2 diet reduced in great extension the body weight while CLA1 was more effective in lowering the concentration of triglycerides in plasma. Liver functions and glycemic status were not affected. The main outcomes of the present research work are that CLA in the form of oil or added to powder milk does not cause toxic effects or alter live functions or glycemia in hamsters. Furthermore, the results suggest that CLA formulated as a skimmed milk powder product can reverse hypercholesterolemic risk factors while high CLA oils is useful for weight control.

1. Introduction

Conjugated linoleic acid isomers (CLA) are fatty acids (FA) characterized by the presence of conjugated double bonds in their 18 carbon atom chain (cis,cis/trans,trans/cis,trans–trans,cis). They are found in the meat (0.12–0.68 g CLA/100 g fat) and mainly in the milk fat (0.34–1.07 g CLA/100 g fat) (Fritsche et al., 1999; Schmid, Collomb, Sieber, & Bee, 2006). Since the last decades exists a high interest in these compounds as have been identified as anticarcinogenic and antiatherogenic agents, modulators of the immune system, able to reduce body fat and to exert anti-inflammatory effects in both, animals and humans (Gonçalves et al., 2010; O'Shea, Bassaganya-Riera, & Mohede, 2004; Shiraishi et al., 2010).

It is stated from extrapolation of animal assays that 3 g CLA/day is the dose to obtain beneficial effects in humans (Ip, Singh, Thompson, & Scimeca, 1994). However that intake from natural sources would lead to very high consumption of these products. Currently, high CLA oils (81% of total FA, RA and 18:2t10,c12, 1:1) produced by alkali isomerization of safflower oil are used in the elaboration of CLA-enriched foods (Ma, Wierzbicki, Field, & Clandinin, 1999; Rodríguez-Alcalá & Fontecha, 2007).

CLA mixtures have been found to significantly reduced total cholesterol, non-high-density lipoprotein cholesterol and triglycerides in plasma as well as also decreased aortic fatty strokes and atherosclerosis lesions by 47% in hamsters and rabbits fed a diet with up to 1% CLA (Eder & Ringseis, 2010; Nakamura, Flintoff-Dye, & Omaye, 2008; Park, 2009). Those authors suggested that CLA acts reducing blood pressure or activating peroxisome proliferator activated receptor (PPAR: involved in lipogenesis), sterol regulatory element-binding proteins (SREBP: involved in cholesterol and fatty acids biosynthesis) and/or stearoyl-CoA desaturase (SCD;involved in the biosynthesis of unsaturated fatty acids). However the true mechanism of action remains unraveled as some other studies reported that CLA isomers replace arachidonic acid in the membrane phospholipids being involved in the eicosanoids metabolism (Park & Pariza, 2007). Nevertheless other works described evidences of gene regulation in lipid metabolism, apoptosis, immune system and inflammatory factors (Akahoshi et al., 2004).

On the other hand, some researchers have reported deleterious effects, such as increases in the LDL/HDL and total cholesterol/HDL ratios

* Corresponding author. Tel.: +55 19 37431766; fax: +55 19 3242 4585.
E-mail address: mtb@ital.sp.gov.br (M.T.B. Pacheco).

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(Wahle, Heys, & Rotondo, 2004), hepatotoxicity (Ramos, Mascarenhas, Duarte, Vicente, & Casteleiro, 2009), alterations in the expression of insulin and glucose metabolism genes (Silveira, Carraro, Monereo, & Tébar, 2007) and oxidative stress (Marineli, Marques, Furlan, & Maróstica, 2012) associated to the C18:2t10,c12 isomer. Thus, according to the existence of previous ambiguous results and effects as well as the fact that the pathway of action of CLA is still unknown, more data from further studies are needed.

The aim of the present research was to determine if high content of CLA oil or CLA-enriched dairy products are able to reverse blood parameters related with atherosclerosis risk factors.

2. Material and methods

2.1. Material

A commercial high CLA oil (Cognis Tonalin® TG80; BASF, Illertissen, Germany) was provided by CAPSA (Capsa Inc., Oviedo, Spain). L-Cystine, choline bitartrate and cholesterol were obtained from Sigma (St. Louis, MO, USA). Powdered whole milk (PWM) and CLA-enriched milk powder (labeled PCM) were shipped from the manufacturer (Capsa Inc., Oviedo, Spain) to the laboratory in isothermal containers at 4 °C. Products were maintained at this temperature until fat extraction. PCM, a non-commercial product, was specially manufactured by Capsa Inc. (Oviedo, Spain) for use in the current study; it contained 10% fat, 6% Tonalin® and 32% protein. It was obtained from skimmed milk, supplemented with CLA oil, dual-homogenized (20,000 ± 1000 kPa) and underwent an indirect UHT process at 142 °C for 6 s. Milk powder was obtained after atomization of CLA-enriched milk. PWM and PCM materials were analyzed for fatty acid composition and CLA isomer distribution.

2.2. Lipid extraction and fatty acid analysis

Milk fat on PWM and PCM products was extracted according to standard methods (ISO, 2001). The fat residue was kept frozen at −20 °C until analysis. Fatty acid methyl esters (FAMEs) were prepared by base-catalyzed methanolysis of the glycerides (KOH 2 N in methanol) according to standard methods (ISO, 2002).

2.3. Fatty acid analysis: gas chromatography–flame-ionization detection

FAMEs were analyzed by gas chromatography–flame-ionization detection (CG-FID) using a Perkin-Elmer Autosystem chromatograph Beaconsfield, UK with a flame-ionization detector (FID) and a fused silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness, CP-Sil 88, Chrompack, Middelburg, the Netherlands). The column was held at 100 °C for 1 min after injection. Then the temperature was increased 7 °C/min to 170 °C, held for 55 min, then increased 10 °C/min to 230 °C, and held there for 33 min. Helium was the carrier gas with a column inlet pressure set at 214 kPa (30 Psig) and a split ratio of 1:20. The injection volume was 0.5 μL. GC-FID was used to determine and identify the CLA isomers by comparing the CLA oil supplement and CLA standards.

2.4. CLA isomers analysis: silver ion-HPLC

Silver ion (Ag+)-HPLC separation of CLA methyl esters was carried out using an HPLC (Shimadzu Vp Series, Duisburg, Germany) equipped with a UV detector operated at 233 nm. Fatty acid methyl esters were eluted using an analytical column (4.6 mm i.d. × 250 mm stainless steel; 5 μm particle size; ChromSpher 5 Lipid column, Varian-Chrompack Int., Middelburg, The Netherlands). The mobile phase was 0.1% acetonitrile in hexane at an isocratic flow rate of 1.0 mL/min. The flow was initiated 0.5 h before the sample injection (10 μL). Pure and mixed CLA FAME isomers from Nu-Chek Prep (Elysian, MN, USA) were used as standards. All the analysis was performed at least in triplicate.

2.5. Experimental design

Male *Golden Syrian* hamsters (9 weeks old, n = 50) weighing 107 ± 2.1 g were housed individually in plastic cages with wood chip bedding and kept at 23 ± 2 °C in a room with a 12 h dark–light cycle. They were fed a commercial diet (Labina®, Agribrands Purina do Brazil Ltda) for a 1-wk adaptation period. Animals had free access to water, and fresh diet was provided daily ad libitum. Body weight was monitored weekly. All experiments were conducted according to the Brazilian Regulations for Animal Experimentation (COBEA), after approval by the Animal Experimentation Ethics Committee of the University of Campinas (protocol no 1676-1-CEEA/UNICAMP).

Diets (Table 1) were formulated according to the specifications of the American Institute of Nutrition (AIN-93M) for rodents in maintenance (Reeves, Rossow, & Lindlauf, 1993). Powdered whole milk (PWM) was used as the only source of lipids, and proteins were used for making the experimental diets. Through the entire experiment, a group of animals was fed a commercial standard diet (CF, n = 15) (Labina®: protein 23%, lipids 4%, humidity and minerals 10%), for reference of normal blood and liver parameters. The assay was divided into two stages. During the first stage, a hypercholesterolemic condition was established. The animals were fed HD diet (n = 35), consisting of PWM, 20% fat content, plus 0.5% cholesterol, for 2 weeks, ad libitum. In the second stage (35 days), the animals were divided into three groups (n = 10) and were fed three different experimental diets in order to evaluate reversal of the hypercholesterolemic condition: (*i*) a control diet (CD) of milk powder (whole and skimmed) with 4% lipid content and 0.1% cholesterol; (*ii*) an enriched CLA diet (CLA1) consisting of PCM containing 2.4% CLA, 4% lipids and 0.1% cholesterol; and (*iii*) an enriched CLA diet (CLA2) consisting of skimmed powder milk with 0.1% cholesterol added, plus 0.6 mL Tonalin® administered orally on a daily basis.

2.5.1. Blood and tissue sampling

At time zero, blood from five hamsters from the CF diet control group was collected via the hamsters' retro-orbital sinus and placed into EDTA blood collection tubes to measure reference parameters. After 15 day, 10 animals, 5 from the control group and 5 from the HD diet group, were starved overnight, anesthetized with pentobarbital (60 mg/kg body weight) and euthanized to measure biological parameters. After 35 days, animals fed experimental diets (CD, CLA1 or CLA2)

Table 1
Composition of the experimental diets.

Ingredient g/kg	HD	CD	CLA1	CLA 2
Centesimal composition (%)				
Protein	19.3	14.0	15.5	14.0
Lipids	20.0	4.0	4.00	0.63
Lactose	23.9	17.9	19.7	19.5
Humidity + minerals	7.0	4.2	4.5	4.8
Powder milk (g)	701.0	410.4	446.4	388.7
Fiber (g)	50.0	50.0	50.0	50.0
Starch (g)	40.0	334.6	298.6	356.3
Dextrin (g)	159.0	159.0	159.0	159.0
L-Cystine (mg)	1.8	1.8	1.8	1.8
Choline bitartrate (mg)	2.5	2.5	2.5	2.5
Mineral mix (g)	35.0	35.0	35.0	35.0
Vitamin mix (g)	10.0	10.0	10.0	10.0
Cholesterol (mg)	5.0	1.0	1.0	1.0
Total (g)	1000.0	1000.0	1000.0	1000.0

HD: hypercholesterolemic diet; CD: Control diet with powder milk (whole and skimmed) with 4% of lipids plus 0.1% cholesterol; CLA1: enriched powder milk (2.4%) with 4% lipids and 0.1% cholesterol; and CLA2: skimmed powder milk plus 0.6 mL Tonalin® (oral fed daily) and 0.1% cholesterol.

were euthanized and 3–5 mL of blood (cardiac puncture) was collected to evaluate serum parameters. Blood samples were centrifuged (15 min, 278 *g*) and serum was stored at −20 °C until analyses. Livers were removed, rinsed in cold saline solution and weighed. One portion was stored at −20 °C for lipid analysis. The adipose visceral tissues were carefully dissected and weighed.

Table 2
Fatty acid composition (%) in powder milk (PWM) and powder CLA enriched milk (PCM).

Fatty acid	PWM		PCM	
	Mean	SD	Mean	SD
C4	4.43	0.47	0.76	0.19
C6	2.96	0.38	0.49	0.13
C8	1.57	0.13	0.28	0.07
C10	3.32	0.31	0.59	0.13
C10:1	0.39	0.03	0.05	0.02
C12	3.65	0.29	0.70	0.12
C14i	0.10	0.01	0.05	0.00
C14	10.69	0.39	2.26	0.24
C15i	0.23	0.02	0.06	0.00
C15ai	0.45	0.01	0.11	0.01
C14:1c	0.90	0.04	0.21	0.02
C15	0.91	0.02	0.21	0.02
C16i	0.24	0.01	0.06	0.00
C16	30.12	0.33	7.37	0.26
C17i	0.35	0.02	0.09	0.01
C16:1 c7	0.17	0.01	0.04	0.01
C17ai	0.44	0.01	0.11	0.00
C16:1 c9	1.25	0.01	0.29	0.01
C17	0.49	0.01	0.13	0.01
C17:1 c9	0.19	0.01	n.d.	–
C18	10.19	0.55	4.14	0.04
C18:1t6–t8	0.20	0.01	0.04	0.01
C18:1t9	0.20	0.02	0.02	0.01
C18:1t10	0.32	0.04	0.06	0.01
C18:1t11	1.02	0.05	0.26	0.01
C18:1t12	0.27	0.02	0.04	0.01
C18:1t13	0.51	0.07	0.09	0.01
C18:1 c9	17.14	0.79	13.21	0.05
C18:1t15	0.49	0.06	n.d.	–
C18:1 c11	0.53	0.00	0.70	0.01
C18:1 c12	0.25	0.02	0.04	0.01
C18:1 c13	0.05	0.01	0.01	0.01
C18:1 c14 + t16	0.23	0.02	0.06	0.01
C18:1 c15	0.05	0.01	n.d.	–
C18:2t,t NMID	0.17	0.01	0.15	0.02
C18:2t9,t12	0.10	0.01	0.07	0.09
C18:2 c9,t13	0.07	0.00	0.12	0.08
C18:2 c9,t12	0.07	0.00	0.10	0.08
C18:2t11,c15	0.10	0.01	0.01	0.01
C18:2 c9,c12	1.98	0.08	0.47	0.01
C20	0.12	0.01	0.03	0.01
C18:3 c9,c12,c15	0.36	0.01	0.10	0.01
CLA c9,t11	0.51	0.03	31.11	0.59
CLA c11,c13	tr	–	0.18	0.04
CLA t10,c12	tr	–	32.03	0.68
CLA c9,c11	tr	–	0.53	0.03
CLA c10,c12	tr	–	0.45	0.03
CLA t9,t11	tr	–	0.39	0.62
C22	0.09	0.02	0.80	0.62
C20:4 n6	0.18	0.01	0.04	0.01
SFA	70.35	1.03	18.23	0.54
MUFA	24.16	0.91	15.10	0.09
PUFA	3.53	0.18	65.74	0.64
Total trans	4.04	0.03	1.12	0.13
Total CLA	0.51	0.03	64.69	0.33
n6	2.15	0.04	0.51	0.01
n3	0.36	0.01	0.10	0.01
n3/n6	6.01	0.03	5.20	0.01

i: iso; ai: ante-iso; t: trans double bond; c: cis double bond; CLA: conjugated linoleic acid; SFA: total of saturated fatty acids; MUFA: total of monounsaturated fatty acids; PUFA: total of polyunsaturated fatty acids; n6: omega 6 fatty acids; n3: omega 3 fatty acids; Tr: trace amounts; and n.d.: not detected.

2.5.2. Biological determinations

Serum glucose, liver enzymes (AST and ALT), total cholesterol, c-HDL, c-LDL, c-VLDL, c-LDL/c-HDL, and triglyceride levels were determined enzymatically using appropriate kits (Labtest Diagnostics®, Lagoa Santa, MG, Brazil). VLDL-c was calculated as the difference between total cholesterol and (HDL + LDL)-c. Liver lipids were extracted with chloroform-methanol (2:1) using the method of Folch, Lees, and Stanley (1957). Cholesterol content was extracted and analyzed as described by Schmarr, Gross, and Shibamoto (1996) by GLC (model 3800, Varian-Chrompack Int., Middelburg, The Netherlands) on a short nonpolar HP5 capillary column (8 m × 0.32 mm i.d. × 0.25 μm film thickness, Hewlett Packard, Palo Alto, CA, USA).

2.5.3. Histological procedures

Livers were removed and weighed and tissues were preserved in 10% neutral-buffered formalin. Paraffin-embedded sections of the liver tissues were sectioned at 6 μm and stained with hematoxylin and eosin following the method of Michalany (1998).

2.6. Statistical analysis

The results were expressed as the mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. A value of $p<0.05$ indicated significance.

3. Results and discussion

3.1. Fatty acid composition and CLA isomer distribution in the assayed powdered milks

The fatty acid compositions of the powdered whole milk (PWM) and CLA-enriched milk powder (PCM) are displayed in Table 2. As expected PWM had a high concentration of saturated fatty acids (SFAs; 70.35%) due to its contents of myristic (10.69%), stearic (30.12%) and palmitic acid (10.19%) content because the whole milk fat. In the CLA enriched skim milk fat those fatty were also detected although in much lower proportion (2.26%; 7.37%; 4.14% respectively). The presence of these fatty acids in PCM as well as short and medium fatty acids chain compounds (butyric, caproic and capric acids), characteristic of milk fat, reveals that although milk was skimmed prior to processing, the processing was not complete. Although oleic acid was the major MUFA compound in both milk samples, the greatest differences between samples were in the PUFA fraction (3.53% for PWM vs. 65.74% for PCM) due to the substitution of the milk fat by CLA enriched oil (Tonalin). Thus, while the CLA contend in PWM was characterized

Table 3
CLA isomers distribution (%) in powdered milk (PWM) and powdered CLA enriched milk (PCM).

	PWM		PCM	
Isomers	Mean	SD	Mean	SD
t,t				
12,14	0.96	0.18	n.d.	
11,13	2.02	0.03	0.99	0.09
10,12	1.20	0.08	0.89	0.07
9,11	1.26	0.04	n.d.	
8,1	0.49	0.02	n.d.	
7,9	1.13	0.03	n.d.	
c,t–t,c				
12,14	0.65	0.04	n.d.	
11,13	2.86	0.17	0.24	0.04
10,12	0.71	0.02	47.81	1.14
9,11	82.00	0.98	48.48	0.45
8,1	1.43	0.02	0.31	0.18
7,9	5.31	0.43	n.d.	
9,11	n.d.		n.d.	0.08
10,12	n.d.		0.57	0.07

t: trans double bond; c: cis double bond; and n.d.: not detected.

Table 4
Body weight (BW), liver weight (g), glucose, triglycerides, lipid profile (mg/dL) of hamsters fed with hypercholesterolemic diet during 2 weeks.

Parameters	Dietary groups	
	CF	HD
BW (g)	108.70 (3.8)^{b}	118.30 (6.4)^{a}
Weight liver (g)	4.14 (0.8)^{b}	6.80 (0.9)^{a}
Plasma parameters (mg/dL)		
Glucose	94.30 (14.2)^{a}	119.60 (32.5)^{a}
Triglycerides	79. 34 (18.6)^{b}	162.55 (23.5)^{a}
Total cholesterol	92.40 (7.5)^{b}	240.6 (3.6)^{a}
LDL-cholesterol	22.80 (3.3)^{b}	134.00 (18.7)^{a}
HDL-cholesterol	32.16 (4.5)^{b}	76.85 (4.2)^{a}
VLDL-cholesterol	15.86 (3.7)^{b}	32.51 (4.7)^{a}

Results expressed as mean (standard deviation), CF: commercial feed; and HD: hypercholesterolemic diet. Superscripts in rows for significant differences ($p<0.05$).

mainly by the presence of rumenic acid C18:2t 9,t11 (RA; 0.51%) in PCM the CLA was constituted by the two major CLA isomers RA and C18:2t10, c12 (31.11% plus 32.03% respectively) and other minor CLA isomers as C18:2 c11,t13 and the cis,cis 9,11 and 10,12 that were also detected.

Ag^{+}-HPLC analysis (Table 3) showed that in addition to RA, C18:2 7t, c9 (5.32 g/100 g CLA), C18:2 c11,t13 (2.86 g/100 g CLA) and C18:2t11, t13 (2.02 g/100 g CLA) were also present in PWM. These fatty acid contents and the distribution of CLA agree with previously reported compositions in powdered milk and CLA-enriched dairy products (Golay, Dionisi, Hug, Giuffrida, & Destaillats, 2007; Rodríguez-Alcalá & Fontecha, 2007).

3.2. Effect of diets

In the present research a high fat diet enriched with cholesterol was used to induce a serum lipid profile associated with hypercholesterolemia and probably atherogenesis. Physical and blood parameters from animals fed control (CF) and hypercholesterolemic (HD) diets for 15 days are presented in Table 4. The HD-fed group in which hypercholesterolemia was induced had greater body and liver weight ($p<0.05$) as well as higher triglycerides, total cholesterol, LDL, HDL and VLDL-cholesterol content, than the control group after the assay time.

In the second stage of the current research work, after hypercholesterolemia was induced on hamsters, one whole milk (CD) and two high CLA diets (CLA1, CLA2) were assayed to study the possible effects on risk factors (Table 5). As can be observed in Fig. 1, both CLA1 and CLA2 diets decreased body gain but in different manners. While CLA1 exerted the effect at day 20 the CLA2 was significantly early (15 days) and more pronounced ($p<0.05$). The Tonalin® oil used in the present study had a total of 81% CLA (rumenic acid and C18:2t10,c12 (1:1)) (Rodríguez-Alcalá & Fontecha, 2007). Previous studies have associated lower body weight values with the action of C18:2t10,c12 (Benjamin & Spener, 2009). Although the metabolic mechanism related with the effect of CLA weight gain reduction in hamsters and humans, it is not fully understood, it has been hypothesized that CLA induces the attenuation of lipid adsorption and transport (Gavino, Gavino, Leblanc, & Tuchweber, 2000). Furthermore, other studies carried out in hamsters fed atherogenic or western-like diets plus 0.5–1% of C18:2t10,c12, found a reduction of fat deposits in the perineal, subcutaneous and white adipose tissue associated with down-regulation of liprotein lipase and leptin, as well as an increase in the oxidation of fatty acids through thermogenesis in skeletal muscle (Ribot, Portillo, Pico, Macarulla, & Palou, 2007; Tarling, Ryan, Bennett, & Salter, 2009; Zabala et al., 2006). Our different obtained results using the CLA1 and CLA2 diets could be related to the employed dose level or the way of feeding effect.

At the end of the assayed time (35 days), visceral adipose tissue weight gain (Table 5) in hamsters fed with CLA (0.96 g/100 g in CLA1;0.86 g/100 g in CLA2) showed similar values to those in the CF group (1.0 g/100 g; $p>0.05$). Glycemia in the fasting period was not altered by either of the diets tested. However, the CLA diets significantly reduced the induced hypertriglyceridemia ($p<0.05$) when compared with CD (91.53 mg/dl in CLA1; 127 mg/dl in CLA2; 240.84 mg/dl in CD). Furthermore, CLA1 provided the highest reduction, reaching triglycerides levels similar to those in hamsters fed the CF diet (71.71 mg/dl) as there were no significant difference.

On the other hand, a recent review of the effects of trans fat on humans pointed out that CLA can increase the LDL/HDL ratio due to the presence of trans double bonds in its atom carbon chain (Brouwer, Wanders, & Katan, 2010). In dairy fat, however, the trans fatty acid profile is completely different from that of industrial oils and there is currently no consistent evidence linking milk and cardiovascular diseases (Chardigny et al., 2008). Furthermore, beneficial effects have been reported in animal trials where both rumenic acid and vaccenic acid were added to diets or occurred naturally in enriched butters (Jacome-Sosa et al., 2010; Lock, Horne, Bauman, & Salter, 2005). Plasma and liver cholesterol in hamsters fed cholesterol together with high-CLA oils decreased up to 40% as CLA curtailed cholesterol absorption (Scher, Pronczuk, Hajri, & Hayes, 2003). Other studies, of *Golden Syrian* hamsters fed an atherogenic meal with added C18:2t10,c12 found no effect on the activity of the HMGCoAR (3-hydroxy-3-methylglutharyl-coenzyme A reductase) enzyme, implicated in the synthesis of cholesterol, on the liver. In contrast, ACAT (cholesterol acyltransferase) activity

Table 5
Biological parameters of hypercholesterolemic hamsters (adults Golden Syrian) fed with experimental diets (CD, CLA1, CLA2) and control diet (CF) after 35 days of treatment.

Biological parameters	Dietary groups			
	CF	CD	CLA1	CLA2
BW (g)	118.0 (8.9)^{b}	129.0 (4.1)^{a}	118.1 (3.8)^{b}	105.0 (12.0)^{c}
Visceral adipose tissue (g)	1.00 (0.29)^{b}	1.93 (0.53)^{a}	0.96 (0.30)^{b}	0.86 (0.26)^{b}
Liver (g)	3.55 (0.32)^{b}	5.03 (0.66)^{a}	4.82 (0.16)^{a,c}	4.12 (0.71)^{b,c}
Liver parameters				
Lipids (g/100 g)	15.90 (2.56)^{b}	23.39 (0.81)^{a}	18.83 (1.55)^{a,b}	22.55 (1.84)^{a}
AST, U/L	32.37(10.54)^{a}	33.66 (11.64)^{a}	26.77 (7.32)^{a}	32.90 (10.70)^{a}
ALT, U/L	44.00 (8.07)^{a}	35.20(12.05)^{a}	27.32(10.25)^{a}	34.36(15.07)^{a}
Plasma parameters (mg/dL)				
Glucose	108.40 (11.1)^{a}	116.40 (11.7)^{a}	117.80 (18.9)^{a}	120.00 (9.9)^{a}
Triglycerides	71.72 (14.3)^{c}	240.84 (28.9)^{a}	91.53 (27.7)^{c,b}	127.08 (20.2)^{b}
Total cholesterol	67.46 (5.9)^{b}	144.41 (16.4)^{a}	129.34 (0.7)^{a}	137.07 (16.6)^{a}
LDL-cholesterol	42.85 (5.6)^{b}	63.90 (15.1)^{a,b}	62.45 (16.8)^{a,b}	75.27 (15.5)^{a}
HDL-cholesterol	10.26 (0.9)^{b}	28.25 (6.8)^{c}	47.16 (11.2)^{a}	22.43 (7.6)^{c}
VLDL-cholesterol	14.36 (2.9)^{b}	48.17 (5.8)^{a}	22.91 (3.2)^{c}	25.42 (7.6)^{c}

Results expressed as mean (standard deviation). Different superscripts letters in the same row mean significant differences by diet ($p<0.05$). HD: hypercholesterolemic diet; CD: control diet with powder milk (whole and skimmed) with 4% of lipids plus 0.1% cholesterol; CLA1: enriched powder milk (2.4%) with 4% lipids and 0.1% cholesterol; and CLA2: skimmed powder milk plus 0.6 mL Tonalin® (oral fed daily) and 0.1% cholesterol.

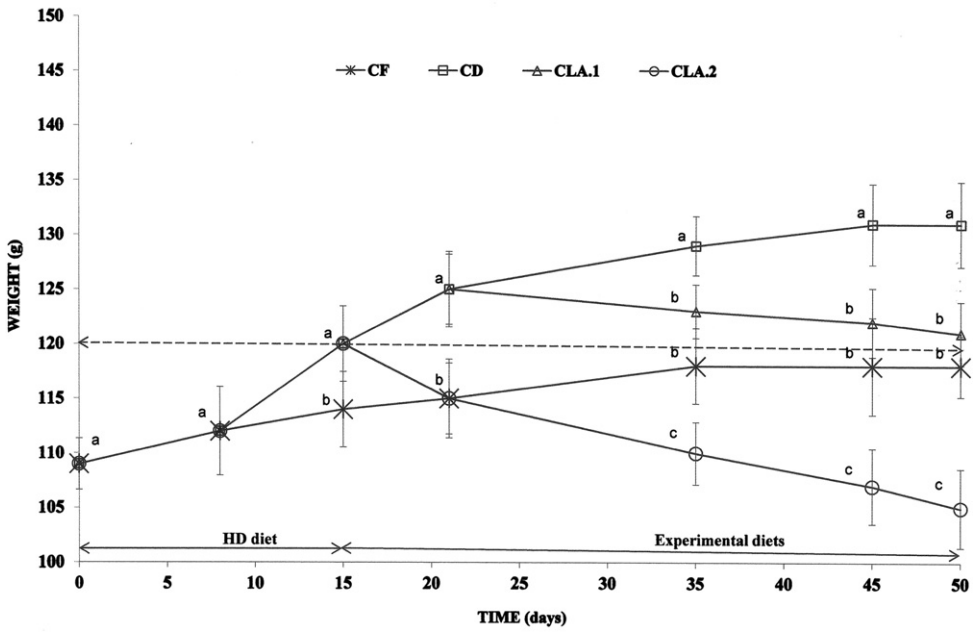

Fig. 1. Body gain evolution from hamsters fed 50 days with the experimental diets. CF: commercial feed; CD: control diet with powder milk (whole and skimmed) with 4% of lipids plus 0.1% cholesterol; CLA1: CLA (2.4%) enriched powder milk with 4% lipids and 0.1% cholesterol and 0.1% cholesterol; CLA2: diet with skimmed powder milk plus 0.6 ml Tonalin® (oral fed daily); and HD: hypercholesterolemic diet.

was reduced, resulting in an increase in CEH (cholesterol ester hydrolase). C18:2t10,c12 CLA also decreased cholesterol ester content and increased free cholesterol in the liver. Although serum remained unchanged, cholesterol fractions showed a significant decrease in VLDL-cholesterol in CLA-fed animals, but HDL or LDL cholesterol levels did not change (Ribot et al., 2007).

In the present study, animals fed milk and cholesterol (CD), showed the highest values in liver lipid content (Table 5; 23.39 g/100 g) even though the value was not significantly different from those of the groups fed CLA diets. Liver weight was significantly lower ($p<0.05$) in hamsters fed the CLA2 diet for 35 days (4.12 g) than the CD control group (5.03 g). Histological liver section evaluation in all the assayed diets showed no tumor formation and no differences between HD and CD among animals fed CF, CLA1 or CLA2 diets (Fig. 2). Previous studies have reported that the increase in liver weight after CLA intake was related to an increase in the number of hepatocytes and that hyperplasia is thus involved in hepatomegaly (Bissonauth et al., 2006; de Deckere, van Amelsvoort, McNeill, & Jones, 1999). In the current study, enzymatic analyses of stress in the liver (AST and ALT) did not show treatment-related differences, and CLA ingestion levels were not toxic.

The CLA enriched milk custom manufactured for this assay and used as diet CLA1 was consumed by the animals gradually throughout the day (ad libitum) and had greater influence on the HDL rate than the CLA2 diet. Also, this diet, with 0.1% cholesterol added, led to a liver lipid level similar to that of the control group fed a commercial standard diet (CF) without added cholesterol.

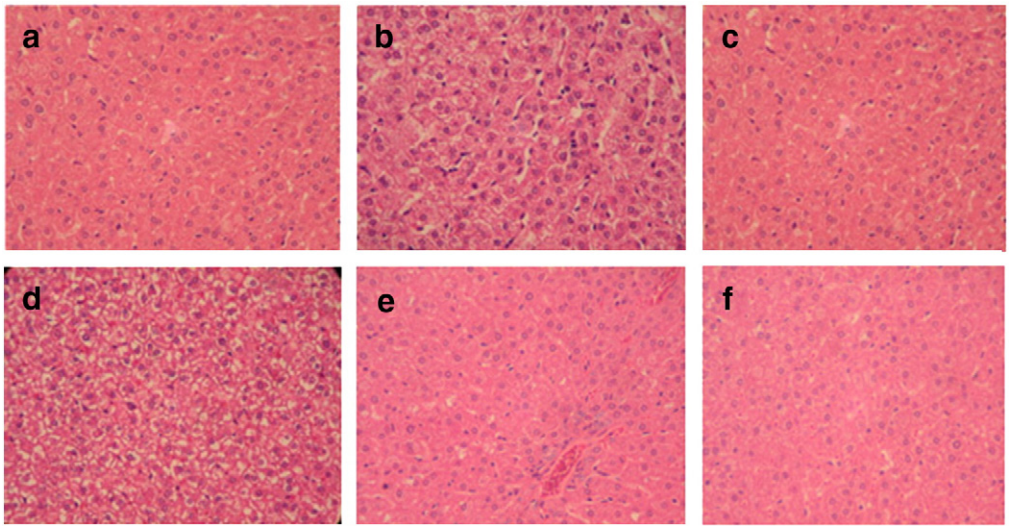

Fig. 2. Histology of liver tissue sections in hamsters (50 days). a) CF: commercial feed (initial control); b) HD: hypercholesterolemic diet (15 days); c) CF: commercial feed; d) CD: control diet with powder milk (whole and skimmed) with 4% of lipids plus 0.1% cholesterol; e) CLA1: CLA (2.4%) enriched powder milk with 4% lipids and 0.1% cholesterol and 0.1% cholesterol; and f) CLA2: diet with skimmed powder milk plus 0.6 ml Tonalin® (oral fed daily) diet. a–f Hematoxylin–eosin staining (400×).

4. Conclusion

The main outcomes of the present research work are that CLA in the form of oil or added to powder milk does not cause toxic effects or alter liver functions or glycemia in hamsters. Furthermore, the results suggest CLA oil incorporated in a skim milk powder formula as a product can reverse hypercholesterolemic risk factors while incorporated as CLA oil seems to be useful for weight control. Results suggest that the effects of CLA are related to lipid metabolism; however, the mechanisms are not entirely clear, so further research is needed.

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