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Effects of dietary conjugated linoleic acid at high-fat levels on triacylglycerol regulation in mice

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Abstract

Objective: Our aim was to investigate the effects of dietary conjugated linoleic acid (CLA) at high-fat (HF) levels on parameters related to triacylglycerol (TG) regulation and some potential impacts on liver damage.

Methods: Growing mice were fed a control diet (7% corn oil), an HF diet containing 20% corn oil, or an HF diet containing 3% CLA (HF + CLA) for 30 d. Tissue and organ weights, plasma and tissue TG levels, and parameters related to their regulation were evaluated. Liver oxidative status was also assessed.

Results: Dietary CLA showed detrimental and beneficial effects. CLA added to the HF diet caused hepatomegaly (+32%) and exacerbated the hepatic TG accumulation (+168%) observed with the HF diet without inducing liver damage; however, it significantly reduced plasma TG concentrations (−37%) and normalized muscular TG content. An increase in glutathione was associated with total normalization of liver lipid peroxidation. In addition, HF + CLA caused dystrophy of epididymal fat pads, even when the HF diet had increased the adipose tissue mass (30%). The biochemical mechanisms involved in the regulation of lipid levels were related to reduced (−20%) hepatic very low-density lipoprotein-TG secretion and decreased muscle (−35%) and adipose (−49%) tissue contributions to the removal of plasma TG by lipoprotein lipase enzymes.

Conclusion: Examination of CLA at HF levels showed hepatomegaly and exacerbation of lipid accretion as a negative impact; however, some positive aspects such as hypotriglyceridemia and protection against oxidative stress were also induced. Even the fat reduction is nutritionally important for weight control; the biochemical mechanisms whereby CLA mediates the potential effects could produce undesirable metabolic alterations.

Keywords: Conjugated linoleic acid; Isomeric fatty acids; Dietary fat; Triacylglycerol regulation; Nutrition; Oxidative status

Introduction

Conjugated linoleic acid (CLA) refers to a mixture of positionally and geometrically conjugated dienoic isomers of linoleic acid (LA) that are generated by biological and/or industrial hydrogenation/isomerization of fatty acid (FA). The *c*9,*t*11-CLA isomer represents approximately 80% of the total isomers in dairy and ruminant fats, whereas *c*9,*t*11- and *t*10,*c*12-CLA are equally abundant (usually 30–40% of each isomer) in commercial mixtures [1].

In recent decades, interest in CLA has increased due to its many bioactive properties related to health. The benefits seem to be very clear, especially in some experimental animal models [2–4], but the effects in humans are still questioned. In some animal models, dietary CLAs reduce carcinogenesis, decrease body fat, increase lean body mass, enhance feed efficiency, protect against oxidative stress, modulate circulating lipids, and prevent impaired glucose tolerance in diabetes. Several of these effects are controver-

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sial; in addition, some adverse results have been demonstrated. For example, in some animal models, hepatomegaly [5], hepatic steatosis [6], lipoatrophy [7], and adipose tissue apoptosis [5] have been noted. The precise actions and the biochemical mechanisms by which CLA mediates the beneficial and/or detrimental effects remain unclear. Furthermore, the effects of CLA are related to the type and level [8] of the specific CLA isomer, the kind [9] and amount [10] of dietary fat, the time of feeding [8], and the animal model [11] used, among other factors.

With regard to modulation of lipid metabolism, dietary CLA has been demonstrated to modify liver [6] and adipose tissue [12] triacylglycerol (TG) contents, and it may also alter plasma TG regulation [13] in mice. Moreover, CLA has been shown to control oxidative status [14,15]. These results for lipid regulation could be metabolically interrelated and dependent on dietary lipid levels, which exert a putative effect. Therefore, the aim of this work was to investigate the effect of dietary CLA at high-fat (HF) levels on parameters related to TG regulation and some potential impacts on liver damage in mice. The present study could strongly affect our understanding of the potential effects of CLA at dietary fat levels similar to those observed in a large proportion of the Western population.

Materials and methods

Materials

Nutrient compounds, including vitamins and minerals for diet preparations, were of food grade or better. The CLA mixture oil was a gift from Lipid Nutrition B.V. (Wormerveer, The Netherlands). Corn oil purchased from a local source (Mazola, Buenos Aires, Argentina) was used as a control for LA-rich oil. Biochemical reagents, enzymes, and standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ^{3}H-triolein was acquired from Perkin Elmer (Boston, MA, USA). Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP) kits were purchased from Wiener Co. (Rosario, Argentina). All solvents and reagents used for the FA quantification were chromatographic grade, and all other chemicals used were at least of American Chemical Society degree.

Animals, diets, and dietary treatment

The experimental procedures were performed according to the principles of our school of biochemistry regulations, compiled according to the Guidelines for the Care and Use of Experimental Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996). Male CF[1] mice were obtained after weaning from the facilities at our university. The mice were housed in animal quarters under controlled conditions (23 ± 2°C and 12-h light/dark cycle) in individual cages. At the beginning of the experiment, mice were randomly divided into three weight-matched groups; they had free access to water and were fed each diet ad libitum for 30 d.

Table 1
Composition of the experimental diets

Ingredient (g/kg diet)	C	HF	HF + CLA
Corn starch	529.5	399.5	399.5
Casein	200	200	200
Sucrose	100	100	100
Corn oil	70	200	170
CLA mix oil	—	—	30
Fiber	50	50	50
Mineral mixture*	35	35	35
Vitamin mixture*	10	10	10
L-cystine/L-methionine	3.0	3.0	3.0
Choline	2.5	2.5	2.5
Energy (MJ/kg)	16.6	19.3	19.3

C, control; CLA, conjugated linoleic acid; HF, high fat

* Vitamin and mineral mixtures were prepared according to Reeves et al. [16].

The three diets were nutritionally balanced, exceeded the essential FA recommendations, and differed in 1) the amount of dietary fat (recommended or HF levels) or 2) the presence or absence of CLA mix oil, which was substituted with 3% LA-rich corn oil (Table 1). The control diet was based on the American Institute of Nutrition Ad Hoc Committee recommendation (AIN-93G diet formulated for the growth, pregnancy, and lactation phases of rodents) [16] and contained the recommended level of fat (70 g of corn oil/kg of diet). The HF diet was enriched in fat by substituting an equal amount of carbohydrate with corn oil, reaching 200 g of fat/kg of diet (38.5% energy as fat). The CLA-rich HF diet (HF + CLA) was similar to the HF diet, with the exception that 3% (w/w) of the CLA mix oil was substituted with 3% (w/w) of the corn oil. Corn oil was used to provide an LA-rich oil, whereas CLA mix oil, formed by an equimolecular mixture of *c*9,*t*11-CLA and *t*10,*c*12-CLA, was the source of isomeric fat. The diets were freshly prepared every 3 d during the experimental period and stored at 4°C.

The FA compositions (Table 2) of corn oil and CLA mix oil, as methyl esters, were evaluated by gas chromatography with a Hewlett-Packard 5890 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame ionization detector. The FA methyl esters were identified by comparison of their retention times with those of commercial standard esters under the same conditions.

Animal weights were recorded three times a week during the experimental period. At the end of the dietary treatments, animals were anesthetized (0900–1100 h) with a mixture of acepromazine and ketamine (1 mg + 100 mg/kg of body weight, respectively) to collect blood and dissected tissues according to the assay proposed or to perform different dynamic experiments. Blood samples were centrifuged at 4°C, and serum or plasma was immediately used or

Table 2
Fatty acid composition of dietary fats*

Fatty acids	Corn oil	CLA mix oil
14:0	ND	ND
16:0	10.78	0.84
c-16:1	0.20	ND
17:0	ND	ND
18:0	2.24	2.67
c-18:1	31.36	14.94
t-18:1	ND	0.09
c,c-18:2	52.85	0.53
*c*9,*t*11-18:2	0.24	34.64
*t*10,*c*12-18:2	0.24	34.93
c,c,c-18:3	0.75	ND
t,c,c-18:3	0.06	ND
20:0	0.52	ND
c-20:1	0.24	ND
t-20:1	0.12	ND
22:0	0.20	ND
24:0	0.20	ND
Others	—	11.4

CLA, conjugated linoleic acid; ND, not detected
* Values are means as a weight percentage (w/w) of total fatty acid methyl esters.

frozen and stored at −80°C until analyzed. Liver, gastrocnemius muscle, and epididymal fat pads were frozen, weighed, and stored at −80°C until processed. For oxidative stress analysis, livers were washed with saline at 4°C.

Serum analysis

Serum TG and cholesterol levels and plasma AST, ALT, and AP activities were assessed by spectrophotometric methods using commercially available kits.

Determination of liver and muscle TG content

Portions of frozen liver and muscle were powdered and homogenized in saline (10%, w/v), and their TG content was determined using a spectrophotometric method [17].

Estimation of "in vivo" TG secretion rate

Animals fasted overnight were anesthetized, as indicated above. Then 600 mg/kg of body weight of Triton WR 1339 in saline solution, an agent known to inhibit peripheral removal of TG-rich lipoproteins, was injected intravenously [18]. Blood samples were taken immediately before and 120 min after the injection of Triton solution for estimation of TG accumulation. The TG secretion rate (SR) was estimated based on plasma TG concentrations at 0 and 120 min, plasma volume, and body weight. Further details have been previously reported [19].

Analysis of lipoprotein lipase activities

Because skeletal muscle and adipose tissue are the main tissues involved in the removal of plasma TG-rich lipoproteins by the enzyme lipoprotein lipase (LPL), the gastrocnemius muscle and epididymal adipose tissue were chosen to measure their enzyme activities.

To assess muscle LPL activity, gastrocnemius muscles were homogenized (10%, w/v) in NH_4Cl/NH_4OH buffer (50 mmol/L), pH 8.6, containing 4 IU/mL of heparin, and incubated for 15 min at 4°C. The crude extracts were incubated at 37°C for 30 min with a glycerol-stabilized [^{3}H]-triolein (specific activity 74 MBq/mmol) emulsion as substrate [20]. The [^{3}H]-FA radioactivity was quantitated with a liquid scintillation counter (Win Spectral 1414, Perkin Elmer). All assays were carried out in triplicate. LPL activity is reported as milliunits per gram of muscle. One milliunit of LPL activity represents 1 nmol of free FA released per minute.

To determine adipose tissue LPL activity, the technique described by Nilsson-Ehle and Schotz [20] and modified by Llobera et al. [21] was used. Briefly, epididymal adipose tissue samples were homogenized in Tris-HCl buffer (0.2 mol/L), pH 8.2, at 4°C and delipidated with cold acetone and diethyl ether three times each. The powders obtained were resuspended and incubated (triplicate samples) at 37°C for 30 min in a similar glycerol-stabilized [^{3}H]-triolein (specific activity 74 MBq/mmol) emulsion [20] as described above, in the absence or presence of 1 mol/L of NaCl. The following steps were the same as those used for muscle LPL activity. Adipose tissue LPL activity was calculated by subtracting non–LPL-dependent activity (high salt) from total lipolytic activity.

Analysis of liver oxidative stress parameters

To quantify liver lipid peroxidation (LPO), malondialdehyde was measured using the thiobarbituric acid method reported by Ohkawa et al. [22]. The amount of malondialdehyde formed from the reaction between thiobarbituric acid and the hydrolyzed, peroxidized lipids was extracted with *n*-butanol, and its intensity was measured at 532 nm using 1,1,3,3-tetramethoxypropane as an external standard. LPO is expressed as nanomoles of malondialdehyde equivalents per gram of liver weight.

Total liver glutathione (GSH) levels, as the main nonprotein sulfhydryl compounds, were measured in 5% trichloroacetic acid sample homogenates according to the method reported by Ellman and Lysko [23]. GSH content is expressed as milligrams of GSH per gram of liver.

Catalase (CAT) and glutathione peroxidase (GSH-Px) activities were assessed in liver samples by the method reported by Aebi [24] and Paglia and Valentine [25], respectively. CAT and GSH-Px activities are expressed as units (micromoles per minute) per milligram of protein. Protein levels in each fraction were determined by the method proposed by Lowry et al. [26].

Histologic analysis of liver

Liver tissues were fixed in 10% buffered formalin and staining procedures for light microscopy using hematoxylin and eosin were carried out on 5-μm-thick tissue sections.

Statistical analysis

Data are expressed as mean ± SE. To determine the effects of CLA at high levels of dietary fat, data were statistically analyzed by one-way analysis of variance. All post hoc multiple comparisons were made using Tukey's critical range test. Statistically significant differences were considered at $P < 0.05$.

Results

The content of fat in the diet and the presence of CLA at HF levels did not alter body weight gain during the 30-d experimental period (Table 3). When the fat level was increased above recommended levels (7%, w/w) compared with those usually found in the human population (20%, w/w, corresponding to 38.5% of calories), no differences were found in liver weight, but a significant increase of approximately 30% was observed in epididymal adipose tissue weight in the HF group. When 3% of the CLA mix oil was substituted for the same amount of corn oil, an increase in liver weight and a very significant reduction in epididymal adipose tissue mass were observed. Gastrocnemius muscle weight did not change in response to the consumption of any of the diets.

As presented in Table 4, the HF diet tended to increase liver (+58%) and plasma (+18%) TG content and significantly augmented gastrocnemius (+57%) TG concentration. The addition of CLA to the HF diet had an important impact on TG content, and this effect depended on the type of tissue considered. In this regard, plasma TG concentrations significantly decreased. In fact, they reached lower values than the control group; gastrocnemius TG content returned to normal values and liver TG content was noticeably higher (+168% versus HF diet and +324% versus control diet). No differences in plasma cholesterol concentrations were found between the experimental groups.

The small increase in plasma TG concentrations in HF-fed mice was related to significantly elevated TG-SR in the circulation, without changes in epididymal adipose tissue LPL activity (Table 5). Intriguingly, gastrocnemius LPL activity was increased more than 120%. This finding did not parallel plasma TG levels, but corresponded with the elevation in gastrocnemius TG content. The decrease in plasma TG levels below normal values by substitution of 3% corn oil with 3% CLA mix was congruent with a moderate but not significant reduction in hepatic TG-SR, but not with a higher TG removal rate. Although CLA increased epididymal LPL activity when expressed per gram of adipose tissue, the contribution of this tissue to the total TG removal rate was significantly lower due to the very small epididymal fad pads. In contrast, gastrocnemius LPL activity returned to normal values when the weight of the muscle was considered ($P = 0.01$). These results were in agreement with the normalized muscle TG levels.

Although there were some differences between groups in plasma AST, ALT, and AP activities (Table 6), the values remained within the normal range. The significant hepatic TG accumulation caused by the HF diet and by CLA at HF levels was not related to hepatic damage. As presented in Table 6, LPO tended to increase and CAT activity was significantly reduced by the HF diet. A normalized hepatic oxidative status (LPO) was accomplished by substitution with the 3% CLA mixture, and this was linked to a significant (85%) increase in GSH content. Because the activity of GSH-Px and CAT were not increased by CLA, these enzymes might contribute minimally to the lower LPO values observed.

Table 3
Body weight gain and liver and epididymal fat pads weights in animals fed C, HF, and HF + CLA diets*

	C	HF	HF + CLA
Body weight gain (g/30 d)	11.0 ± 0.89	11.10 ± 1.85	9.80 ± 1.40
Liver weight			
g	2.22 ± 0.14^{ab}	1.95 ± 0.06^{a}	2.59 ± 0.09^{b}
g/100 g body weight	6.57 ± 0.44^{ab}	5.91 ± 0.27^{a}	7.63 ± 0.34^{b}
Epididymal fat pad weight			
g	0.32 ± 0.02^{a}	0.41 ± 0.03^{b}	0.06 ± 0.01^{c}
g/100 g body weight	0.94 ± 0.05^{a}	1.25 ± 0.11^{b}	0.17 ± 0.03^{c}
Gastrocnemius muscles weight			
g	0.3 ± 0.02	0.3 ± 0.04	0.27 ± 0.02
g/100 g body weight	0.87 ± 0.04	0.89 ± 0.09	0.78 ± 0.06

C, control; CLA, conjugated linoleic acid; HF, high fat

* Values are means ± SEMs for six, six, and five animals in the C, HF, and HF + CLA groups, respectively. Unalike superscript letters indicate statistical differences by one-way analysis of variance followed by Tukey's test ($P < 0.05$).

Table 4
Effects of CLA on plasma lipid concentrations and tissular TG contents*

	C	HF	HF + CLA
Plasma concentrations (mmol/L)			
TG	0.39 ± 0.04^{ab}	0.46 ± 0.05^{b}	0.29 ± 0.02^{a}
Cholesterol	3.83 ± 0.43	3.98 ± 0.38	4.39 ± 0.19
Liver TG content (μmol/g)	14.58 ± 0.70^{a}	23.03 ± 2.63^{b}	61.86 ± 8.85^{c}
Gastrocnemius TG content (μmol/g)	4.56 ± 0.32^{a}	7.14 ± 0.54^{b}	4.04 ± 0.69^{a}

C, control; CLA, conjugated linoleic acid; HF, high fat; TG, triacylglycerol

* Values are means ± SEMs for five, seven, and seven animals in the C, HF, and HF + CLA groups, respectively. Unalike superscript letters indicate statistical differences by one-way analysis of variance followed by Tukey's test ($P < 0.05$).

Table 5
Effects of CLA on plasma and tissue parameters related to TG regulation*

	C	HF	HF + CLA
TG-SR (nmol/min per 100 g)	256.88 ± 41.02[a]	487.55 ± 38.61[b]	392.64 ± 9.76[b]
LPL activities			
mU/g epididymal WAT	113.71 ± 12.39[a]	106.86 ± 18.21[a]	372.60 ± 12.46[b]
mU/epididymal WAT (×2)	36.43 ± 4.90[ab]	41.82 ± 4.21[a]	21.57 ± 3.70[b]
mU/g gastrocnemius	37.33 ± 2.03[a]	84.71 ± 18.62[b]	54.57 ± 6.11[ab]
mU/gastrocnemius (×2)	11.21 ± 1.14[a]	26.48 ± 4.92[b]	14.18 ± 1.37[a]

C, control; CLA, conjugated linoleic acid; HF, high fat; LPL, lipoprotein lipase; TG, triacylglycerol; TG-SR, triacylglycerol secretion rate; WAT, white adipose tissue

* Values are means ± SEMs for six animals in each group. Unalike superscript letters indicate statistical differences by one-way analysis of variance followed by Tukey's test ($P < 0.05$).

The histopathologic analysis did not show abnormalities in the livers of animals fed the HF diet. However, the animals that consumed CLA presented small and large vacuoles typical of lipid accumulation (Fig. 1).

Discussion

To our knowledge, this is the first study to investigate the potential effect of dietary CLA at high dietary fat levels on very low-density lipoprotein (VLDL)-TG-SR and removal rate and liver damage in mice. However, many researchers have studied the effects of CLA on different aspects of lipid regulation in several distinct experimental animal models.

There is evidence that CLAs might alter plasma lipid concentrations, increase TG liver accumulation, and reduce TG stores in adipose tissue in different animal models. However, there is no agreement about the impact of CLA on plasma TG concentrations and, consequently, on the mechanisms involved in their regulation. The controversial results might be due to several factors, such as the experimental animal model, type and dose of isomers, type and amount of dietary fats, and feeding time. Various investigators have agreed that the effects on lipids are mainly due to the *t*10,*c*12-CLA isomer [27–29], and that mice are a highly sensitive species to these actions [30,31]. Nevertheless, diverse effects were observed in animals fed a CLA mixture containing similar amounts of *c*9,*t*11- and *t*10,*c*12-CLA [13,32,33], which is available for human consumption. In our animal model, cholesterol levels were not modified by the amount of dietary fat with or without CLA addition. These data are in agreement with those reported by Warren et al. [27] and Javadi et al. [34], but differ from the results published by Corino et al. [35] and Lee et al. [36] who demonstrated a hypocholesterolemic effect of CLA in normal mice. Moreover, the slight but insignificant increase in plasma TG concentrations induced by the HF diet was significantly reduced by CLA supplementation. Consistent with our results, Faulconnier et al. [33] and Stangl [37] examining normal dietary fat levels and Liu et al. [13] assessing high dietary fat levels showed a hypotriglyceridemic effect of CLA. Furthermore, other investigators have reported different outcomes on plasma TG concentrations in mice [27,34] and in other animal species [32,38].

Our results also showed liver TG accretion based on chemical analysis in animals fed the HF diet, although it did not reach statistical significance. This increase was significantly abrogated by CLA supplementation. This effect of CLA was confirmed by histopathologic analysis, because vacuoles were observed only in this group of animals. These

Table 6
Effects of CLA on hepatic damage and oxidative stress parameters*

	C	HF	HF + CLA
Index of hepatic damage enzyme			
Plasma AST activity (U/L)	22.59 ± 1.18[a]	16.77 ± 1.48[b]	23.15 ± 1.66[a]
Plasma ALT activity (U/L)	15.61 ± 0.68[a]	16.61 ± 0.72[ab]	20.62 ± 0.36[b]
Plasma AP activity (U/L)	201.62 ± 11.71[a]	118.58 ± 13.47[b]	141.95 ± 6.18[b]
Hepatic oxidative stress parameters			
LPO (nmol MDA/g)	167.34 ± 14.72	195.62 ± 12.86	161.45 ± 10.32
GSH content (μmol/g)	2.87 ± 0.13[a]	2.71 ± 0.07[a]	5.00 ± 0.69[b]
GSH-Px activity (U/mg protein)	0.045 ± 0.003	0.046 ± 0.004	0.036 ± 0.002
CAT activity (U/mg protein)	53.45 ± 2.75[a]	41.33 ± 2.86[b]	33.40 ± 1.09[b]

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CAT, catalase; CLA, conjugated linoleic acid; GSH, glutathione; GSH-Px, glutathione peroxidase; HF, high fat; LPO, lipid peroxidation; MDA, malondialdehyde

* Values are means ± SEMs for six animals in each group. Unalike superscript letters indicate statistical differences by one-way analysis of variance followed by Tukey's test ($P < 0.05$).

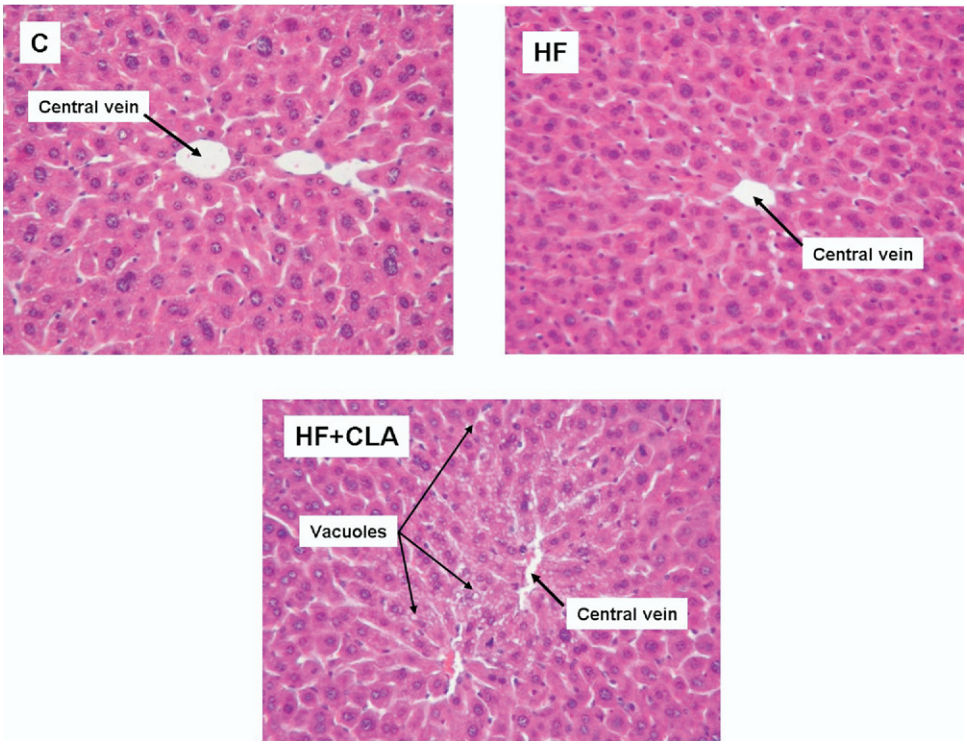

Fig. 1. Representative histologic images from livers of mice fed the C or HF diet show normal morphology, whereas those from mice fed the HF + CLA diet show small and large vacuoles typical of lipid accumulation (arrows). Hematoxylin and eosin stain; original magnification 200×. C, control; CLA, conjugated linoleic acid; HF, high-fat.

alterations were insufficient to provoke significant hepatic damage, as reflected by the normal levels of AST, ALT, and AP. However, these findings were associated with the characteristic organ alterations previously described, such as hepatomegaly and adipose tissue dystrophy. Liver TG accumulation and hepatomegaly have been previously reported by several researchers in mice [6,7], as has decreased fad pad mass [2,27]. Conversely, some investigators using different animal models found a decrease in hepatic steatosis [39,40], and others have reported that liver enlargement could be related to an increase in the number of hepatocytes [41]. Other changes attributed to CLA, such as increased muscle mass [19,42] and reduced body weight gain [39,43], were not observed in the present study. Such differences could be due to the mouse strain, level of dietary fat, or type of CLA isomer assessed.

Diverse reports in different animal models have contributed to the interpretation of these characteristic abnormalities induced by CLA, and it is hypothesized that lipodystrophic syndrome could be involved [7]. Our data, in a systematic and novel way, contribute to explaining the TG regulation in the plasma and some tissues, such as liver, skeletal muscle, and adipose tissue, exerted by CLA intake at HF levels. The variations in plasma and tissue TG could be explained, among other possibilities, by alterations in VLDL-TG synthesis, SR, and removal rate. The slight increase in plasma TG observed in HF-fed rats could be related to a high hepatic TG-SR, which was only partly compensated for by the high TG removal rate of muscle LPL enzyme. CLA supplementation at HF levels significantly reduced plasma TG levels, which was associated with a decline in hepatic TG-SR, but not with changes observed in plasma TG removal rates. The moderate but not significant reduction in liver TG-SR could contribute to hepatic lipid accumulation. Liver steatosis and hypotriglyceridemia induced by *t*10,*c*12-CLA was not linked to decreased liver secretion of VLDL by Degrace et al. [44]; in fact, they observed hypersecretion of VLDL-apolipoprotein-B. Several investigators have focused on other biochemical aspects related to the hepatic lipid accretion caused by CLA and reported increased FA uptake [13,45], FA synthesis [44], and alterations in VLDL-TG assembly [46]. Some of these observations and other hepatic alterations can be related to the role of CLA as a peroxisome proliferator-activated receptor ligand [13].

Prevention of increased muscular TG content by CLA was associated with a decrease in muscular LPL activity. However, this enzymatic activity did not reach the levels observed in the control group, whereas the TG content did, suggesting a potential increase in β-oxidation. In this manner, Park et al. [2] and Zabala et al. [38] reported increased carnitine palmitoyltransferase-1 activity in mice and hamsters, respectively. Because CLAs are potent peroxisome proliferator-activated receptor ligands [13,47,48], upregulation of the enzymes related to muscular β-oxidation could be expected. In agreement with muscle LPL activity, adi-

pose tissue did not contribute to plasma TG variations in the HF or HF + CLA diet groups. The 28% increase in adipose tissue fat pad weight observed in the HF diet group was not associated with a rise in LPL activity. This could be due to reduced lipolysis and β-oxidation and elevated TG biosynthesis in adipose tissue.

The significant adipose tissue dystrophy produced by CLA in the HF diet is clearly related to TG catabolism rather than lower FA uptake and TG synthesis. Several researchers have reported that CLAs decrease the size rather than the number of adipocytes [8,49]. These findings are consistent with our results demonstrating high adipose tissue LPL activity per gram of tissue, which did not correlate with the low-fat mass observed. The latter modestly contributed to the plasma TG removal mentioned above. Our results concerning the effect of CLA on LPL activity differed from those of other researchers [2,50]. Moreover, the data regarding enzyme expression and activity are controversial [2,5,50,51] and might be related, among others, to disparities between species, differences between in vitro and in vivo experiments, and/or age of the animals. The biochemical mechanisms related to TG regulation in adipose tissue are beyond the scope of our study. However, there are many references that explain the reduction in white adipose tissue depots. Pariza et al. [11] suggested a model for the effects of *t*10,*c*12-CLA on adipocytes and preadipocytes based on various studies. Specifically, they proposed that reduced lipid uptake is due to low expression and enzymatic activity of stearoyl coenzyme A desaturase and LPL, and decreased lipogenesis and increased lipolysis are associated with decreased adipocyte differentiation and proliferation and increased apoptosis. This hypothesis was supported and later extended by others [50,52–54] using in vivo and in vitro models.

The increased liver TG depots induced by the HF diet rich in LA, as expected, slightly altered the oxidative status. This could be due to a reduction in CAT activity and the high availability of unsaturated FAs, which are prone to oxidation. The 3% substitution of corn oil with the CLA mixture fully normalized the oxidative status without increasing CAT activity. The mechanism by which CLA compensated for the slight increase in LPO induced by HF was related to an 84% increase in GSH content. These results are in agreement with those obtained by Arab et al. [55] who reported that CLA is the only polyunsaturated FA able to induce GSH synthesis and that its hairpin structure could be determining the induction of the enzyme involved in GSH biosynthesis. The ability of CLA to prevent LPO has been reported by other investigators based on other mechanisms. Livisay et al. [14] reported that CLA does not act as an antioxidant, but its ability to improve membrane FA stability could decrease the formation of lipid oxidation compounds. In support of this mechanism, Kim et al. [4] reported that CLA reduces LPO by lowering the levels of oxidation-susceptible polyunsaturated FAs in vitamin E–deficient rats.

Conclusion

By focusing on CLA at HF levels we found hepatomegaly and exacerbation of lipid accretion to be a negative impact; however some positive aspects such as hypotriglyceridemia and protection against oxidative stress were also induced. Even the fat reduction is nutritionally important for weight control; the biochemical mechanisms whereby CLA mediates the potential effects could produce undesirable metabolic alterations.

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