RESEARCH ARTICLE
Linoleic Acids
European Journal of Lipid Science and Technology
www.ejlst.com

Effect of Conjugated Linoleic Acids on Nutritional Status and Lipid Metabolism in Rats Fed Linoleic-Acid-Deprived Diets

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This study aims to investigate the effect of conjugated linoleic acid (CLA) on nutritional parameters and triacylglycerol (TAG) regulation in male Wistar rats fed linoleic acid (LA)-deprived (−LA) diets compared to LA-enriched (+LA) diets. In both +LA and −LA groups, CLA are incorporated into the tissues, showing higher levels in the adipose tissue. However, different metabolic and nutritional effects are observed depending on the LA status. CLA markedly reduces fat depots in the −LA group, associated with an increased lipoprotein lipase (LPL) and lipogenic enzyme activities as compensatory mechanisms. Moreover, CLA restores the hepatic TAG levels in −LA animals, associated with a normalized triacylglycerol-secretion rate (TAG-SR), an increased lipogenic enzyme activity and higher mRNA levels of fatty acid synthase. Serum TAG levels are not affected by CLA in the +LA group. However, in the −LA group, CLA decreases the TAG levels associated with a reduced TAG-SR and a higher adipose tissue LPL activity. Thus, the CLA effects on the nutritional parameters and TAG metabolism differs depending on the LA status. CLA causes certain beneficial biological and nutritional effects in LA-deprived but not in LA-enriched animals.
***Practical Applications*: The approach by the authors involve growing animals in healthy physiological conditions fed with diets containing recommended levels of dietary fats, moderate amounts of commercial CLA mixture obtained from industrial synthesis (equimolecular amounts of 9c,11t- and 10t,12c-isomers), and unbalanced LA levels. These variables constitute a situation observed in the human population. The present study might contribute to understanding the role of CLA on nutritional parameters and TAG metabolism depending on the nutritional milieu.**

1. Introduction

In the last century and especially in Western countries, the human population has drastically increased the consumption of vegetable oils rich in linoleic acid (LA; 9c,12c-18:2) with a parallel decrease of α-linolenic acid (ALA; 9c,12c,15c-18:3), shifting the n-6:n-3 fatty acid (FA) ratio to 15–50:1.[1] Some studies[2] have related a high n-6:n-3 FA ratio to increased levels of arachidonic acid (AA; 5c,8c,11c,14c-20:4)-derived eicosanoids, that could contribute to a proinflammatory state, an endothelial dysfunction, and a gradual fat mass enhancement. Moreover, in experimental animal models, high n-6 polyunsaturated FA (PUFA) have been related to impaired serum lipid levels, eicosanoid production, and other metabolic alterations.[3] However, if compared with saturated FA intake, n-6 PUFA reduces liver fat and modestly improves the metabolic status, without weight loss.[4] On the other hand, LA-deprived (−LA) animals have shown several lipid alterations including lower serum concentrations of triacylglycerol (TAG), cholesterol, and phospholipids[5] as well as changes in FA composition[6] and fat accretion in liver.[7]

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers that are derived from octadecadienoic acids conjugated with double bonds. 9c,11t-CLA, or rumenic acid, is the main isomer produced by ruminants (≈80%), whereas commercial CLA, which is obtained from industrial synthesis, contains equimolecular amounts of a 9c,11t-CLA and 10t,12c-CLA mixture (≈37–40% each). The interest in CLA has increased in the recent decades because of its potential preventive effects on different diseases. In some experimental animal models, CLA have shown to possess anticancer,[8,9] antiatherogenic, and antiobesogenic[10,11] properties. Likewise, it has been observed that CLA improve circulating lipid levels and lipoprotein profiles[12] and increases the lean body mass.[13] As a result, either industrially synthesized or natural, CLA is currently used in functional foods or directly

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DOI: 10.1002/ejlt.201800362

consumed in capsule form. However, adverse effects have also been reported due to CLA consumption, such as insulin resistance, hyperinsulinemia, and massive hepatic steatosis in mice.[11,14,15] On the other hand, the effects of the two main CLA isomers differ among animal models depending on multiple factors, such as species, age, dosage, treatment duration, and nutritional state.[16]

Considering that CLA might act as an alternative substrate in the LA metabolic pathways producing metabolites with different and/or unknown biological functions,[17] the hypothesis of the present work is the following: CLA at different levels of dietary LA have distinct effects on TAG regulation. Therefore, this study aimed to investigate the effect of CLA on the nutritional parameters and TAG regulation of rats fed LA-deprived diets compared to rats fed LA-enriched diets.

2. Experimental Section

2.1. Materials and Diets

Nutrients and other chemical compounds, vitamins, and minerals for the diet preparations were of chemical grade or better, with the exception of corn oil (Arcor, Córdoba, Argentina), sucrose, cellulose, and corn starch, which were locally obtained. CLA-enriched oil was obtained from Lipid Nutrition BV (Wormerveer, The Netherlands) and consisted of an equimolecular mixture of 9c,11t-CLA and 10t,12c-CLA. Corn oil was used as an unsaturated cis-FA source rich in LA (51% of total FA). Coconut oil was used to produce an EFA-deprived status and CLA-enriched oil was used as CLA source. All solvents and reagents used for the FA quantification were of chromatography grade, and all other chemicals used were of at least American Chemical Society grade. The standard FA were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Nu-Chek (Nu-Chek Prep, Inc., Elysian, MN, USA). TAG commercial test kit was obtained from Sociedad de Bioquímicos (Santa Fe, Argentina).

The composition of the diets is presented in **Table 1** and was based on the American Institute of Nutrition ad hoc writing committee recommendation (AIN-93G),[18] except for the FA source that was based on AIN-76A.[19] All diets were isoenergetic, theoretically providing 16.6 kJ g^{-1}. The +LA diet contained 7% of corn oil as a dietary fat source. The −LA diet contained 7% of coconut fat. CLA supplementation was achieved by replacing 1% of corn oil (+LA+CLA) or 1% of coconut fat (−LA+CLA) by 1% of CLA rich oil (equimolecular mixture of 9c,11t-CLA and 10t,12c-CLA −39.0% and 38.8%, respectively). Except for the type of fats, all diets were identical. Each diet was freshly home-made; meals were prepared every 3 days throughout the experimental period and conserved at 4 °C. In order to control the homogeneity between the different pools of the prepared diets, centesimal composition (water, protein, fats, carbohydrates, and fiber) was determined after each preparation.[20]

The FA composition of dietary fats (**Table 2**) was determined by gas chromatography with a Shimadzu chromatograph (GC 2014) equipped with a flame ionization detector. The FA methyl esters (FAME) were obtained from the base-catalyzed methanolysis of the glycerides (KOH in methanol), after dissolving the lipid extract in high-performance liquid chromatography

Table 1. Composition of the experimental diets.

Dietary constituent [g kg^{-1} diet]	+AL	−AL	+AL+CLA	−AL+CLA
Corn starch	529.5	529.5	529.5	529.5
Casein	200.0	200.0	200.0	200.0
Sucrose	100.0	100.0	100.0	100.0
Corn oil	70.0	–	60.0	–
Coconut fat	–	70.0	–	60.0
CLA-rich oil	–	–	10.0	10.0
Fibre	50.0	50.0	50.0	50.0
Vitamin mixture	35.0	35.0	35.0	35.0
Mineral mixture	10.0	10.0	10.0	10.0
L-Cystine-L-methionine	3.0	3.0	3.0	3.0
Choline	2.5	2.5	2.5	2.5
Energy (kJ g^{-1})	16.6	16.6	16.6	16.6

+LA, enriched with linoleic acid; −LA, deprived of linoleic acid; +LA+CLA, +LA supplemented with CLA-enriched oil; −LA+CLA, −LA supplemented with CLA-enriched oil; CLA, conjugated linoleic acid.
Diets were prepared according to AIN-93G,[18] except for the FA source, which was based on AIN-76A.[19]

Table 2. Fatty acid composition of dietary fats.

Fatty acids [%]	Corn oil	Coconut fat	CLA-rich oil
6:0	ND	0.49	ND
8:0	ND	6.76	ND
10:0	ND	5.64	ND
11:0	ND	0.02	ND
12:0	ND	47.67	ND
13:0	ND	0.02	ND
14:0	0.03	17.46	ND
16:0	12.21	9.21	5.85
16:1	0.12	ND	ND
18:0	1.93	12.53	1.20
9c-18:1	31.95	0.05	9.05
11c-18:1	0.54	ND	0.41
9c,12c-18:2	51.26	0.01	1.08
20:0	0.50	0.14	ND
11c-20:1	0.25	ND	ND
9c,12c,15c-18:3	0.88	ND	ND
22:0	0.16	ND	0.35
24:0	0.15	ND	ND
9c,11t-CLA	ND	ND	38.99
11c,13t-CLA	ND	ND	1.54
10t,12c-CLA	ND	ND	38.76
$\sum$ NI	ND	ND	3.11

CLA, conjugated linoleic acid; ND, non detected; NI, other fatty acids non identified.
All values are presented as weight percentages of total fatty acid methyl esters.

quality hexane.[21] FAME were separated on a capillary column CP Sil 88 (100 m, 0.25 mm film thickness), according to the AOCS Official Method Ce 1j-07.[22] FAME were identified by comparison of their retention times relative to those of commercial standards. Values were expressed as percentage of total FAME.

2.2. Animals and Experimental Design

The experimental procedures were approved by the Ethics Committee of our School of Biochemistry and compiled according to the Guide for the Care and Use of Laboratory Animals.[23] Male Wistar rats were housed in an animal facility under controlled conditions (23 °C ± 2 °C and a 12-h light–dark cycle). After reaching 100–120 g, the rats were assigned to 1 of 4 weight-matched groups ($n = 6$ per group) and fed ad libitum for 60 days with one of the following diets: +LA, −LA, +LA supplemented with CLA-rich oil (+LA+CLA), or −LA supplemented with CLA-rich oil (−LA+CLA).

Rats were weighed and food intake was recorded three times a week during the whole dietary treatment. Food intake was adjusted for waste by collecting food spillage. On the morning of day 60 the animals were sacrificed under anesthesia (1 mg of acepromazine +100 mg of ketamine/kg of body weight), the body was shaved and the abdomen was cut open to remove visceral organs. The carcasses were weighed, chopped, and frozen at −80 °C until compositional evaluation. A second series of animals were treated under the same conditions and sacrificed as described above, with the purpose of collecting blood and dissected tissues according to the intended purpose. Serum was obtained by centrifugation immediately after blood collection. Liver, gastrocnemius muscle, epididymal adipose tissue (EAT), and retroperitoneal adipose tissue (RPAT) were frozen, weighed, and stored at −80 °C until analysis. A third series of rats were employed to estimate the in vivo hepatic triacylglycerol-secretion rate (TAG-SR).

2.3. Nutritional and Body Composition Parameters

Nitrogen in samples from the carcass homogenate was converted to $(NH_4)_2SO_4$ by the Kjeldahl method[20] and its content was determined. Carcass protein levels were estimated by multiplying their nitrogen contents by 6.25. Water content was determined by drying aliquots of the carcass and food to a constant weight in an oven at 60 °C. Total fat in the dried samples of carcasses, foods, and feces was extracted with light petroleum-ether.[20] The fat extract was evaporated in a vacuum system and the total fat was gravimetrically measured. The energy intake was calculated by multiplying the weight of the dry food consumed daily by the number of kJ g^{-1} of dry diet. Body weight gain efficiency was estimated as percentage of body weight gained (g) divided by energy intake (kJ/experimental period) during 60 days.

2.4. Serum and Tissue Fatty Acids Analysis

The extraction of total lipids was performed by the method described by Bligh and Dyer,[24] followed by FAME obtaining from the base-catalyzed methanolysis of the glycerides[21] and the analysis of FA by gas chromatography as described above. From the serum, liver, EAT, and gastrocnemius muscle FA composition, the following parameters were estimated: (i) biomarkers of EFA status: LA, ALA, mead acid levels, and triene/tetraene ratio; (ii) the sum of saturated fatty acids (SFA) ($\sum$ SFA), monounsaturated fatty acids (MUFA) ($\sum$ MUFA), and PUFA ($\sum$ PUFA); and (iii) the isomeric FA incorporation.

2.5. Triacylglycerol Levels in Serum, Liver, and Muscle

TAG levels in serum were determined by spectrophotometric methods using a commercially available test kit. Liver and muscle TAG levels were determined by the method of Laurell.[25] Portions of frozen tissues (0.2 g) were powdered and homogenized in distilled water 1:10 (w/v) for TAG content quantification.

2.6. Hepatic Triacylglycerol Secretion Rate

Rats, fasted overnight, were anesthetized as indicated above. Then, 600 mg kg^{-1} of body weight of triton WR 1339 in saline solution, an agent known to inhibit the peripheral removal of TAG-rich lipoproteins, was injected intravenously.[26] Blood samples were taken immediately before and 120 min after the injection of the Triton solution for the estimation of TAG accumulation in serum. Hepatic TAG-SR was estimated based on serum TAG concentration at 0 and 120 min, plasma volume and body weight. Further details have been previously reported.[27]

2.7. Lipoprotein Lipase Activity in Adipose Tissue and Gastrocnemius Muscle

The removal capability of TAG-rich lipoproteins was evaluated by lipoprotein lipase (LPL) activities in the main tissues responsible for the uptake of TAG: adipose tissue and muscle. The enzymatic activity of adipose tissue LPL was quantified in EAT acetone powder by the fluorometric method of Del Prado et al.[28] Briefly, EAT samples were delipidated by a double extraction with cold acetone followed by a double extraction with diethyl ether. The powders obtained were resuspended and incubated in a buffer (25 mM NH_4Cl, pH 8.1 containing 1 UI mL^{-1} of heparin). The enzymatic reaction was carried out in a medium containing dibutiryl fluorescein (DBF) as enzyme substrate. The quantification of LPL activity was performed measuring the increase in fluorescence ($\lambda_{excitation} = 490$ ηm; $\lambda_{emission} = 530$ ηm). In parallel, an identical assay was carried out in the same samples but in the presence of NaCl during incubation to inhibit specific enzyme activity. LPL activity was estimated as the difference between non-specific lipolitic and the total lipolitic activity. Values were expressed as ηmol fluorescein/min/total EAT. To assess muscle LPL activity, gastrocnemius muscle samples were homogenized in NH_4Cl/NH_4OH-Heparin buffer. Then, the quantification of LPL activity in muscle was performed as previously described for adipose tissue. The measured activity was expressed as ηmol fluorescein/min/total muscle.

2.8. Lipogenic Enzyme Activities

Liver and EAT samples were homogenized in a buffer solution (pH 7.6 containing 150 mM KCl, 1 mM $MgCl_2$, 10 mM N-acetyl-cysteine, and 0.5 mM dithiothreitol). After centrifugation at 100 000 g for 40 min and 4 °C, the supernatant fraction was used for the quantification of enzyme activities. Fatty acid synthase (FAS; EC 2.3.1.85), malic enzyme (ME; EC 1.1.1.40), and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) activities were measured by the methods of Lynen,[29] Hsu and Lardy,[30] and Kuby and Noltmann,[31] respectively. Enzyme activities were expressed either as ηmol NADPH consumed (FAS) or as ηmol NADPH produced (G6PDH and ME)/min/mg of protein (1 mU = 1 ηmol NADPH/min). Protein content was determined by Lowry et al.'s technique[32] using bovine serum albumin as standard.

2.9. Carnitine Palmitoyl Transferase-I Activity

Carnitine palmitoyl transferase-1a (CPT-1a) and carnitine palmitoyl transferase-1b (CPT-1b) (EC 1.3.99.3) activities were assessed in the mitochondrial fraction by the method of Bieber et al.[33] Liver and muscle samples were homogenized in a buffer (pH 7.4 containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris–HCl). Homogenates were centrifuged at 700 g for 10 min and 4 °C, and supernatant fluid was again centrifuged at 12 000 g for 15 min and 4 °C. Pellets were resuspended in a buffer (pH 7.4 containing 70 mM sucrose, 220 mM mannitol, 1 mM EDTA, 2 mM HEPES). Mitochondria were incubated for 5 min at 30 °C in a Tris–HCl buffer (pH 8 containing 116 mM Tris–HCl, 2.5 mM EDTA, 0.11 mM DTNB, and 0.2% Triton X–100) with 75 µL of homogenization buffer, 0.05 mM palmitoil Co-A and 0.11 mM L(−) carnitine. Reaction was started with 25 µL of homogenate and the activity was evaluated by CoA liberation (through CoASH formation) at 412 ηm for 3 min. In parallel and under similar conditions, unspecific activity was assessed in absence of L(−) carnitine. The result was expressed as the difference between activity in presence and in absence of L(−) carnitine. The pellet protein content was determined as described above. The CPT-1 activities were expressed as mU mg^{-1} of protein (1 mU = 1 ηmol CoA/min).

2.10. Hepatic mRNA Levels of Enzymes Involved in Lipogenesis and β-Oxidation

Total RNA was isolated from liver using Trizol according to the manufacturer's instructions. RNA samples were then treated with a DNA-free kit (Applied Biosystems, Foster City, CA, USA) to remove any contamination with genomic DNA. The yield and quality of the RNA were assessed by measuring absorbance at 260, 270, 280, and 310 nm and by electrophoresis on 1.3% agarose gels. 1.0 µg of total RNA of each sample was reverse-transcribed to first-strand complementary DNA (cDNA) using a M-MLV Reverse Transcriptase. Relative mRNA levels were quantified using real-time PCR with a StepOne 18 TM Real-Time PCR Detection System (Applied Biosystems). Sequence-specific primers were used (Genbank: acetyl-CoA carboxylase -ACC-, NM_022193.1; FAS, NM_017332.1; sterol regulatory element-binding protein-1c -SREBP-1c-, NM_001276708.1; CPT-1a, NM_031559.2; peroxisome proliferator-activated receptor-α -PPAR-α-, NM_013196.1; β-Actin, NM_031144.3 and ubiquitin C -UBC-, NM_017314.1), commercially synthesized (Invitrogen Custom Primers) and the sequences were: ACC: 5′-AAC AGT GTA CAG CAT CGC CA-3′ (forward), 5′-CAT GCC GTA GTAG GTT GAG GT-3′ (reverse); FAS, 5′-CAG AAC TCT TCC AGG ATAG TCA ACA -3′ (forward), 5′- GTC GCC CTAG TCA AGG TTC AG -3′ (reverse); SREBP-1c, 5′-GGA GCC ATAG GAT TAGC ACA TT-3′ (forward), 5′- GCT TCC AGA GAG GAG CCC AG-3′ (reverse); PPAR-α, 5′- CCC CAC TTAG AAG CAG ATAG ACC-3′ (forward), 5′- CCC TAA GTA CTAG GTA GTC CGC-3′ (reverse); β-actin 5′- CAT GAA GAT CAA GAT CAT TAGC TCC T-3′ (forward), 5′-CTAG CTT GCT GAT CCA CAT CTAG-3′ (reverse); UBC 5′- ACACCAAGAAGGTCAAA-CAGGA-3′ (forward), 5′- CACCTCCCCATCAAACCCAA-3′ (reverse). Standard curves for each primer were generated on separate runs using several serial dilutions (1/10–1/1000) of pooled cDNA samples. The corresponding primer efficiency (E) of one cycle in the exponential phase was calculated according to equation $E = 10^{[-1/\text{slope}]}$. All the efficiencies of the primers were 100 ± 10%. Target genes were normalized with the geometric mean of two housekeeping genes, β-Actin and UBC. Relative expression ratios were calculated using the recommended $2^{-\Delta\Delta Ct}$ method.[34]

2.11. Statistical Analysis

Values were expressed as mean with their standard errors (SE) of six animals per group. The minimum sample size needed to detect a statistically significant difference ($p < 0.05$) was calculated. A sample size of six had an 80% power ($p = 0.05$). Statistical differences between mean values were established by two-way ANOVA (2 × 2) using LA status and CLA supplementation as independent variables. All post hoc multiple comparisons were made using Scheffe's critical range test. For 9c,11t-CLA and 10t,12c-CLA isomers levels, statistical differences between means were established by unpaired Student's t-test. Differences were considered statistically significant at $p < 0.05$.[35]

3. Results

3.1. Physiological Status and Nutritional Parameters

During the experimental period, all the animals showed a healthy condition. Even though the biochemical markers, mead acid and triene/tetraene ratio, indicated an EFA deficiency, no physical symptoms or signs of deficiency were observed.

The average daily energy intake, the body weight gained and the carcass energy retention did not differ among the groups. The −LA+CLA group showed lower EAT and RPAT weights (**Table 3**).

3.2. Serum and Tissue Fatty Acid Composition

Under our experimental conditions, the sum of SFA levels was significantly higher in both −LA and −LA+CLA groups in

Table 3. Effect of CLA on nutritional and body composition parameters in rats fed LA-enriched or LA-deprived diets.

	Experimental groups				ANOVA		
	+LA	-LA	+LA+CLA	−LA+CLA	LA	CLA	LA×CLA
Energy intake [kJ d^{-1}]	293.32 ± 8.29	310.02 ± 5.97	312.00 ± 20.40	300.86 ± 12.84	NS	NS	NS
Body weight gain [g]	209.75 ± 7.31	226.25 ± 7.08	201.92 ± 3.95	203.58 ± 6.60	NS	S	NS
Carcass energy retention [kJ d^{-1}]							
Total	44.60 ± 1.87	48.13 ± 3.01	45.70 ± 1.97	39.15 ± 2.02	NS	NS	NS
Fat	27.56 ± 1.17	29.91 ± 2.81	28.15 ± 0.51	24.64 ± 1.78	NS	NS	NS
Protein	17.04 ± 0.86	18.22 ± 0.66	17.55 ± 1.55	14.51 ± 0.49	NS	NS	NS
Energy efficiency [%]	15.29 ± 0.95	15.56 ± 1.09	15.07 ± 1.69	13.02 ± 0.48	NS	NS	NS
Relative tissues weight [g/100 g]							
Liver	6.90 ± 0.97^{a}	5.04 ± 0.19^{b}	6.29 ± 0.09^{a}	6.72 ± 0.10^{a}	S	S	S
Gastrocnemius muscle	0.66 ± 0.05	0.65 ± 0.07	0.76 ± 0.05	0.75 ± 0.02	NS	NS	NS
EAT	4.24 ± 0.17^{a}	4.64 ± 0.37^{a}	4.51 ± 0.36^{a}	3.01 ± 0.20^{b}	NS	S	S
RPAT	4.27 ± 0.34^{a}	3.67 ± 0.35^{a}	4.24 ± 0.49^{a}	2.50 ± 0.24^{b}	S	NS	NS

+LA, enriched with linoleic acid; −LA, deprived of linoleic acid; +LA+CLA, +LA supplemented with CLA-enriched oil; −LA+CLA, −LA supplemented with CLA-enriched oil.
CLA, conjugated linoleic acid; EAT, epididymal adipose tissue; RPAT, retroperitoneal adipose tissue.
Values are mean ± SE. Significant differences were established by 2-way ANOVA (2 × 2) followed by the Scheffe test. For the ANOVA test, S indicates $p < 0.05$ and NS indicates $p \geq 0.05$. For the Scheffe test, values in the same row with different letters (a or b) are significantly different ($p < 0.05$).

serum, liver, adipose tissue, and gastrocnemius muscle, and total MUFA showed greater levels in serum as a result of the FA composition of the LA-deprived diet. In EAT, MUFA levels were increased in the −LA group but CLA supplementation reduced them in +LA+CLA and −LA+CLA versus the +LA and −LA groups, respectively. In liver, MUFA levels were not modified by the different diets but the gastrocnemius muscle showed greater levels in both LA-deprived groups. The sum of PUFA levels was markedly reduced because of the LA-deprivation in the serum, liver, adipose tissue, and gastrocnemius muscle (**Table 4**). LA levels showed a significant reduction in both LA-deprived groups independently of the CLA supplementation and tissue considered. Moreover, CLA supplementation also reduced LA levels in the +LA+CLA group in liver and EAT. Likewise, ALA levels were reduced by LA-deprivation and CLA supplementation in liver and EAT, but only by LA-deprivation in gastrocnemius muscles. Mead acid (5c,8c,11c-20:3), a well-known EFA deficiency biomarker derived from the exacerbated synthesis of long chain PUFA of n-9 FA in EFA deficiency, was detected in serum, liver and gastrocnemius muscle of animals fed with −LA and −LA+CLA diets. As a consequence of the detected levels of mead acid, the triene/tetraene ratio could be estimated in serum, liver, and gastrocnemius muscle of animals fed both LA-deprived diets.

Both dietary CLA isomers were incorporated into serum and tissues, and the levels were related to the tissue considered, the type of isomer and LA status (Table 4). In general terms, the adipose tissue showed the highest isomer levels, independently of the LA status. Moreover, although CLA supplementation provided equimolecular amounts of 9c,11t-CLA and 10t,12c-CLA isomers, 9c,11t-CLA showed higher levels than 10t,12c-CLA in serum and tissues, independently of LA levels. Furthermore, in the adipose tissue, LA-deprivation reduced both isomer levels in the −LA+CLA group.

3.3. Serum and Tissue Triacylglycerol Contents and Their Bioregulation

Serum TAG levels were decreased by LA-deprivation and this effect was more pronounced with the CLA supplementation (−LA+CLA group). The liver TAG content was reduced in the −LA group and increased by CLA supplementation in the LA-deprived vs. −LA group. However, the levels of this parameter reached in −LA+CLA were similar to those found in the +LA group. Hepatic TAG-SR was significantly increased in the −LA group, and was normalized by CLA supplementation in the LA-deprived group (**Table 5**). LPL activity in EAT, expressed as total tissue weight, was significantly increased by the LA-deprivation and CLA supplementation, and this effect was exacerbated in the −LA+CLA group. Muscle LPL activity did not show differences among the groups.

The hepatic FAS, ME, and G6PDH activities were raised by the LA-deprivation, and this effect was exacerbated by CLA supplementation in the LA-deprived group (**Table 6**). In EAT, ME and G6PDH activities were increased in the −LA versus +LA group. Additionally, CLA supplementation increased all the measured lipogenic enzyme activities and this effect was exacerbated in the −LA+CLA group.

The activity of the muscular key enzyme on β-oxidation, CPT-1b, did not show difference among the groups.

The liver expression of some key genes and transcriptional factors related to FA biosynthesis (ACC, FAS, and SREBP-1c) and β-oxidation (CPT-Ia, PPAR-α) are shown in **Table 7**. The

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Table 4. Effect of CLA on serum and tissue fatty acid composition on rats fed LA-enriched or LA-deprived diets.

	Experimental groups				ANOVA		
	+LA	−LA	+LA+CLA	−LA+CLA	LA	CLA	LA×CLA
Serum							
9c,12c-18:2	20.20 ± 0.59^{a}	7.89 ± 0.53^{b}	19.19 ± 1.15^{a}	13.42 ± 0.30^{c}	S	S	S
9c,12c,15c-18:3	0.22 ± 0.01	0.20 ± 0.01	0.24 ± 0.02	0.19 ± 0.03	NS	NS	NS
5c,8c,11c-20:3	0.00 ± 0.00^{a}	0.36 ± 0.05^{b}	0.00 ± 0.00^{a}	0.32 ± 0.07^{b}	S	NS	NS
9c,11t-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.74 ± 0.10^{b}	0.89 ± 0.11^{b}	NS	S	NS
10t,12c-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$0.55 \pm 0.06^{b*}$	$0.42 \pm 0.11^{b*}$	NS	S	NS
Triene/Tetraene ratio	0.00 ± 0.00^{a}	0.04 ± 0.00^{b}	0.00 ± 0.00^{a}	0.04 ± 0.00^{b}	S	NS	NS
∑ SFA	35.46 ± 1.22^{a}	44.97 ± 1.10^{b}	37.40 ± 0.82^{a}	47.21 ± 1.66^{b}	S	NS	NS
∑ MUFA	13.30 ± 1.01^{a}	26.70 ± 1.78^{b}	13.96 ± 1.12^{ac}	20.40 ± 1.32^{c}	S	NS	S
∑ PUFA	48.30 ± 0.93^{a}	21.60 ± 0.35^{b}	43.89 ± 1.39^{a}	27.00 ± 0.90^{c}	S	NS	S
Liver							
9c,12c-18:2	21.10 ± 0.63^{a}	5.13 ± 0.20^{b}	15.78 ± 1.27^{c}	7.40 ± 0.52^{b}	S	NS	S
9c,12c,15c-18:3	0.29 ± 0.03^{a}	0.12 ± 0.01^{b}	0.15 ± 0.02^{b}	0.07 ± 0.01^{b}	S	S	S
5c,8c,11c-20:3	0.00 ± 0.00^{a}	0.27 ± 0.04^{b}	0.00 ± 0.00^{a}	0.22 ± 0.03^{b}	S	NS	NS
9c,11t-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.86 ± 0.13^{b}	0.89 ± 0.01^{b}	NS	S	NS
10t,12c-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$0.33 \pm 0.04^{b*}$	$0.23 \pm 0.03^{b*}$	NS	S	NS
Triene/Tetraene ratio	0.00 ± 0.00^{a}	0.03 ± 0.00^{b}	0.00 ± 0.00^{a}	0.03 ± 0.00^{b}	S	NS	NS
∑ SFA	34.30 ± 0.44^{a}	49.94 ± 0.92^{b}	39.84 ± 0.71^{c}	48.90 ± 0.85^{b}	S	S	S
∑ MUFA	25.30 ± 1.45^{ab}	29.12 ± 1.07^{a}	22.57 ± 0.89^{b}	25.25 ± 1.18^{ab}	S	S	NS
∑ PUFA	38.13 ± 1.42^{a}	21.25 ± 1.02^{b}	34.39 ± 1.04^{a}	23.12 ± 0.95^{b}	S	NS	S
Epididymal adipose tissue							
9c,12c-18:2	34.30 ± 1.00^{a}	1.86 ± 0.08^{b}	28.92 ± 1.27^{c}	2.52 ± 0.42^{b}	S	S	S
9c,12c,15c-18:3	0.67 ± 0.05^{a}	0.00 ± 0.00^{b}	0.48 ± 0.06^{c}	0.00 ± 0.00^{b}	S	S	S
5c,8c,11c-20:3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	NS	NS	NS
9c,11t-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.71 ± 0.09^{b}	2.39 ± 0.10^{c}	NS	S	NS
10t,12c-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$1.23 \pm 0.07^{b*}$	$0.61 \pm 0.05^{c*}$	NS	S	NS
∑ SFA	25.07 ± 0.87^{a}	42.82 ± 0.47^{b}	30.91 ± 1.22^{c}	49.87 ± 1.27^{b}	S	S	S
∑ MUFA	38.55 ± 0.32^{a}	45.36 ± 0.22^{b}	34.33 ± 0.34^{c}	41.85 ± 1.62^{a}	S	S	NS
∑ PUFA	35.85 ± 1.06^{a}	1.86 ± 0.08^{b}	33.59 ± 1.49^{a}	5.52 ± 0.59^{b}	S	NS	S
Gastrocnemius muscle							
9c,12c-18:2	21.36 ± 1.34^{a}	6.72 ± 0.52^{b}	23.39 ± 2.01^{a}	9.20 ± 0.99^{b}	S	NS	NS
9c,12c,15c-18:3	0.16 ± 0.02^{a}	0.06 ± 0.02^{b}	0.17 ± 0.04^{a}	0.02 ± 0.00^{b}	S	NS	NS
5c,8c,11c-20:3	0.00 ± 0.00^{a}	0.08 ± 0.02^{b}	0.00 ± 0.00^{a}	0.03 ± 0.01^{c}	S	NS	NS
9c,11t-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.13 ± 0.05^{b}	1.34 ± 0.03^{b}	NS	S	NS
10t,12c-CLA	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$0.60 \pm 0.03^{b*}$	$0.47 \pm 0.08^{b*}$	NS	S	NS
Triene/Tetraene ratio	0.00 ± 0.00^{a}	0.02 ± 0.00^{b}	0.00 ± 0.00^{a}	0.01 ± 0.00^{c}	S	S	S
∑ SFA	33.04 ± 0.46^{a}	43.16 ± 0.03^{b}	33.51 ± 1.49^{a}	41.81 ± 1.16^{b}	S	NS	NS
∑ MUFA	21.10 ± 1.05^{a}	38.72 ± 3.00^{b}	25.63 ± 2.94^{a}	34.30 ± 0.38^{b}	S	NS	NS
∑ PUFA	41.68 ± 0.79^{a}	15.62 ± 2.28^{b}	40.16 ± 2.33^{a}	21.22 ± 1.32^{b}	S	NS	NS

+LA, enriched with linoleic acid; −LA, deprived of linoleic acid; +LA+CLA, +LA supplemented with CLA-enriched oil; −LA+CLA, −LA supplemented with CLA-enriched oil.

CLA, conjugated linoleic acid; ND, non detected; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Values are mean ± SE. Significant differences were established by 2-way ANOVA (2 × 2) followed by the Scheffe test. For the ANOVA test, S indicates $p < 0.05$ and NS indicates $p \geq 0.05$. For the Scheffe test, values in the same row with different letters (a, b, or c) are significantly different ($p < 0.05$). Significant differences ($p < 0.05$) between CLA-isomers levels were established by Student's *t*-test and identified by * between 9c,11t-CLA versus 10t,12c-CLA.

Table 5. Effect of CLA on triacylglycerol contents and their bioregulation in rats fed LA-enriched or LA-deprived diets.

	Experimental groups				ANOVA		
	+LA	−LA	+LA+CLA	−LA+CLA	LA	CLA	LA×CLA
TAG levels							
Serum [g l^{-1}]	1.27 ± 0.17^{a}	0.82 ± 0.06^{b}	1.21 ± 0.09^{a}	0.58 ± 0.03^{c}	S	NS	NS
Liver [μmol g^{-1}]	19.85 ± 1.06^{ac}	15.90 ± 1.17^{b}	23.04 ± 1.72^{c}	19.82 ± 1.55^{ac}	S	S	NS
Gastrocnemius muscle [μmol g^{-1}]	4.63 ± 0.44	3.64 ± 0.32	5.02 ± 0.36	3.71 ± 0.16	S	NS	NS
TAG-SR [ηmol/min/100 g]	160.78 ± 4.83^{a}	240.54 ± 11.06^{b}	155.62 ± 6.18^{a}	169.55 ± 10.07^{a}	S	S	S
Epididymal adipose tissue LPL [ηmol fluorescein/minutes/total EAT]	0.53 ± 0.09^{a}	1.73 ± 0.09^{b}	1.44 ± 0.12^{b}	3.04 ± 0.10^{c}	S	S	S
Gastrocnemius muscle LPL [ηmol fluorescein/minutes/total tissue]	3.56 ± 0.39	3.99 ± 0.51	4.30 ± 0.31	4.20 ± 0.17	NS	NS	NS

+LA, enriched with linoleic acid; −LA, deprived of linoleic acid; +LA+CLA, +LA supplemented with CLA-enriched oil; −LA+CLA, −LA supplemented with CLA-enriched oil.
CLA, conjugated linoleic acid; TAG, triacylglycerol; TAG-SR, triacylglycerol secretion rate; LPL, lipoprotein lipase; EAT epididymal adipose tissue.
Values are mean ± SE. Significant differences were established by 2-way ANOVA (2 × 2) followed by the Scheffe test. For the ANOVA test, S indicates $p < 0.05$ and NS indicates $p \geq 0.05$. For the Scheffe test, values in the same row with different letters (a, b, or c) are significantly different ($p < 0.05$).

mRNA of FAS, CPT-1a, and PPAR-α were significantly increased in the −LA+CLA group.

4. Discussion

CLA might act as an alternative substrate in the LA metabolic pathways producing metabolites with unknown and/or different biological functions. The present study provides evidence of some unknown effects of CLA at a moderated dose on nutritional parameters, serum and tissue TAG levels, and their regulation in rats fed LA-deprived diets compared with rats fed LA-enriched diets. It is important to note that our interest was focused on the effects of dietary CLA supplementation at limited LA-disposal comparing with a LA-enriched diet mimicking the FA profile of the Western diet (rich in LA). In this regard, diets were based on Reeves's[18] recommendations with the modification of the oil source, as previously recommended in the AIN-76A diet.[19] It is worth mentioning that, despite the low ALA levels and the unbalance of the n-6:n-3 FA ratio in corn oil, we did not detect the EFA deficiency biomarkers: Mead acid and triene/tetraene ratio in animals fed +LA and +LA+CLA diets. In contrast, these biomarkers were present in both LA-deprived groups and did not show differences because of the CLA supplementation in liver and serum. In agreement with other authors,[36] these results show that in response to the marginal EFA deficiency (less than 2% of energy as LA), long chain PUFA biosynthesis was exacerbated, driving to a high synthesis of Mead acid (5c,8c,11c-20:3) and, as a consequence, to a higher triene/tetraene ratio. Even though the adipose tissue can

Table 6. Effect of CLA on lipogenic and oxidative enzyme activities in rats fed LA-enriched or LA-deprived diets.

	Experimental groups				ANOVA		
	+LA	−LA	+LA+CLA	−LA+CLA	LA	CLA	LA×CLA
Liver [mU mg^{-1} protein]							
FAS	9.79 ± 1.53^{a}	14.15 ± 0.93^{b}	12.86 ± 0.01^{a}	19.88 ± 0.16^{c}	S	S	S
ME	36.18 ± 1.31^{a}	66.74 ± 2.34^{b}	39.83 ± 3.15^{a}	105.52 ± 0.58^{c}	S	S	S
G6PDH	40.98 ± 2.28^{a}	125.90 ± 8.44^{b}	49.11 ± 2.72^{a}	262.00 ± 10.21^{c}	S	S	S
CPT-1a	1.23 ± 0.12	0.89 ± 0.11	0.97 ± 0.12	1.01 ± 0.11	NS	NS	NS
EAT [mU mg^{-1} protein]							
FAS	9.31 ± 3.04^{a}	13.52 ± 2.01^{a}	14.24 ± 0.72^{b}	16.95 ± 0.31^{b}	S	S	S
ME	164.66 ± 9.12^{a}	228.47 ± 6.63^{b}	193.04 ± 6.91^{c}	316.47 ± 1.51^{c}	S	S	S
G6PDH	141.34 ± 8.65^{a}	175.96 ± 5.54^{b}	161.30 ± 10.47^{ab}	556.62 ± 6.66^{c}	S	S	S
Muscle-CPT-1b [mU mg^{-1} protein]	1.35 ± 0.21	1.28 ± 0.01	1.55 ± 0.23	1.43 ± 0.18	NS	NS	NS

+LA, enriched with linoleic acid; −LA, deprived of linoleic acid; +LA+CLA, +LA supplemented with CLA-enriched oil; −LA+CLA, −LA supplemented with CLA-enriched oil.
CLA, conjugated linoleic acid; FAS, fatty acid synthase; ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; EAT, epididymal adipose tissue; CPT-1, carnitine palmitoyltranferase-1.
Values are mean ± SE. Significant differences were established by 2-way ANOVA (2 × 2) followed by the Scheffe test. For the ANOVA test, S indicates $p < 0.05$ and NS indicates $p \geq 0.05$. For the Scheffe test, values in the same row with different letters (a, b, or c) are significantly different ($p < 0.05$).

Table 7. Effect of CLA on hepatic mRNA levels of enzymes and transcriptional factors involved in lipogenesis and β-oxidation in rats fed LA-enriched or LA-deprived diets.

	Experimental groups				ANOVA		
	+LA	−LA	+LA+CLA	−LA+CLA	LA	CLA	LA×CLA
ACC	1.00 ± 0.25	2.09 ± 0.42	1.76 ± 0.09	1.91 ± 0.27	S	NS	NS
FAS	1.00 ± 0.10^{a}	3.37 ± 1.01^{ab}	2.26 ± 1.22^{a}	12.14 ± 6.18^{b}	NS	S	NS
SREBP-1c	1.00 ± 0.31	1.97 ± 0.63	1.62 ± 1.11	2.14 ± 0.77	NS	NS	NS
CPT-1a	1.00 ± 0.20^{a}	1.53 ± 0.11^{a}	3.29 ± 0.06^{b}	3.71 ± 0.55^{b}	NS	S	S
PPAR-α	1.00 ± 0.46^{a}	1.34 ± 0.12^{ab}	1.50 ± 0.95^{ab}	4.49 ± 1.11^{b}	NS	NS	NS

+LA, enriched with linoleic acid; −LA, deprived of linoleic acid; +LA+CLA, +LA supplemented with CLA-enriched oil; −LA+CLA, −LA supplemented with CLA-enriched oil.

CLA, conjugated linoleic acid; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SREBP-1c, sterol regulatory element-binding protein-1c; CPT-1, carnitine palmitoyltranferase-1; PPAR-α, peroxisome proliferator-activated receptor-α.

Values are mean ± SE. Significant differences were established by 2-way ANOVA (2×2) followed by the Scheffe test. For the ANOVA test, S indicates $p < 0.05$ and NS indicates $p \geq 0.05$. For the Scheffe test, values in the same row with different letters (a or b) are significantly different ($p < 0.05$).

synthesize Mead acid, it has not been detected in the present study. Thus, it is probable that the adipose tissue tends to compensate the low LA levels releasing PUFA into circulation.[37] It is important to consider that, even though the 5c,8c,11c-20:3 is not a substrate for the formation of eicosanoids with known biological functions, it can be released to circulation to substitute n-6 and n-3 PUFA in the biological membranes.[37]

In the present work, higher levels of total CLA were observed in adipose tissue. These results confirm the preferential incorporation of CLA in tissues rich in neutral lipids.[38] On the other hand, the LA status differentially influences the isomer content. While in liver, muscle, and serum both isomers had similar levels regardless of the LA status, in epididymal adipose tissue the content of both isomers 9c,11t-CLA and 10t,12c-CLA, was lower in animals receiving LA-deprived than LA-enriched diet. This might be explained by higher levels and expression of uncoupling protein-I in adipose tissue in animals deprived of EFA, as described by Portillo.[39] In addition, taking into account the similarity of the CLA with LA and the EFA demand by tissues in the LA-deprived groups, an increase of CLA isomers flow from adipocytes to circulation, might be occurring.

The 9c,11t-CLA and 10t,12c-CLA content in tissues and serum depends on diverse factors such as amount of isomer ingested, dietary treatment time, affinity for tissue incorporation, secretion rate of the esterified isomer, and oxidation capacity. In all the analyzed tissues, we found that the 9c,11t-CLA content was higher than 10t,12c-CLA, and this difference can be attributed to the higher oxidation capacity of 10t,12c-CLA isomer and to a greater tendency of 9c,11t-CLA to follow metabolic elongation and desaturation pathways for incorporation into lipids. These effects have been demonstrated by other authors.[17,40]

CLA supplementation reduced the levels of LA and ALA in the liver and adipose tissue of animals fed the LA-enriched diet. It is probable that CLA compete with both FA for the tissue and organs absorption/incorporation. Moreover, a higher mitochondrial and peroxisomal β-oxidation of PUFA could exist induced by 10t,12c-CLA, which exacerbated the LA oxidation lowering these FA levels.[11,17,41]

In the present study, we did not observe changes in the body weight gained during the experimental period. Nevertheless, CLA supplementation showed a different behavior on nutritional parameters, depending on the LA status. Thus, in animals fed LA-enriched diets, CLA did not change the liver, EAT, and RPAT weights. On the contrary, in animals fed LA-deprived diets, CLA reduced EAT and RPAT and increased liver weight. These changes were not associated with a lower intake of food or energy, but were associated with lower energy efficiency. Similar effects due to CLA intake have been observed by other authors,[11,42–45] mainly in mice. In contrast, little effects or lack of them have been observed in rats or other animals models.[40,46] Concerning the nutritional status, other author have observed a greater tendency to loss of body fat induced by CLA in mice, due to the consumption of diets containing coconut fat or fat free diets.[47–49] Very little data exists regarding the possible mechanisms by which coconut oil or EFA deprived diets emphasize the antiobesogenic CLA response.[48,49]

According to our findings, the regulatory mechanisms involved in the CLA effect on adipose tissue appear to depend on the LA status. Thus, in the LA-enriched group, CLA increased LPL activity in the epididymal adipose tissue. A similar effect has been observed in 3T3-L1 cells culture by other authors.[50] Nevertheless, a decrease in LPL activity has also been reported in cell cultures[51] as well as in vivo studies, the 10t,12c-CLA isomer being responsible for this effect.[52,53] In addition to the higher LPL activity, we found greater FAS and G6PDH activities in adipose tissue but, surprisingly, these results were not accompanied by changes in the tissue weight. Probably there exists in parallel a higher hormone sensitive-lipase activity, which generates a greater lipolysis, as it has been demonstrated to occur in cell cultures by CLA supplementation.[9,52] On the other hand, in the LA-deprived group, LPL and lipogenic enzyme activities were also increased but, in this case, the effect could be a compensatory mechanism resulting from the marked reduction in the adipose tissue induced by CLA. Similar results have been observed in different animal models by other authors.[53] In our experience, these mechanisms tending to increased lipid accretion seem not to compensate the higher

lipolysis and β-oxidation that could be occurring as a result of the CLA supplementation.[9,52]

In the liver, the CLA supplementation increased TAG levels only in animals fed the LA-deprived diet compared with its respective control group. This effect was associated with a decreased TAG-SR and this could be related to a decreased synthesis of apolipoprotein B-100, as demonstrated by Lin et al.[50] in HepG2 cells due to the 10t,12c-CLA isomer consumption. Moreover, the higher FAS, ME, G6PDH activities and the increased mRNA levels of ACC with normal CPT-Ia activity clearly demonstrated an unbalance between lipogenesis and β-oxidation. Nevertheless, mRNA levels of CPT-1a and PPAR-α were increased; probably, this is a compensatory effect in response to the higher lipogenesis. Other authors have also found an increase in liver TAG levels associated with a higher lipogenesis in mice[54] and this effect has been attributed to the 10t,12c-CLA isomer.[14,15] On the contrary, Purushotham et al.[55] in rats and Wang et al.[56] in genetically obese rats reported that CLA supplementation reduced liver TAG levels by different mechanisms. We have not investigated the possible factors that could drive to this higher lipogenesis, but other authors have described the lipoatrophic syndrome induced by the CLA, associated with a chronic hyperinsulinemia, factor that favors fat deposition in the liver through an induction of a higher FA uptake and lipogenic pathway.[13–15] We cannot deny that some of these mechanisms could be involved in our experiment. Furthermore, in a previous work we studied glucose utilization in isolated skeletal muscle and demonstrated that CLA lowered the insulin response capacity for glucose utilization at both dietary LA levels, effect that could be mediated by leptin and adiponectin.[57]

There are controversial reports about the effect of CLA isomers on serum TAG levels. We observed a differential effect depending on the LA dietary levels. Specifically, in rats fed diets enriched in LA, the CLA did not affect TAG levels, showing a correlation with the normal TAG-SR and the muscle removal by LPL. On the contrary, in the LA-deprived group, CLA decreased the TAG levels associated with a reduced TAG-SR and a higher removal by the LPL in adipose tissue. These TAG reduced levels are in agreement with those reported by other authors.[58] Finally, the muscle TAG levels were not modified by CLA, and this effect was accompanied by a normal TAG uptake and FA oxidation, represented by LPL and CPT-1b activity, respectively.

5. Conclusions

The effect of CLA on nutritional parameters and TAG metabolism differed depending on the dietary LA status. CLA in the −LA group induced a marked reduction in fat depots with an increased LPL and lipogenic enzyme activities as compensatory mechanisms. In the liver, the CLA supplementation normalized TAG levels in LA-deprived animals, associated with a decreased TAG-SR and increased lipogenesis. Notoriously, in LA-deprived animals, CLA markedly reduced the serum TAG levels, associated with a higher TAG removal from adipose tissue with normal hepatic TAG-SR. In the +LA group, the CLA were incorporated into tissues without causing significant changes in the evaluated parameters. Thus, CLA showed certain beneficial biological and nutritional effects in LA-deprived animals. Probably, a low LA intake could result in an increased sensitivity to the CLA, or in diets rich in LA, the CLA do not compete with the metabolism of n-6 PUFA, leading to differential effects in both metabolic situations. This does not preclude the fact that different levels of bioactive metabolites can be generated from the CLA depending on the LA status.

Abbreviations

AA, arachidonic acid; ACC, acetyl-CoA carboxylase; ALA, α-linolenic acid; CLA, conjugated linoleic acid; CPT, carnitine palmitoyl transferase; EAT, epididymal adipose tissue; EFA, essential fatty acids; FA, fatty acids; FAME, fatty acid methyl esters; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; LA, linoleic acid; LPL, lipoprotein lipase; ME, malic enzyme; MUFA, monounsaturated fatty acids; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; RPAT, retroperitoneal adipose tissue; SFA, saturated fatty acids; SREBP, sterol regulatory element-binding protein; TAG, triacylglycerol; TAG-SR, triacylglycerol-secretion rate; UBC, ubiquitin C.

Acknowledgements

The authors wish to thank Walter DaRú for his technical assistance. The authors also wish to acknowledge the support received from Universidad Nacional del Litoral – Cursos de Acción para la Investigación y Desarrollo (CAI + D 2011 Tipo II. #501 201101 00165 LI) and from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP CONICET # 112-201101-00786).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

conjugated linoleic acid, linoleic acid, lipid metabolism, nutrition, triacylglycerol bioregulation

Received: August 22, 2018
Revised: December 18, 2018
Published online:

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