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Conjugated linoleic acid improves glucose utilization in the soleus muscle of rats fed linoleic acid–enriched and linoleic acid–deprived diets

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ABSTRACT

The effect that conjugated linoleic acid (CLA) has on glucose metabolism in experimental animals depends on nutritional conditions. Therefore, we hypothesized that CLA improves glucose utilization and insulin sensitivity in rats fed different levels of dietary linoleic acid (LA). We investigated the effect of CLA on the uptake, incorporation, and oxidation of glucose and glycogen synthesis in the soleus muscle of rats who were fed either LA-enriched ($^{+}$LA) or LA-deprived ($^{-}$LA) diets, under basal conditions and in the absence or presence of insulin and/or palmitate. For 60 days, male Wistar rats were fed 1 of 4 diets consisting of $^{+}$LA, $^{-}$LA, or $^{+}$LA and $^{-}$LA supplemented with CLA. Nutritional parameters and soleus glucose metabolism were evaluated. Under basal conditions, CLA enhanced soleus glucose oxidation, whereas increased glucose uptake and incorporation were observed in the $^{-}$LA + CLA group. Conjugated linoleic acid–supplemented rats presented a lower response to insulin on glucose metabolism compared with non–CLA-supplemented rats. Palmitate partially inhibited the effect of insulin on the uptake and incorporation of glucose in the $^{+}$LA and $^{-}$LA groups but not in the $^{+}$LA + CLA or $^{-}$LA + CLA groups. Dietary CLA increased glucose utilization under basal conditions and prevented the palmitate-induced inhibition of glucose uptake and incorporation that is stimulated by insulin. The beneficial effects of CLA were better in LA-deprived rats. Conjugated linoleic acid may also have negative effects, such as lowering the insulin response capacity. These results demonstrate the complexities of the interactions between CLA, palmitate, and/or insulin to differentially modify muscle glucose utilization and show that the magnitude of the response is related to the dietary LA levels.

Abbreviations: AIN, American Institute of Nutrition; ALA, α-linolenic acid; CLA, conjugated linoleic acid; c9,t11, *cis*-9,*trans*-11; EFAs, essential fatty acids; FAs, fatty acids; FAMEs, fatty acid methyl esters; GLUT-4, glucose transporter-4; LA, linoleic acid; PUFAs, polyunsaturated fatty acids; t10,c12, *trans*-10,*cis*-12.

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1. Introduction

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers that are derived from octadecadienoic acids conjugated with double bonds. *Cis*-9,*trans*-11 (*c*9,*t*11)–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 *c*9, t11-CLA and *trans*-10,*cis*-12 (*t*10,*c*12)–CLA mixture (~37%-40% each). The interest in CLA has increased in recent decades because of its potential preventive effects for different chronic diseases [1–3]. As a result, either industrially synthesized or natural CLA is currently used in functional foods or directly consumed in capsule form.

Conjugated linoleic acid exerts numerous beneficial effects in humans and experimental animal models, such as body fat reduction; protection from cancer, atherosclerosis, diabetes, and glucose intolerance; and the improvement of certain immune functions. The effects of the 2 main CLA differ among animal models depending on multiple factors, such as species, age, dosage, treatment duration, and pathophysiologic state. Regarding carbohydrate metabolism, CLA has improved glucose intolerance and normalized hyperinsulinemia in different experimental rat models (prediabetic, obese-diabetic insulin resistant, and those fed with high-fat diet) [4,5]. These effects have also been observed in female mice fed high-fat diets and in genetically obese mice (lep^{ob}/lep^{ob}). Nevertheless, partially positive effects [6] and a lack thereof [7,8] have also been observed in mice fed a linoleic acid (LA)–enriched diet that was supplemented with CLA ($^{+}$LA + CLA). In addition, the literature describes the deterioration of glycemic control and insulin sensitivity associated with lipodystrophy [9–12].

Under normal conditions, insulin increases glucose uptake and metabolism in skeletal muscle by activating the phosphatidylinositol-3-kinase/Akt pathway and inactivating Akt substrate 160 (AS160), which translocates glucose transporter-4 (GLUT-4) from the intracellular vesicles to the plasma membrane to elevate glucose metabolism [13]. However, these effects are significantly impaired under insulin resistant conditions, including obesity, type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease [14,15]. Although the mechanisms involved in the development of insulin resistance in skeletal muscle are not completely understood, some authors suggest that the elevated disposal of saturated nonesterified fatty acids (FAs), such as palmitic and stearic acids, is involved [16,17]. In fact, when skeletal muscle or isolated myocytes are exposed to saturated long-chain FAs, insulin sensitivity is decreased [15,17,18]. To the best of our knowledge, the effects of CLA on the palmitate-induced insulin resistance in skeletal muscle remain unknown. Accordingly, they constitute the focus of this investigation.

Among other factors, the CLA effects depend on the dietary LA levels, which share the metabolic pathways of polyunsaturated FA (PUFA) biosynthesis and eicosanoids formation. Recently, we demonstrated that male Wistar rats that were fed a diet deprived of LA but supplemented with a 1% CLA mixture showed an important reduction in the weight of adipose tissue [19]. This could be related to an increase in glucose metabolism, mainly in the skeletal muscle. Thus, we hypothesized that CLA improves glucose utilization and insulin sensitivity of rats fed different levels of dietary LA. Consequently, we aimed to investigate the CLA effects on glucose uptake, incorporation, and oxidation, as well as glycogen synthesis in the soleus muscle of rats fed a diet either enriched or deprived of LA under basal conditions and in the absence and/or presence insulin and palmitate, which are 2 key modulators of glucose metabolism.

2. Methods and materials

2.1. Materials

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), hydrogenated coconut oil without TFA (Castoroil, Buenos Aires, Argentina), sucrose, cellulose, and corn starch, which were locally obtained. Conjugated LA–enriched oil was obtained from Lipid Nutrition BV (Wormerveer, The Netherlands) and consisted of an equimolecular mixture of 9*c*,11*t*-CLA and 10*t*,12*c*-CLA. Corn oil was used as an unsaturated *cis*-FA source enriched with LA, to resemble the FA composition of the Western diet. Coconut oil was used to produce an essential FA (EFA)–deficient status. 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 FAs were purchased from Sigma Chemical Co (St Louis, MO, USA). The glucose test kits were commercially obtained from Sociedad de Bioquímicos (Santa Fe, Argentina). The insulin used was Humulin R (U-100), which was acquired from Eli Lilly (Indianapolis, IN, USA). Amersham International (Bucks, UK) provided the 2-desoxi-[2,6-^{3}H]D-glucose and [U-^{14}C]D-glucose.

2.2. Animals, diets, and experimental design

The experimental procedures were approved by the Ethics Committee of the School of Biochemistry and by the Ethics Committee of the Institute of Biomedical Sciences, University of Sao Paulo. Both followed the Guide for the Care and Use of Laboratory Animals [20].

Male Wistar rats were housed in an animal facility under controlled conditions (23°C ± 2°C and a 12-hour light-dark cycle). After reaching 100 to 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: enriched with LA ($^{+}$LA), deprived of LA ($^{-}$LA), $^{+}$LA supplemented with CLA-enriched oil ($^{+}$LA + CLA), or $^{-}$LA supplemented with CLA-enriched oil ($^{-}$LA + CLA).

The ingredient composition of the diets is presented in Table 1. The diets are based on the American Institute of Nutrition ad hoc writing committee recommendation (AIN-93G) [21], except for the FA source that was based on AIN-76 [22]. All diets were isoenergetic, theoretically providing 16.6 kJ/g. The $^{+}$LA diet contained 7% corn oil (20% of energy) as a dietary lipid source and was considered the control group for the comparisons. In the $^{-}$LA diet, the corn oil was replaced with 7% of hydrogenated coconut fat. Conjugated LA supplementation

Table 1 – Ingredient and FA composition of the experimental diets fed to rats [a]

Ingredient [b]	$^{+}$LA	$^{-}$LA	$^{+}$LA + CLA	$^{-}$LA + CLA
Corn starch	529.5	529.5	529.5	529.5
Casein	200	200	200	200
Sucrose	100	100	100	100
Fiber	50	50	50	50
Vitamin mixture	35	35	35	35
Mineral mixture	10	10	10	10
L-Cystine–L-methionine	3.0	3.0	3.0	3.0
Choline	2.5	2.5	2.5	2.5
Corn oil	70	0	60	0
Coconut fat	0	70	0	60
CLA rich oil	0	0	10	10
FA composition [c]				
6:0	ND	0.49	ND	0.42
8:0	ND	6.76	ND	5.79
10:0	ND	5.64	ND	4.83
11:0	ND	0.02	ND	0.02
12:0	ND	47.67	ND	40.86
13:0	ND	0.02	ND	0.02
14:0	0.031	17.46	0.03	14.97
16:0	12.21	9.21	11.30	8.73
16:1	0.12	ND	0.10	ND
18:0	1.93	12.53	1.83	10.91
9c-18:1	31.95	0.05	28.68	1.34
11c-18:1	0.54	ND	0.52	0.06
9c,12c-18:2	51.26	0.01	44.09	0.16
20:0	0.50	0.14	0.43	0.12
11c-20:1	0.25	ND	0.21	ND
9c,12c,15c-18:3	0.88	ND	0.75	ND
22:0	0.16	ND	0.19	0.05
24:0	0.15	ND	0.13	ND
9c,11t-CLA	ND	ND	5.57	5.57
11c,13t-CLA	ND	ND	0.22	0.22
10t,12c-CLA	ND	ND	5.54	5.54
∑NI	ND	ND	0.44	0.44
Energy (kJ/g)	16.6	16.6	16.6	16.6

[a] Diets were prepared according to AIN-93G [21], except for the FA source, which was based on AIN-76 [22]. Diets: $^{+}$LA, enriched with LA; $^{-}$LA, deprived of LA; $^{+}$LA + CLA, $^{+}$LA supplemented with CLA-enriched oil; $^{-}$LA + CLA, $^{-}$LA supplemented with CLA-enriched oil. ND, nondetected; NI, other nonidentified FAs.
[b] Grams per kg diet.
[c] Weight percentage of total FAs.

was achieved by replacing 1% of corn oil ($^{+}$LA + CLA) or 1% of coconut fat ($^{-}$LA + CLA) with CLA-enriched oil. The only difference between the diets was the type of lipid. Each diet was freshly prepared every 3 days, throughout the experimental period.

2.3. Experimental protocol

Throughout the entire dietary treatment period, the rats were weighed and the food intake was recorded 3 times per week. Food was removed at 6:00 AM on the morning of day 60, and the rats were anesthetized (1 mg of acepromazine + 100 mg of ketamine/kg of body weight) 4 hours later (at 10:00 AM). The blood and soleus muscle were extracted for the assays. Because of the high metabolic and oxidative capacity, soleus muscles were used to measure the insulin responsiveness related to glucose metabolism.

2.4. Fatty acids composition in serum and dietary lipids

The total lipids in the serum (n = 6 per group) were extracted, using the method described by Bligh and Dyer [23]. The FAs in the serum and dietary lipids were analyzed by gas chromatography. The FA methyl esters (FAMEs) were obtained from the base-catalyzed methanolysis of the glycerides (KOH in methanol), after dissolving the lipid extract in high-performance liquid chromatography quality hexane [24]. The total FA profile was recorded by analyzing the FAME on a gas-liquid chromatograph (Shimadzu GC 2014, Kyoto, Japan) with an autoinjector (AOC-20i) Split/Splitless equipped with a flame ionization detector and a CP Sil 88 fused silica capillary column (100 m × 0.25 mm × 0.2 μm, film thickness; Varian, Lake Forest, CA, USA), according to the American Oil Chemists' Society Official Method [25]. The FAMEs were identified by comparing their retention times to those of commercial standards. The values were expressed as a percentage of the total FAME. The detection limit for the main FAME identified ranged from 0.01% to 0.03%.

2.5. Biomarkers of EFA status and CLA incorporation

The EFA deficiency generated from the dietary treatment in the $^{-}$LA and $^{-}$LA + CLA groups was evaluated using the decrease in the LA and α-linolenic acid (ALA; 9c,12c,15c-18:3) levels, as well as the increase in the levels of mead acid (5c,8c,11c-20:3) and the triene/tetraene (5c,8c,11c-20:3/5c,8c,11c,14c-20:4) ratio in the serum. The incorporation of CLA isomers in the $^{+}$LA + CLA and $^{-}$LA + CLA groups was evaluated based on the individual isomer levels in the serum.

2.6. Glucose levels in serum

The glucose levels in the serum of rats (n = 6 per group) fasted for 4 hours were determined by spectrophotometry, using commercially available test kits (Sociedad de Bioquímicos).

2.7. Glucose metabolism studies

2.7.1. Preincubation of strips of soleus muscle

The soleus muscles (n = 4 per group) were rapidly and carefully isolated and preincubated, as previously described [26–28]. Briefly, isolated soleus muscles were split longitudinally into equal strips weighing 25 to 35 mg, attached to stainless steel clips to maintain resting tension, and preincubated for 4 hours at 35°C in Krebs-Ringer buffer at pH 7.4 (containing 5 mM glucose and 1 % wt/vol bovine serum albumin) that was gassed with 95% O_2/5% CO_2 in the presence or absence of 400 μM palmitate. This protocol has been previously used to induce muscle insulin resistance with palmitate [29]. After this period, the incubation was performed as described below.

2.7.2. Glucose uptake, incorporation and oxidation, and glycogen synthesis measurements

Soleus muscle strips were transferred to other vials containing the same buffer supplemented with 0.2 μCi/mL [U-^{14}C] D-glucose and 0.2 μCi/mL 2-deoxy-[2,6-^{3}H]D-glucose. They were then incubated for 1 hour in the absence or presence of 7 nM insulin. The [U-^{14}C]D-glucose oxidation was estimated

based on the $^{14}CO_2$ production, according to Leighton et al [30]. A microtube containing filter paper embedded in 2 N NaOH was inserted into the vials to absorb the $^{14}CO_2$ released during the incubation period. The ^{14}C radioactivity from the filter paper was then quantified to determine the $^{14}CO_2$ content. To determine the 2-deoxy-[2,6-^{3}H]D-glucose uptake, [U-^{14}C]D-glucose incorporation, and ^{14}C-glycogen synthesis, the muscles were digested with 1N KOH at 70°C for 20 minutes at the end of the incubation [31,32]. An aliquot of the muscle homogenate was used to quantify the ^3H and ^{14}C radioactivity, in order to determine the 2-deoxy-[2,6-^{3}H]D-glucose uptake and [U-^{14}C]D-glucose incorporation, respectively. Another aliquot of the muscle homogenate was used to measure the ^{14}C-glycogen synthesis. Briefly, the total muscle glycogen was precipitated using a saturated solution of Na_2SO_4 and nonlabeled glycogen solution in a cold ethanolic solution (–20°C). The samples were centrifuged at 400 × *g*, and glycogen was resuspended in distilled water. The ^{14}C radioactivity was then quantified to determine the ^{14}C-glycogen synthesis [32].

2.8. Statistical analyses

The values are expressed as the means ± SE of 4 or 6 animals per group. The minimum sample size needed to detect a statistically significant difference ($P < .05$) was calculated [33]. A sample size of n = 6 had an 80 % power ($P = .05$), whereas a sample size of n = 4 had a 70 % power ($P = .05$). Significant differences between mean values were established with a 2-way analysis of variance (ANOVA; 2 × 2), using the LA levels and CLA supplementation as independent variables. All post hoc multiple comparisons were made using Scheffe critical range test. To compare the effect of insulin and palmitate in each group with its basal value, significant differences between means were established with an unpaired Student *t* test. Differences were considered statistically significant at $P < .05$ [34].

3. Results

3.1. Physiological status, nutritional parameters, and FA composition

During the experimental period, all animals appeared healthy without any physiological manifestations of EFA deficiency or pathological signs due to the treatments received. As shown in Table 2, the average daily food intake and body weight variation did not differ between the groups. Conjugated LA decreased the glucose levels, but this difference was only significant between the $^+$LA + CLA and $^+$LA (control) groups.

The EFA deficiency protocol used in this study, but not CLA, significantly increased the serum biomarkers of EFA deficiency, mead acid levels, and the triene/tetraene ratio (Table 3). The serum levels of FA—12:0, 14:0; LA, 9c,11t-CLA, and 10t,12c-CLA, but not 18:0, 9c-18:1, and ALA—correlated with the FA profile of the diet. Conjugated LA attenuated the lower serum LA levels in the $^-$LA group, but the arachidonic acid (5c,8c,11c,14c-20:4) levels were equally reduced in the serum of both groups of LA-deprived rats. The 2 independent dietary variables, CLA and LA, did not modify the n-3 PUFA levels.

3.2. Glucose metabolism

Glucose uptake, incorporation, and oxidation, as well as glycogen synthesis in the soleus muscle, at either basal conditions or in the presence of insulin, palmitate, and insulin plus palmitate are shown in Table 4. At basal conditions, glucose uptake increased in the $^-$LA + CLA group compared with the $^+$LA group. Independently of the dietary treatments, insulin stimulation increased glucose uptake when compared with the basal condition. Nevertheless, compared with the $^+$LA group, this parameter was lower in the $^+$LA + CLA group. These changes were more evident when the response to insulin was presented as relative units in relation to its basal value (+Insulin/Basal). In this regard, significant differences were found in the CLA supplemented ($^+$LA + CLA and $^-$LA + CLA) groups compared with the $^+$LA group. The palmitate treatment did not inhibit glucose uptake; however, the prevailing differences between the groups observed at basal conditions are not presented. This finding is reflected in the lack of differences in the glucose uptake response with respect to basal conditions (+Palmitate/Basal). Palmitate treatment in the presence of insulin (+Insulin + Palmitate) partially, but significantly, inhibited the effect on glucose incorporation stimulated by insulin (+Insulin) in $^+$LA and $^-$LA but not in the $^+$LA + CLA and $^-$LA + CLA groups. The relative response in relation to its basal value (+Insulin + Palmitate/Basal) decreased in the $^-$LA + CLA group compared with the $^+$LA group.

The glucose incorporation at basal conditions was higher in the $^-$LA + CLA group than in the $^+$LA group. As expected, insulin stimulation increased glucose incorporation in all

Table 2 – Effect of CLA on growth and plasma glucose in rats fed LA-enriched or LA-deprived diets

	Experimental groups				ANOVA		
	$^+$LA	$^-$LA	$^+$LA + CLA	$^-$LA + CLA	LA	CLA	LA × CLA
Food intake (g/d)	17.71 ± 0.50	18.70 ± 0.35	18.80 ± 1.22	18.16 ± 0.76	NS	NS	NS
Initial weight (g)	141.8 ± 23.4	130.0 ± 4.8	129.0 ± 19.8	140.5 ± 29.7	NS	NS	NS
Final weight (g)	350.8 ± 25.0	352.7 ± 5.9	337.8 ± 22.8	345.3 ± 16.6	NS	NS	NS
Plasma glucose (g/L)	1.32 ± 0.09^a	1.21 ± 0.05^{ab}	0.98 ± 0.08^b	1.06 ± 0.08^{ab}	NS	S	NS

Experimental groups: $^+$LA, enriched with LA; $^-$LA, deprived of LA; $^+$LA + CLA, $^+$LA supplemented with CLA-enriched oil; $^-$LA + CLA, $^-$LA supplemented with CLA-enriched oil.
Data are means ± SE; n = 6 per group. Significant differences were established by 2-way ANOVA (2 × 2) followed by Scheffe test. For the ANOVA test, S indicates $P < .05$ and NS indicates $P > .05$. For Scheffe test, values in the same row with different superscript letters are significantly different ($P < .05$).

Table 3 – Effect of CLA on serum FAs levels in rats fed LA-enriched or LA-deprived diets *

	Experimental groups				ANOVA		
	$^{+}$LA	$^{-}$LA	$^{+}$LA + CLA	$^{-}$LA + CLA	LA	CLA	LA × CLA
12:0	ND^{a}	1.41 ± 0.19^{b}	ND^{a}	1.01 ± 0.24^{b}	S	NS	NS
14:0	0.31 ± 0.03^{a}	2.35 ± 0.28^{b}	0.48 ± 0.05^{a}	1.48 ± 0.16^{c}	S	NS	S
16:0	20.06 ± 0.31	20.66 ± 0.96	21.98 ± 0.68	20.13 ± 0.69	NS	NS	NS
9c-16:1	0.90 ± 0.22^{a}	4.37 ± 0.13^{b}	1.69 ± 0.38^{a}	3.21 ± 0.35^{b}	S	NS	S
18:0	15.09 ± 1.16^{a}	16.95 ± 1.35^{ab}	14.73 ± 1.06^{a}	21.37 ± 1.42^{b}	S	NS	NS
9c-18:1	9.65 ± 0.80^{a}	17.03 ± 1.52^{b}	10.04 ± 0.58^{a}	12.85 ± 1.03^{ab}	S	NS	S
11c-18:1	2.66 ± 0.17^{a}	5.14 ± 0.21^{b}	2.09 ± 0.36^{a}	4.36 ± 0.18^{b}	S	NS	NS
9c,12c-18:2	20.20 ± 0.59^{a}	7.89 ± 0.53^{b}	19.91 ± 1.15^{a}	13.42 ± 0.30^{c}	S	S	S
6c,9c,12c-18:3	0.09 ± 0.02^{a}	0.26 ± 0.02^{b}	0.19 ± 0.02^{b}	ND^{a}	NS	S	S
11c-20:1	0.11 ± 0.01^{a}	0.16 ± 0.03^{a}	0.14 ± 0.01^{a}	ND^{b}	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
9c,11t-CLA	ND^{a}	ND^{a}	0.74 ± 0.10^{b}	0.89 ± 0.11^{b}	NS	S	NS
10t,12c-CLA	ND^{a}	ND^{a}	0.55 ± 0.06^{b}	0.42 ± 0.11^{b}	NS	S	NS
11c,14c-20:2	0.45 ± 0.03^{a}	0.16 ± 0.04^{b}	0.29 ± 0.03^{c}	0.30 ± 0.03^{c}	S	NS	S
22:0	ND^{a}	3.60 ± 0.17^{b}	0.21 ± 0.03^{a}	3.22 ± 0.36^{b}	S	NS	NS
8c,11c-20:2	ND^{a}	0.41 ± 0.03^{b}	ND^{a}	0.32 ± 0.07^{c}	S	S	S
5c,8c,11c-20:3	ND^{a}	0.36 ± 0.05^{b}	ND^{a}	0.32 ± 0.07^{b}	S	NS	NS
8c,11c,14c-20:3	0.46 ± 0.04^{a}	1.12 ± 0.07^{b}	0.46 ± 0.04^{a}	1.33 ± 0.11^{b}	S	NS	NS
11c,14c,17c-20:3	ND^{a}	0.35 ± 0.08^{b}	ND^{a}	ND^{a}	S	S	S
5c,8c,11c,14c-20:4	24.95 ± 0.76^{a}	9.50 ± 0.56^{b}	20.37 ± 1.06^{a}	7.70 ± 0.30^{b}	S	S	S
4c,7c,10c,13c,16c,19c-22:6	1.96 ± 0.18	1.70 ± 0.07	1.14 ± 0.18	2.11 ± 0.34	NS	NS	NS
∑NI	1.97 ± 0.15^{a}	1.43 ± 0.11^{a}	2.09 ± 0.31^{a}	0.44 ± 0.06^{b}	S	S	S
Triene/Tetraene ratio	ND^{a}	0.04 ± 0.00^{b}	ND^{a}	0.04 ± 0.01^{b}	S	NS	NS

Data are means ± SE; n = 6 per group. Significant differences were established by 2-way ANOVA (2 × 2) followed by Scheffe test. For the ANOVA test, S indicates $P < .05$ and NS indicates $P > .05$. For Scheffe test, values in the same row with different superscript letters are significantly different ($P < .05$).

* Weight percentage of total FAs. Experimental groups: $^{+}$LA, enriched with LA; $^{-}$LA, deprived of LA; $^{+}$LA + CLA, $^{+}$LA supplemented with CLA-enriched oil; $^{-}$LA + CLA, $^{-}$LA supplemented with CLA-enriched oil. ND, nondetected; NI, other nonidentified FAs.

groups. Similar to the effect on glucose uptake, glucose incorporation in the presence of insulin decreased in the CLA-supplemented ($^{+}$LA + CLA and $^{-}$LA + CLA) groups compared with the $^{+}$LA group. As a consequence, the response to insulin in relation to the basal value (+Insulin/Basal) was markedly reduced in the $^{-}$LA, $^{+}$LA + CLA, and $^{-}$LA + CLA groups compared with the $^{+}$LA group. Again, the addition of palmitate did not inhibit glucose incorporation at basal conditions, which is similar to the effect on the glucose uptake. The palmitate treatment in the presence of insulin (+Insulin + Palmitate) partially inhibited the effect on the glucose incorporation stimulated by the hormone (+Insulin) in the $^{+}$LA and $^{-}$LA groups but not in the $^{+}$LA + CLA and $^{-}$LA + CLA groups. However, the dietary groups and the relative response did not differ from the basal levels (+Insulin + Palmitate/Basal).

Glucose oxidation in the soleus muscle was higher in the $^{+}$LA + CLA and $^{-}$LA + CLA groups at basal conditions. The addition of insulin increased glucose oxidation in the $^{+}$LA group only. This response was clearly more pronounced in the + Insulin/Basal ratio. The addition of palmitate tended to reduce glucose oxidation only in the $^{+}$LA group, as compared with the basal value. Under these conditions, glucose oxidation was higher in the $^{+}$LA + CLA and $^{-}$LA + CLA groups than in the $^{+}$LA and $^{-}$LA groups. The palmitate treatment in the presence of insulin (+Insulin + Palmitate) significantly inhibited the insulin effect (+Insulin) in the $^{+}$LA group, but it did not modify the modest effects observed in the remaining experimental groups.

Glycogen synthesis in the soleus muscle did not differ between the groups at basal conditions. Insulin stimulation significantly increased the glycogen synthesis. However, the final values were lower in the $^{-}$LA, $^{+}$LA + CLA, and $^{-}$LA + CLA groups compared with the $^{+}$LA group. Moderate effects on glycogen synthesis were observed in response to palmitate treatment, showing a reduction in the $^{-}$LA group vs the basal values only. The palmitate treatment in the presence of insulin (+Insulin + Palmitate) attenuated the stimulation of glycogen synthesis by this hormone in the $^{+}$LA group; however, the inhibitory effect of palmitate was not observed in the $^{-}$LA, $^{+}$LA + CLA, and $^{-}$LA + CLA groups.

4. Discussion

In the present study, we demonstrate that CLA might positively affect glucose utilization in skeletal muscle depending on the metabolic state of the experimental animal. However, it appears that CLA may also have negative effects, lowering the insulin response capacity on glucose metabolism.

Conjugated LA has been predominantly related to beneficial properties on glucose metabolism in a variety of metabolic disorders in rats [35]. Nevertheless, deleterious effects on glucose metabolism due to CLA supplementation have been observed in several animal models [6,11]. Recently, we demonstrated [19] that rats fed a diet deprived of LA and supplemented with a CLA mixture presented diverse alterations in lipid metabolism. These alterations were associated with changes in glucose utilization and glycogen synthesis, even with normal levels of glucose. Although the animals fed

Table 4 – Effect of CLA on glucose uptake, incorporation and oxidation, and glycogen synthesis in soleus muscle of rats fed LA-enriched or LA-deprived diets

	Experimental groups				ANOVA		
	$^{+}$LA	$^{-}$LA	$^{+}$LA + CLA	$^{-}$LA + CLA	LA	CLA	LA × CLA
Glucose uptake (μmol/g)							
Basal	1.88 ± 0.05^{a}	2.22 ± 0.17^{ab}	2.21 ± 0.08^{ab}	2.65 ± 0.15^{b}	S	S	NS
+Insulin	5.30 ± 0.15^{a*}	4.75 ± 0.35^{ab*}	4.13 ± 0.25^{b*}	4.43 ± 0.18^{ab*}	NS	S	NS
+Palmitate	1.96 ± 0.09	1.96 ± 0.07	2.11 ± 0.15	2.11 ± 0.06	NS	NS	NS
+Insulin + Palmitate	3.88 ± 0.07$^{*\#}$	3.37 ± 0.09$^{*\#}$	3.52 ± 0.36*	3.67 ± 0.27*	NS	NS	NS
+Insulin/Basal	2.82 ± 0.10^{a}	2.18 ± 0.19^{ab}	1.88 ± 0.15^{b}	1.69 ± 0.13^{b}	S	S	NS
+Palmitate/Basal	1.05 ± 0.07	0.90 ± 0.06	0.97 ± 0.09	0.81 ± 0.06	NS	NS	NS
+Insulin + Palmitate/Basal	2.07 ± 0.07^{a}	1.55 ± 0.11^{ab}	1.61 ± 0.19^{ab}	1.40 ± 0.13^{b}	S	S	NS
Glucose incorporation (μmol/g)							
Basal	2.02 ± 0.09^{a}	2.18 ± 0.15^{ab}	2.22 ± 0.13^{ab}	2.62 ± 0.10^{b}	S	S	NS
+Insulin	5.33 ± 0.19^{a*}	4.21 ± 0.26^{ab*}	4.07 ± 0.21^{b*}	4.02 ± 0.39^{b*}	NS	S	NS
+Palmitate	2.01 ± 0.12	1.78 ± 0.15	2.16 ± 0.10	2.29 ± 0.26	NS	NS	NS
+Insulin + Palmitate	3.40 ± 0.24$^{*\#}$	3.23 ± 0.07$^{*\#}$	3.87 ± 0.14*	3.77 ± 0.31*	NS	S	NS
+Insulin/Basal	2.67 ± 0.17^{a}	1.94 ± 0.12^{b}	1.84 ± 0.10^{b}	1.53 ± 0.15^{b}	S	S	NS
+Palmitate/Basal	1.00 ± 0.05	0.83 ± 0.10	0.99 ± 0.09	0.87 ± 0.07	NS	NS	NS
+Insulin + Palmitate/Basal	1.71 ± 0.18	1.49 ± 0.07	1.75 ± 0.07	1.44 ± 0.14	NS	NS	NS
Glucose oxidation (μmol/g)							
Basal	0.40 ± 0.06^{a}	0.46 ± 0.08^{a}	0.71 ± 0.09^{b}	0.95 ± 0.10^{b}	NS	S	NS
+Insulin	1.08 ± 0.03^{a*}	0.43 ± 0.10^{b}	0.95 ± 0.06^{a}	1.15 ± 0.18^{a}	NS	S	S
+Palmitate	0.28 ± 0.04^{a}	0.40 ± 0.03^{a}	0.77 ± 0.11^{b}	0.80 ± 0.11^{b}	NS	S	NS
+Insulin + Palmitate	0.39 ± 0.02$^{a\#}$	0.40 ± 0.10^{a}	0.84 ± 0.11^{ab}	1.13 ± 0.15^{b}	NS	S	NS
+Insulin/Basal	2.51 ± 0.19^{a}	1.12 ± 0.31^{b}	1.47 ± 0.18^{b}	1.42 ± 0.37^{b}	S	NS	S
+Palmitate/Basal	0.84 ± 0.20	1.18 ± 0.42	1.23 ± 0.26	0.87 ± 0.10	NS	NS	NS
+Insulin + Palmitate/Basal	1.23 ± 0.34	0.91 ± 0.20	1.24 ± 0.16	1.24 ± 0.18	NS	NS	NS
Glycogen synthesis (μmol/g)							
Basal	0.45 ± 0.02	0.50 ± 0.07	0.42 ± 0.01	0.64 ± 0.07	S	NS	NS
+Insulin	2.00 ± 0.06^{a*}	1.09 ± 0.07^{b*}	1.36 ± 0.14^{b*}	1.22 ± 0.21^{b*}	S	NS	S
+Palmitate	0.48 ± 0.03	0.39 ± 0.04*	0.37 ± 0.03	0.45 ± 0.09	NS	NS	NS
+Insulin + Palmitate	0.90 ± 0.03$^{*\#}$	0.81 ± 0.10*	1.21 ± 0.25*	1.24 ± 0.13*	NS	S	NS
+Insulin/Basal	4.45 ± 0.17^{a}	2.27 ± 0.20^{bc}	3.26 ± 0.38^{b}	1.94 ± 0.31^{c}	S	S	NS
+Palmitate/Basal	1.08 ± 0.11	0.81 ± 0.12	0.88 ± 0.08	0.70 ± 0.09	S	NS	NS
+Insulin + Palmitate/Basal	2.01 ± 0.05^{ab}	1.63 ± 0.06^{a}	2.93 ± 0.43^{b}	2.02 ± 0.22^{ab}	S	S	NS

Experimental groups: $^{+}$LA, enriched with LA; $^{-}$LA, deprived of LA; $^{+}$LA + CLA, $^{+}$LA supplemented with CLA-enriched oil, $^{-}$LA + CLA: $^{-}$LA supplemented with CLA-enriched oil. +Insulin/Basal, +Palmitate/Basal, and + Insulin + Palmitate/Basal ratios represent the response of + Insulin, +Palmitate, and + Insulin + Palmitate, respectively, relative to basal conditions. Data are means ± SE; n = 4 per group. Statistical analyses between different groups were established with a 2-way ANOVA (2 × 2), followed by Scheffe test. For the ANOVA test, S indicates $P < .05$ and NS indicates $P > .05$. For the Scheffe test, values in the same row with different superscript letters are significantly different ($P < .05$). Significant differences between different experimental conditions in each group were established with Student t test and identified at $P < .05$ by * between + Insulin vs Basal, +Palmitate vs Basal and + Insulin + Palmitate vs Basal, and by # between + Insulin + Palmitate vs +Insulin.

a diet with a low 18:2 n-6/16:0 ratio showed deteriorated insulin sensitivity and glucose transport via a reduction in membrane fluidity [36], studies focusing on the effects of CLA on glucose metabolism in experimental animal models fed an unbalanced diet of LA have not been published, to the best of our knowledge. We focused on the effects of CLA during 2 different dietary treatments on the LA contents: the first dietary treatment aimed to mimic the FA profile of the western diet (rich in LA), and the second treatment was designed to limit LA disposal (low in LA). Specifically, the diets were based on the recommendations by Reeves et al [21] while modifying the oil source, as previously recommended in the AIN-76 diet [22]. Lien et al [37] concluded that feeding rats either an AIN-93G or AIN-76 diet for 13 weeks promoted normal growth and development and did not cause toxicological effects. These results agree with the results of the present work, as we did not find toxicological manifestations in any of the experimental groups. In addition, we observed that the average daily food intake and body weight gain were similar among the animals. Although the ALA level is low and the ratio of LA/ALA is not the recommended one in the corn oil [21], the control animals did not show biomarkers of EFA deficiency according to the mead acid and the ratio of triene/tetraene. In contrast, animals fed coconut fat showed significant changes in these parameters. Previously, we found [19] similar results in deficiency biomarkers in other tissues, such as the liver, adipose tissue, and brain. In addition, the type of dietary lipids did not affect CLA incorporation. The serum CLA levels mainly correlated with the proportion of these isomers in the diets. Although CLA isomers were incorporated into the serum lipids, they did not induce major changes in the levels of the main FA, with the exception of the LA levels whose reduction was attenuated by CLA. This effect could be associated with a lower metabolization or utilization of LA in the presence of CLA. In addition, CLA supplementation reduced the ratio of arachidonic acid/LA (–45%) in LA-deprived rats, and this effect could be associated with decreased hepatic n-6 PUFA biosynthesis [38]. In

contrast, the 2 dietary independent variables, CLA and LA, did not appear to modify the n-3 PUFA biosynthesis.

At basal conditions, CLA increased glucose oxidation in the soleus muscle of animals fed diets either enriched with or deprived of LA. Nevertheless, CLA differentially increased oxidation, uptake, and incorporation of glucose and raised glycogen synthesis in the LA deprived group. These potentially beneficial effects of CLA, which were mainly observed in isolated soleus muscles from rats characterized by an EFA deficiency status, agree with the previously indicated CLA effects on glucose utilization observed in the experimental models of metabolic alterations [39,40]. In this regard, CLA improved the glycemic control and glucose tolerance in diabetic-obese Zucker rats [4] and in other obese and insulin-resistant rodent models induced by high-fat diets [41]. However, controversial studies have been published regarding this issue, with some showing negative and others reporting no effects in diverse experimental models. Numerous authors [10,11,42] have not observed any alterations in serum glucose levels, either in normal animals fed CLA or in diabetic mice treated with *t*10,*c*12-CLA [8]. Recently, we demonstrated [6] that the *c*9,*t*11- + *t*10,*c*12-CLA mixture partially prevents changes in the glycolytic pathway in the liver but not in the muscle of mice fed a high-fat diet. This effect was associated with deleterious consequences, such as hyperglycemia and the hepatic accumulation of lactate. Specifically, the normalization of triacylglycerol contents in the gastrocnemius muscle of these mice was associated with an inhibition of the glycolytic pathway, likely via the intracellular glucose-FA cycle [16,43]. Instead, CLA increased glucose oxidation in the soleus muscles of rats fed a LA-enriched diet in our experimental model, which was characterized by normal gastrocnemius muscle triacylglycerol levels [19] and oxidative enzymes (data not shown). Moreover, this change was even more pronounced in the soleus muscles of rats fed the LA-deprived diet. The higher glucose oxidation may have increased the cellular glucose demand, which increased glucose uptake and incorporation in the muscle, but left glycogen synthesis unchanged.

Conjugated LA supplementation increased glucose utilization at basal conditions. However, the animals supplemented with this lipid presented a lower insulin-stimulated glucose uptake, incorporation and oxidation, and glycogen synthesis than nonsupplemented animals. These results agree with Tsuboyama-Kasaoka et al [11], who observed that female mice fed a diet supplemented with 1% CLA presented insulin resistance and hyperinsulinemia, despite the elevation in the GLUT-4 expression as well as the increase in glucose uptake and utilization in skeletal muscle. They also observed that insulin resistance was associated with low serum leptin levels and abolished by leptin infusion. This observation agrees with the decreased insulin response noted in the isolated muscles of animals that were fed a diet supplemented with CLA in the present study. Regarding the effect of CLA on glucose metabolism, *t*10,*c*12-CLA is well known to reduce circulating adiponectin levels [42], as well as leptin and adiponectin gene expression in normal mice [10]. Similar results have been reported in mice fed a high-fat diet supplemented with mix-CLA [12]. However, in contrast to our results, various authors demonstrated a beneficial effect of CLA on glucose uptake and glycogen synthesis in the presence of insulin in obese-diabetic rats [4,35]. These effects were associated with an increase in adiponectin levels, which reduced the IRS-1 phosphorylation in serine residues, thus resulting in decreased insulin resistance [44,45]. Furthermore, the improvement in insulin sensitivity observed in mice supplemented with CLA has been previously related to the peroxisome proliferator–activated receptor α and γ levels in response to long-term but not short-term treatments [5].

Preincubation with palmitate reduces glucose uptake and incorporation in skeletal muscle in basal and insulin-stimulated conditions [29,46], mimicking an in vivo insulin resistance state induced by a high disposal of plasma free FAs. This state occurs in several pathological conditions, including obesity, type 2 diabetes mellitus, and metabolic syndrome, as well as animals fed a high-fat diet, and it leads to insulin resistance and hyperinsulinemia [16,17,47]. This effect depends on the type of dietary FA and on potential changes in membrane phospholipids, as well as on the bioactive lipid metabolites generated by the different FAs [48,49]. For example, long-chain saturated FAs, including palmitate and stearate, are associated with an impairment in insulin sensitivity in skeletal muscle and isolated muscle cells via a decrease in the PUFA content in membrane phospholipids and an increase in the intramyocellular lipid derivatives; these changes are associated with deleterious effects on the skeletal muscle cells [49,50]. In our study, palmitate addition did not inhibit the studied glucose metabolism parameters in the absence of insulin in the control or the CLA-supplemented animals. However, palmitate clearly impaired glucose uptake and metabolism stimulated by insulin. The response between the LA-enriched and LA-deprived groups differed in that palmitate inhibited the action of insulin on glucose uptake and incorporation, but had no effect in both groups of animals fed CLA-supplemented diets. These results indicate that CLA prevented the palmitate inhibition of muscle glucose uptake and incorporation stimulated by insulin. These effects may be attributed to muscle plasma membrane modifications and lipid derivative production, which are known to modulate glucose uptake and may be present in animals fed CLA-supplemented diets. Similar effects were observed based on the membrane enrichment with PUFA, which increased the GLUT-1 and GLUT-4 contents in the plasma membrane as well as the membrane fluidity [51]. It also improved insulin signaling, among several other proposed mechanisms [52].

Based on the present work, we accept the hypothesis that CLA improves glucose metabolism in rats fed different dietary levels of LA in the presence and absence of effectors in the soleus muscle. This finding is supported by the following: (1) CLA improved glucose uptake and incorporation as well as glycogen synthesis via an increase in glucose oxidation, which enhanced the glucose utilization at basal conditions, and (2) CLA exerted beneficial muscular effects by attenuating or preventing the palmitate-induced inhibition of glucose uptake and incorporation stimulated by insulin; this action was likely mediated by an effect on the muscle plasma membranes. These beneficial effects of CLA on glucose metabolism were better in LA-deprived rats. Nevertheless, because CLA lowered the insulin response capacity for glucose utilization, which could be mediated by leptin and adiponectin, we reject the

hypothesis that CLA can improve insulin sensitivity in rats fed different dietary levels of LA.

As expected, this study does have some limitations. Glucose utilization was investigated in isolated skeletal muscles, which does not necessarily reflect in vivo glucose metabolism. Further studies to extend the present observations will be conducted in vivo in the near future. The importance of different key regulators, such as leptin, adiponectin, and insulin, will be considered to elucidate potential mechanisms or interactions with canonical signaling pathways involved in regulating skeletal muscle glucose utilization. In addition, the FA composition of muscle membrane phospholipids was not measured; thus, we cannot correlate changes in the structural lipids of membranes with the sensitivity of insulin action. Finally, the ability to extrapolate the CLA effects on glucose utilization in LA-enriched or deprived rats to humans remains to be determined.

Ultimately, the present results demonstrate the complexities of how CLA, palmitate, and/or insulin interact to differentially modify muscle glucose utilization depending on the dietary levels of LA. These findings could help to understand some potential physiological conditions that could be useful for the prevention or treatment of glucose metabolism alterations.

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