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Adipocyte membrane glycerol permeability is involved in the anti-adipogenic effect of conjugated linoleic acid

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ABSTRACT

Conjugated linoleic acid (CLA), a group of minor fatty acids from ruminant origin, has long been recognized as a body fat lowering agent. Given the *trans*(*t*)10,*cis*(*c*)12-CLA well documented interference on lipolysis, we hypothesized for adipocytes altered permeation to glycerol when supplemented with this isomer. 3T3-L1 murine differentiated adipocytes were medium supplemented with linoleic acid (LA) and individual or combined *c*9,*t*11 and *t*10,*c*12-CLA isomers. Adipocytes treated with the *t*10,*c*12-CLA isomer and CLA mixture showed reduced triacylglycerols content ($p < 0.001$), re-enforcing the *t*10,c12-CLA as the anti-adipogenic CLA isomer. This finding was supported by decreased Δ9-desaturase index and adipocyte differentiation markers for the *t*10,*c*12-CLA group ($p < 0.001$), which suggest reduced lipogenesis and differentiation, respectively. The glycerol permeability was higher in all CLA treated cells compared to control and LA groups ($p < 0.05$). The increase in glycerol permeability agrees with both reduced triacylglycerols and non-osmotic cellular volume in the *t*10,c12-CLA and CLA mixture groups. Taken together, our data suggest that the increased adipocyte plasma membrane glycerol fluxes may be part of the anti-adipogenic response to CLA treatments.

1. Introduction

In developed countries obesity and its associated metabolic and cardiovascular complications are increasing to endemic levels contributing to human morbidity and premature mortality [1]. Facing this worldwide public health problem, scientific research is struggling for the discovery of new compounds from natural origin, which could mitigate or even reverse such scenario. Conjugated linoleic acid (CLA) has been one of most studied natural compounds in the last decades as a body fat reducing agent. CLA is a generic term for a group of fatty acids (FA) originated from linoleic acid (LA, 18:2*n* − 6) with conjugated double bonds, in either *cis* (*c*) or *trans* (*t*) configuration [2] naturally found in milk, dairy products and meat of ruminants [3]. The most studied and biologically active isomers of CLA are the *c*9,*t*11 and the *t*10,*c*12. The majority of CLA in the human diet occurs as *c*9,*t*11, accounting for 85–90% of the total CLA content in dairy products [2], while dietary CLA supplements usually contain a 50:50 mixture of *c*9,*t*11 and *t*10,*c*12-CLA isomers. The *t*10,*c*12-CLA isomer seems to be responsible for the fat-lowering properties of CLA by affecting lipid metabolism, but the molecular mechanisms of action are not yet fully understood and conflicting data have been reported [4]. Decreased lipogenesis [5] and/or increased fatty acid oxidation [6] are some of the mechanisms appointed for *t*10,*c*12-CLA effects in adipocytes. In an attempt to unveil CLA effects on fat deposition and adipocyte membrane biophysical properties, the effects of CLA isomers on membrane composition, permeability and fluidity were characterized using visceral adipose total membranes from Zucker rats [7]. However, results assessing plasma membrane function of metabolically active cells rather than whole tissue homogenates are greatly needed for a clearer insight into CLA contribution as an anti-adipogenic supplement.

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Under conditions of negative energy balance, adipocytes rapidly liberate FA and glycerol into the circulation, which are then used in thermogenesis and gluconeogenesis, respectively [8]. The main glycerol channel, aquaglyceroporin-7 (AQP7), regulates glycerol accumulation in adipocytes [9,10] and prevents acute rise in intracellular osmotic pressure when glycerol is rapidly produced during lipolysis [11]. This process is tightly coordinated by catecholamines, through their action on both lipolytic and anti-lipolytic adrenoreceptors [12]. It has been reported that short-term treatment with isoproterenol, a non-selective beta-adrenergic agonist and lipolysis stimulator, induces AQP7 translocation to plasma membrane [12], whereas long-term stimulation reduces the expression of *Aqp7* in 3T3-L1 adipocytes [13]. Attending to the *t*10,*c*12-CLA well documented interference on the process of lipolysis, we hypothesized for adipocytes altered plasma membrane permeation to glycerol when supplemented with this isomer. To test our hypothesis, 3T3-L1 mouse (pre) adipocytes were medium supplemented with individual or combined *c*9,*t*11 and *t*10,*c*12-CLA isomers and determined the intracellular triacylglycerol content. On the pursuing of CLA anti-adipogenic effects, we also assessed the effect of CLA isomers on Δ9-desaturase activity and quantified adipocyte differentiation markers. Finally, adipocyte plasma membrane permeability to glycerol was evaluated in order to associate glycerol fluxes with the increased lipolysis induced by the *t*10,*c*12-CLA isomer.

2. Material and methods

2.1. Cell cultures

3T3-L1 preadipocytes (CCL 92.1; American Type Culture Collection, Manassas, VA) were grown to confluence and induced to differentiate as described [14]. Mature adipocytes were used 10–15 days after initiation of differentiation. The FA treatments were LA (90150, Cayman, Ann Arbor, MI, USA), *c*9,*t*11-CLA (90370, Cayman), *t*10,*c*12-CLA (90145, Cayman) with approximately 98% purity and the mixture of both CLA isomers (50:50) dissolved in dimethyl sulfoxide (DMSO, 276855, Sigma) as 100 mM stock solutions. FA were introduced throughout the differentiation at a physiological concentration of 50 μM (in the mixture treatment, *c*9,*t*11- and *t*10,*c*12-CLA were at 25 μM each). Control cells received the same volume of DMSO.

2.2. Measurement of triacylglycerol content

Cells were harvested with RIPA buffer (R0278, Sigma), stirred for 30 min, scrapped and syringed. TAG content was measured using the TRIGS kit (Randox, Crumlin, UK), following on protein (Bicinchoninic Acid Protein Assay kit, Sigma), normalization and expressed as μg TAG/μg protein.

2.3. Total cholesterol and fatty acid profile

Cells were washed in PBS, scrapped and syringed. For the cholesterol content, samples were lyophilized and submitted to a direct saponification with saturated methanolic KOH solution. Total cholesterol was identified by high performance liquid chromatography [7] and expressed as μg/g dry cells. FA were extracted in isopropanol and *n*-hexane by sonication (3 min, 4 × 10%), as reported [15]. FA methyl esters (FAME) were obtained through a combined base/acid methylation method with NaOH in anhydrous methanol (0.5 M) followed by HCl:methanol (1:1 v/v), at 50 °C during 30 and 10 min, respectively [16]. The resulting FAME were analyzed using a HP7890A chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a flame-ionization detector (GC–FID) and a fused silica capillary column (CP-Sil 88; 100 m × 0.25 mm i.d. × 0.20 μm of film thickness, Agilent Technologies Inc., Santa Clara, CA, USA). For total FAME quantification nonadecanoic acid (19:0) was used as internal standard. Results for each FA were expressed as a percentage of total FA.

2.4. RNA extraction and quantitative real time PCR (qPCR)

Total RNA was isolated using the RNeasy Mini kit (Qiagen, CA, USA), according to the manufacturer's instructions. RNA evaluation was performed on a NanoDrop® ND-2000c spectrophotometer (Thermo Scientific, Wilmigton, DE, USA). 300 ng of total RNA were used for cDNA synthesis using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol.

For SYBR Green assay, *PPARG*, *GLUT4*, *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *RPS29* (ribosomal protein S29) primers were designed with Primer Express 3.0 software (Applied Biosystems) and Primer3 (http://frodo/wi.mit.edu/primer3/), and purchased from NZYTech (Lisbon, Portugal). A set of five candidate housekeeping genes was evaluated using geNorm and NormFinder, as described by Refs. [17] and [18], respectively. *GADPH* and *RPS29* were selected as controls for normalization. The specific primers used were: *GLUT4* (5′-ATGGCTGTCGCTGGTTTCTC-3′ and 5′-AAGCAGGAGGACGGCAAATA-3′); *PPARG* (5′-GAGGGCGATCTTGACAGGAA-3′ and 5′-GATGGCCACCTCTTTGCTCT-3′); *GAPDH* (5′-CGTGTTCCTACCCCCAATGT-3′ and 5′-GCCTGCTTCACCACCTTCTT3′) and *RPS29* (5′-GGAGTCACCCACGGAAGTTC-3′ and 5′-CATGTTCAGCCCGTATTTGC-3′). Relative quantification was performed as described [19]. *AQP7* transcript was quantified using TaqMan gene expression assay Mm00431839_m1 (Applied Biosystems). Ct-values were normalized to the mean of two endogenous reference genes: Rplp0 (Mm01974474_gH) and β-Actin (Mm02619580_g1). All reactions were carried out on a StepOnePlus 96-well format (Applied Biosystems), using standard thermal cycling conditions.

Relative expression levels were calculated as a variation of the Livak method [20] corrected for variation in amplification efficiency, as described [21].

2.5. Permeability assays and volume measurements

Glycerol permeability coefficient (P_{gly}) was measured in individual adherent cells on a coverslip, following the protocol designed in Ref. [10]. 3T3-L1 adipocytes were loaded with 5 μM calcein acetoxymethyl ester (calcein-AM) (Sigma) (a volume sensitive fluorescence probe) for 90 min at 37 °C. Coverslips with adhered cells were mounted in a closed perfusion chamber (Warner Instruments, Hamden, USA) on the stage of a Zeiss Axiovert 200 inverted microscope. Fluorescence was excited at 495/10 nm wavelength and the emission fluorescence was collected with a 535/25 nm bandpass filter coupled with a 515 nm dichroic beam splitter. Images were captured using a ×40/1.6 epifluorescence oil immersion objective and a digital camera (CoolSNAP EZ, Photometrics, USA) and recorded by the Metafluor software (Molecular Devices, USA).

For P_{gly} assessment, cells were equilibrated for 60 s in isotonic solution containing 200 mM mannitol (200 mM mannitol, 35 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, 5 mM Hepes, pH 7.4, $(osmout)_o = 300$ mosM) and suddenly exposed to the same perfusate where mannitol was replaced by glycerol. Under these conditions, no osmotic shock was applied ($\Lambda = 1$).

Cell volume V was measured at selected time points from 2D images obtained during the permeability assay to evaluate the initial volume (V_o volume prior to the osmotic challenge) and final

volume (final equilibrium volume). For each experimental condition, three coverslips were assayed (40–50 cells analyzed per condition). The cross sectional area of calcein-AM loaded cells was measured using the Image J software and cells were assumed as spherical for volume calculations.

P_{gly} was calculated as before [10]. Briefly, the time dependent volume changes were used for P_{gly} estimation. The relative non-osmotic volume $\beta = V_{Nosm}/V_o$ was considered in all calculations. Parameters (P_{gly} and β) were calculated by numerical integration and curve fitting the time dependent V/V_o data, using the model equations detailed in Ref. [10] and the Berkeley Madonna software (http://www.berkeleymadonna.com/).

2.6. Statistics

Statistical analyses were carried out with the Statistical Analysis Systems software, version 9.2 (SAS Institute, Cary, NC, USA). Once normality was tested (Kolmogorov–Smirnov test), all statistical analyses were performed based on a one-way analysis of variance (ANOVA) using the PROC GLM. Differences between groups were considered significant at a p-value less than 0.05. The results were expressed as mean ± standard error of the mean (SEM).

3. Results and discussion

3.1. Adipocyte TAG content is decreased by t10,c12-CLA and CLA mixture

After the differentiation period, the intracellular TAG content was quantified (Fig. 1) and found similar in the *c*9,*t*11-CLA group ($p > 0.05$) relative to non-CLA treated groups. However, adipocytes treated with the *t*10,*c*12-CLA isomer showed a marked reduction on lipid accumulation when compared with the control, LA and *c*9,*t*11-CLA groups ($p < 0.001$). The same effect was observed for the CLA mixture group, which can be attributed to the *t*10,*c*12-CLA isomer. Interestingly, the mixture treatment was equally effective to the *t*10,*c*12-CLA alone in reducing TAG content, despite containing half the *t*10,*c*12-CLA concentration. This indicates that *t*10,*c*12-CLA isomer at a concentration of 25 μM is able to elicit almost up to 50% adipocytes delipidation compared with non-CLA treated groups. These results confirm the ability of this individual isomer to impair TAG accumulation in adipocytes and to modulate body fat composition [22].

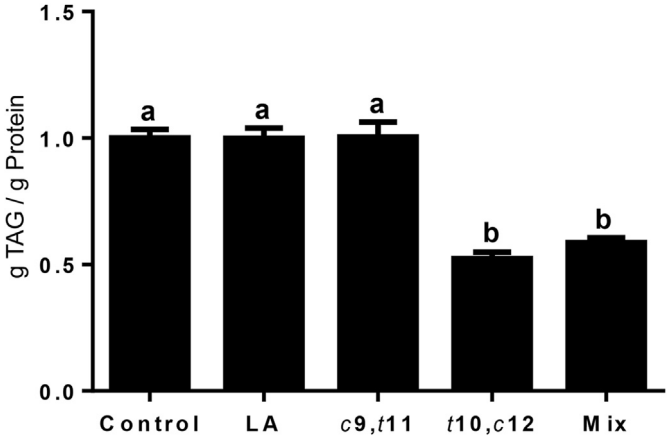

Fig. 1. Effect of linoleic acid (LA) and CLA isomers on TAGs content in 3T3-L1 adipocytes. Values are relative to control and "mix" is a mixture of *c*9,*t*11- and *t*10,*c*12-CLA isomers in equal proportions. Data are means ± SEM (n = 6). Means without a common letter differ at $p < 0.05$.

3.2. t10,c12-CLA decreases Δ9-desaturase activity

The molecular mechanisms by which the *t*10,*c*12-CLA isomer exerts its anti-adipogenic effects remain to be fully understood. Modulation of Δ9-desaturase activity, the limiting enzyme of MUFA synthesis, by CLA has been appointed as one of the mechanisms by which the *t*10,*c*12-CLA reduces body fat in animals [23,24].

The adipocyte lipid composition, including total cholesterol and fatty acid profile upon CLA treatments, are shown in Table 1 and reflects primarily the FA treatments. No significant differences were observed among groups for the total cholesterol content ($p > 0.05$). To evaluate the influence of CLA isomers on Δ9-desaturase enzyme activity, the Δ9–16 and –18 indexes were calculated. As shown in Table 1, Δ9–16 and –18 indexes were significantly decreased in the *t*10,*c*12-CLA group when compared to the other groups ($p < 0.001$), indicating decreased MUFA synthesis and lipogenic activity. This finding agrees with decreased TAG content in cells treated with the *t*10,*c*12-CLA isomer (Fig. 1) and with increased SFA/MUFA and decreased PUFA/SFA ratios ($p < 0.001$) (Table 1). A *cis*-12 double bond appears to be a key structural feature for inhibiting Δ9-desaturase activity, especially when coupled with a *trans*-10 double bond [23]. By applying half of the *t*10,*c*12-CLA concentration

Table 1
Effect of LA and CLA isomers on cholesterol content (μg/g dry cells) and fatty acid profile (g/100 g total FA) in 3T3-L1 adipocytes.

	Control	LA	*c*9,*t*11	*t*10,*c*12	Mix	SEM	Significance
Cholesterol	2.38	2.31	2.16	2.12	2.23	0.185	ns
Fatty acid profile							
14:0	2.61^{b}	2.59^{bc}	2.50^{c}	2.92^{a}	2.66^{b}	0.041	***
14:1*c*9	1.35^{a}	1.19^{b}	1.28^{a}	0.53^{d}	0.95^{c}	0.031	***
15:0	13.9^{a}	13.0^{ab}	14.0^{a}	12.2^{b}	13.8^{a}	0.415	*
16:0	18.7	18.9	18.7	20.1	19.3	0.553	ns
16:1*c*9	27.1^{a}	23.9^{c}	25.5^{b}	17.8^{e}	24.0^{d}	0.431	***
17:0	2.24^{c}	2.21^{c}	2.20^{c}	3.06^{a}	2.62^{b}	0.056	***
17:1*c*9	1.51^{b}	1.82^{b}	1.21^{b}	3.30^{a}	1.33^{b}	0.252	***
18:0	1.04^{c}	1.48^{b}	1.57^{b}	2.44^{a}	1.41^{bc}	0.181	***
18:1*c*9	7.75^{b}	8.06^{b}	9.03^{ab}	10.0^{b}	8.53^{b}	0.459	*
18:1*c*11	2.22^{b}	1.94^{c}	2.23^{b}	2.79^{a}	2.32^{b}	0.048	***
18:2*n* – 6	0.368^{c}	2.14^{a}	0.655^{b}	0.365^{c}	0.333^{c}	0.097	***
18:3*n* – 6	nd	0.250	nd	nd	nd	0.010	***
18:3*n* – 3	0.107^{a}	0.067^{a}	nd	nd	nd	0.014	***
CLA c9,t11	0.310^{c}	0.496^{bc}	1.46^{a}	0.418^{c}	1.14^{ab}	0.229	**
CLA t10,c12	nd	nd	nd	1.19^{a}	0.588^{b}	0.050	***
20:4*n* – 6	0.331^{b}	0.532^{a}	0.193^{cd}	0.142^{d}	0.306^{bc}	0.042	***
22:5*n* – 3	0.191^{a}	0.202^{a}	nd	nd	nd	0.016	***
22:6*n* – 3	0.193^{a}	0.215^{a}	nd	0.062^{b}	nd	0.030	***
Others	20.1	21.1	19.5	22.6	20.6	1.19	ns
Sums							
SFA	38.5	38.1	39.0	40.8	39.8	0.880	ns
MUFA	39.9^{a}	36.9^{b}	39.3^{a}	34.5^{c}	37.2^{b}	0.576	***
PUFA	1.19^{b}	3.41^{a}	0.848^{c}	0.569^{c}	0.639^{c}	0.105	***
CLA	0.310^{b}	0.496^{b}	1.46a	1.60^{a}	1.73^{a}	0.240	***
n – 3	0.491^{a}	0.484^{a}	nd	0.062^{b}	nd	0.035	***
n – 6	0.700^{b}	2.926^{a}	0.848^{b}	0.507^{c}	0.639^{bc}	0.104	***
Ratios							
SFA/MUFA	0.964^{c}	1.03^{bc}	0.992^{c}	1.18^{a}	1.07^{b}	0.019	***
PUFA/SFA	0.031^{b}	0.089^{a}	0.023^{bc}	0.014^{d}	0.016^{cd}	0.003	***
18:1*c*9/18:2*n* – 6	21.3^{b}	3.77^{c}	17.0^{b}	29.4^{a}	17.9^{b}	2.91	***
Desaturation indexes							
Δ9-index16	0.592^{a}	0.559^{bc}	0.577^{ab}	0.471^{d}	0.555^{c}	0.006	***
Δ9-index18	0.882^{a}	0.847^{b}	0.856^{b}	0.807^{c}	0.859^{b}	0.008	***

SEM, standard error of the mean; ns, not significant; nd, not detected.
[a,b,c]Means without a common letter differ at *p < 0.05, **p < 0.01, ***p < 0.001.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid isomers. Sum (SFA) = sum of 14:0, 15:0, 16:0, 17:0 and 18:0; Sum (MUFA) = sum of 14:1*c*9, 16:1*c*9, 17:1*c*9, 18:1*c*9 and 18:1*c*11; Sum (PUFA) = sum of 18:2*n* – 6, 18:3*n* – 6, 18:3*n* – 3, 20:4*n* – 6, 22:5*n* – 3 and 22:6*n* – 3; Sum (CLA) = sum of *c*9,*t*11-CLA and *t*10,*c*12-CLA; Sum (*n* – 6) = sum of 18:2*n* – 6, 18:3*n* – 6 and 20:4*n* – 6; Sum (*n* – 3) = sum of 18:3*n* – 3, 22:5*n* – 3 and 22:6*n* – 3; Δ9-index16 = 16:1/(16:0 + 16:1*c*9); Δ9-index18 = 18:1/(18:0 + 18:1*c*9).

(mixture group), a distinct Δ9-desaturase activity pattern was obtained. Since an equivalent effect on TAG reduction was observed for both *t*10,*c*12-CLA and CLA mixture groups (Fig. 1), these data suggest that lowered Δ9-desaturase activity may not be the only factor contributing to impaired TAG content.

3.3. *t10,c12-CLA inhibits adipogenesis*

The conversion of preadipocytes into adipocytes involves the activation of key transcription factors that activate genes responsible for the adipocyte phenotype, including the accumulation of lipid droplets [25]. There is much evidence showing that CLA isomers modulate adipogenic factors activity and interfere with adipocyte differentiation [5,26]. An anti-adipogenic effect has been attributed to the *t*10,*c*12-CLA isomer [27,28], whereas either no effect [28] or a pro-adipogenic activity [29,30] have been reported for the *c*9,*t*11-CLA isomer.

To clarify the effect of CLA isomers on adipogenesis, we quantified the gene expression level of specific markers of adipocyte differentiation (peroxisome proliferator-activated receptor γ, PPARγ; glucose transporter type 4, Glut4 and aquaporin 7, AQP7) in 3T3-L1 cells under FA treatments (Fig. 2). Adipocytes treated with the *c*9,*t*11-CLA isomer had similar expression of PPARγ and Glut4 in regard to non-CLA treated groups ($p > 0.05$) (Fig. 2A and B), and only slightly decreased AQP7 expression (Fig. 2C). On the contrary, the *t*10,*c*12-CLA isomer had a significant down-regulation of all markers in comparison with all other groups ($p < 0.001$), which clearly indicates a decrease on cell differentiation.

Although PPARγ decrease could be associated to the same effect on Glut4 expression for the *t*10,*c*12-CLA treatment, it is worth mentioning that CLA may antagonize PPARγ activity at multiple levels: expression, degradation and DNA binding affinity, all contributing to the final anti-adipogenic outcome (reviewed by Ref. [5]). This might be the reason why the mixture group with a lower content of *t*10,*c*12-CLA isomer, showed comparable differentiation markers levels to control and LA ($p > 0.05$) groups, except for the Glut4 ($p < 0.001$), indicating that differentiation is affected but not at the same extent as having the t10,*c*12-CLA isomer alone. In addition, our results indicate that inhibition of PPARγ expression alone does not by itself justify the lowering effect in TAG content exerted by the *t*10,*c*12-CLA isomer.

3.4. *CLA supplementation increases membrane glycerol permeability*

Increased lipolysis is another mechanism proposed for the *t*10,*c*12-CLA isomer anti-adipogenic activity [4]. During lipolysis, TAGs are hydrolyzed into glycerol and FA and both products are released into the bloodstream. Several membrane proteins have been related to FA transport [31], whereas only one protein, AQP7, candidates as the major transporter for glycerol in adipocytes [32]. Therefore, despite AQP7 reduced mRNA levels in the *t*10,*c*12-CLA treatment (Fig. 2C), increased glycerol effluxes are expected in adipocytes with stimulated lipolysis.

Reduced AQP7 expression by the *t*10,*c*12-CLA isomer comes in line with the already reported down-regulation of AQP7 by catecholamines [13], which are also potent activators of lipolysis [33]. In addition, catecholamines are described to promote AQP7 translocation from intracellular stores to the plasma membrane [9], confirming their involvement in the fine-tuning of glycerol release by adipocytes. Thus, we hypothesized a similar mechanism for the anti-adipogenic effect of *t*10,*c*12-CLA isomer.

To further investigate the changes of AQP7 activity in adipocyte plasma membranes subjected to different fatty acid treatments, we assessed the overall glycerol permeability in these cells. We started

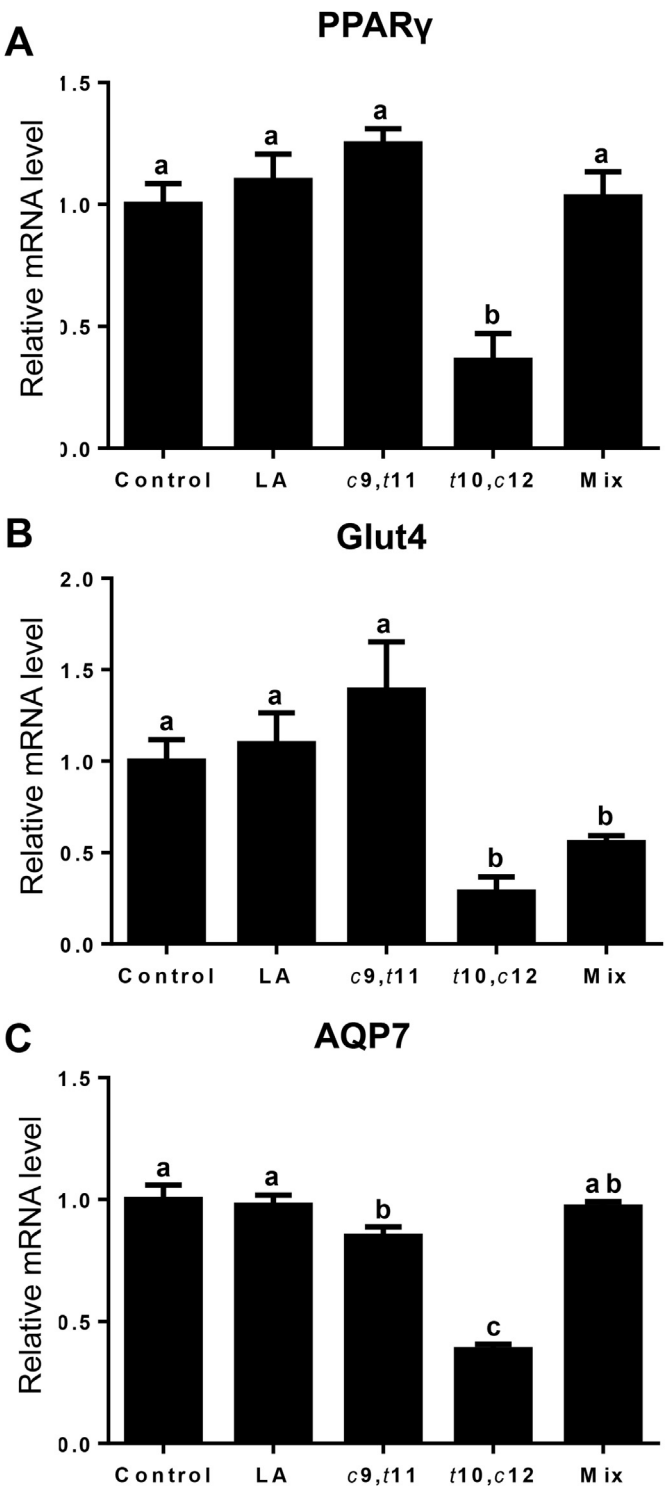

Fig. 2. Effect of linoleic acid (LA) and CLA isomers on mRNA expression levels of 3T3-L1 adipocytes differentiation markers PPARγ (A), Glut4 (B) and AQP7 (C). Values are relative to control and "mix" is a mixture of *c*9,*t*11- and *t*10,*c*12-CLA isomers in equal proportions. Data are means ± SEM (n = 3). Means without a common letter differ at $p < 0.05$.

by measuring the equilibrium cell volume before and after osmotic challenges in adipocytes [10] to assess the relative non-osmotic volume (β) values. The relative non-osmotic volume represents the fraction of the cell that is osmotically unresponsive greatly due to lipid droplet content, as shown in Fig. 3A [10]. In Fig. 3B, it is observed that no significant differences regarding the β values were

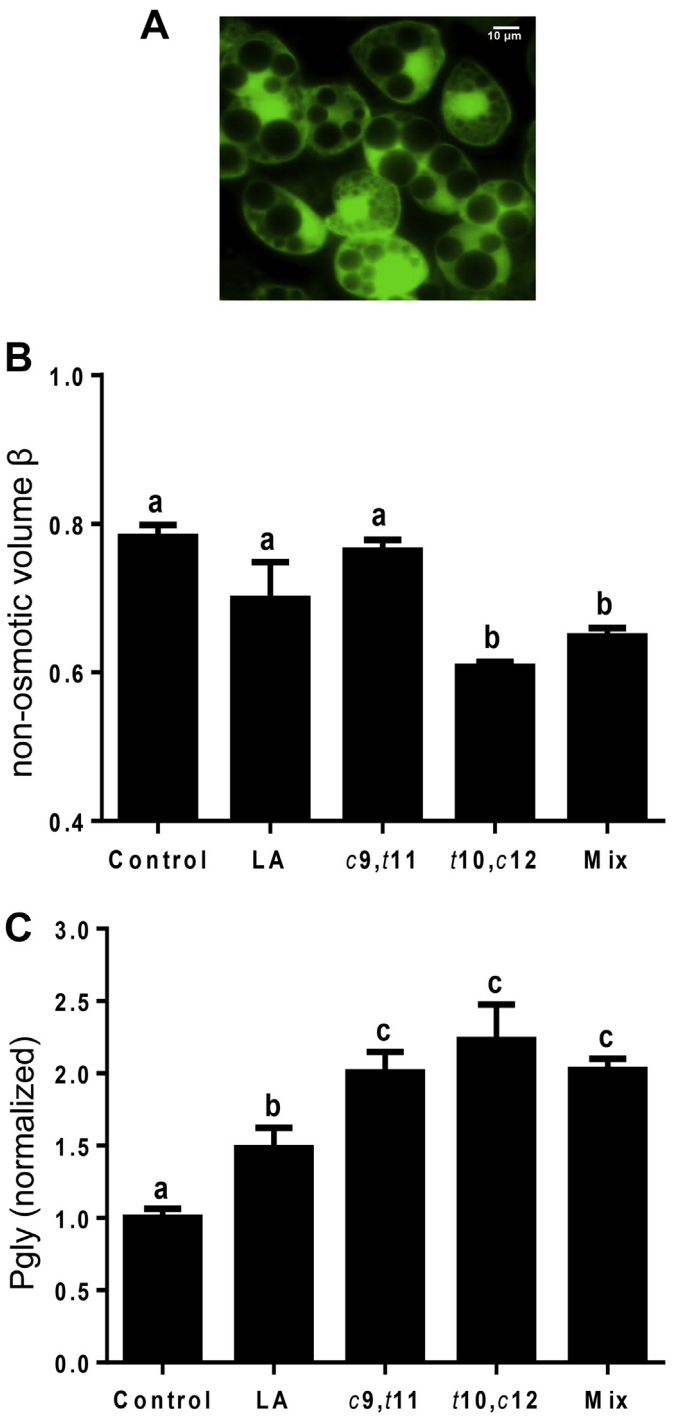

Fig. 3. Permeability assays. Representative illustration of fluorescence loaded adipocytes (A) and the effect of linoleic acid (LA) and CLA isomers on non-osmotic volume β (B) and glycerol permeability (P_{gly}) (C) of 3T3-L1 adipocytes. Permeability values are relative to control and "mix" is a mixture of *c*9,*t*11- and *t*10,*c*12-CLA isomers in equal proportions. Data are means ± SEM (n = 3). Means without a common letter differ at $p < 0.05$.

found for control, LA and *c*9,*t*11-CLA groups ($p > 0.05$). However, adipocytes treated with the *t*10,*c*12-CLA isomer showed a significant marked reduction on β values ($p < 0.001$), which relates with decreased TAG content (Fig. 1). This same effect was observed for the CLA mixture group ($p < 0.001$), and it can be fairly attributed to the *t*10,*c*12-CLA isomer.

The effect of fatty acid treatments on glycerol membrane permeability (P_{gly}) is depicted in Fig. 3C. The CLA treated groups (*c*9,*t*11-CLA, *t*10,*c*12-CLA and mixture) showed the highest P_{gly} ($p < 0.05$) relatively to non-CLA treated groups. The increased permeability for all CLA treated groups, either as a mixture or individual isomers, but not for the LA group, supports an increase in AQP7 activity at the plasma membrane. Regarding the *t*10,*c*12-CLA isomer, its effect is probably similar to the one described for catecholamines repressing AQP7 expression [13] and at the same time increasing the remaining AQP7 translocation to the plasma membrane [9]. Surprisingly, P_{gly} was also increased for the *c*9,*t*11-CLA group despite no increase in lipolysis is expected. The CLA mixture group also showed increased P_{gly} in accordance with decreased TAGs content (Fig. 1).

In conclusion, this work confirms the *t*10,*c*12-CLA isomer as responsible for adipocyte TAG depletion and impairment of adipocyte differentiation. Its lipolytic action may share some common mechanisms with catecholamines. The mixture, which represent the dietary CLA supplements, only partially match the *t*10,*c*12-CLA individual effects. The half concentration of t10,*c*12-CLA in the mixture is sufficient to fully attain some of the effects promoted by the individual *t*10,*c*12-CLA supplementation, in particular the reduced intracellular TAG content.

Each of the investigated CLA isomers have unique physiological outcomes when supplemented to biological systems, as demonstrated by *c*9,*t*11- and *t*10,*c*12-CLA unequal actions in TAG accumulation, Δ9-desaturase enzyme activity and adipogenesis. But as far as insulin function is concerned, both isomers seem to be responsible for insulin resistance in humans [26]. Increased glycerol effluxes promoted by the most biologically active CLA isomers may underlie increased glycerol release by adipocytes, with subsequent increase in hepatic gluconeogenesis and circulating glucose levels. This warrants further elucidation.

Conflict of interest

The author declares no conflict of interest.

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