Dietary trans-10,cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse

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Abstract Conjugated linoleic acids (CLA) are a class of positional, geometric, conjugated dienoic isomers of linoleic acid (LA). Dietary CLA supplementation results in a dramatic decrease in body fat mass in mice, but also causes considerable liver steatosis. However, little is known of the molecular mechanisms leading to hepatomegaly. Although c9,t11- and t10,c12-CLA isomers are found in similar proportions in commercial preparations, the respective roles of these two molecules in liver enlargement has not been studied. We show here that mice fed a diet enriched in t10,c12-CLA (0.4% w/w) for 4 weeks developed lipoatrophy, hyperinsulinemia, and fatty liver, whereas diets enriched in c9,t11-CLA and LA had no significant effect. In the liver, dietary t10,c12-CLA triggered the ectopic production of peroxisome proliferator-activated receptor γ (PPARγ), adipocyte lipid-binding protein and fatty acid transporter mRNAs and induced expression of the sterol responsive element-binding protein-1a and fatty acid synthase genes. In vitro transactivation assays demonstrated that t10,c12- and c9,t11-CLA were equally efficient at activating PPARα, β/δ, and γ and inhibiting liver-X-receptor. Thus, the specific effect of t10,c12-CLA is unlikely to result from direct interaction with these nuclear receptors. Instead, t10,c12-CLA-induced hyperinsulinemia may trigger liver steatosis, by inducing both fatty acid uptake and lipogenesis.

Conjugated linoleic acids (CLA) are a class of positional and geometric conjugated dienoic isomers of linoleic acid (LA) found in dairy products, in bovine and ovine meat, and in partially hydrogenated vegetable oils. Although c9,t11-CLA (also known as rumenic acid) is the main CLA isomer present in food, it is found in almost equal proportions with the t10,c12-CLA isomer in commercial CLA preparations. The effects of CLA have been thoroughly studied in various animal models (1, 2). CLA have anticarcinogenic properties in both mice and rats (3), and delay the onset of atherosclerosis in rabbits (4) and hamsters (5). CLA-enriched diets also cause rapid, massive changes in body composition, particularly in the mouse, in which a decrease in fat stores associated with an increase in lean body mass has been reported (6, 7). Much is now known about the physiological basis of the CLA-induced decrease in adipose tissue mass. CLA supplementation leads to an increase in energy expenditure (7), which may be secondary to a stimulation of sympathetic nervous activity (8). CLA reduce lipid uptake and storage in 3T3-L1 adipocytes by inhibiting lipoprotein lipase (9, 10) and stearoyl-CoA desaturase-I (11) activities. Finally, it has been suggested that the decrease in adipose tissue involves an apoptotic mechanism linked to an increase in tumor necrosis factor α production (12). Recent studies with purified isomers have strongly suggested that CLA-induced fat loss is dependent on the t10,c12-CLA isomer in the mouse (13).

Another consequence of dietary CLA supplementation in mice is massive liver enlargement (12, 14–16). However, the cellular and molecular mechanisms involved in this process are unknown. It has been suggested that the effects of CLA on the liver may be partially controlled by...
peroxisome proliferator-activated receptor α (PPARα) (17), a nuclear receptor known to regulate lipid metabolism in this organ (18). Indeed, both c9,t11-CLA and t10,c12-CLA have been shown to activate PPARα in transfection assays (17). Consistent with this finding, an isomeric CLA mixture induced the expression of typical PPARα target genes encoding proteins involved in hepatic lipid transport (liver fatty acid-binding protein or L-FABP) and catabolism (acyl-CoA oxidase, and cytochrome P450 4A1) (19). However, the role of PPARα in CLA-mediated steatosis remains to be clarified.

Other transcription factors in addition to PPARα, such as liver-X-receptors (LXRs) and sterol responsive element-binding protein 1 (SREBP1), play a critical role in hepatic lipid metabolism by controlling de novo fatty acid synthesis (20, 21). It was recently suggested that the balance within the cell between oxysterols and polyunsaturated fatty acids (PUFA), which interfere with LXR activation in vitro, is a crucial determinant of hepatic lipogenesis (22). It has also been established that SREBP1 is a major regulator of this pathway.

This study was designed to explore the effects of dietary supplementation with purified CLA isomers. The effects of purified c9,t11-CLA and t10,c12-CLA were investigated in mice fed an isomer enriched-diet for 4 weeks. We found that the t10,c12-CLA isomer was responsible for CLA-induced lipotoxaphy and liver steatosis. A profound change in the pattern of hepatic gene expression, favoring lipid accumulation, was observed in mice fed a diet rich in t10,c12-CLA. In vitro transactivation assays showed that this effect on gene expression was not mediated by the direct activation of PPARα or LXRs. Instead, it may have been triggered by the marked increase in circulating insulin levels induced by dietary t10,c12-CLA.

### MATERIALS AND METHODS

#### Experimental protocols

French guidelines for the use and care of laboratory animals were followed. C57Bl/6j mice weighing 22.5 ± 0.1 g at the beginning of the experiment were individually housed in a controlled environment (constant temperature, humidity, and darkness from 8 AM to 8 PM). The food intake and body mass gain of each mouse were monitored at regular intervals.

To explore the effects on body composition of the two main CLA isomers found in commercial preparations, female mice were fed ad libitum for 4 weeks on a semi-synthetic diet (UAR, France) containing either 2.4% sunflower oil (control diet), or 2% sunflower oil plus 0.4% linoleic acid (LA diet; Sigma), or highly purified CLA isomers (i.e., c9,t11-CLA, or t10,c12-CLA diets; Natural Lipids Ltd, Norway) (Table 1). The diets were freshly prepared every day. We used females rather than males because they are more responsive to CLA supplementation (6). Anesthetized animals were bled by sectioning auxiliary vessels. The mice were killed, and liver and perirenal white adipose tissue (WAT) was collected, weighed, then rapidly frozen in liquid nitrogen and stored at −80°C.

#### Northern blotting

Total RNA was extracted from liver and WAT by the phenol-chloroform-LiCl method (23). It was subjected to electrophoresis in a 1% agarose gel and transferred to a GeneScreen membrane (NEN) as previously described (24). cDNA probes were obtained from various sources: the liver fatty acid-binding protein (L-FABP) cDNA was obtained from J. I. Gordon (Washington University, St Louis MO), adipocyte lipid-binding protein (ALBP) cDNA and fatty acid transporter (FAT/CD36) cDNA were obtained from P.A. Grimaldi (INSERM U460, Nice, France), fatty acid synthase (FAS) cDNA was obtained from P. Ferré (INSERM U465, Paris, France) and phosphoenolpyruvate carboxykinase (PEPCK) cDNA was obtained from C. Forest (INSERM U530, Paris, France). The probes were labeled with [α-32P]dCTP (3000 Ci/mmol; Amersham) using a megaprime kit (Amersham). A 24-residue oligonucleotide specific for rat 18S rRNA was used as a control to ensure that equivalent amounts of RNA were loaded and transferred. This oligonucleotide was 5′-end-labeled using T4 polynucleotide kinase and [γ-32P]ATP (3000 Ci/mmol, Amersham).

#### Real-time quantitative RT-PCR

cDNA was synthesized by reverse transcription of 5 μg of total RNA in a total volume of 20 μl using random hexamers and murine Moloney leukemia virus reverse transcriptase (Life Technologies). Real-time quantitative RT-PCR was then performed with 50 ng of reverse transcription products (diluted in 5 μl of 1X Sybr Green buffer), with 200 nM sense and antisense primers (Genset) in a final volume of 25 μl, using Sybr Green PCR core reagents in an ABI PRISM 7700 Sequence Detection System instrument (Applied Biosystems). As we used Sybr Green to determine amplification-associated fluorescence for real-time quantitative RT-PCR, it was important to check that the fluorescence generated was not overestimated due to contamination resulting from residual genomic DNA amplification (using controls without reverse transcriptase) and/or from the formation of primer dimers (controls with no DNA template or reverse transcriptase). RT-PCR products were also analyzed by electrophoresis in an ethidium bromide-stained agarose gel to check that a single amplicon of the expected size was indeed obtained. 18S rRNA and glyceraldehyde-3-phosphate dehydrogenase amplifications were used to assay variability in the initial quantities of cDNA. Relative quantification for any given gene, expressed as fold variation over control, was calculated by determining the difference between the cycle threshold (Ct) of the given gene in the control (A) and treated (B) samples, using the 2ΔΔCt formula, according to manufacturer’s protocol. CT values are expressed as means of triplicate measurements. The sense and antisense primers used were: GGGAGGCCGTGAGAAGCCGC and GGGTG-GGGAGTGGGTAATTT for 18S, GCCCATCCACAGTTCTCGG and ACCACAGTCCATGCATCAGTCCA for GAPDH, GGGC-

### Table 1. Composition of diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>LA</th>
<th>c9,t11-CLA</th>
<th>t10,c12-CLA</th>
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<tr>
<td>Sunflower oil</td>
<td>2.4</td>
<td>2.0</td>
<td>2.0</td>
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<tr>
<td>Fatty acids LA</td>
<td></td>
<td>0.4</td>
<td></td>
<td></td>
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<tr>
<td>(99% of purity)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>c9,t11-CLA</td>
<td>0.4</td>
<td></td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>(91.6% of purity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t10,c12-CLA</td>
<td>0.008</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>(96.2% of purity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
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<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
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</tr>
<tr>
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<td>3.3</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Mineral+Vitamin mix</td>
<td>4</td>
<td>4</td>
<td>4</td>
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</table>
CATGGACGAGCTG and TTGGCAACCTGGGCCGCT for SREBP1a, GGAGCCATGATTGCACATT and GCTTCCAGAGAGAGGAGCAG for SREBP1c, CCGTGTATCGGGCGA for DEF, pGal4-PPAR activation assays (pG5TkpGL3), and 100 ng/well of expression vector (pGal4-binding domain of human PPARα), involving incubation for 2 h with RPR120535B in serum-free medium. The medium was then replaced with DMEM supplemented with 10% FCS and various concentrations of LA, c9,11-CLA, or t10,c12-CLA (10 to 100 μM), or the ethanol vehicle alone. The cells were incubated for 16 h, after which we added 10 μM 22(R)-hydroxycholesterol (22R-CS) and incubated the cells for a further 20 h. Cell extracts were prepared and assayed for luciferase activity. Results were standardized on the basis of β-galactosidase activity. All points correspond to triplicate determinations.

Biochemical assays

The total lipid content of livers was determined by Delsal’s method (31). Blood glucose concentration was determined by enzymatic methods (Biotrol Diagnostics). Plasma insulin and leptin levels were determined by radioimmunoassay (CIS Bio and Linco, respectively).

Statistical analysis

The results are expressed as means ± SE. The significance of differences between groups was determined by carrying out Student’s t-test.

RESULTS

CLA effects on energy intake and body mass

CLA supplementation led to a significant decrease in daily energy intake (15.6 ± 0.3 Kcal/mouse/day for c9,11-CLA, and 16.5 ± 0.7 for t10,c12-CLA versus 23.3 ± 1.5 in controls, P < 0.001). This effect has been reported in previous studies (9) and is not CLA-specific. Indeed, a similar decrease was also found in mice fed a diet supplemented with LA (16.9 ± 0.7 Kcal/mouse/day, P < 0.05 vs controls). The addition of LA and CLA as fatty acids rather than as triglycerides might lead to sensorial and/or post-ingestive problems, resulting in partial aversion for these diets (7). A short-term decrease in body mass occurred in mice fed a diet rich in t10,c12CLA, but no significant difference was found at the end of the experiment (data not shown).

CLA-mediated changes in body composition are specific to the t10,c12-CLA isomer

In contrast to the results obtained for LA- and c9,11-CLA-enriched diets, the diet enriched in t10,c12-CLA resulted in a dramatic decrease in the mass of peri-uterine WAT (Fig. 1A). The abundance of the mRNAs encoding adipocyte lipid-binding protein (ALBP, also termed aP2) and fatty acid synthase (FAS), two proteins known to be involved in fatty acid uptake and accumulation in the adipocyte, was also markedly lower in mice fed this diet than in other mice (Fig. 1B). Similarly, only the t10,c12CLA diet triggered a massive enlargement of the liver (3.1-fold increase, P > 0.001), which displayed the typical features of a fatty liver: pale color and accumulation of intracellular lipids (Fig. 2).
Fig. 1. *t*10,*c*12-CLA is responsible for changes in white adipose tissue (WAT). Female C57Bl/6J mice fed for 4 weeks on diets containing 2.4% (w/w) sunflower oil alone (Control), or 2% sunflower oil plus 0.4% linoleic acid (LA), or 0.4% *c*9,*t*11-CLA, or 0.4% *t*10,*c*12-CLA. A: Typical gross changes and relative periuteral WAT mass. B: Northern blots and bar graph indicating changes in adipocyte lipid-binding protein (ALBP) and fatty acid synthase (FAS) mRNA levels. The changes with respect to the control were calculated after correcting for loading differences on the basis of 18S rRNA levels. Means ± SE, n = 5. *P < 0.05; ***P < 0.01.

Fig. 2. Liver steatosis is induced by *t*10,*c*12-CLA supplementation. Female C57Bl/6J mice were fed for 4 weeks on diets containing 2.4% (w/w) sunflower oil alone (Control), or 2% sunflower oil plus 0.4% linoleic acid (LA), 0.4% *c*9,*t*11-CLA, or 0.4% *t*10,*c*12-CLA. A: Typical gross changes. B: Relative liver mass and hepatic lipid content. Means ± SE, n = 8. ***P < 0.01.

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**LA, t10,c12-CLA and c9,t11-CLA exert similar effects on PPARα, β/δ, and γ and LXRα activation**

We investigated whether the isomer-specific CLA-mediated effect on adipose tissue and liver lipid metabolism was due to the direct activation of PPARs by means of a sensitive and specific assay involving chimeric proteins comprising the DNA-binding site of the yeast transcription factor Gal4 fused to the ligand-binding domain of each of the three known PPAR isoforms (PPARα, PPARβ/δ, and PPARγ) and a reporter gene system driven by five copies of the Gal4 response element inserted upstream from the luciferase gene (30). LA and the two CLA isomers were found to be potent PPARα activators, whereas PPARβ/δ was activated to a lesser extent and the effect on PPARγ activation was negligible (Fig. 3). Consistent with the results obtained by Moya-Camarena et al. (17), c9,t11-CLA appeared to be more efficient than t10,c12-CLA at activating PPARα. Indeed, 200 μM t10,c12-CLA was required to obtain the same level of PPARα activation obtained with 50 μM c9,t11-CLA (Fig. 3). Thus, the direct activation of PPARs by t10,c12-CLA cannot account for the induction of fatty liver by this compound. However, CLA may upregulate typical PPARα target genes. We investigated this possibility by cotransfecting Caco-2 cells with a construct consisting of the L-FABP promoter cloned upstream from a CAT reporter gene and PPAR expression vectors. The PPARα isoform gave the greatest increase in L-FABP promoter activity. Moreover, slightly higher levels of PPARα-mediated transactivation of the L-FABP promoter were obtained in the presence of c9,t11-CLA than with t10,c12-CLA or LA (Fig. 4A). In vivo, both c9,t11-CLA- and t10,c12-CLA-enriched diets induced a significant increase in liver L-FABP mRNA levels, whereas the LA diet did not (Fig. 4B). The accumulation of CLA in the liver and/or the transformation of CLA into more active metabolites may account for this difference.

PUFA are known to inhibit the lipogenic pathway. The molecular basis of this regulation was recently described and involves competitive inhibition between physiological LXR agonists (oxysterols) and PUFA for the LXR ligand-binding domain, leading to the inhibition of SREBP1c induction by LXR, a crucial step in lipogenesis (22). We investigated the effects of CLA on LXR activity by cotransfecting cells with a construct encoding a Gal-4 DNA-binding domain fused to the ligand-binding domain of LXRα and a reporter plasmid in which expression of the reported gene was driven by a Gal4-responsive element. The efficiency of this assay was assessed. We observed a 2.5-fold increase in luciferase activity if the specific physiological LXR agonist 22(R)-hydroxycholesterol (22R-CS) was used alone, and 42% inhibition if this molecule was used in association with LA (Fig. 5). Dose-dependent inhibition of reporter gene transactivation by 22R-CS was observed in the presence both c9,t11-CLA and t10,c12-CLA,

![Fig. 3](image-url)  **Effects of linoleic acid (LA), c9,t11-CLA, and t10,c12-CLA on the activity of PPARα, PPARβ/δ and PPARγ chimeras.** COS-1 cells were cultured and transfected with plasmids expressing the Gal4-hPPARα, Gal4-hPPAR, and Gal4-hPPARγ chimeras and a reporter plasmid carrying five copies of the DNA-binding element of the yeast transcription factor Gal4 cloned upstream from the thymidine kinase promoter and the luciferase reporter gene. Cells were treated for 36 h with vehicle (0.1% DMSO, v/v), LA, c9,t11-CLA, or t10,c12-CLA (1 to 200 μM). Cell extracts were then analyzed for luciferase activity and protein content.
demonstrating that the two CLA exerted inhibitory effects on LXR activation similar to those of LA (Fig. 5).

### t10,c12-CLA supplementation modifies the expression of hepatic genes

Substantial t10,c12-CLA-mediated modifications of hepatic gene expression may be responsible for liver steatosis. We investigated this possibility by studying the levels of expression of genes encoding transcription factors, lipid-binding proteins, and enzymes known to play a significant role in lipid metabolism. LA- and c9,t11-CLA-enriched diets had no significant effect on the pattern of expression of the genes studied. By contrast, supplementation with the t10,c12-CLA isomer quadrupled PPARγ mRNA levels. This upregulation was accompanied by the robust induction of two typical PPARγ target genes: those encoding the fatty acid transporter (FAT/CD36) and the ALBP, known to be involved in LCFA uptake in adipocytes. A slight but significant increase in liver sterol responsive element-binding protein 1a (SREBP1a) mRNA levels was also observed. By contrast, expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene, which encodes a key gluconeogenic enzyme, decreased significantly following t10,c12-CLA treatment (Fig. 6). Finally, other mRNAs, encoding PPARα, PPARβ/δ, SREBP1c, SREBP2 (Fig. 6), and liver-X-receptors (LXRα, LXRβ, data non shown), showed no clear change in level.

### t10,c12-CLA-fed mice are markedly hyperinsulinemic

The hepatic phenotype developed by mice fed the t10,c12-CLA-enriched diet (fatty liver associated with the overproduction of PPARγ and FAS) is very similar to that found in fat-depleted transgenic aP2/SREBP1c (32), A-ZIP/F1 (33), and aP2/DTA (34) mice. Expression of the PPARγ (35) and FAS genes is inducible by insulin and an inverse correlation between plasma leptin and insulin levels has been found in these lipoatrophic mouse models. We therefore decided to assay these two hormones. Leptin is secreted by both white and brown adipose cells as a function of fat accumulation. As expected, the depletion of fat stores specifically mediated by t10,c12-CLA supplementation was associated with a significant decrease in plasma leptin levels, which was not reproduced in mice fed the LA- or c9,t11-CLA-enriched diets (Fig. 7A). A dramatic increase in non fasting plasma insulin levels was observed in mice fed the t10,c12-CLA-enriched diet, these mice displaying insulin concentrations 10 times higher than mice fed control, and LA- or c9,t11-CLA-enriched diets (Fig. 7B). Despite this marked hyperinsulinemia, plasma glucose concentration remained within the normal range in these mice (Fig. 7C).

### DISCUSSION

The possible beneficial effects of CLA supplementation in decreasing body fat mass have received a great deal of
and ALBP, was observed in mice fed the normal liver, such as those encoding PPARγ, 
was added 16 h later and cells were incubated for a further 20 h. Luciferase activity
micromoles of 22(R)-hydroxycholesterol (22R-CS) was added 16 h later and cells were incubated for a further 20 h. Luciferase activity was measured and standardized according to β-galactosidase activity. Means ± SE, n = 3, **P < 0.01 versus 22R-CS.

Fig. 5. CLA inhibits LXR activation by 22R-hydroxycholesterol. HEK293 cells were cotransfected with the Gal4-driven luciferase reporter construct, a plasmid encoding the Gal4 DNA-binding domain fused to the ligand-binding domain of LXR, and a control plasmid, pSV-βGal. The cells were then incubated with 0 μM, 10 μM, or 100 μM linoleic acid (LA), c9,t11-CLA or t10,c12-CLA. Ten micromoles of 22(R)-hydroxycholesterol (22R-CS) was added 16 h later and cells were incubated for a further 20 h. Luciferase activity was measured and standardized according to β-galactosidase activity. Means ± SE, n = 3, **P < 0.01 versus 22R-CS.

attention, but the potentially adverse effects of CLA on the liver and insulin balance have been largely ignored. This is paradoxical because CLA-mediated hepatomegaly and/or hyperinsulinemia have been observed in several animal studies (2) and a trend toward an increase in insulin levels has been reported in humans (36). The effects of CLA are especially dramatic in the C57Bl/6J mouse strain, in which chronic supplementation with a 1% equimolar mixture of the c9,t11-CLA and t10,c12-CLA isomers induces a marked loss of body fat and massive fatty liver accompanied by marked hyperinsulinemia (12). This study provides the first demonstration that the t10,c12-CLA isomer is responsible for this remarkable phenotype, which closely resembles the lipoatrophic diabetes syndrome found in transgenic fat-depleted aP2/SREBP1c (32), aP2/DTA (33), and A-ZIP mice (34). It also provides evidence for profound t10,c12-CLA-mediated changes in the pattern of hepatic gene expression, contributing to fat accumulation in the liver. Indeed, the strong and specific induction of genes expressed at only very low levels in the normal liver, such as those encoding PPARγ, FAT/CD36, and ALBP, was observed in mice fed the t10,c12-CLA-enriched diet. Similar hepatic overexpression of the PPARγ gene has also been reported in fat-less A-ZIP/F-1 and aP2/DTA transgenic mice and in ob/ob mice, suggesting that it is a specific feature of steatotic livers (37). As FAT/CD36 and ALBP are cellular lipid-binding proteins, their overproduction is likely to increase fatty acid uptake capacity in the liver. The increase in FAS mRNA levels in t10,c12CLA fed mice demonstrates that the lipogenic activity of the liver is also specifically induced by this CLA isomer. This effect may be accounted for by the concomitant induction of the SREBP1a gene, which is known to be involved in regulation of the hepatic lipogenic program (38).

It was recently suggested that the observed CLA-mediated changes in body composition result from the direct activation of PPARs (17). The lack of reproduction of a lipoatrophic diabetes-like syndrome in mice fed a diet enriched in c9,t11-CLA, even though this isomer can also bind and activate PPARα and PPARβ/δ, is not consistent with this hypothesis. Indeed, the upregulation of typical PPARα and PPARβ/δ target genes, such as L-FABP (25), by both t10,c12CLA and c9,t11-CLA clearly dissociates this activity from hepatic fat accumulation. This conclusion is consistent with recent data obtained in PPARα-null mice. Indeed, although the CLA-dependent activation of PPARα target genes is not reproduced in the liver of PPARα−/− mice, the absence of PPARα does not preclude the reduction of body fat mass and liver enlargement (39). LXR is another transcription factor known to be involved in regulation of the rate-limiting enzymes of lipogenesis. LXRα activation assays have demonstrated that t10,c12CLA-induced fat storage in the liver cannot be accounted for by a specific agonist effect of this CLA isomer. Moreover, both the c9,t11-CLA and t10,c12-CLA isomers inhibit LXRs in a similar manner to PUFA (22). Thus, our data demonstrate that t10,c12-CLA-mediated liver steatosis is not dependent on the specific activation/inhibition of PPARα, β/δ and γ, or LXRα.

Thus, the train of events leading to t10,c12-CLA-induced alterations in the liver remains unclear, but is probably indirect. Several lines of evidence strongly suggest that the liver steatosis occurring in t10,c12-CLA-fed mice is secondary to hyperinsulinemia, which causes high levels of FA uptake and synthesis (Fig. 7). First, fatty liver was not observed in c9,t11-CLA-fed mice, which remained normoinsulinemic (Figs. 2 and 7). In these conditions, levels of expression of the PPARγ and FAS genes were low and similar to those in mice fed the control diet. Second, in CLA-fed mice and in aP2-SREBP1c and A-ZIP/F1 fat-less transgenic mice, fat deposition in the liver is reversed if blood insulin and leptin levels are normalized by systemic leptin infusion (12, 40, 41). Third, hyperinsulinemia is associated with the induction of PPARγ gene expression in the liver and with liver steatosis in several mouse models (37). Fourth, insulin is known to upregulate PPARγ gene expression in adipocytes (35) and to induce FAS gene expression in the liver (42, 43). Finally, and most importantly, the downregulation of PEPCK strongly suggests that the livers of t10,c12-CLA-fed mice remain sensitive to insulin.
The cause of the dramatic hyperinsulinemia triggered by the \textit{t}10,\textit{c}12-CLA-enriched diet remains to be determined. Further experiments are required to determine whether CLA supplementation alters insulin secretion by pancreatic β-cells.

CLA have been found to affect body composition in several animal models including mice, rats, hamsters, rabbits, chickens, and pigs. However, it is unclear whether the lipoatrophic effects of \textit{t}10,\textit{c}12-CLA isomer found in the C57Bl/6J mouse can be extrapolated to other species. Indeed, the response to CLA appears to be highly species-specific, with mice generally more sensitive than other rodent species (44). Moreover, within a single species, differences between strains may be observed. For instance, lev-
els of fat accumulation in the liver appear to be higher in C57BL/6J, CD-I, and AKR/J mice than in SENCAR mice (12, 14, 43, and data presented here). In a recent review, Pariza et al. attributed these differences in CLA responsiveness to species-specific body fat turnover, which may be higher in mice than in larger mammals. Indeed, CLA-mediated fat loss appears to be largely dependent on fat turnover because the action of CLA on adipose tissue results in the inhibition of fatty acid uptake by the adipocyte, with no change in the lipolytic activity of the cell (1). Therefore, the lack of a clear effect of CLA on body fat mass in some species may be due to low levels of fat turnover during the duration of the experimental period (1).

In humans, few clinical studies have been carried out and the results available are not readily comparable. For instance, no significant change in body fat mass and energy expenditure was found in healthy women (45) subjected to CLA supplementation (3 g/d for 64 d). By contrast, a more intense CLA treatment (3.4 or 6.8 g/d for 12 weeks) was found to be positively associated with a significant decrease in body fat mass in overweight and obese humans (46). Regarding the detrimental effects of t10,c12CLA on the liver demonstrated in the C57BL/6J mouse strain, the lack of reliable data for humans necessitates further investigations before any conclusions can be drawn as to the possible clinical value of CLA supplementation with a commercial mixture as a means of weight management. 10

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