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## **Conjugated linoleic acids: all the same or to everyone its own function?**

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**Abstract** — Conjugated linoleic acid (CLA) is a generic term referring to a mixture of geometrical and positional isomers of linoleic acid in which up to 16 members have been identified. Many potentially beneficial health effects have been ascribed to these fatty acids when consumed as a mixture, and where generally 2 isomers dominate, e.g. the 9c,11t-isomer, the so-called rumenic acid, and the 10t,12c-isomer: anti-carcinogenic, immune modulator, anti-atherosclerotic, and anti-obesity among the most spectacular. The question arises as to whether the pleiotropic biological activity is supported by one or several of the isomers. Recent studies using pure individual isomers have started to elucidate this issue, but many others are required to ascribe a respective role to each CLA isomer (the main ones as well as the minor ones), such as those occurring in some complex mixtures already commercially available, or even in foodstuff. The aim of the present study was to focus on the CLA-isomer specific effects depicted in the literature up to now.

**CLA isomers / rumenic acid / cancer / obesity / atherosclerosis**

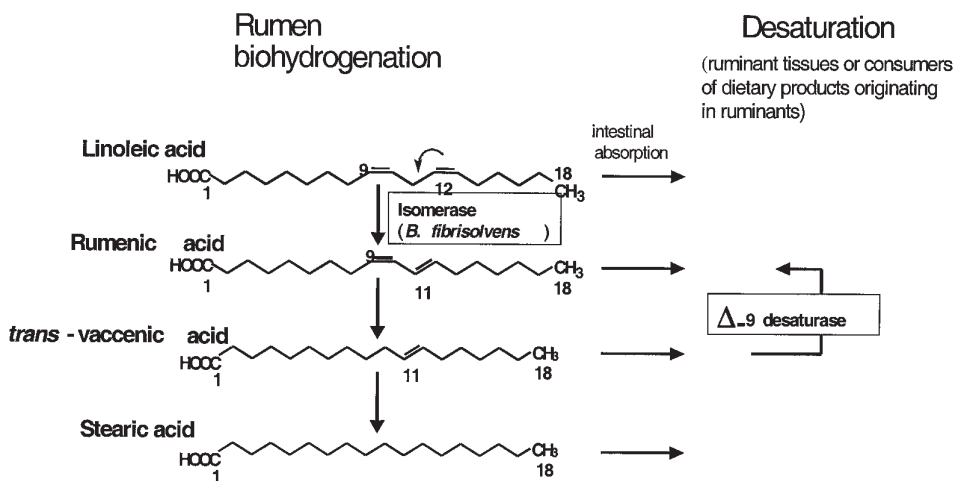
### **1. INTRODUCTION**

A great deal of concern has arisen from the study of conjugated linoleic acids (CLA), because of their considerable pleiotropic effects: anti-carcinogenic, immune modulator, anti-diabetic, anti-obesity, anti-thrombotic and anti-atherogenic [4, 5, 47]. CLA

are 18 carbon chain-length fatty acids with 2 double bonds. These are therefore isomers of linoleic acid, but to the contrary to this essential fatty acid where the double bonds are methylene-interrupted, they are consecutive (e.g. conjugated) in CLA (Fig. 1). The double bond system is localized on carbons 7,9; 8,10, etc. up to carbon 12,14 of the

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**Figure 1.** Natural origin of ruminic acid: rumenal synthesis from linoleic acid, and endogenous occurrence from delta-9 desaturation of trans-vaccenic acid (from [26], with permission).

olefinic chain, including all possible geometrical combinations (*cis/cis*, *cis/trans*, *trans/cis* et *trans/trans*). As many as 16 members have been identified thus far in marketed products [38]. The latter are obtained by alkaline isomerization of vegetable oil enriched with linoleic acid (safflower oil, sunflower oil) and sold as food supplements. In this example, the CLA isomeric distribution is generally dominated by 2 main isomers, e.g. the 9*c*,11*t*-isomer and 10*t*,12*c*-isomer, including in some preparations the 8*t*,10*c*- and 11*t*,13*c*-isomers. Conversely, the 9*c*,11*t*-isomer is the main CLA occurring naturally in foodstuff (up to 80% of total isomers) [23, 43], although the other isomers are also present in minor amounts and should therefore be considered as “natural” compounds [43]. Importantly, the balance between the various isomers is not the same in synthetic CLA products and those occurring from natural sources. Since most of the CLA intake and therefore the 9*c*,11*t*-isomer arise from ruminant products, this isomer is called ruminic acid. It is a by-product of microbial biohydrogenation that takes place in the rumen from linoleic acid (and  $\alpha$ -linolenic acid) occurring from plants

and ingested by ruminants (Fig. 1). Some of the ruminic acid formed escapes total hydrogenation and is taken up by the intestines and reaches milk and muscle lipids. Trans-vaccenic acid (18 carbons long, one *trans*-double bond located in the  $\Delta$ -11 position, another by-product of the biohydrogenation reaction), can also undergo delta-9 desaturation in the intestines, liver, mammary gland, and adipose tissue, and there it forms ruminic acid endogenously [25] (Fig. 1). In humans, trans-vaccenic acid occurring from the intake of ruminant products can be similarly converted to the 9*c*,11*t*-isomer [1, 48]. This comes in addition to the daily 200–400 mg of pre-formed ingested ruminic acid [15].

When dealing with the bioactivity of CLA, it is likely that their structural peculiarities underly some of their radically different actions when compared to linoleic acid (reviewed in [4, 47]). Nevertheless, only a few studies have addressed the discrete potency of each isomer (Tab. I), or the particular synergistic or competitive isomers-effects of several isomers present together in the same mixture.

**Table I.** Summary of the biological effects involving selected isomers of CLA.

Biological effects	9 <i>c</i> ,11 <i>t</i> -CLA	10 <i>t</i> ,12 <i>c</i> -CLA	Other isomers	Comments
Anti-cancer	+	+	?	Strong evidence for the 9 <i>c</i> ,11 <i>t</i> - in animal models
Decrease of fat body mass	0	+	0 (9 <i>t</i> ,11 <i>t</i> -)	Efficiency is species-dependent (requires confirmation in humans)
Anti-atherosclerosis	+(?)	+(?)	?	Needs further confirmation
Insulin resistance	0	+ or –	?	In rats, not in humans, 10 <i>t</i> ,12 <i>c</i> -CLA may improve glucose tolerance or induce strong insulin resistance depending on the initial physiological status
Immune modulation	?	?	?	No study reported with individual isomers
Fatty acid desaturation	– (delta 6)	– (delta 9) + (delta 5)	?	
Eicosanoid synthesis	–	–	–	9 <i>c</i> ,11 <i>t</i> - is more potent for the constitutive PGH synthase, followed by 9 <i>c</i> ,11 <i>c</i> , 9 <i>t</i> ,11 <i>t</i> , and 10 <i>t</i> ,12 <i>c</i> . All of these isomers are equally potent for COX-2 (inducible)
Pro-inflammatory agents (cytokines and NO)	–	–	–	9 <i>c</i> ,11 <i>t</i> ; 9 <i>c</i> ,11 <i>c</i> ; 9 <i>t</i> ,11 <i>t</i> ; and 10 <i>t</i> ,12 <i>c</i> - isomers equally potent
PPARα ligand & activator	+	+	+ (9 <i>t</i> ,11 <i>t</i> -)	9 <i>c</i> ,11 <i>t</i> - is more potent
PPARγ activator	+	+	+(9 <i>c</i> ,11 <i>c</i> -; 9 <i>t</i> ,11 <i>t</i> -)	

+: positive effect; –: negative effect; 0: neutral.

For further details, refer to appropriate references and text section.

## 2. CANCER AND CLA: WHICH ISOMER IS POTENT?

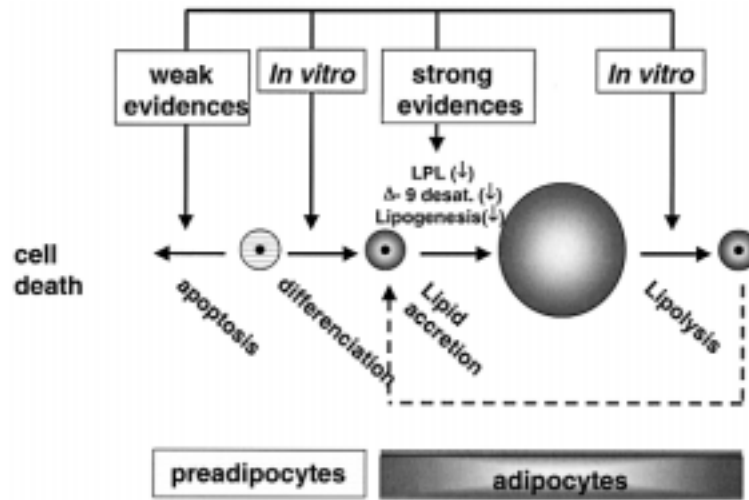
Fifteen years ago Pariza et al. [17] found that CLA of a fried ground beef extract was highly potent in reducing epidermal tumor incidence of mice topically treated with 12-*O*-tetradecanoylphorbol-13-acetate. This suggests that the anticarcinogenic effect was due to one isomer, since the CLA in ruminant products are mainly made up of 9*c*,11*t*-CLA. The potency of this isomer has been further confirmed by us [22] and Ip [19] in another carcinogenic model, e.g. NMU-induced rat mammary carcinogenesis. In these experiments, female rats were injected with a pro-carcinogen and fed for 6 months with diets containing either a CLA mixture (complex mixture), or a chemically-prepared 9*c*,11*t*-CLA [22], or a butter diet naturally enriched in the 9*c*,11*t*-CLA, or artificially increased with a CLA mixture (Ip study). In both experiments, CLA amounted to 1% by weight. The cancer risk was decreased in all CLA-diets, including those enriched with only the 9*c*,11*t*-isomer, thereby indicating a high anticarcinogenic potency for this isomer, the so-called rumenic acid. In addition, a recent case-control study carried out on Finish women [3] demonstrated that breast cancer risk is decreased by 2.5 fold (odds ratio of 0.4) in the women with the highest serum 9*c*,11*t*-CLA concentrations as compared to those with the lowest concentrations. On the contrary, in vitro studies have also demonstrated that the 10*t*,12*c*-isomer is even more potent than the 9*c*,11*t*-isomer against colorectal cancer proliferation [33]. In conclusion, there is a strong indication that the 9*c*,11*t*-isomer has an anticarcinogenic effect; there is also a good indication that the 10*t*,12*c*-isomer is also a potent anticarcinogen.

## 3. CLA ISOMERS IN THE MANAGEMENT OF FAT BODY MASS

There is now strong evidence showing that the 10*t*,12*c*-isomer is mostly responsi-

ble for the fat reduction observed upon CLA treatment [34]. This was demonstrated effectively by a mouse study in which different purified isomers were used (10*t*,12*c*-, 9*c*,11*t*-, 9*t*,11*t*) and in which the 10*t*,12*c*-isomer was the most efficacious in decreasing body fat mass [35]. According to Pariza [34], CLA and specifically the 10*t*,12*c*-isomer blocks body fat gain, but does not necessarily reduce the body fat level which had accumulated prior to the CLA administration (Fig. 2).

We reached the same conclusion in our experiments. We fed hamsters a lipid-enriched diet (33% in energy) for 8 weeks, supplemented or not with CLA (1% by weight). Only the CLA diet containing the 10*t*,12*c*-isomer prevented the accretion of body triglyceride over time, while the diet containing the sole 9*c*,11*t*-isomer failed to do so [8]. A recent human study examined the effects of feeding obese men with the metabolic syndrome for 12 weeks 3.4 g of either 10*t*,12*c*-CLA, or a CLA mixture containing equal amounts of both the 9*c*,11*t*- and 10*t*,12*c*-CLA [40]. The sagittal abdominal diameter and % body fat (determined by bioelectrical impedance analysis) decreased similarly in both CLA groups compared to the baseline values, but not compared to the placebo values at the completion of the study. It is noteworthy that the treatment with 10*t*,12*c*-CLA, but not with the CLA mixture, worsened the insulin sensitivity in that population. Other human data performed on populations free of the metabolic syndrome have reported the use of more or less complex mixtures in which the bioactive isomer in fat management, e.g. the 10*t*,12*c*-isomer, is diluted among the other isomers (10*t*,12*c*-isomer ranging from less than 20% [52] to up to 45% [44] of the overall CLA isomers). This variable dilution of the 10*t*,12*c*-isomer among the preparations might explain the fact that some authors have found an effective but moderate fat reduction upon CLA supplementation [29, 39, 44], whereas others have failed [52]. Taken together, these studies indicate



**Figure 2.** Putative mechanisms underlying fat reduction by CLA (from [34], with permission). LPL: lipoprotein lipase;  $\Delta 9$  desat: delta-9 desaturase.

that the  $10t,12c$ -isomer is likely the biologically active isomer in fat management in humans, but its effect is more modest than that observed in animal models such as mice. Also, the other isomers present in the most complex mixtures are apparently not effective in reducing body fat.

In conclusion, there is strong evidence showing that the  $10t,12c$ -isomer is the active isomer in eliciting body fat reduction, whereas  $9c,11t$  is not. A more precise evaluation of the  $10t,12c$ -isomer on populations free of the metabolic syndrome would help to more precisely define the efficiency of CLA in body fat management.

#### 4. CLA ISOMERS AND ATHEROSCLEROSIS

There are four important well-recognized causes leading to a pejorative atherosclerotic phenotype: (1) the plasma lipid profile, (2) the lipid deposition in vessels, (3) the platelet aggregation feature, and (4) the inflammatory status in the arterial wall.

#### 4.1. CLA isomers and lipid status

A hamster study in which the  $10t,12c$ - or the  $9c,11t$ -isomer (0.66% by weight) were exchanged for linoleic acid in the diet revealed that only the  $10t,12c$ -CLA (or a CLA mixture containing this isomer) decreased the fasting value of LDL- and HDL-cholesterol (18 and 11%, [13]) and increased VLDL-TG (61%), while the  $9c,11t$ -isomer did not display such an effect. On the contrary, we demonstrated that when either the  $9c,11t$ -isomer (0.5% by weight) or a CLA mixture ( $10t,12c$ - &  $9c,11t$ -isomer, 50:50, 1% by weight) were added to a lard based-diet *without any substitution for linoleic acid*, only the  $9c,11t$ - additioned diet favorably increased the HDL-cholesterol (32%) as well as the HDL-/LDL-cholesterol ratio (55%), with no modification of the VLDL-TG content (+ 268% on the contrary for the CLA mixture) (unpublished results). The apparent discrepancy between the aforementioned studies may arise from the strain of hamsters used ( $F_1B$  in one case, LPN Golden Syrian in the other study), the based-diet, and the way CLA were added

to it (substitution of linoleic acid with CLA in one case, simple addition in the other one with no substitution), or a combination of the above reasons. In addition, it should be said that in one case the cholesterol content of the based-diet amounted to 0.01% (by weight), whereas it reached up to 0.06% in the second study, which makes the comparison of the studies difficult. With this in mind, in our study both CLA diets (9*c*,11*t*- and CLA mix) increased the lipoprotein scavenger receptor mass (e.g. SR-BI and LDL-r) in a similar manner in the liver. Thus, from our data it appeared that the 9*c*,11*t*-isomer is potent in reducing the atherosclerotic risk, whether provided alone or together with the 10*t*,12*c*-isomer in a mixture. Although the CLA mixture appears effective in preventing aortic lipid deposition and in increasing fatty streak regression in both hamster and rabbit models [21, 50] (but not in mice [32]), there are no in vivo studies so far dealing with this specific issue while using individual isomers in animals. An in vitro study examined the lipid secretion by a human hepatocyte-like cell line (HepG2) treated with either 9*c*,11*t*- or 10*t*,12*c*-CLA [24]. The VLDL-TG secretion rate was decreased only by the 10*t*,12*c*-CLA treatment. Nevertheless, this is not consistent with the observation of Riserius et al. [40] in obese humans with the metabolic syndrome, in which on the contrary, the VLDL-TG increased while feeding 3.4 g of the 10*t*,12*c*-CLA for 12 weeks. Feeding a CLA mixture induced no changes at the same time. This is consistent with other human studies using a CLA mixture which constantly demonstrate no changes in VLDL-TG in populations free of the metabolic syndrome [6, 7, 44]. Also importantly, in the population with the metabolic syndrome, HDL-C decreased in the 10*t*,12*c*-CLA group [40], which together with the rise in VLDL-TG do not appear favorable in the etiology of atherosclerosis. Therefore, the studies using purified isomers give inconsistent results, probably because both the models and the experimental settings were

different. More definitive conclusions as to the effect of the various CLA isomers on plasma lipids is therefore certainly required to compare experiments designed with a common background, such as animal model / population, CLA amounts and isomer profiles, metabolic status, ...

#### **4.2. CLA isomers and platelet aggregation**

Both the 9*c*,11*t*- and 10*t*,12*c*-isomers are highly potent in inhibiting the calcium ionophore, arachidonic acid- and collagen-induced Human platelet aggregation compared to linoleic acid [45]. In addition, they both inhibit the formation of the pro-aggregatory cyclooxygenase-catalyzed product, TxA<sub>2</sub> [45]. Therefore, both isomers seemingly possess similar antithrombotic properties, at least in vitro, on human platelets.

#### **4.3. CLA isomers, inflammation and macrophage differentiation**

In addition, these isomers may also individually inhibit the formation of the pro-inflammatory prostaglandin PGF<sub>2</sub>α by 40% in human saphenous vein endothelial cells stimulated by a calcium ionophore [49]. This can also contribute favorably to an antiatherogenic effect.

In an in vitro study using murine RAW macrophages [51], the authors also found that in this cell line, the 9*c*,11*t*-; 9*c*,11*c*-; 9*t*,11*t*-; and 10*t*,12*c*- isomers all identically decreased the production of the pro-inflammatory nitric oxide (NO) through inhibition (in a dose dependent manner) of the inducible nitric oxide synthase (iNOS) mRNA and iNOS gene promoter activity. In addition, they also decreased the production of TNFα by these macrophages, both at the protein and mRNA level, as well as the production of IL-1b, and IL-6. These latter anti-inflammatory effects might be seen as favorable in the etiology of atherosclerosis.



On the contrary, the 9,11-isomers only (9*c*,11*t*-; 9*c*,11*c*-; and 9*t*,11*t*-) were all potent in increasing the differentiation of HL60 cells into monocytes and macrophages [51], which seems contradictory with their anti-atherogenic role, since macrophages may ultimately lead to foam cell formation. Nevertheless, further studies are required to determine the net effect of CLA isomers among the anti-inflammatory role and macrophage differentiation on the pathogenesis of atherosclerosis.

In conclusion, both the 9*c*,11*t*- and the 10*t*,12*c*- isomers of CLA can be efficient in modulating the severity of atherosclerosis, either at the circulating lipid level or at the thrombotic and endothelial levels. On the contrary, all of the CLA isomers tested indistinctively decreased the level of pro-inflammatory agents produced in cell cultured-macrophages, whereas the 9,11-isomers only, and not the 10*t*,12*c*-isomer, induced macrophage differentiation. No human studies evaluating these potencies *in vivo* are available so far for the individual isomers.

## 5. CLA ISOMERS AND INSULIN RESISTANCE

In the mouse model, the ingestion of a CLA mixture has been reported to increase insulin secretion and to decrease glucose clearance, leading to the paroxystic conditions of the lipodystrophic syndrome [46]. This effect has been unambiguously ascribed to the 10*t*,12*c*-isomer [12] in female mice. Paradoxically, this is not the case in the diabetic *fa/fa* obese Zucker rat (ZDF) in which CLA administrated as a mixture improved glucose tolerance [41] as much as the anti-diabetic drugs, thiazolidinediones [18]. We observed that hamsters fed with a CLA mixture containing only the 9*c*,11*t*- and the 10*t*,12*c*-isomer (50:50 mixture) displayed an insulin resistance phenotype, whereas those fed with the 9*c*,11*t*-isomer did not (Unpublished results). In ZDF rats, only the 50:50 isomeric mixture is able to improve

glucose tolerance, whereas the 9*c*,11*t*-isomer alone fails [41]. Therefore, in the animal model it seems that the 10*t*,12*c*-isomer is responsible for both insulin resistance and glucose tolerance according to the basal metabolic feature of the animal, and that the 9*c*,11*t*-isomer is neutral. Human studies published thus far have not reported insulin resistance following supplementation with the CLA mixture. Nevertheless, this is not the case in a recent study comparing the effect of the 10*t*,12*c*-isomer to that of a CLA mixture on insulin-resistant obese humans, where the authors observed a 19% loss of insulin sensitivity merely in the 10*t*,12*c*-treated individuals compared to the placebo [40]. In conclusion, the 10*t*,12*c*-isomer seems to support the insulin-resistant phenotype even in humans. Nevertheless, such an effect is not observed when this isomer is included in a mixture [40]. One could underline the great care that should be taken both in the consumption of CLA by a selected population, and in the choice of the CLA isomer used, as well as in the interaction between both.

## 6. BIOAVAILABILITY OF CLA ISOMERS

### 6.1. Intestinal availability

One of the first questions when dealing with nutrients is their bioavailability. There is seemingly no selectivity in the intestinal absorption of either CLA isomers, at least when ingested as triacylglycerol [27]. Especially, all the geometrical isomers of 9,11- and 10,12-CLA displayed an identical lymph recovery, similar to that of linoleic acid.

### 6.2. Esterification in complex lipids and cell processing

This is not the case for their incorporation into complex lipids. In most cases, when a



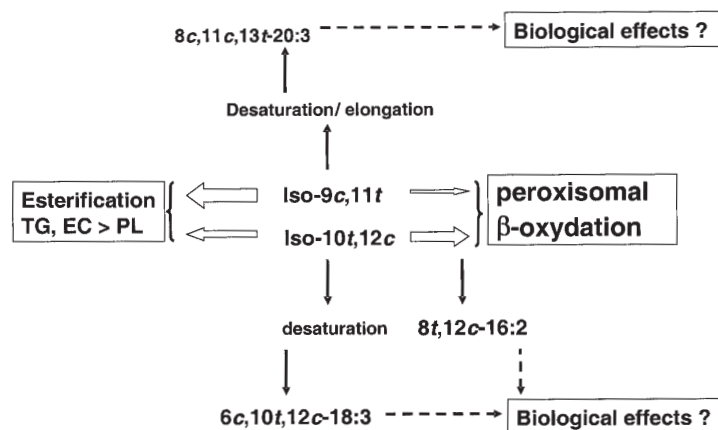
mixture of CLA is fed (up to 13 different isomers), the 9*c*,11*t*-isomer is almost always the main isomer found in tissue lipids, and the 10*t*,12*c*-isomer the least one [4, 20, 47]. A noticeable exception is in the heart phospholipids where the 11*c*,13*t*-isomer greatly accumulates over the other isomers and over its relative content in the diet [20]. Also, when rats were given either 9*c*,11*t*-, 9*t*,11*t*-, 10*t*,12*c*-, or 10*t*,12*t*- as TG and for 6 days, the 9,11 isomers generally accumulated in higher amounts in tissue lipids (liver, kidneys, brain, retroperitoneal adipose tissue and heart) than their 10,12 homologues [2]. These observations partly rely on differences in the post-absorptive metabolism among the isomers. This seems to be the case for the oxidative degradation pathways in cell peroxisomes. For instance, the 9*c*,11*t*-isomer oxidative breakdown is lower in these organites than for the 10*t*,12*c* isomer [16, 28]. This lesser breakdown would explain why the 9*c*,11*t*-isomer is found esterified in higher amounts in complex lipids (triacylglycerol mainly). In addition, if indeed most of the CLA might be oxidized or esterified, a small amount may undergo another metabolic fate: whereas the 9*c*,11*t*-isomer can be desaturated and chain-elongated, 10*t*,12*c*-CLA can be chain-shortened in the peroxisomes into a 16 carbon chain length

conjugated fatty acid, or else simply delta-6 desaturated merely to get a conjugated isoform of linolenic acid [42] (Fig. 3). It is of course likely that all of these metabolites can be biologically potent [4, 34].

## 7. MODULATION OF CELL METABOLISM BY SELECTED ISOMERS OF CLA

### 7.1. Fatty acid desaturation

Interestingly, both the 9*c*,11*t*- and the 10*t*,12*c*-CLA can interfere with the metabolism of the other fatty acids through modulation of the desaturase activities. However, the in vitro delta-6 desaturase activity is preferentially decreased by the 9*c*,11*t*-isomer [9, 11], with no change in the delta-6 mRNA expression [11]. On the contrary, the ingestion of the 10*t*,12*c*-isomer by rats (male Sprague Dawley) seems to increase the apparent delta-5 desaturation activity in the liver microsomes [42]. As a result, the C22 polyunsaturated fatty acid content in the liver membrane increases [42]. Moreover, the 10*t*,12*c*-isomer is able to decrease the in vitro and in vivo (apparent) delta-9 desaturase activity [9, 42] and gene expression [36], leading to less oleic acid



**Figure 3.** Cellular metabolism and bioavailability of the 2 main CLA isomers.

in tissue lipids. On the contrary, the human hepatocyte-like cell line (HepG2) treated with the 10*t*,12*c*-isomer have reduced apparent  $\Delta 9$ ,  $\Delta 6$ , and  $\Delta 5$  desaturase activities, whereas those treated with the 9*c*,11*t*-isomer have only reduced apparent  $\Delta 9$  activity [14]. Briefly, both isomers have a distinct effect on the desaturation of MUFA and PUFA, with the 10*t*,12*c*-isomer consistently reducing the  $\Delta 9$  desaturase. This, might be consequential to modulate the synthesis of eicosanoids as well as membrane fluidity and peroxydability. A decrease of the estimated  $\Delta 6$  and  $\Delta 9$  activities and an increase in the  $\Delta 5$  desaturase one were observed in humans consuming a CLA mixture [44]. Most generally, the biological consequence of the modification of the fatty acid profile by CLA isomers should be more thoroughly investigated.

### 7.2. Synthesis of eicosanoids

In addition to modulating the availability of the parent fatty acids involved in eicosanoid synthesis, CLA might also inhibit the *in vitro* prostaglandin synthesis through inhibition of the constitutive cyclooxygenase prostaglandin H synthase (PGHS) of Ram seminal vesicles. The 9*c*,11*t*-isomer appears to be the most effective, followed by the 9*c*,11*c*-, the 10*t*,12*c*-, and finally the 9*t*,11*t*-isomers [10]). A related finding has been observed with the inducible cyclooxygenase (COX-2 of the PGHS-2 complex) of cultured murine RAW macrophages [51], where several CLA isomers (e.g. 9*c*,11*t*-, 9*c*,11*c*-, 9*t*,11*t*-, and 10*t*,12*c*-) all identically decreased the mRNA expression of this enzyme. Nonetheless, it should be said that linoleic acid displayed the same effect. Consistent with the mRNA expression, the reaction product of COX-2, PGE<sub>2</sub>, decreased in a dose dependent manner along with 9*c*,11*t*- administration [51]. These observations are of major concern, since eicosanoids and especially prostaglandins are involved in most of the effects in which CLA are also

potent (e.g. carcinogenesis, atherosclerosis, obesity, immune function). This could contribute to explain some differences in the biological activity of individual CLA isomers.

### 7.3. Modulation of gene expression by selected CLA isomers through transcription factors

It has also been reported that individual CLA isomers are able to modulate gene expression since they can bind and activate transcription factors such as PPARs [30, 31, 51]. CLA isomers were shown to be ligands for human PPAR $\alpha$  with a rank order of potency of 9*c*,11*t* > 10*t*,12*c* > 9*t*,11*t*-, with the 9*c*,11*t*-isomer being the most efficacious PPAR $\alpha$  activator in a transfected cell model (COS-1) [31]. It was demonstrated that the 9*c*,11*t*-isomer was as potent as Wy-14,643, a well-known peroxisome ligand and activator, to activate PPAR $\alpha$  [30], consistent with the results of others [12]. On the contrary, CLA isomers do not always demonstrate a phenotypic feature of current peroxisome proliferators, such as in rats [28, 30] or hamsters [13] for instance. In addition, although CLA isomers and especially the 9*c*,11*t*-isomer are potent ligands and activators of PPARs, their lipid lowering effect still remains in PPAR $\alpha$  knock-out mice [37]. This indicates that the biological activities of either CLA isomers cannot be only ascribed to their activation of PPARs, and especially PPAR $\alpha$ . The PPAR $\gamma$  pathway is another pathway which is affected by CLA, as found in cultured murine RAW macrophages [51]. In this example, several isomers of CLA (9*c*,11*t*-, 9*c*,11*c*-, 9*t*,11*t*-, 10*t*,12*c*-CLA) shown the same PPAR $\gamma$  activation properties in these cells, which account for the above reported effects in the production of the pro-inflammatory agents by macrophages, as well as macrophage differentiation. Other pathways regulated by many other transcription factors need to be explored (such as C/EBP, LXR, HNF4 $\alpha$ , SREBP, NF $\kappa$ B) using the individual isomers of CLA to get

a clearer picture of the effect of CLA on these important aspects of gene regulation.

## 8. CONCLUSION

A summary of the main effects of the individual isomers thus far evaluated is listed in Table I. Of course, these effects in the selected issues reported need to be further explored, especially the effects of the individual isomers on gene expression. In addition, most of the published results deal with both the 9*c*,11*t*- and 10*t*,12*c*-isomers, and explain many of the already described effects obtained with complex mixtures of CLA. From this data, the claim that the 9*c*,11*t*-isomer is the only biologically active CLA is not supported. In addition, some distinct activities related to selected isomers are now appearing. Nonetheless, other isomers need to be evaluated, such as 11*c*,13*t*- sometimes present in high amounts in commercial preparations, or the 7*c*,9*t*-, present in dairy products, the 10*t*,12*t*- formed in heated oil, etc. The limitation is the cost of the marketed products. Also, the efficiency of individual isomers in humans must be assessed so that the respective role of CLA in health as well as their possible toxicological effects are known. Such an approach could help to delineate the balance between the risk and the desired benefit while consuming either isomer, with the knowledge of the possible side-effects.

## ACKNOWLEDGEMENTS

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### List of abbreviations

C/EBP: CAAT enhancer binding protein, CLA: conjugated linoleic acids, COX: cyclooxygenase, HDL: high density lipoproteins, HNF4 $\alpha$ : human nuclear factor 4  $\alpha$ , IL: interleukines, LDL: low density lipoproteins, LDL-r: VLDL/LDL receptor, LXR: liver X receptor, MUFA: monounsaturated fatty acids, NF $\kappa$ B: nuclear factor  $\kappa$ B, NMU: N-methyl-nitrosourea, NO: nitric oxide, PGE2: prostaglandin E2, PGF2 $\alpha$ : prostaglandin F2 $\alpha$ , PGHS: prostaglandin H synthase, PPAR: peroxisome proliferator activated receptors, PUFA: polyunsaturated fatty acids, SR-BI: scavenger receptor type I, SREBP: sterol regulatory element binding protein, TG: triacylglycerols, TNF $\alpha$ : tumor necrosis factor  $\alpha$ , TxA2: thromboxane A2, VLDL: very low density lipoproteins.