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

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(Received 14 June 2002; accepted 6 November 2002)

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
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 9c,11t-isomer and 10t,12e-isomer, including in some preparations the 8t,10c- and 11t,13e-isomers. Conversely, the 9c,11t-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 9c,11t-isomer arise from ruminant products, this isomer is called rumenic acid. It is a by-product of microbial biohydrogenation that takes place in the rumen from linoleic acid (and α-linolenic acid) occurring from plants and ingested by ruminants (Fig. 1). Some of the rumenic 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 Δ-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 rumenic acid endogenously [25] (Fig.1). In humans, trans-vaccenic acid occurring from the intake of ruminant products can be similarly converted to the 9c,11t-isomer [1, 48]. This comes in addition to the daily 200–400 mg of pre-formed ingested rumenic 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.

Figure 1. Natural origin of rumenic acid: rumenal synthesis from linoleic acid, and endogenous occurrence from delta-9 desaturation of trans-vaccenic acid (from [26], with permission).
Table I. Summary of the biological effects involving selected isomers of CLA.

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

+: 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 9c,11t-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 9c,11t-CLA [22], or a butter diet naturally enriched in the 9c,11t-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 9c,11t-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 9c,11t-CLA concentrations as compared to those with the lowest concentrations. On the contrary, in vitro studies have also demonstrated that the 10t,12c-isomer is even more potent than the 9c,11t-isomer against colorectal cancer proliferation [33]. In conclusion, there is a strong indication that the 9c,11t-isomer has an anticarcinogenic effect; there is also a good indication that the 10t,12c-isomer is also a potent anticarcinogen.

3. CLA ISOMERS IN THE MANAGEMENT OF FAT BODY MASS

There is now strong evidence showing that the 10t,12c-isomer is mostly responsible for the fat reduction observed upon CLA treatment [34]. This was demonstrated effectively by a mouse study in which different purified isomers were used (10t,12c-, 9c,11t, 9t,11t) and in which the 10t,12c-isomer was the most efficacious in decreasing body fat mass [35]. According to Pariza [34], CLA and specifically the 10t,12c-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 10t,12c-isomer prevented the accretion of body triglyceride over time, while the diet containing the sole 9c,11t-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 10t,12c-CLA, or a CLA mixture containing equal amounts of both the 9c,11t- and 10t,12c-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 10t,12c-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 10t,12c-isomer, is diluted among the other isomers (10t,12c-isomer ranging from less than 20% [52] to up to 45% [44] of the overall CLA isomers). This variable dilution of the 10t,12c-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...
Biological effect of CLA isomers

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-addeditioned 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 (F1 B in one case, LPN Golden Syrian in the other study), the based-diet, and the way CLA were added.

![Diagram](url)

**Figure 2.** Putative mechanisms underlying fat reduction by CLA (from [34], with permission). LPL: lipoprotein lipase; Δ9 desat: delta-9 desaturase.
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 (9c,11t- 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 9c,11t-isomer is potent in reducing the atherosclerotic risk, whether provided alone or together with the 10t,12c-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 9c,11t- or 10t,12c-CLA [24]. The VLDL-TG secretion rate was decreased only by the 10t,12c-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 10t,12c-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 10t,12c-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 9c,11t- and 10t,12c-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, TxA2 [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 PGF2α 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 9c,11t-; 9c,11c-; 9t,11r-; and 10t,12c- 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-1β 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 (9c,11t; 9c,11c; and 9t,11t-) 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 9c,11t- and the 10t,12c– 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 indiscriminately decreased the level of pro-inflammatory agents produced in cell cultured-macrophages, whereas the 9,11-isomers only, and not the 10t,12c-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 paroxysmic conditions of the lipodystrophic syndrome [46]. This effect has been unambiguously ascribed to the 10t,12c-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, thiazolinediones [18]. We observed that hamsters fed with a CLA mixture containing only the 9c,11r- and the 10t,12c-isomer (50:50 mixture) displayed an insulin resistance phenotype, whereas those fed with the 9c,11t-isomer did not (Unpublished results). In ZDF rats, only the 50:50 isomeric mixture is able to improve glucose tolerance, whereas the 9c,11r-isomer alone fails [41]. Therefore, in the animal model it seems that the 10t,12c-isomer is responsible for both insulin resistance and glucose tolerance according to the basal metabolic feature of the animal, and that the 9c,11t-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 10t,12c-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 10t,12c-treated individuals compared to the placebo [40]. In conclusion, the 10t,12c-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 9c,11t-isomer is almost always the main isomer found in tissue lipids, and the 10t,12c-isomer the least one [4, 20, 47]. A noticeable exception is in the heart phospholipids where the 11c,13t-isomer greatly accumulates over the other isomers and over its relative content in the diet [20]. Also, when rats were given either 9c,11t-, 9t,11t, 10t,12c-, or 10t,12t- 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 9c,11t-isomer oxidative breakdown is lower in these organites than for the 10t,12c isomer [16, 28]. This lesser breakdown would explain why the 9c,11t-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 9c,11t-isomer can be desaturated and chain-elongated, 10t,12c-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 9c,11t- and the 10t,12c-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 9c,11t-isomer [9, 11], with no change in the delta-6 mRNA expression [11]. On the contrary, the ingestion of the 10t,12c-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 10t,12c-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

![Diagram](image-url)

**Figure 3.** Cellular metabolism and bioavailability of the 2 main CLA isomers.
Biological effect of 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 PPARα with a rank order of potency of 9c,11t > 10t,12c > 9t,11r, with the 9c,11t-isomer being the most efficacious PPARα activator in a transfected cell model (COS-1) [31]. It was demonstrated that the 9c,11t-isomer was as potent as Wy-14,643, a well-known peroxisome ligand and activator, to activate PPARα [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 9c,11t-isomer are potent ligands and activators of PPARs, their lipid lowering effect still remains in PPARα 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 PPARα. The PPARγ pathway is another pathway which is affected by CLA, as found in cultured murine RAW macrophages [51]. In this example, several isomers of CLA (9c,11r; 9c,11c; 9t,11r; 10t,12c-CLA) shown the same PPARγ 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, HNF4α, SREBP, NFκB) 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 9c,11t- and 10t,12c-isomers, and explain many of the already described effects obtained with complex mixtures of CLA. From this data, the claim that the 9c,11t-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 11c,13t-sometimes present in high amounts in commercial preparations, or the 7c,9t-, present in dairy 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

Dr Amir Ravandi is fully acknowledged for his language expertise.

REFERENCES

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List of abbreviations