ASPECTS TECHNOLOGIQUES ET DEVELOPPEMENTASPECTS TECHNOLOGIQUES ET DEVELOPPEMENT

Technological and development aspects Combining linoleic acid and functional food?

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Résumé : L'acide linoléique conjugué (CLA) est présent dans les aliments d'origine animale, en particulier ceux issus des ruminants. Le CLA peut aussi être synthétisé au laboratoire par isomérisation alcaline de l'acide linoléique. Alors que l'isomère naturel est le 18 : 2 9c,11t, les produits de synthèse contiennent principalement deux isomères, les 18 : 2 9c ,11t et 10t,12c. De nombreuses études menées sur des modèles animaux, le plus souvent avec des mélanges synthétiques, montrent des effets potentiellement bénéfiques pour la santé (anticarcinogènes, protecteurs vis-à-vis de l'athérosclérose, modulateurs de la composition corporelle...) alors que la plupart des études menées chez l'homme concernent les effets des CLA sur la composition corporelle. À ce jour, les données concernant les effets sur la composition corporelle chez l'homme semblent indiquer un bénéfice potentiel, mais les résultats restent controversés. Par ailleurs, bien qu'un effet anticarcinogène soit démontré chez l'animal, aucune étude chez l'homme n'a pu établir de relation entre CLA et cancer du sein. De plus, peu d'études traitant des aspects sécuritaires liés à la consommation à long terme ont été publiées. Par conséquent, il est nécessaire de disposer de plus de données en particulier chez l'Homme pour garantir l'innocuité de ces acides gras afin de protéger le consommateur, avant d'envisager l'utilisation de CLA dans des aliments fonctionnels.

Mots-clés : acide linoléique conjugué, aliments fonctionnels, cancer, composition corporelle, athérosclérose, immunité, sécurité alimentaire.

Summary : Conjugated linoleic acid (CLA) is found naturally in many animal products especially those from ruminant sources. CLA can also be synthesized in the laboratory by isomerisation of linoleic acid. While the "natural" isomer is the 9c,11t-, synthetic mixtures contain mainly two isomers, the 9c,11t- and the 10t,12c-18:2. Many experiments carried out on animal models mainly using the synthetic isomers have shown different health benefits (anticarcinogenic, antiatherosclerotic effects, modulation of body composition...) while most of the studies carried out on human concern the effects of CLA on body composition. At the moment, the data on the effects on body composition in human seem to indicate a potential benefit but are still controversial. Furthermore even if anticarcinogenic effects have been demonstrated in animals, studies so far carried out on human have failed to demonstrate a relation between CLA and breast cancer and very few data dealing with

the safety of using CLA in long term feeding studies have been so far published. Consequently, before using CLA in functional food, it will be necessary to obtain more human data and to be able to guarantee the safety of these fatty acids in order to protect the consumer.

Keywords : conjugated linoleic acid, functional food, cancer, body composition, atherosclerosis, immunity, food safety.

ARTICLE

Due to their physiological effects, mainly demonstrated in animals, conjugated linoleic acid (CLA) has been stimulated research for the past few years.

CLA is mainly found in dairy products and meat from ruminants [1, 2]. In this case, the 9c,11t- isomer represents 85-90% of the total CLA isomers [3, 4]. It is produced by microbial conversion in the rumen and delta9 desaturation of vaccenic acid in the mammary gland of lactating cows [5].

However, CLA mixtures have also lately being produced by the industry [6]. The starting material in most cases is linoleic acid or oils rich in linoleic acid (sunflower, safflower). In general, the fatty acids are converted into conjugated forms with strong alkali [7]. In this case, a mixture of isomers is obtained in which the 10t,12c- and the 9c,11t- each represents about 40% of the total CLA isomers. These are accompanied by other ct/tc isomers (8,10; 11,13).

So far, many studies have been carried out using these "synthetic mixtures", which are now well defined considering the development of analytical techniques based on the combination of gas liquid chromatography, and silver-nitrate high performance liquid chromatography [3, 8].

This paper will review the research so far carried out on CLA and the potentialities of enriching foodstuff in CLA and/or using CLA in functional food.

Potential beneficial effects of CLA in animals

CLA and cancer prevention

Prevention of cancer by CLA in animals is with certainty the field of research which has been the most deeply investigated since the early 1990s. We do not aim to review exhaustively all the experiments carried out on CLA and cancer, extensive papers have already been published so far [9, 10]. Rather we aim to give in this section a brief general overview on CLA and mammary cancer prevention in animals.

The role of CLA was evaluated *in vitro* and *in vivo* in rat or mouse models of chemically-induced cancers of the mammary gland, of forestomach, intestine, prostate and skin. Concerning mammary carcinogenesis, a typical experiment follows a design in which CLA is added to the diet during gland maturation (52d post-wean-ing in the rat), the carcinogen is then added and tumor progression is analyzed in term of number and size of tumors and tumor incidence along the following weeks. Two carcinogens were used by Ip and co-workers in 1994 [11]: 7,12-dimethylbenz(a)anthracene (DMBA) or methylnitrosourea (MNU). CLA given to rats at 1% of the diet was able to reduce the number of tumors and tumor incidence by about 35% in both chemically-induced models. This finding was of

importance as it suggests that CLA would reduce tumorigenesis by interefering in the metabolism of DMBA and/or either by preventing the direct alkylating action of MNU on DNA. While this study was carried out with a mixture of CLA consisting of 9c,11t-18:2, 10t,12c-18:2 in equal proportion and other minor isomers, one other trial was performed with rumenic acid (9c,11t-18:2). Compared to the CLA mixture, rumenic acid was as effective to reduce the number and incidence of mammary tumors [12] *(Table)*. How do CLA act? One mechanism would be by reducing the mass of the mammary epithelium and the terminal end bud density in the mammary gland [12]. In such a way, the maturation of the mammary epithelium would be down-regulated, and would explain the CLA-induced reduction of mammary cancer.

CLA and body composition

CLA exhibit beneficial effects on lean to fat mass composition in animals. CLA-induced reduction of fat to protein mass was reported to occur in rats [13], mice [14-19] or pigs [20, 21]. Again more or less complex mixtures of CLA isomers were used for these experiments. However, the 10t,12c-18:2 isomer was consistently associated with this effect [14, 15]. The mechanism that underlies this action may be found in the dual effect of CLA on carnitine palmitoyltransferase and lipoprotein lipase activities. By enhancing the former enzyme in muscle and inhibiting the latter in adipose tissue [17], CLA may reduce the intracellular fatty acid concentration, and as a consequence would explain the reduction of fat mass.

CLA also exhibit one other interesting action in connection with growth. Animals fed with CLA showed significant reduction of feed intake without affecting consistently body weight [14, 18]. This would result from a better feed efficiency.

CLA, blood lipids and atherosclerosis

So far few studies have evaluated the role of the different CLA isomers taken alone on blood lipids. Nevertheless, the lipid lowering effect of CLA mixtures [22, 23] can be assigned to 10t,12c-18:2, but not to 9c,11t-18:2 [22, 24]. In this field, assuming that the reduction of cholesterol and triacylglycerols levels in blood lipids would represent a less favourable atherosclerotic profile, dietary CLA may thus prevent such a disease in animals. However, caution should be taken when looking at the results of a study performed in C57/BL6 mice by Munday *et al.* [25]. Mice were fed 15 weeks an atherogenic diet containing 10% of cholesterol and 2.5 or 5% of CLA. Serum triacylglycerol level was lowered and the ratio of HDL- to total cholesterol was increased in the CLA treated mice. These data are consistent with the results reported above and in-line with the correlation between CLA consumption and a less atherogenic profile. However, aortic fatty streak area has also been analyzed, and these authors reported increased values in mice fed 2.5% of CLA. One may thus contradict the potential benefits of CLA in connection with the blood lipids, if not considering the great variability of the data within the different groups: $0.13 \pm 0.13 mm^2$ in the control group, $0.33 \pm 0.27 mm^2$ in the 2.5%-CLA group (mean ± SD). In view to extrapolate the effects observed in animals to humans, other models which physiology is more comparable to humans, rabbits and pigs were investigated. Rabbits fed daily 0.5g of CLA and a pro-atherogenic diet (0.1% cholesterol), showed a significant lower LDL- to HDL-cholesterol ratio after 22 weeks and thicker aortic fatty lesions, although the difference in this parameter failed to reach significance [26]. Later this study was re-evaluated to test whether CLA would prevent also regression of fatty streaks. At dietary levels of 0.1% CLA inhibited atherosclerosis in rabbits, and at 1% CLA substantially caused a 30% regression of established lesions [27]. Adult

female swines were fed 6 weeks a diet containing 1% of CLA (34.6% rumenic acid plus 9t,11c-18:2, 18.4% 10t,12c-18:2 and minor isomers). Compared to the control animals fed sunflower oil, triacylglycerol and cholesterol contents of VLDL and LDL were increased by CLA. The difference reached significance for the level of triacylglycerols in LDL fractions only (+58%) and for the 26% increase of the LDL- to HDL-cholesterol ratio [28]. This is in contrast to other animal studies since it would suggest a positive correlation between CLA consumption and atherogenesis.

CLA and the immune response

During an immune stimulation macrophages are activated, leading to the production of cytokines like IL-1, and eicosanoids like prostaglandin E_2 from arachidonic acid. IL-1 induces PGE_2 release which in turn down regulates IL-1 production. Application of cytokines and PGE_2 to muscle leads to its degradation, called cachexia. When included in the diet, CLA prevented immune-induced weight loss [29]. This effect has positively been correlated to the inhibition of PGE_2 production in response to lipopolysaccharides in a macrophage cell line [30]. CLA has also been shown to enhance immune function by increasing circulating and splenic T-lymphocytes [31].

Human studies

Only limited data are available on CLA supplementation in humans and most of the studies published are short term experiments dealing with the effect of CLA on body composition.

One study done in Norway [32] on overweight or obese volunteers using 3.4g of CLA for 12 weeks showed a reduction of mean body weight and body fat mass in the CLA group compared to the placebo group (olive oil), but the data were not significant. However, in another experiment, the same group [33] found a reduction of body fat in the CLA group when it was administered at 1.8g/day to healthy exercising humans for 12 weeks. In studies report-ed by Vessby *et al*. [34] using CLA (mixture of 9c,11t- and 10t,12c-18:2), or purified 10t,12c-18:2 at 3.4g/day for 3 months, indications of reduction of body fat in the CLA groups were found but the differences were of border line significance. However, while the CLA mixture did not affect serum lipids or glucose metabolism, the 10t,12c- isomer caused an "impairment of the peripheral insulin sensitivity, blood glucose concentrations and HDL levels in serum".

Studies carried out in USA also using a CLA mixture [35], showed that supplementation of CLA for 2 months at 3g/d did not affect body composition or energy expenditure in adult women. In a similar 9 weeks experiment Medina *et al*. [36] demonstrated that 3g of CLA mixture given to the same population decreased circulating leptin concentrations in the first part of the study without any detectable changes of body fat mass while it returned to baseline levels over the last two weeks of supplementation. Furthermore, supplementation did not affect appetite, and the authors concluded that "these results are counter to what would be expected if CLA were able to reduce body fat in humans as it does in animals".

Two other human studies looking at the effect of CLA on blood parameters (platelet function, blood coagulation) were published recently [37]. It looks like a short term (93d) consumption of CLA (mixture of isomers) did not induce any changes on blood coagulation and platelet function in healthy adult females. Furthermore, CLA administration for 63 days did not change the level of plasma cholesterol, low density or high density lipoprotein cholesterol and triglycerides. On the

contrary to animal studies previously described CLA did not seem to have any health benefits as far as the prevention of atherosclerosis is concerned. However, we must be careful when drawing conclusions as these are short term studies using a very complex mixture of isomers.

To our knowledge, only the results of one study dealing with breast cancer were recently reported and are available as an abstract [38]. While extensive data have demonstrated that CLA have anticarcinogenic properties in animals, no evidence was found to document the potential of CLA to influence breast cancer presentation at diagnosis or to decrease the risk of metastases after the treatment.

Two studies also reported the effect of CLA on the human immune status. One study (93d) done on young healthy women showed that CLA (complex mixture of isomers) did not have beneficial effect on their immune status [39]. On the contrary, a study carried out in the Netherlands showed that a specific mixture of CLA (50% 9c,11t-, 50% 10t,12c-) stimulates the humoral immune response as reflected by an increase of the seroprotection rate (SPR) while a 80/20 CLA mixture did not affect the SPR [40].

Safety aspects

Animal studies

Among the numerous studies reporting physiological effects of CLA in animals, only a few of them are dealing with safety aspects. Only one experiment was conducted in order to study specific toxicological effects of CLA supplementation on male Fischer 344 rats [41]. It was reported that feeding CLA induced no modification of the organ weight, except thymus and no modification of the haematological profile. However, some other papers report some data on safety, even if it was not the major topic of the study.

Rat and mice studies

Most of the rat studies were carried out using the Sprague-Dawley strain. As previously described by Scimeca, several authors reported that CLA feeding did not induce any significant modifications of liver weight [13, 42, 43]. Sugano *et al.* [44] reported in 1997 that feeding CLA (1% in the diet) did not induce any modification of the weight of liver, heart, kidney, lung, spleen, brain and perirenal adipose tissue. In a further study [45], an increase of the liver weight (+9%) concomitantly with a decrease of the perirenal adipose tissue weight (-31%) was mentioned. No significant effects on the weight of other tissues were described. An increase in the liver lipid content has also been reported [46], but only after feeding 1.5% CLA. In the same study, Moya-Camarena *et al*. observed a decrease of the liver lipid content in female mice (but not in male) after feeding 0.5% CLA. Lipid droplets in the liver tissues were also observed by Yamasaki *et al*. [47] after feeding 2% CLA.

Stangl *et al.* [23] reported that feeding CLA up to 5% in the diet did not induce any modification of plasma protein, urea, ASAT and ALAT. However, albumin and creatinine levels were increased after 5 weeks of a diet containing 5% of CLA.

Some studies reported an enlargement of the liver [18, 25, 48], together with a modification of the colour of the organ and a hepatic lipidosis. These results were confirmed by Tsuboyama-Kasaoka *et al.* [19] who observed a strong decrease of the adipose tissues (white and brown) weights

concomitantly with an enlargement and lipidosis of the hepatic tissue. However, these data along with those of Munday *et al*., were obtained with C57/BL6 mice. It seems that this strain of mice is more sensitive to the dietary treatment. Feeding AKR/J mice for 39d with 1% CLA-supplemented diets, resulted in an increase of liver (+28%) and spleen (+18%) weight, but no lipid accumulation was observed in the hepatic tissue [16].

Besides these effects on the liver size, Belury's group [49] also reported a peroxisome proliferator like response, as CLA feeding induced an increase in ACO-, CYP4A1- and FABP-mRNA. On the other hand, Jones *et al.* concluded that such effects were not observable in rats, suggesting a specie-dependent response [50].

Other animal studies

CLA treatment also were evaluated on pigs and hamsters. F1B hamsters have been shown to present an increase in liver and kidney weight [24] after CLA supplementation. However, the kidney was heavier only after supplementation with the 10t,12c-CLA isomer. This point is important as all the other studies were carried out using CLA mixtures from different origins, which means different CLA isomer profiles. In the same study, the 10t,12c-isomer was also reported to induce a liver hypertrophy, as illustrated by a decrease of the number of nuclei in the histological analysis. On the other hand, hamsters did not present a peroxisome proliferator-like response, as indicated by the lack of increase of the related enzyme activities. Again, different responses may be observed according to the animal species used. To our knowledge, only one study in pigs mentioned some safety aspects. Feeding a CLA mixture for 6 weeks, Stangl *et al.* [28] reported no modification of T3 and T4, albumin, creatinine, urea and glucose in the plasma.

Taken all together, it is difficult to conclude about the safety of CLA supplementation, as observations may vary according to the different species and strains of animals. Moreover, the composition of the CLA mixtures used may also explain some differences. The results reported on C57BL6 mice are the most interesting, but well-conducted safety studies according to international guidelines are mandatory before taking any conclusions.

Human studies

Besides these animals studies, few human studies have also been so far reported and some of them mentionned some safety aspects. However, all the published studies used CLA mixtures from different origins. Consequently, final conclusions are difficult to draw as the effects of each isomer is not reported.

We can mention here the publication from Basu *et al.* [51] who reported that feeding CLA (4.2g/d, 12wk) induced an increase in the lipid peroxidation status in men, as illustrated by the urinary isoprostane content. On the other hand, it is stated that "No participant experienced any side effects during the study period". It was also demonstrated [52] that even if CLA induce both non-enzymic and enzymic lipid peroxidation in men, the lipid peroxidation parameters had returned to their basal levels 2 weeks after stopping the CLA intake *(Figure)*.

A human double-blind placebo-controlled safety study was recently reported [32]. The control subjects received olive oil where as the treated ones ingested CLA (3.4g/d, 12wk). No modification of blood parameters (blood lipids, hematology, liver enzymes, electrolytes, creatinine, LDH) were observed and the paper concluded on the safety of CLA under these experimental conditions.

Another human study was reported by Kelley *et al.* [39]. Young healthy women were fed 3.9g/d CLA for 63d. No modification of circulating white cells and no change in lymphocyte proliferation were observed. The latter differs from results obtained in different animal species, i.e. mice, rats and chicken [29, 53, 54].

These data seem to indicate that CLA supplementation is safe in humans, but long term studies with isolated isomers are needed before drawing definitive conclusions, as some data from animal studies indicate the CLA may have some adverse effects.

CONCLUSION

From the data which have been so far published, we may conclude that the results obtained from the animal experiments are much more convincing than the human data. However, most human experiments have been short term studies, using complex mixtures of CLA isomers and we think that it is rather difficult to conclude at the moment. We need more human studies using supplementation with pure isomers and we also need more data on the safety of CLA supplementation on long term basis. At the moment our laboratory is in collaboration with AFSSA determining the CLA content of different food items sold on the french market in order to be able evaluate the consumption of CLA by the consumer and to further carry out epidemiological studies. No doubt that all the studies which are planned in different laboratories working on CLA will permit to determine soon if utilisation of CLA in functional food is beneficial for the consumer.

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Illustrations

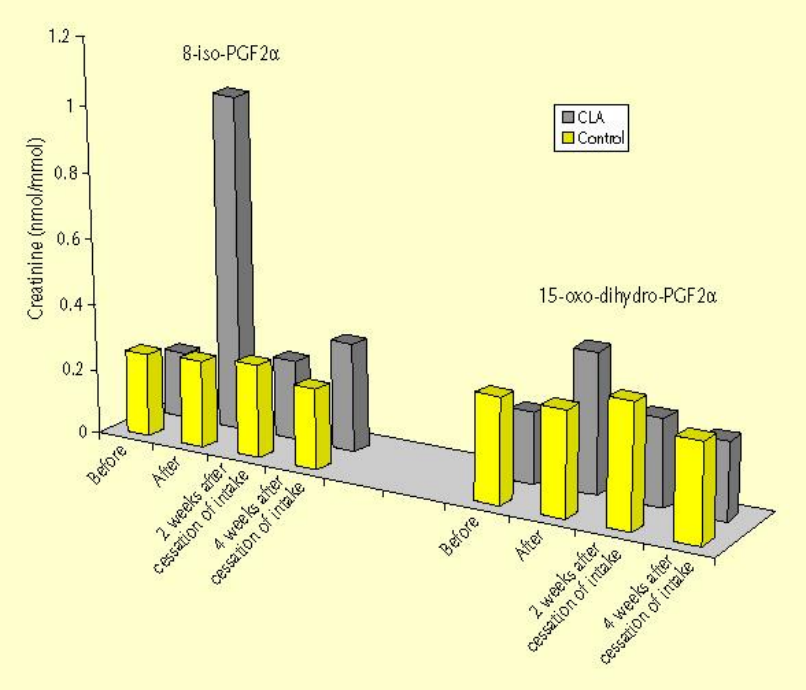

Figure. *Urinary levels of 8-iso-PGF2alpha* and *15-oxo-dihydro-PGF2alpha in control subjects and subjects treated with CLA for 1 month (from Basu* et al. *[52]).*

Table. *Bioassay of mammary cancer prevention in rats fed CLA (adapted from Ip et al. [12]).*

Group	CLA in diet ($g.L^{-1}$)	CLA composition	Tumor incidence	Total tumors (n)
Control	0.1	9c,11t- 83% 7t,9c- 6%	28/30	92
Butter CLA	0.8	9c,11t- 92% 7t,9c- 5%	15/30	43
CLA mixture	0.8	9c,11t- 25% 10t,12c- 37% 11c,13t- 18% 8t,10c- 15%	17/30	48