

CLAs, nature, origin and some metabolic aspects

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Abstract: CLA (conjugated linoleic acid) is a generic term for several isomers of linoleic acid with conjugated double bonds. They have been reported since 1935 in butter fat, but the major natural isomer (9cis,11trans-18:2) was identified in 1977 and further named « ruminic acid ». This fatty acid is formed in the rumen as a product of biohydrogenation. Tissues may also produce ruminic acid from vaccenic acid, which is a further intermediate of ruminal biohydrogenation. Interest for CLAs started with a report on beneficial effects of CLA from grilled beef on skin tumours. CLA was produced as mixtures of isomers from chemically modified vegetable oil. As a metabolic point of view, it has been shown that ruminic acid is bioconverted like linoleic acid and beta-oxidised. CLA isomers may also interfere with the metabolism of other fatty acids. Other targets than skin tumours have also been identified. These aspects will be developed in other sections of the present issue.

Key words: CLA, rumen, milk, meat, metabolism

Introduction

For several years, conjugated linoleic acid (CLA) isomers have been extensively studied, due to their potential effects on various biological functions and disorders (cancer, cardiovascular diseases, obesity, etc.) [1]. This interest is clearly illustrated by the increasing number of publications on CLA research since the mid' 90s. One can have a look at the exhaustive list of papers dealing with CLA research which is available at the <http://www.wisc.edu/fri/clarefs.htm> web address. As an example, more than 200 papers have been published per year in 2002 and 2003.

This short paper will review the nature and origin of different CLA isomers and will summarize CLA metabolism.

History of CLA

The CLA story started as early as 1935 when Booth reported that butterfat contained fatty acids which presented absorption at 230 nm [2]. This characteristic was then associated with the occurrence of conjugated double bonds associated with 18 carbon chains [3]. Later, it was reported that biohydrogenation occurs in the rumen. Unsaturated fatty acids were reported to be transformed in less unsaturated fatty acids with *trans* double bonds [4, 5]. In 1967, Kepler [6] reported that a ruminal bacteria, *Butyrivibrio fibrisolvens*, was able to produce conjugated 18:2 from linoleic acid. On the other hand, Kuzdal-Savoie and coworkers identified various *cis*, *trans* and *trans,cis* 18:2 isomers by using AgNO₃-TLC [7], and finally

Parodi [8] identified the major CLA isomer in dairy fat as 9cis,11trans-18:2. This fatty acid was then called "ruminic acid" by Kramer and Parodi in 1998, instead of "bovinic acid" which was considered as too restrictive [9].

Origin of CLA

The occurrence of ruminic acid in milk fat is now fully understood (figure 1). Dietary linoleic acid is hydrogenated in the rumen. Ruminic (9cis,11trans), vaccenic (11trans) and stearic (saturated) acids are formed as a result of hydrogenation, and vaccenic acid may also result from the hydrogenation of dietary α -linolenic acid (see the paper of Chilliard *et al* in this issue for more details). These fatty acids are made available for peripheric tissues. Among them, the mammary gland is able to

produce ruminic acid from vaccenic acid by a $\Delta 9$ desaturation. This pathway is now considered as the major source of ruminic acid in milkfat, as its contribution may represent about 60% of the ruminic acid content of milk [10]. Consequently, dietary α -linolenic acid of ruminants is a precursor of milk ruminic acid by this metabolic pathway.

Starting interest for CLA

Biological effects of CLA have been discovered when studying a lipid fraction from grilled beef, and not from milk and dairy products. At the end of the 80's, Ha and coworkers [11] studied the effects of different lipid fractions from grilled beef on chemo-induced skin tumours. They isolated a fraction containing CLA isomers and this fraction was shown to be able to

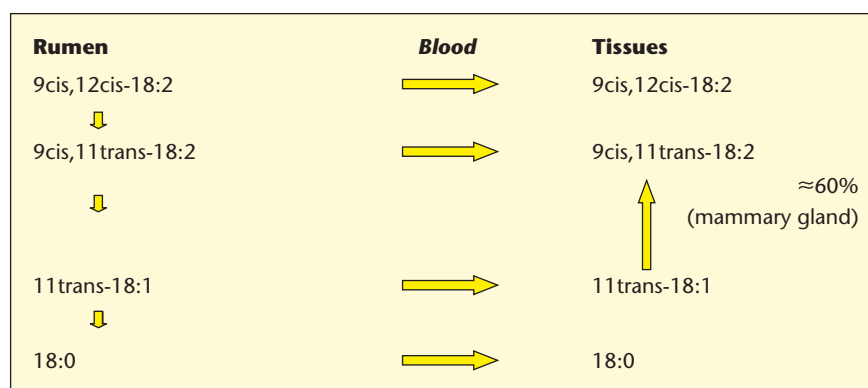


Figure 1. Metabolic pathways involved in the biosynthesis of ruminic acid in ruminants (adapted from Griinari, Corl *et al.* 2000 [10]).

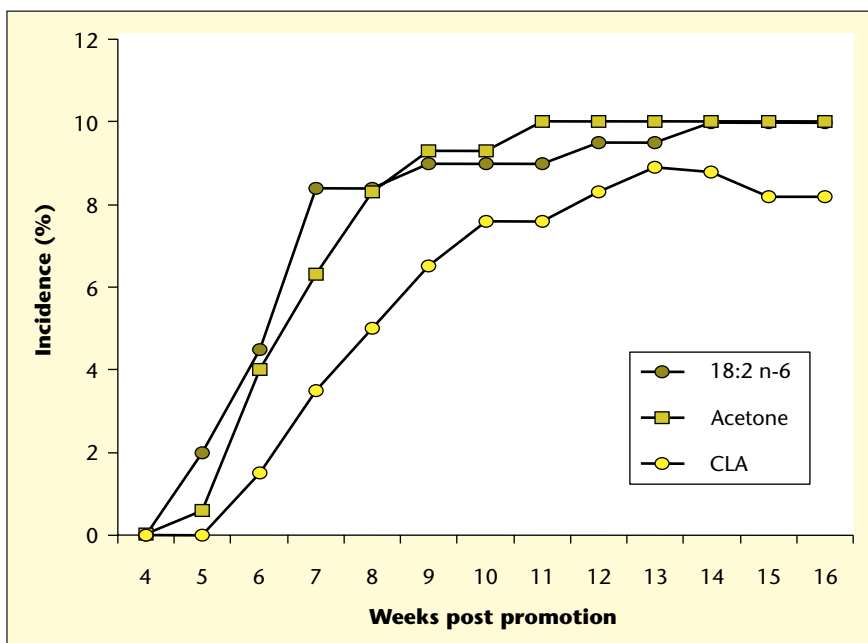


Figure 2. Cumulative incidence of skin tumours after induction by DMBA in mice fed CLA from grilled beef, linoleic acid of vehicle (acetone) adapted from Ha, Grimm *et al.* 1987 [11]).

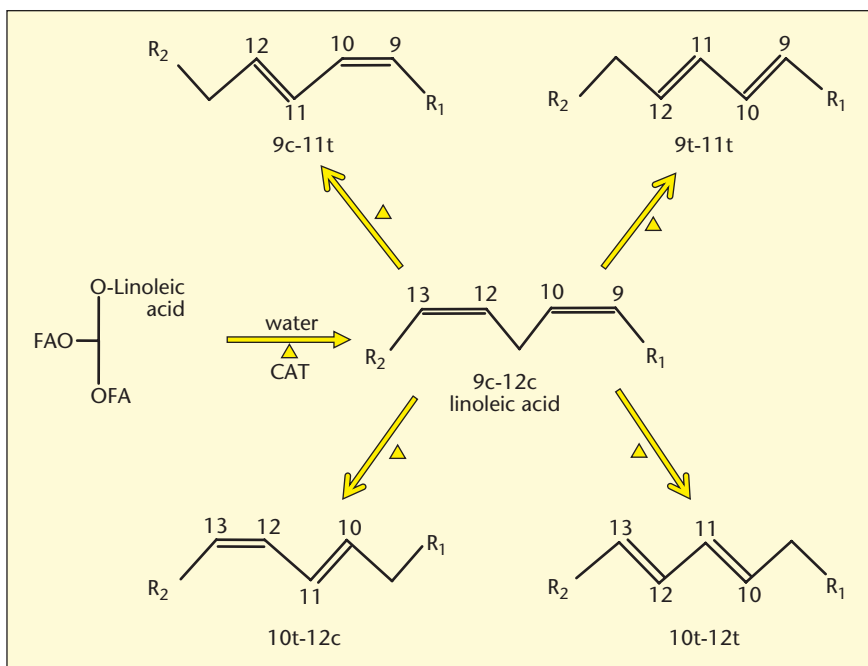


Figure 3. Formation of CLA isomers by radical oxidation of linoleic acid (adapted from Ha, Grimm *et al.* 1987 [11]).

reduce the tumour incidence in skin, when chemically induced by DMBA (figure 2). It was suggested that CLA isomers were formed during heating by radical isomerization of linoleic acid (figure 3), which produced several CLA isomers.

As a consequence, industrial production of CLAs was initiated, and mixtures of isomers were made available for various studies. These

were generally obtained by chemical isomerisation of linoleic acid-rich oils (e.g. safflower oil). These mixtures may contain more than 10 geometrical and positional isomers. However, most mixtures used in experiments mainly contain rumenic acid and the 10trans,12cis isomer (1:1 w/w), which together represent between 60 and 95% of the fatty acid content of the product. On the other hand, almost pure

rumenic acid can be obtained by deshydration of ricinoleic acid from castor oil [12].

CLA in food

Due to its biological origin, CLA is present in food as rumenic acid as the major isomer (> 80% of total CLAs). The main dietary sources are ruminant fat, including milk, dairy products, and meat. As an example, milkfat generally contains about 1% of total fatty acids as rumenic acid. Consequently, rumenic acid intake is estimated to be less than 500 mg per day in most countries.

However, it is important to underline that the CLA content in food depends of various parameters, including food intake of ruminants, seasons, etc. These aspects are developed in the papers by Y. Chilliard *et al.* and N. Combe in the present issue.

CLA in human tissues

Few studies reported the occurrence of CLA in human biopsies before any supplementation. Recently, we analysed abdominal adipose tissue from men and women in the surroundings of Tours (France). The rumenic acid content ranged from 0.19 and 0.66 of total fatty acids (mean 0.40) [13]. Similar data were obtained in mammary adipose tissue in French women (see the paper of P. Bougnoux *et al.* in this issue). In 10 human milk samples collected 4 days *post partum*, we found about 0.35% of total fatty acids as rumenic acid. We also found some 9cis,11cis- and 9trans,11trans-18:2, less than 0.05 each), but some conjugated 20:3 fatty acid was detected, probably resulting from bioconversion of rumenic acid (see below). Similar data were obtained in human plasma samples (unpublished data).

Metabolism of CLA isomers

Linoleic acid (9cis,12cis-18:2) is converted by successive desaturations and elongation in arachidonic acid (figure 4). Rumenic acid is now known to be converted by similar metabolic pathways. Such bioconversion has been reported in rats and lambs, but the occurrence of conjugated 20:3 in human samples (see above), suggests that this pathways also occurs in men. The significance of the last step of conversion (conjugated 20:3 \Rightarrow conjugated 20:4) is less documented and occurs at a low extent, as illustrated by the relative accretion of conjugated 20:3. Banni *et al.* studied the specific incorporation of these different conjugated metabolites in various lipid classes [14]. They suggested that unlike linoleic acid and its 18:3 (n-6) and 20:3 (n-6) metabolites which

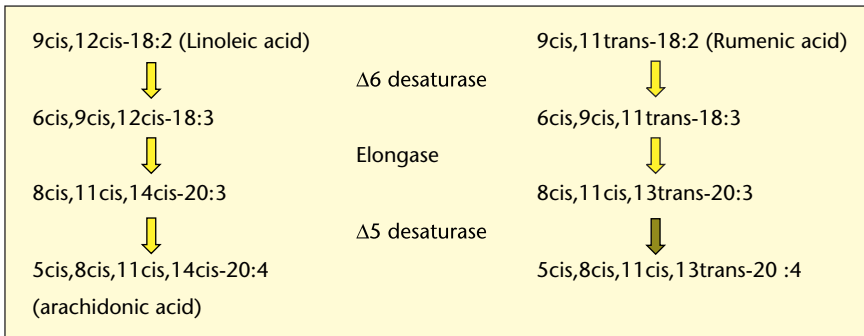


Figure 4. Desaturations and elongation of linoleic and rumenic acids.

are mainly incorporated in phospholipids, rumenic acid as well as conjugated 18:3 and 20:3 metabolites are mainly incorporated in neutral lipids. This means that rumenic acid behave more as oleic acid than as linoleic acid regarding incorporation and acylation channelling. On the other hand, conjugated 20:4 was mainly incorporated in phospholipids, but not in the same classes than arachidonic acid. The authors questioned if this metabolite may modulate eicosanoid metabolism, as conjugated 20:4 is readily incorporated in phosphatidylinositol, a major substrate for phospholipase A2 and eicosanoid biosynthesis. However, the ratio between arachidonic acid and conjugated 20:4 remains as high as about 100:1, but influence of CLA intake on prostaglandins biosynthesis is well documented. In the same paper, it was reported that rumenic acid intake did not extensively modify the fatty acid profile in the liver. This was similar to our

previous results in rats [15]. On the other hand, the trans10,cis12 isomer, as well as a mixture of both isomers induced an increase of 18:0 at the expense of 18:1 n-9. This may be related to *in vitro* data on the influence of both conjugated fatty acids on the Δ9 desaturation of stearic acid [16]. It was concluded that the trans10,cis12-18:2 isomer was an inhibitor of the stearoyl CoA desaturase (SCD) activity (figure 5). Others also reported a decrease on the expression of the SCD by the 10trans,12cis CLA isomer [17].

Being a fatty acid, rumenic acid has also to be considered as an energy source. Using 1-¹⁴C-radiolabelled molecules, we showed that in rats, rumenic acid, as well as 10trans,12cis-CLA, were used for β-oxidation. Both conjugated fatty acids were more oxidised than linoleic acid used as control. On the other hand, conjugated fatty acids are less incorporated in most tissues [18]. A similar study with

1-¹³C-labelled CLA isomers given as TAG to overweight humans will be reported elsewhere.

In conclusion, CLA are channelled as other fatty acids between different metabolic pathways. However, they behave differently compared to non conjugated fatty acids and may interfere with their metabolism. On the other hand, it has to be underlined that the major natural CLA isomer is rumenic acid. For a quantitative aspect, the second one is the cis7,trans9 isomer, which has not yet been extensively studied. Most studies consider the trans10,cis12 isomer together with rumenic acid in animals or humans. For the latter, the major endpoint is body composition, and the data obtained so far are still controversial in humans. For example, the first report by Blankson *et al.* [19] described a dose dependent effect of the CLA mixture, whereas we did not found any effect of pure isomers fed as TAG (1.5 or 3 g/d) for 16 weeks [20] on body composition. This particular point will be discussed in the paper by Quignard-Boulangé in the same issue.

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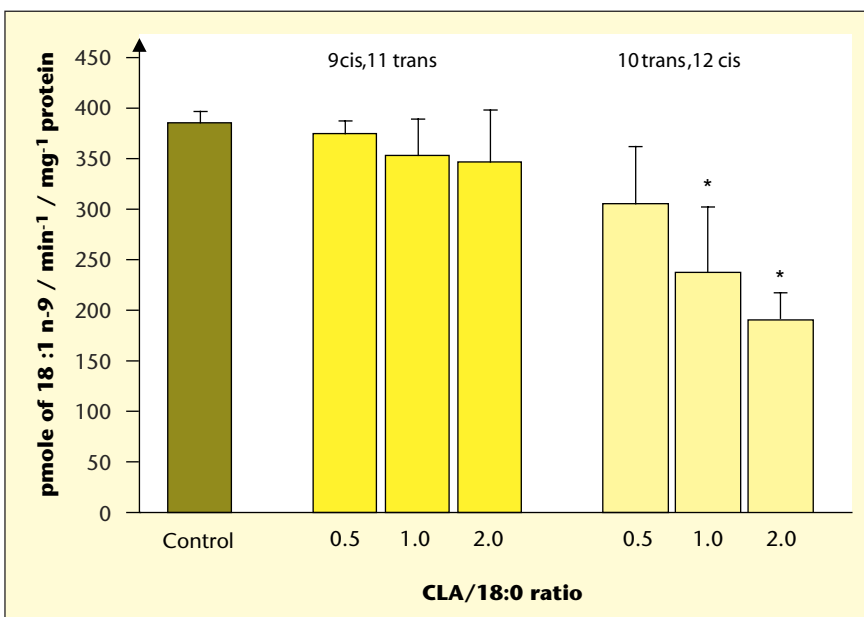


Figure 5. Inhibition of the Δ9 desaturase of [1-¹⁴C]-18:0 in rat liver microsomes by CLA isomers compared to control (adapted from Bretillon, Chardigny *et al.* 1999 [16]).

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