UPDATE ON CONJUGATED LINOLEIC ACIDS (CLA)

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INTRODUCTION

The CLA story began with the discovery by Pariza and co-workers that fat extract from ruminants contained compounds that possessed anticarcinogenic activity. They subsequently identified these compounds as conjugated dienoic isomers of linoleic acid and coined the term conjugated linoleic acid or CLA (see review 1). Over the last decade the published research on the biology of CLA has increased exponentially and many additional health benefits have been identified (Table 1; 2). The range of physiological processes affected by CLA is impressive and raises interesting questions as to mechanisms.

Table 1. Beneficial health effects of CLA reported from biomedical studies with animal models[a].

Biological effect
Anticarcinogenic effects (*in vivo* and *in vitro* studies)
Antiatherogenic properties
Altered nutrient partitioning and lipid metabolism
Antidiabetic (type II diabetes) and reduced hyperglycemia
Immune modulation
Improved bone mineralization

[a]Adapted from Bauman et al. (3).

The CLA in human diets originate almost exclusively from food products of ruminant origin and we presented our first paper on CLA at this conference in 1996 (4). Our interest related to identifying micro-nutrients that provide a beneficial health effect in foods beyond their traditional nutritional value. This concept is often referred to as "functional foods" and offers the potential to design foods in ways that improve their healthfulness. In general, the physiological effects of CLA have been identified using biomedical studies with animal models and chemically synthesized CLA supplements that contain a variety of isomers. Many CLA isomers are possible and they can differ in both the position of the conjugated double bonds (e.g. 8-10, 9-11, 10-12, etc.) and the configuration of the double bonds (*cis-cis, cis-trans, trans-cis*, or *trans-trans*). The predominant isomer in milk and beef fat is *cis*-9, *trans*-11 CLA, but sophisticated analytical techniques have revealed that ruminant fat contains minor or trace amounts of over 20 CLA isomers (5). Thus, it is of obvious interest to establish the relationship of specific isomers to the various biological effects observed when commercial supplements of CLA are fed. It is clear that the *cis*-9, *trans*-11 CLA isomer possesses anticarcinogenic activity. Of special significance are our recent studies showing that this isomer is also effective when consumed as a natural component of foods – in this case we used a rat model for breast cancer and included a dietary supplement of butter that had been naturally enriched in *cis*-9, *trans*-11 CLA (6). The *trans*-10, *cis*-12 CLA isomer has a clear effect on lipid metabolism thereby reducing milk fat synthesis in lactating animals and body fat accretion in growing animals (see review 7). In addition, our recent work suggests that the *trans*-10, *cis*-12 isomer is responsible for the anti-diabetic effects (8).

Understanding the biology of specific CLA isomers continues to be an area of active research. From the perspective of ruminants, the focus has been on two CLA

isomers – *cis*-9, *trans*-11 and *trans*-10, *cis*-12. Enhancing ruminant fat content of the former is of interest because of the demonstrated role of *cis*-9, *trans*-11 CLA as an anticarcinogen, whereas *trans*-10, *cis*-12 CLA is of interest because of its effects on lipid metabolism. In the following sections we will summarize recent developments related to these two CLA isomers. For more comprehensive summaries of the ruminant dimension of CLA we refer readers to recent reviews (3, 9, 10).

CIS-9, *TRANS*-11 CLA AND MILK FAT

The uniqueness of ruminant-derived foods as a source of CLA relates to rumen biohydrogenation. CLA is an intermediate in the rumen biohydrogenation of linoleic acid and it had been assumed this was the sole source of the CLA found in milk and beef fat. However, Griinari and Bauman (9) challenged this based on the kinetics of rumen biohydrogenation and observations that dietary polyunsaturated fatty acids other than linoleic also increased the CLA content of milk fat. We suggested an alternative source could be endogenous synthesis involving the enzyme Δ^9-desaturase. The substrate for this reaction would be *trans*-11 $C_{18:1}$, another intermediate formed in rumen biohydrogenation. In a series of studies, we manipulated the supply of *trans*-11 $C_{18:1}$ and altered the activity of Δ^9-desaturase under different experimental conditions (11, 12). Overall, our results indicate that endogenous synthesis is the major source accounting for over two-thirds of the CLA in milk fat (Table 2). Thus, considering rumen production of *trans*-11 $C_{18:1}$ is critical in attempts to increase the CLA content of milk and beef fat.

Previous studies investigating dietary influences on CLA production have demonstrated a wide range in CLA content of milk fat among individual cows on a given diet (e.g. see 13-17). These observations combined with the data on the contribution of endogenous synthesis to milk fat CLA discussed above, led us to investigate the sources of variation in CLA production in dairy cows (18). Briefly, our study consisted of three groups of animals (n = 10 per group); one maintained on a standard TMR, one maintained on a diet that included extruded full-fat soybeans as a slow-release source of linoleic acid designed to elevate milk fat CLA, and a third group that was switched between these diets at 3 week intervals over the course of the 12 week study.

The diets gave the expected difference in CLA content of milk fat with the extruded full-fat soybean diet averaging about twice that of cows fed the TMR diet. Similar to previous studies we observed a 2- to 3-fold range in CLA concentration in milk fat among individuals on each of the diets. A key observation was the remarkable consistency in hierarchy of CLA content among cows over the 12 week period. The consistency was similar for all 3 groups. We have included a graphic representation for the treatment group that switched between the two diets at 3 week intervals because the data illustrate that the rank order was maintained not only over time, but also across dietary shifts (Figure 1). Also evident in this treatment group was a range in the magnitude of response to dietary shifts among cows; some cows had a minimal response to diet shifts while others increased milk fat content of CLA more than double.

Table 2. Importance of endogenous synthesis of *cis*-9, *trans*-11 CLA in milk fat.

Diet	Milk Fat CLA in Control (mg/g fatty acid)	Endogenous Synthesis of *c*9, *t*11 CLA[a]	Reference
Total mixed ration	4.2	64%	Griinari et al. (11)
Total mixed ration	6.5 \ 7.6	78%	

+ PHVO[b]			Corl et al. (12)
Pasture	15.5	71%	Kay et al. (unpublished)

[a]Estimated by use of sterculic oil as a source of cyclopropene fatty acids to block Δ^9-desaturase.
[b]Partially hydrogenated vegetable oil.

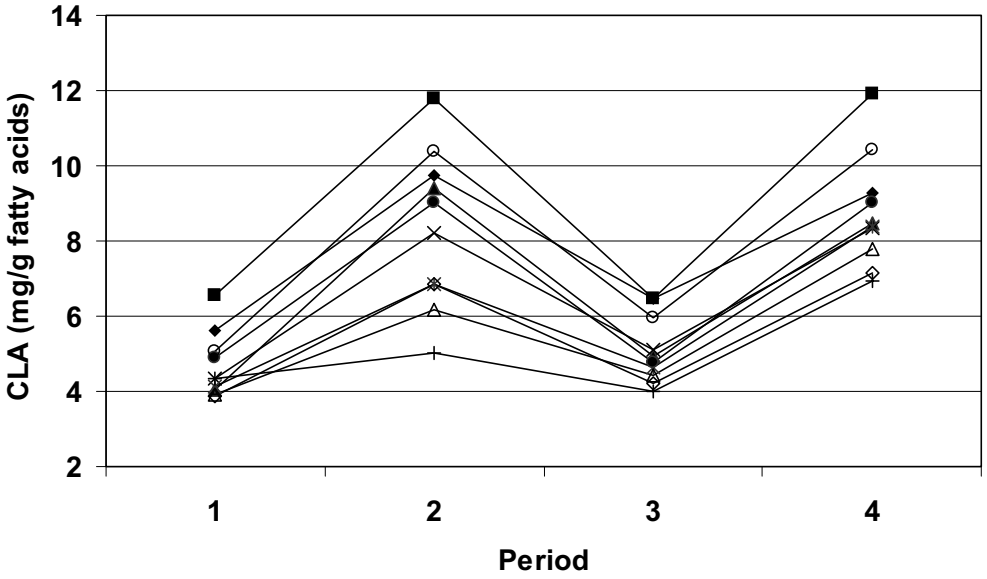

Figure 1. Temporal pattern of the hierarchy in milk fat CLA for cows alternated between high and standard CLA diets in successive periods (3 wk intervals). Adapted from Kelsey et al. (18).

The second facet addressed in our investigation involved analyzing the variation associated with endogenous synthesis of CLA and Δ^9-desaturase. There are four main indices that represent a proxy of desaturase activity and we refer to them as the desaturase index. These are fatty acid pairs that represent a product:substrate ratio for Δ^9-desaturase, and they include the myristoleic ratio ($C_{14:1}/C_{14:0}$), the palmitoleic ratio ($C_{16:1}/C_{16:0}$), the oleic ratio ($C_{18:1}/C_{18:0}$), and the CLA ratio (*cis*-9, *trans*-11 CLA/*trans*-11 $C_{18:1}$). Of these four, the myristoleic ratio is of particular value because the only source of myristic acid (substrate) is mammary *de novo* synthesis and therefore all myristoleic acid present is a product of mammary Δ^9-desaturase activity. All three of the other indices are confounded by possible contribution from the diet and/or Δ^9-desaturase present in other tissues, and are therefore less rigorous in terms of indicating mammary Δ^9-desaturase activity. Results were similar for all three treatment groups and we have presented data from the group that switched between diets as an illustration (Figure 2). Again we observed a highly consistent hierarchy among individuals over time as well as a similar 2- to 3-fold range across animals within each group. Our study demonstrates that there is significant animal-to-animal variation in response to a CLA-enhancing diet and in Δ^9-desaturase activity, although individual animals remain quite consistent over time and across diets (18). Thus, dietary manipulation of milk fat CLA acts in concert with individual animal traits to culminate in the observed content of CLA in milk fat.

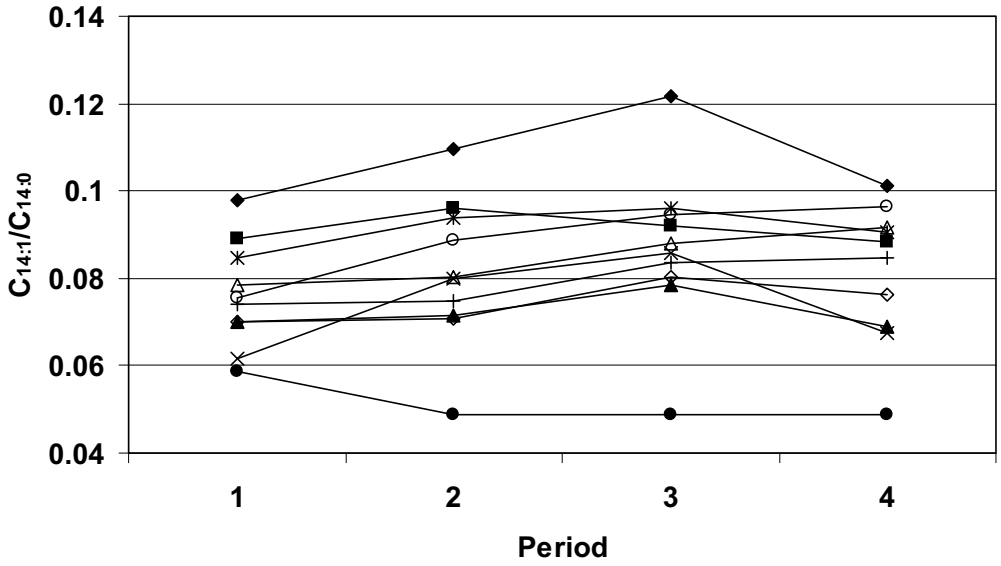

Figure 2. Temporal pattern of the $C_{14:1}/C_{14:0}$ desaturase index hierarchy for cows alternated between a high and standard CLA diet in successive periods (3 wk intervals). Adapted from Kelsey et al. (18).

Advances in understanding the biology of CLA in ruminants have been sufficient to allow formulation of strategies to increase the content of *cis*-9, *trans*-11 CLA in milk fat. A schematic to illustrate potential strategies was developed by M. Griinari and it is presented in Figure 3. Four key sites have been listed where intervention would increase fat content of CLA. The first two involve alterations in the rumen that would increase the rumen production of substrate for use in endogenous synthesis of CLA. Increasing the supply of C18 polyunsaturated fatty acids would be beneficial (Figure 3, #1) because the biohydrogenation of both linoleic acid and linolenic acid results in the production of *trans*-11 $C_{18:1}$ as an intermediate. At the same time, the outflow of *trans*-11 $C_{18:1}$ from the rumen would be markedly enhanced if the terminal reductase step in biohydrogenation is inhibited (Figure 3, #2). We have suggested that diet supplements of fish oil increase milk fat content of CLA because they result in a less complete biohydrogenation sequence through an inhibition of the reductase reaction (3). The third site highlighted is to prevent an isomerase shift in rumen biohydrogenation (Figure 3). This often occurs with the addition of oils and while the proportion of biohydrogenation via an alternate pathway is minor, it has pronounced undesirable effects. The *trans*-10, *cis*-12 CLA isomer causes a reduction in total milk fat and also inhibits Δ^9-desaturase, thereby reducing endogenous synthesis of *cis*-9, *trans*-11 CLA. The final site listed is to enhance endogenous synthesis via effects on the activity or gene expression of Δ^9-desaturase (Figure 3). The regulation of Δ^9-desaturase has been extensively investigated using the enzyme from rodent liver and results show it is affected by diet, hormonal balance and physiological state (3). Presently, several groups are extending this work to ruminants and to the mammary enzyme. Nevertheless, the variation cited above in desaturase index measures offer clear evidence that genetic differences exist in the gene expression and/or activity kinetics for Δ^9-desaturase.

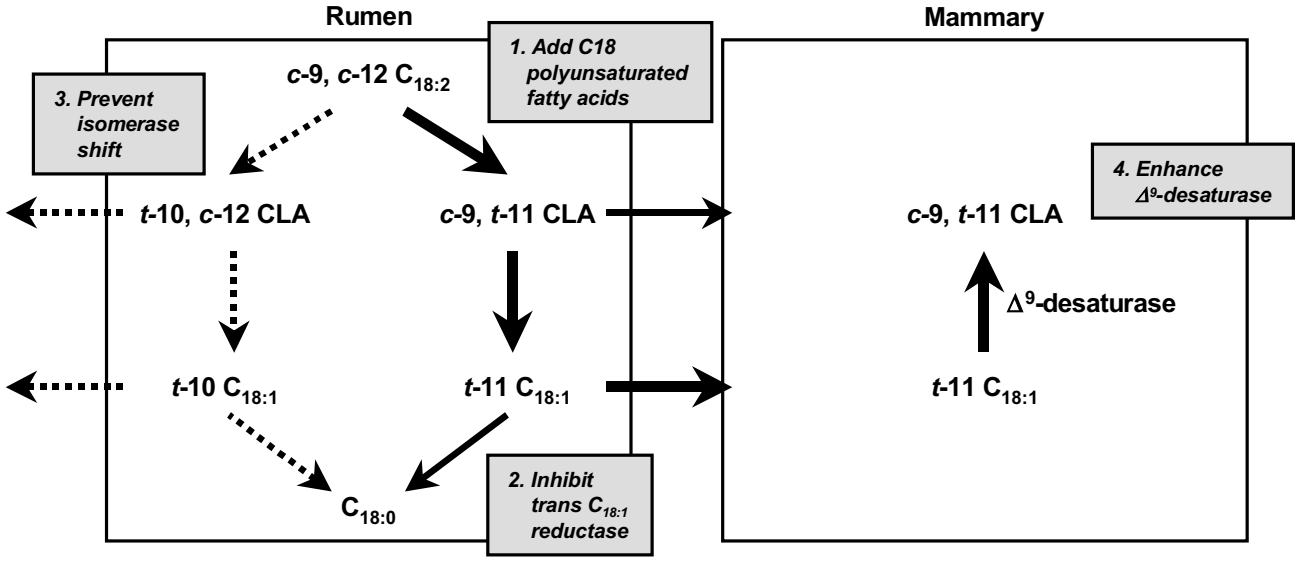

Figure 3. Strategies to increase CLA content of milk products. Adapted from M. Griinari (unpublished).

TRANS-10, *CIS*-12 CLA AND MILK FAT SYNTHESIS

Commercial supplements of CLA cause a reduction in milk fat yield when abomasally infused or fed as rumen-protected salts. As summarized by Baumgard et al. (7), effects are specific for milk fat and generally there are little or no effects on the yield of milk or other milk components. We also demonstrated that the reduction in milk fat was specific for the *trans*-10, *cis*-12 isomer as *cis*-9, *trans*-11 CLA had no effect (19). We recently completed a dose response study of the effects of *trans*-10, *cis*-12 CLA on milk fat yield (20) and these results are presented in Figure 4 along with data points from other more recent unpublished studies by our group. A hyperbolic dose curve is apparent and it is clear that *trans*-10, *cis*-12 CLA is a very potent inhibitor of milk fat synthesis. As little as 3.5 g/d (0.016% DMI) results in a 25% reduction in milk fat yield (20). The mechanism by which *trans*-10, *cis*-12 CLA is able to reduce milk fat yield is of interest. Our initial results indicated that this CLA isomer causes a reduction in gene expression (mRNA abundance) for key enzymes involved several aspects of milk fat synthesis including mammary uptake and transport of preformed fatty acids, *de novo* fatty acid synthesis, desaturation of fatty acids needed for maintenance of milk fat fluidity, and esterification of fatty acids into milk fat triglycerides (21). Further investigations on the mechanisms and signaling systems is an active area of investigation by our group and others.

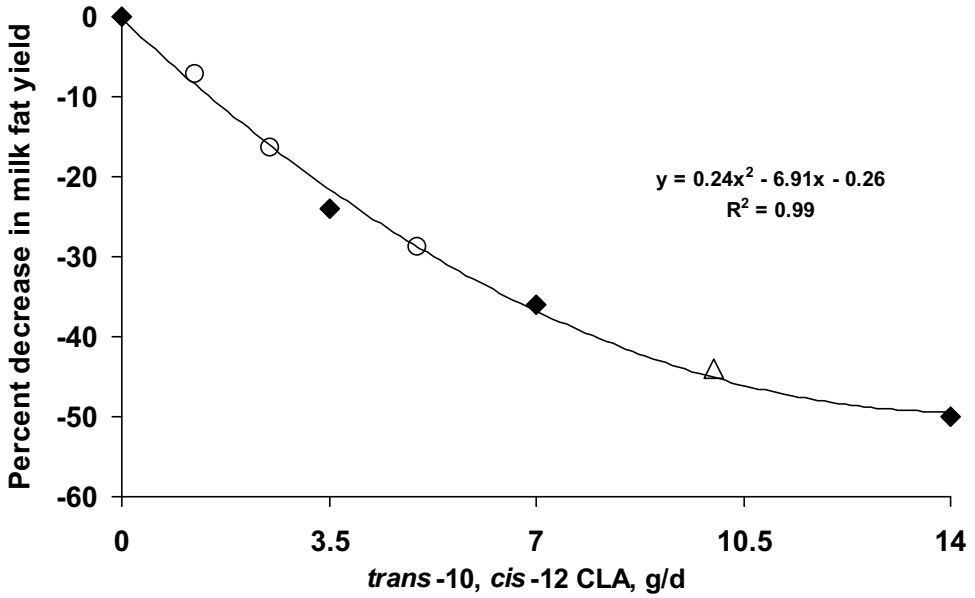

Figure 4. Effects on milk fat yield (percent decrease) from various doses of abomasally infused *trans*-10, *cis*-12 CLA. Closed diamonds are from Baumgard et al. (20), open triangle is from Baumgard et al. (19), and open circles represent more recent results (Peterson et al., unpublished).

One interesting aspect of the CLA story is the relationship between the *trans*-10, *cis*-12 CLA isomer and classical diet-induced milk fat depression (MFD). Dairy producers have observed a reduction in milk fat yield with a number of different types of diets and dietary conditions, and this has been an active area of research for over half a century. Results to date were summarized at the last conference (22) but a more extensive review has recently been published (23). We demonstrated that diet-induced MFD coincides with an increase in milk fat content of *trans*-10, *cis*-12 CLA. Based on this and the aforementioned results with CLA isomers, Bauman and Griinari (23) postulated the "biohydrogenation theory" to explain MFD. This theory hypothesizes that "under certain dietary conditions the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates which are potent inhibitors of milk fat synthesis" (23). *trans*-10, *cis*-12 CLA represents one example of such an intermediate, but it seems likely that other unique fatty acid intermediates formed during rumen biohydrogenation might also be inhibitory. For example, by analogy to the formation of *trans*-10, *cis*-12 CLA from linoleic acid, we would expect the *trans*-10, *cis*-12, *cis*-15 $C_{18:3}$ isomer formed from linolenic acid could also be a potent inhibitor of milk fat synthesis (23). As the "biohydrogenation theory" is investigated and extended by others, it will be interesting to see if it represents a unifying theory that explains the basis for MFD across all diets.

In addition to implications related to understanding diet-induced MFD, there are a number of situations where it might be of commercial value to consider reducing milk fat yield. Over the last decade the price paid to producers for milk components has shifted with a decrease in the value of milk fat and an increase in the value of milk protein. Thus, any use of CLA to reduce milk fat yield would need to be considered in terms of the economic benefit to producers. One situation of interest relates to producers regulated by a quota system based on milk fat; dairy farmers in Canada and EU are examples. We have just completed a longer-term study in which pregnant, well-fed cows received rumen-protected CLA over the last 20 weeks of lactation (24). We observed a 23% decrease in milk fat yield with no change in the yield of milk or milk protein; cow health and well-being were unaffected (Table 3). Another circumstance

where a reduction in milk fat yield would be of possible interest is in the transition cow (25) and this will be discussed in Dr. Overton's presentation. Other situations are where nutrients are limiting either due to feedstuff availability (e.g. summer pasture), physiological state (early lactation) or environmental factors (e.g. heat stress). All of these are areas of active research where the potential benefits include performance and animal health and well-being; the first longer term results from feeding rumen-protected CLA have been presented as abstracts. Medeiros et al. (26) demonstrated the energy limitation response when they observed that rotationally grazed cows receiving rumen-protected CLA had reduced milk fat whereas milk protein yield was increased by 13%. Likewise, a benefit by overcoming an energy limitation in early lactation was observed when feeding rumen-protected CLA resulted in an increase in milk yield corresponding to the reduction in milk fat (27).

Table 3. Performance of pregnant dairy cows treated with rumen-protected CLA over the last 20 weeks of lactation[a].

Variable	Control (n=12)	CLA (n=11)	SE	*P-value*
DMI, kg/d	22.8	22.6	0.5	0.78
Milk, kg/d	30.4	30.8	1.3	0.85
Fat				
%	3.8	2.9	0.1	<0.001
Yield, g/d	1201	927	35	<0.001
Protein				
%	3.1	3.2	0.1	0.61
Yield, g/d	992	1004	26	0.73
BCS	3.4	3.4	0.1	0.72
BW, kg	655	652	6	0.70
Net EB, Mcal/d	4.6	5.8	1.3	0.53

[a]Cows received rumen-protected CLA (42.8 g/d of CLA) or EnerG II (Control). Adapted from Perfield II et al. (24).

CONCLUSION

CLA are micronutrients found in food products derived from ruminants that have many potential beneficial health effects. Two CLA isomers are of particular interest, *cis*-9, *trans*-11 because of its anticancer effects and *trans*-10, *cis*-12 because of its ability to inhibit fat synthesis. Substantial animal variation exists in milk fat content of *cis*-9, *trans*-11 CLA, and potential strategies to enhance levels of this isomer are detailed. The *trans*-10, *cis*-12 CLA isomer is a potent inhibitor of fat synthesis and recent studies have characterized dose-response relationships and effects of rumen-protected CLA on milk fat yield. These results demonstrate that dietary supplements of CLA have the potential to be utilized as a management tool to regulate milk lipid secretion, enhance productivity and improve animal well-being.

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