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REVIEWS

REVIEW: Conjugated Linoleic Acid: Historical Context and Implications[1]

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Abstract

*Conjugated linoleic acids (**CLA**) are implicated as anti-carcinogenic, anti-atherosclerosis, and anti-inflammatory agents in a variety of experimental model systems. However, evidence of dietary CLA protection against human mammary cancer risk is mixed and comes from European countries where the death rate from mammary cancer is relatively scarce. Unfortunately, epidemiological data are inconclusive, especially from retrospective studies. Prospective human study evidence will take more time. European values as great as 1.9% CLA in the fat of milk products from grass-fed ruminants has been reported; ordinary monogastric meat and egg products contain substantially less CLA in fat (0.3%). It is now recognized that the principle CLA in ruminant meat and milk is the natural diene, cis (**c**)-9, trans (**t**)-11 isomer (rumenic acid) of C18:2 (octadecadienoic acid). Another isomer, C18:2 t-10, c-12, also contributes to the unique biological activity of CLA, but does not readily accumulate in ruminant lipids and is found only in commercial preparations of mixed CLA isomers. Evidence in humans suggested that the role of the dietary mixed isomer CLA in the loss of body fat mass (**BFM**) was only modest compared with the results from animal model studies, and urine metabolites of prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**), indicative of lipid oxidation stress, have been elevated during supplementation. In addition, the fatty acid C18:1 t-11 (vaccenic) is now believed to be the principle precursor of endogenous c-9, t-11 CLA in both ruminants and monogastrics. This finding helps explain the discrepancy between measured c-9, t-11 CLA originating from the rumen and that secreted in cow's milk. Manipulation of ruminant meat and milk by feeding marine or vegetable oils is clearly associated with increases in vaccenic acid as well. This relationship requires a re-examination of human foods for vaccenic acid content and quantitative measures of CLA endogenous synthesis in humans as well to formulate dietary strategies to capture CLA's potential protective health benefits.*

(Key Words: Conjugated, Linoleic, Vaccenic, Anti-Carcinogen, Inflammation.)

Introduction

The current notoriety and dramatic increase in scientific articles addressing the merits of conjugated linoleic acid (CLA) can hardly be overlooked by nutritional practitioners and others closely related to domestic animal agriculture. The CLA is a purported protector of human health and is a naturally occurring food entity in meat and milk produced from ruminants. Although food animal producers consider CLA a potential marketing trait, a greater awareness and the importance of the research context need to be maintained regarding CLA, lest, in the confusion, it is passed over as merely another food fad. It is now widely recognized that CLA is not a single chemical entity but a mixture of positional and geometric isomers. This is a subset of octadecadienoic acids (a fatty acid with 18 carbons, four of which are unsaturated [i.e., two double bonds]). It is not surprising that the biological effects also reflect this complexity. Of the four principal isomers, two of them are believed to be responsible for its biological activity (the 18:2 *cis* (*c*)-9, *trans* (*t*)-11 CLA and the 18:2 *t*-10, *c*-12 CLA; Ip et al., 1994).

Whether the primary food animal producer can benefit economically

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from this knowledge remains uncertain and will depend on both consumer knowledge and perception (Greenberg and Klasma, 2002). Dietary manipulations to achieve target human dietary needs via animal food products may be easily supplanted by direct human supplementation of isolated and enriched CLA. In fact, a survey of the US Patent and Trademark Office web site (2002) reveals that 30% of 75 patents (from 1996 to 2002) directly pertaining to CLA deal specifically with CLA isomer synthesis and the enrichment processes. Several others are use patents for direct incorporation into human foods as ingredients or spreads. Currently, human CLA supplements are already being sold nationally as human health aids. A few use patents also deal with specific CLA enrichments in eggs, milk, and meat (fat) by diet supplementation; 11% (eight patents) are confined to specific CLA enrichments in animal feeds. Paradoxically, the number of use patent claims is almost inverse to the number of scientific papers published on each issue. More than 15% of use patents deal with BW loss, 12% with topical applications for skin care, and 7% with immunity or inflammation modulation claims. Only a few claims each are made for specific benefits involving cancer, bone-related disease, diabetes, and atherosclerosis. This review is intended to restate the purported benefits of CLA, yet inform the reader of the context of the research from which it comes. As more direct trials with human subjects have now been published, ubiquitous and far-reaching claims for human health must be narrowed and refined.

Review

Animal food products are highly regarded nutrient sources in the human diet worldwide. However, in western developed country populations where they are most readily available, a stigma remains associated with animal fats, especially because of the prevalence of coronary heart disease (Hu et al., 2001), the epidemiological association of cancer to dietary fats (Howe, 1994), and mutagenic heterocyclic amines known to be present in cooked meats (Nagao et al., 1994).

It is ironic, then, that CLA anti-cancer effects became known as a consequence of isolation and identification experiments from cooked beef that was screened for bacterial mutagens. The scientists were looking for heterocyclic amines, an unrelated class of chemicals (Hargraves and Pariza, 1983). The isolated CLA fraction was shown to have some anti-cancer effects in a two-stage epidermal cancer mouse model [90% incidence and 60% papilloma number, CLA *vs* linoleic acid (18:2 *c*-9, *c*-12) or acetone control; Ha et al., 1987]. In addition, CLA gave a dose-response inhibition to cytochrome P-450 enzymes in a rat-microsomal in vitro assay, blocking a hypothesized mechanism for heterocyclic amine metabolite generation believed responsible for mutagenesis (Ha et al., 1987).

Ip et al. (1994) summarized results from two additional cancer models: benzo(a)pyrene-induced forestomach tumor in mice and dimethylbenz(a)anthracene (**DMBA**)-induced mammary tumor in rats. These experiments demonstrated the oral effectiveness of isolated CLA and established the effective range (up to 1.5% CLA by weight in the diet, with 10 mg DMBA). As little as 0.1% CLA in the diet produced measurable tumor inhibition; beneficial effects reached a plateau around 1.0% dietary CLA. At lesser levels of mutagen (5 mg DMBA), less CLA was needed to achieve similar tumor incidence inhibition (58% inhibition at 0.5% diet CLA; Ip et al., 1994). The isolated CLA contained three predominant isomers (18:2 *c*-9, *t*-11; *t*-9, *c*-11; and *t*-10, *c*-12), accounting for 85% of the total and therefore believed responsible for CLA biological effects (Ip et al., 1994). These dietary CLA levels became the focal point for much conjecture and extrapolations for application to human diets.

Summary of Biological Effects. Parodi (1994) summarized the relevance of CLA as an anti-cancer agent, outlining evidence gained from eight different models or enzyme assays. In addition to those previously mentioned are CLA incorporation into cell membrane phospholipid fractions (*c*-9, *t*-11 CLA), CLA antioxidant properties, CLA inhibition of a cell-signaling cascade involving protein kinase C, CLA inhibition of ornithine decarboxylase activity (a cell proliferation indicator induced by the tumor promoter 12-o-tetradecanoylphorbol-13-acetate), and CLA cytotoxicity in additional in vitro cell models for malignant melanoma, colorectal, and breast cancers. Belury (1995) reiterated much of the same, but pointed out the evidence and potential contradiction of CLA having a pro-oxidant (*vs* anti-oxidant) role in the mechanism of cancer cell cytotoxicity. In addition, CLA had roles in the inhibition of inflammatory processes via the arachadonic acid generation of eicosinoids and subsequent inflammatory prostaglandins (Pariza et al., 2000).

The proceedings of a symposium held in 1999 addressing the health benefits of CLA (Scimeca and Miller, 2000) contained reviews that broaden the implications of CLA benefits from cancer and atherosclerosis to bone biology (Watkins and Seifert, 2000), where the beneficial effects are elicited via inhibition of inflammation processes.

Evidence for a role in athrosclerosis was given by Kritchevsky et al. (2000) using a rabbit model. Diets containing 1% CLA (commercial source of mixed isomers, predominantly *c*-9, *t*-11 and *t*-10, *c*-12 CLA) showed 30% regression of the experimentally induced aorta athrosclerosis, although serum cholesterol and high density lipoprotein cholesterol were elevated. Khosla and Fungwe (2001) reviewed a greater body of animal model data (including mice, hamster, and pig) investigating CLA impacts and

concluded that the evidence pointed to the *t*-10, *c*-12 CLA isomer as that which had the biologically active role in modulating blood lipid profiles. However, they remained skeptical about any meaningful implications for humans.

MacDonald (2000) reviewed the evidence for mice, rats, and chickens where as little as 0.5% dietary CLA resulted in short-term (1 to 2 mo) body fat reductions of 57 to 70%, 23%, and 22%, respectively. Others have also reviewed this effect (Pariza et al., 1999; Dyck, 2000; Whigham et al., 2000). Most concur that a lack of human experiments is still problematic.

However, in food animal production, the most unequivocal evidence for CLA involvement in lipid metabolism was the dramatic effect of abomasally infused *c*-10, *t*-12 CLA inhibition on dairy cow milk fat secretion (Baumgard et al., 2000). As little as 10 g/d (0.05% diet equivalents) reduced both milk fat percentage and fat yield (42 and 44%, respectively). Just as dramatic were experiments with laying hens fed mixed isomers of CLA (0.5% of diet), which resulted in 100% mortality of fertilized eggs compared with 16% mortality of fertilized eggs from laying hens fed corn oil control diets. Feeding olive oil (10%) or refeeding the control diet reversed the effects within 6 d (Aydin et al., 2001). The CLA mixed isomers increased saturated fatty acids (16:0 and 18:0) and reduced mono-unsaturates (16:1 and 18:1), where oleic acid (18:1 *c*-9) was particularly affected, dropping to 26.8% of lipids *vs* 39% for the controls. The researchers noted other chemical changes in the egg, suggesting that the egg yolk membrane after CLA feeding was more fragile, resulting in mineral osmotic and pH changes between yolk and albumin as well.

CLA Evidence from Human Subjects. Direct health benefits of CLA in humans will be difficult to come by because of limitations in experimental protocol and management challenges with free, living subjects. For example, human evidence for the anti-cancer claim will require large studies and yet will always be limited by accuracy in measurement of CLA intake because of the long timeframe constituent of this disease process.

Controversial evidence has slowly started to appear. Aro et al. (2000), in a case-control study of Finnish women (approximately 200 per group), found a significant effect of CLA intake with reduced mammary cancer risk when intake was >200 mg/d, as assessed by a food frequency questionnaire (**FFQ**; significant where the 95% confidence interval for odds ratio fell below 1.00; that is, when greatest intake quintile risk was divided by least intake quintile risk, and the odds ratio for the least intake quintile was set to 1.0). The greatest quintile serum CLA, myristic acid (14:0), and vaccenic acid groups were also considered at significantly less risk. However, Chajes et al. (2002) found no association between actual CLA mammary adipose tissue content and incidence of invasive mammary cancer in French women. Mammary CLA concentrations ranged from 0.14 to 0.75%, and mean values for cases (n = 213) *vs* controls (n = 84) were 0.44 and 0.43%, respectively. Overall, the concentrations were negatively associated with body mass index. Zlatanos et al. (2002) pointed out that, by European standards, both Finland and France have lesser death rates associated with mammary cancer, and per capita cheese consumption is considered great in France (173% of Finland) at 23.3 kg/yr. Those researchers suggested a reduced risk in countries where cheese consumption is greater (such as France and Greece).

Oral supplementation of mixed-isomer CLA to humans in the 3- to 4-g/d range did show measurable levels in the plasma lipids, but primarily as *c*-9, *t*-11 CLA (Benito et al., 2001a). Supplementation of mixed isomers (3.9 g/d) given to women for 63 d had no impact on peripheral blood mononuclear cell biochemical indexes of immune function (Kelley et al., 2001). These young healthy women also had no altered response to any of the indices of immune status following influenza vaccine (Kelley et al., 2000). With a similar protocol in the same laboratory, oral CLA supplementation had no effect on blood coagulation of blood platelet functions (Benito et al., 2001b).

In other studies, urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 15-keto-dihydro-$PGF_{2\alpha}$ were detected after 1 and 3 mo of supplementation of a mixed-isomer CLA (4.2 and 3.4 g/d, respectively) to obese men (Basu et al., 2000a; Riserus et al., 2002). A similar response was measurable in healthy subjects (Basu et al., 2000b) after 3 mo of supplementation. These biochemicals serve as markers of oxidative stress caused by lipid peroxidation and seemed to be specifically related to the *t*-10, *c*-12 CLA isomer (Riserus et al., 2002). A similar detection of 8-iso-$PGF_{2\alpha}$ was also made by Turpeinen et al. (2002) after oral supplementation with vaccenic acid (a precursor to *c*-9, *t*-11 CLA).

Terpstra (2001) has reviewed the early work in humans (seven positive results) regarding CLA supplementation and its impact on body fat mass (BFM) and contrasted it to the experimental evidence in mice. After standardization of the data set for CLA dose and duration, they concluded that modest BFM changes (approximately 0.5 kg/mo per 70 kg of BW) in humans are in line with expectations derived from mice, if the same ratio of metabolic rate is conserved (where mice are approximately seven times human).

The data of Zambell et al. (2000) on humans was included in the analysis despite its statistically insignificant results for BW and BFM when women were supplemented with 3 g/d of mixed-isomer CLA for 64 d under controlled metabolic conditions. Riserus et al. (2001) supplemented obese men with 4.2 g/d of mixed isomer CLA for 1 mo with only modest changes in sagittal

abdominal diameter. Noone et al. (2002) found no changes in BW after 8 wk of 3-g/d mixed-isomer CLA supplementation. However, longer duration studies (>3 mo) such as those of Blankson et al. (2000) and Smedman and Vessby (2001) were able to show significant reductions in BFM and percentage of body fat, respectively. The subjects in the Blankson et al. (2000) experiments incorporated some physical training; yet only mixed-isomer CLA intakes >3.0 g/d achieved significant BFM reductions. All CLA treatments together averaged only 1.1 kg of BFM loss over the 3 mo; lean body mass increased 0.9 kg, illustrating the often-confounded components of observing only BW change.

Evidence in isolated human preadipocytes of *t*-10, *c*-12 CLA, which inhibited lipogenesis, resulting in lessened triglyceride (**TG**) levels (Brown et al., 2001), would seem to be consistent with altered fat synthesis in the mammary gland of dairy cows (Piperova et al., 2000), whereas *c*-9, *t*-11 CLA seemed to have little effect. Noone et al. (2002) showed similar evidence of this CLA isomer specificity when mixed-isomer CLA with greater *t*-10, *c*-12 CLA decreased plasma TG compared with controls or mixed isomer with greater *c*-9, *t*-11 CLA. Smedman and Vessby (2001) also showed altered profiles of serum lipid after mixed-isomer CLA supplementation. Altered lipids suggested a decrease in delta-9 desaturase activity, and such results were in agreement with evidence in laying hens where mixed isomer CLA dramatically reduced 16:1 and 18:1 in egg yolks (Aydin et al., 2001). This finding was inconsistent with the evidence that *t*-10, *c*-12 CLA altered milk lipid composition with milk fat depression diets fed to dairy cows; 18:1 did not seem altered, but milk fat depression diets contained added soybean oil rich in 18:1 *c*-9 (Piperova et al., 2000).

Much of the lack of effects on plasma lipid profiles (Benito et al., 2001a), glycerol, and free fatty acid kinetics (Zambell et al. 2001) and other energy-partitioning modulators (Madina et al., 2000) might be due to the choice of mixed isomer CLA, where the fraction of *t*-10, *c*-12 CLA was less and varied from <15 up to 23%, a reflection in changed CLA source. Others have reported batch-to-batch variation in mixed isomer CLA distributions (Adlof et al., 2001).

It seems that using mixed isomer CLA sources in human studies results in some impact on lipid metabolism, but effects are less than dramatic compared with animal or in vitro biochemical measures. Implications for natural sources of enriched CLA foods must wait for resolution of the complexity of evidence coming from mixed isomer CLA sources.

CLA Analytical Challenges and CLA Food Content. Although several reported values for CLA in milk or butterfat were published prior to the 1980s, it is only recently that an appreciation of positional and geometric isomers of CLA has gained wide recognition. The early work with CLA had been overshadowed by the attention and importance of the then newly identified essential fatty acids (Reaney et al., 2002). It is now generally recognized that the principle CLA in ruminant products is the 18:2 *c*-9, *t*-11 isomer (also called rumenic acid), but early reported values are plagued with analytical difficulty. Ha et al. (1989) reported the CLA isomer composition of cheeses, milk, and beef (raw and grilled). Later researchers from the same lab supplanted this work with a more comprehensive list of foods (nearly 100), utilizing a different analytical method (Chin et al., 1992). It was pointed out that the use of harsh acidic methylation methods (14% BF_3 or 4% H_2SO_4) prior to GLC elution caused losses of the *c*-9, *t*-11 CLA isomer (57 and 28%, respectively) and the formation of *t*-9, *t*-11 and *t*-10, *t*-12 CLA isomers. Chin et al. (1992) reported <5% loss of *c*-9, *t*-11 CLA using a 4% HCl/methanol methylating reagent (60°C; 20 min). Others have also done extensive evaluation of CLA analytical methods. Park et al. (2001) has thoroughly investigated the time and temperature influences on CLA isomerization, loss, and analytical artifacts for the HCl/methanol, BF_3/methanol, and $NaOCH_3$(sodium methoxide) methylation methods. Kramer et al. (1997) investigated the problem for milk fats and rumen fatty acids. Both are in agreement that harsh methods are problematic, and, as interest grows in the other minor CLA isomers, better preparation and standardization becomes a necessity. Alternative and more sophisticated methods are becoming commonplace (Kramer et al., 1997; Ostrowska et al., 2000).

CLA Food Content and Human Intake Levels. Even though the necessary level of mixed isomer CLA in experimental rat diets, either 0.5 or 1.0% (depending on the level of carcinogenic challenge) secured optimal mammary tumor suppression (Ip et al., 1994), an equivalent standard in the human diet remains elusive. Parodi (1994) speculated that 500 to 1500 mg/d of CLA intake was achievable by Australians eating milk and meat products from ruminants. This intake should provide humans from one-third up to the entire dose extrapolated to achieve measurable human beneficial effects.

In Finland, FFQ and 14-d food diaries (**FD**) were used to assess CLA intake (and many other dietary parameters) of women chosen as part of a case-control breast cancer epidemiology study. The greatest quintile consumed approximately 200 mg of CLA/d, and the least quintile consumed only 70 mg/d (Aro et al., 2000). Ritzenthaler et al. (2001) concluded that FD and FFQ methodologies underestimated CLA intake compared with measured intakes (mean values of 212 mg/d for men and 151 mg CLA/d for women) in a northwest U.S. population. German students reported 246 and 323 mg of CLA/d using FFQ and 7-d FD, respectively (Fremann et al., 2002). In addition, those researchers found plasma phospholipid CLA content and plasma TG CLA content only useful as short-term (daily) and

medium-term (weekly) biomarkers of CLA intake.

Information on the CLA content of human foods has become more common, but often the data were expressed as milligrams of CLA per gram of fat as an analytical convenience (Chin et al., 1992; Rule et al., 2002). The work of Fogerty et al. (1988), Ma et al. (1999), and Zlatanos et al. (2002) includes CLA expressions as milligrams per 100 g of sample or milligrams per serving, a more convenient form for human dietetic purposes.

From the Australian work of Fogerty et al. (1988), it was clear that, under normal feeding conditions, the *c*-9, *t*-11 CLA content of monogastric food products (pork and chicken) is less than that of ruminant products (lamb, beef, and dairy) when expressed as a fraction of the fat. In work from the U.S., Shantha et al. (1994) reported data for steaks and ground beef (cooked and uncooked) that were in agreement with Fogerty et al. (1988), where *c*-9, *t*-11 CLA was 0.23 to 1.25% in the fat, respectively. However, U.S. ground beef delivered 105 to 152 mg of total CLA, and the Australian beef cuts only contained 6 to 43 mg of CLA/100 g of serving, most likely because of fat content variations.

Zlatanos et al. (2002) reported *c*-9, *t*-11 CLA values for sheep and goat cheeses. All have minimum content near 0.4 to 0.5% CLA in the fat, but upper ranges vary according to cheese type (hard or soft) and aging (source was always confounded). Values as great as 1.9% CLA in the fat are common place, delivering 60 to 340 mg of CLA from feta-type cheeses and 130 to 560 mg of CLA from hard (aged) cheeses per 100-g portions.

Ma et al. (1999) reported CLA values on 20 dairy products and five cuts of beef (cooked and uncooked). For the dairy products, all were in close agreement with minimum values of Zlatanos et al. (2002) expressed as a percentage in the fat, thus delivering only approximately 100 mg of CLA/100 g for a typical cheese. Milk, whether whole, 1%, or 2% fat, deliverd 10 to 25 mg/250-mL (8-oz) serving. Yogurt and cottage cheese contained 43 and 27 mg of CLA per serving (175 and 150 mL), respectively. Cream cheeses, sour, and processed cheeses contained only 10 mg of CLA in a 15-mL serving. Butter, with its greater fat content, delivered 65 mg (in a 15-g serving).

Shantha et al. (1994) and Ma et al. (1999) are in agreement that no uniform influence on CLA content existed as a result of beef preparation when expressed as percentage in the fat. Shantha et al. (1994) pointed out, however, that cooking does affect final fat content and yield (because of moisture loss) and, therefore, CLA content per serving. High temperature baking (80°C) of ground beef delivered the most CLA per 100-g serving (152 mg).

Chin et al. (1992) was in general agreement with the dairy and meat values cited here (expressed as a percentage in the fat). However, the Canadian beef values of Ma et al. (1999) have very low unexplained CLA values compared with the other references cited. Surprisingly, elk meat CLA (range fed) was low, 0.10 to 0.19% CLA in the fat, and intermediate between chicken breast and other ruminant values (Rule et al., 2002).

CLA Manipulation in Ruminant Food Products. From an anthropological view, the advent of agriculture and the domestication of animals have led to dramatic changes in the lipid profile of human diets (Cordain et al., 2002). Both the choice of species eaten and their precursor diets impact the sensory appeal, biochemical composition, nutritional value, and implications for health (Geay et al., 2001; Moloney et al., 2001). This change was readily apparent in the CLA content of milks from confinement-fed *vs* grazing dairy cattle. Loor et al. (2002) moved dairy cattle from total mixed ration preliminary diets to three supplemented grazing treatments (cool-season grasses and clover mixed pastures) supplemented with 6.7 kg/d of concentrates high in soybean meal. Milk *c*-9, *t*-11 CLA increased from 0.3 to 0.88, 1.14 and 1.26% in milk fat of the grazing cows. Molkentin (2000) reviewed the data of several studies, and the evidence suggests a clear doubling or more in CLA expressed as a percentage in milk fat when cows move from confinement to grazing diets. A study from the U.S. Southeast also supported this difference. Where cows grazed a warm-season grass, CLA content in milk fat was 0.65% *vs* 0.36% for a typical total mixed ration based on alfalfa and corn silage (White et al., 2001). Milk and butter values from grazing European cows, however, were more commonly near 1.0% CLA in milk fat (Molkentin, 2000).

Dairy diets with marine oils also are known to increase milk CLA (Jones et al., 2000). Although 2% fat added as menhaden fish oil reduces overall milk and milk fat yields, CLA values can be driven as great as 2.0% in milk fat (Whitloock et al., 2002). Lesser levels of fish oil blended with extruded soybeans (Whitlock et al., 2002) or with tallow (Jones et al., 2000) helped counter negative effects. Extruded soybean blends with fishmeal also boosted milk fat CLA to 1.59% (Abu-Ghazaleh et al., 2002). Ward et al. (2002) conducted experiments with vegetable oils (canola and flaxseed) compared with a control diet of 2% fishmeal. All diet treatments resulted in milk fat CLA values >1.2%.

Direct infusions of *c*-9, *t*-11 CLA into the abomasum of dairy cows (10 g/d) influenced milk fat *c*-9, *t*-11 CLA (0.88% *vs* 0.49% for controls). However, *t*-10, *c*-12 CLA infusions did not influence *c*-9, *t*-11 CLA, only the *t*-10, *c*-12 CLA content (0.39% *vs* <0.01% for controls) (Baumgard et al., 2000). Feeding up to 100 g/d of mixed CLA from an enriched source gives the composite result of the previously cited treatments, milk fat *c*-9, *t*-11 CLA increased to 0.6% *vs* 0.49% for controls, and *t*-10, *c*-12 CLA increased to 0.13% *vs* 0.03% for

controls (Giesy et al., 2002). Boylston and Beitz (2002) fed soy oil (0 or 5%) and mixed isomer CLA (0%, 1% as acid, or 1% as salt) to dairy cattle in a 2 × 3 factorial experiment. The resulting yogurt product neutral lipid fractions were enriched in response to both treatments, but as much as 200 g/d of dietary CLA did not move yogurt fat CLA content beyond 0.71%. Isolated phospholipid fraction CLA contents were only slightly more CLA-enriched. This direct CLA supplementation approach was quite an inefficient process (<5% efficiency) in dairy cattle, most likely because of biohydrogenation loss in the rumen.

Rule et al. (2002) contrasted range and feedlot environmental differences. The CLA content of feedlot beef and bison was less than range-fed animals; the differences were greatest for the beef supraspinatus muscle (0.31% *vs* 0.52% CLA in the fat for feedlot *vs* range-fed, respectively). In a more controlled experimental setting, French et al. (2000) obtained a more dramatic effect as grass DMI increased from 1 kg/d to 100% of intake while concentrates were reduced from 8 to 0 kg/d. The CLA content in fat rose from 0.37 to 1.08% (longissimus).

Madron et al. (2002) reported finishing crossbred Angus steers at 111 d with extruded full-fat soybeans (**ESB**). Dietary fat levels were 3.9, 5.8, and 7.8% for the control, 12% ESB, and 25.6% ESB diets, respectively. The *c*-9, *t*-11 CLA concentrations in fatty acids for three cuts of meat (longissimus, round, and chuck) were averaged and not reported separately. The means, averaged across all three cuts, were 0.66, 0.69, and 0.77% for the three diets, respectively (the later was statistically different, $P<0.05$). This report was in contrast to that of Beaulieu et al. (2002), who fed steers 5% soybean oil in a finishing experiment for 102 d. Meat *c*-9, *t*-11 CLA values were 0.32 to 0.35% of fat and not different from controls.

Bolte et al. (2002) fed lambs from weaning to slaughter (120 to 150 d) with diets that contained 5% supplemental fat from high oleic acid (18:1 *c*-9) safflower and normal (high linoleic 18:2 *c*-9, *c*-12) safflower oil. Several different adipose depots and muscles were sampled, and all showed dietary treatment responses. The muscle lipid *c*-9, *t*-11 CLA concentration was greatest for the linoleic (0.87%), followed by oleic (0.57%), and then the control dietary treatments (0.34%). The same pattern held for adipose tissues but at slightly greater CLA. Clearly time on treatment diets made up a significant portion of the lambs' life span, allowing for maximum effects not as likely to occur in beef finishing regimes. These studies have shown that CLA was clearly maniputable in the lipid products of ruminants. High forage diets and vegetable and marine oils have a clear impact on dairy product CLA, but time on feed and choice of meat cut are also important variables for beef and lamb products.

Ruminal CLA and Post-Absorptive Synthesis. Kepler and Tove (1967) described the formation of a conjugated diene (18:2 *c*-9, *t*-11 CLA) and a conjugated triene as intermediates in the biohydrogenation pathway of linolate and linolenate (18:3 delta 9,12,15) respectively, by the anaerobe Butyrivibrio fibrisolvens (strain A38). They established that the conjugated triene was further hydrogenated to a non-conjugated diene and eventually to the monoene 18:1 t11 (vaccenic acid) as was the *c*-9, *t*-11 CLA. The kinetics of the required delta-12-*c*, delta-11-*t* isomerase was described, and its pH optimum was established between 7.0 and 7.2 after isolation. Kim et al. (2000) further investigated this same strain and established its sensitivity to linoleic acid and CLA concentrations and the linkage of the isomerization and reduction steps in linoleic acid biohydrogenation. The CLA would only accumulate and persist when biohydrogenation was interrupted (by aerobic incubation), leading the authors to conclude that anaerobe inactivation or death is needed for a productive flow of CLA from the rumen.

In contrast, the same lab (Kim et al., 2002) identified strains of Megasphaera elsdenii capable of significant *t*-10, *c*-12 CLA production. The strain was isolated from enriched mixed ruminal microbes harvested from a grain-fed cow and, thus, suggests a probable source of *t*-10, *c*-12 CLA now viewed as responsible for milk fat depression of dairy cattle fed high grain diets (Peterson et al., 2002).

Work measuring duodenal flows of fatty acids from the rumen suggested a shortfall in CLA yield compared with the secretory yield in milk by the mammary gland (Kucuk et al., 2001; Piperova et al., 2002). This follows from the work of Griinari et al. (2000), who reported that abomasal infusions of 18:1 *t*-11 increased CLA in milk fat by 31%. In addition, infusions of sterculic oil (an inhibitor of delta-9 desaturase) decreased CLA in milk fat 45% and altered other delta-9 desaturase-dependent milk components. Griinari et al. (2000) hypothesized that 18:1 *t*-11, another ruminal biohydrogenation intermediate, in conjunction with an endogenous delta-9 desaturase, was the primary source and accounted for over 60% of milk fat CLA.

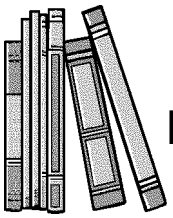
The previously mentioned evidence combined gives meaning to the high correlation between milk fat *c*-9, *t*-11 CLA and the 18:1 *t*-11 isomer (Chilliard et al., 2000, 2001). This correlation was also noted when measured in beef (Madron et al., 2002). Although Mosley et al. (2002) has shown that oleic acid can be a precursor for mixed rumen microbes to generate *trans* 18:1 fatty acids, the evidence of Bolte et al. (2002) suggested that it was likely a less efficient source than linoleic. It seems evident now that any attempt to drive up CLA content of ruminant products using dietary unsaturated fats is accompanied by an associated increase in *trans* fats.

Trans fats like CLA are not a single entity. As a group however, *trans* fats are quite negatively perceived.

Epidemiological evidence exists for their association with increased coronary heart disease, clinical evidence of elevated blood lipid profiles [apolipoprotein (a), TG, and low density lipoprotein-cholesterol], lessened plasma high density lipoprotein-cholesterol (good cholesterol), interference with essential fatty acid metabolism, and the promotion of insulin resistance in humans (Hu et al., 2001). Clearly, health risk information about specific *trans* isomers such as 18:1 *t*-11 is needed if CLA manipulation in animal food products becomes a marketable production process. Turpeinen et al. (2002) fed healthy humans 1.5, 3.0, or 4.5 g of vaccenic acid (18:1 *t*-11)/d for 9 d and measured serum fatty acid fractions of both vaccenic and rumenic acid (*c*-9, *t*-11 CLA). A positive linear response in plasma CLA was evidence for human delta-9 desaturase conversion of the vaccenic acid precursor and must be considered in the future when computing the benefits of ruminant-based or various other CLA-enriched food products.

Implications

Conjugated linoleic acids have been implicated as anti-carcinogenic, anti-atherosclerosis, and anti-inflammatory agents in a variety of experimental model systems, but the same evidence in humans is still limited. Prospective human study evidence will take more time. Evidence in humans suggested that the role of dietary mixed isomer CLA in body fat mass loss was only modest compared with the evidence from animal model studies. Urine metabolites of $PGF_{2\alpha}$, indicative of lipid oxidation stress, have been elevated during supplementation. The CLA was clearly maniputable in the lipid products of ruminants. High forage diets and vegetable and marine oils have clear impacts on dairy product *c*-9, *t*-11 CLA, but time on feed and choice of meat cut are also important variables for beef and lamb products. In addition, the fatty acid C18:1 *t*-11 (vaccenic) is now believed to be the principle precursor of endogenous *c*-9, *t*-11 CLA in both ruminants and monogastrics. This relationship requires a re-examination of human foods for vaccenic acid content and quantitative measures of CLA endogenous synthesis in humans as well to formulate dietary strategies to capture CLA's potential protective health benefits.



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